Introduction

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MS channels of bacteria

Mechanosensitive (MS) ion channels commonly play a role as transducers, converting mechanical stimuli into electrical or chemical signaling, thus allowing the cell to regulate its behavior in response to changing environment conditions. MS channels participate in sensation of orientation and sound in inner ear (hair cells (Hackney and Furness, 1995; Liang et al., 2003)), in osmoregulation of bacteria (Martinac et al., 1987; Sukharev et al., 1993; Sukharev et al., 1997) or in touch sensation (Chalfie et al., 1985; Goodman and Schwarz, 2003). This wide range of functional and environmental requirements leads to a greater structural diversity of MS ion channels (Sukharev and Corey, 2004) as compared to ligand or voltage gated channels which are commonly considered to have evolved from common ancestors (Cockcroft et al., 1995; Ortells and Lunt, 1995).

Originally, bacterial MS channels were classified by their conductance. The three principal families are mechanosensitive channels of large (MscL, ~3 nS), small (MscS ~1 nS) and mini (MscM $\leq$ 0.3 nS) conductance. The channels are gated primarily by the tension of the membrane; however, MscS homologs are also regulated by voltage (Martinac et al., 1987). Tension sensitivity of the channels correlates with their conductance, allowing the bacteria to regulate their response appropriately to the severity of the osmotic shock.

Of these families, MscL and MscS channels have received the most attention. Early identification of their genes and availability of the crystal structure prompted a range of studies of mechanosensation in bacteria: biochemical, electrophysiological and computational.

Comparatively little is known about the third family of bacterial MS channels, MscM. As for other mechanosensitive channels, currents with a corresponding conductance of 100-350 pS can be activated by hypoosmotic solution or application of suction to the patch pipette and reversibly

inhibited by Gd^{3+}. These currents can be observed in triple-null (MscL, *YggB/KefA*) mutants or in fractions of inner bacterial membrane reconstituted into artificial liposomes (Berrier et al., 1989; Berrier et al., 1996), thus ruling out the possibility to attribute these observations to a substate activity of MscS or MscL ion channels.

MscL channel family

After the early pioneering studies of mechanosensitive activity in *E. coli* (Martinac et al., 1987; Zoratti and Petronilli, 1988), the first MS channel to be cloned and successfully reconstituted in artificial liposomes, while preserving its function, was the MscL channel of *Escherichia coli* (Sukharev et al., 1993). Cloning and reconstitution has opened the possibility for molecular biological and electrophysiological characterization of bacterial MS channels. Later, the crystal structure of the *M. tuberculosis* MscL homolog was determined at the resolution of 3.5 Å (Chang et al., 1998).

M. tuberculosis MscL is a homopentameric channel. Each MscL subunit consists of 151 amino acid residues (136 for *E. coli* MscL) and has two transmembrane domains, one extracellular loop and a C-terminal cytoplasmic α–helix (Figure 1). The channels have 3 nS conductance at physiological salt concentrations (300 mM) and a correspondingly large estimated open state pore diameter of ~ 30 Å (Sukharev et al., 1997; Sukharev et al., 1999; Sukharev et al., 2001a). Interestingly, despite such large conductance and open pore diameter, these channels are smaller than most channel proteins. Nonetheless, formation of such a large pore provides opportunities for side chain and domain arrangements with the channel partially conducting, observed as multiple conductive substates, a feature rarely observed among mammallian ion channels. The observed number of MscL substates increased as more electrophysiological data became available; from 5

(Sukharev et al., 1999) to 7 (Sukharev et al., 2001a) and finally 9 (Chiang et al., 2004) in the latest analysis of *E. coli* MscL activity in spheroplasts. Furthermore, the author suggests (Chapter 5) that in order to describe observed single-channel kinetics of MscL channels it may be advantageous to apply schemes that emphasize continuum models (Levitt, 1989) over regular Markovian discrete-state approach.

MscL channels are only known to be gated by the tension of the membrane, however occupancy of the observed substates is, in addition, modulated by voltage applied across the membrane (Chiang et al., 2004). The *E. coli* MscL activation threshold is 11.8 ± 0.8 dyn/cm (Sukharev et al., 1999). *M. tuberculosis* MscL gates at higher tension, nearing the rupture threshold of the plasma membrane, complicating successful acquisition of the activity of this channel. Nonetheless, its tension sensitivity has been measured, albeit with less precision, yielding an activation threshold ~ 2x higher than that of the *E. coli* MscL ion channel (Moe et al., 2000).

Figure 1 shows a structure of MscL channel in a ribbon representation as well as a molecular surface rendering. TM1 helices in the core of the transmembrane bundle make up the main gate of the channel. The pattern of regularly placed glycine residues in the TM1 segments permits tight packing of the five central helices, which form a narrow (~4 Å diameter) hydrophobic constriction predicted to be completely dehydrated in the closed state (Gullingsrud and Schulten, 2003). Hydrophobic TM2 helices on the periphery of the barrel face the lipid molecules (Figure 1B,C).

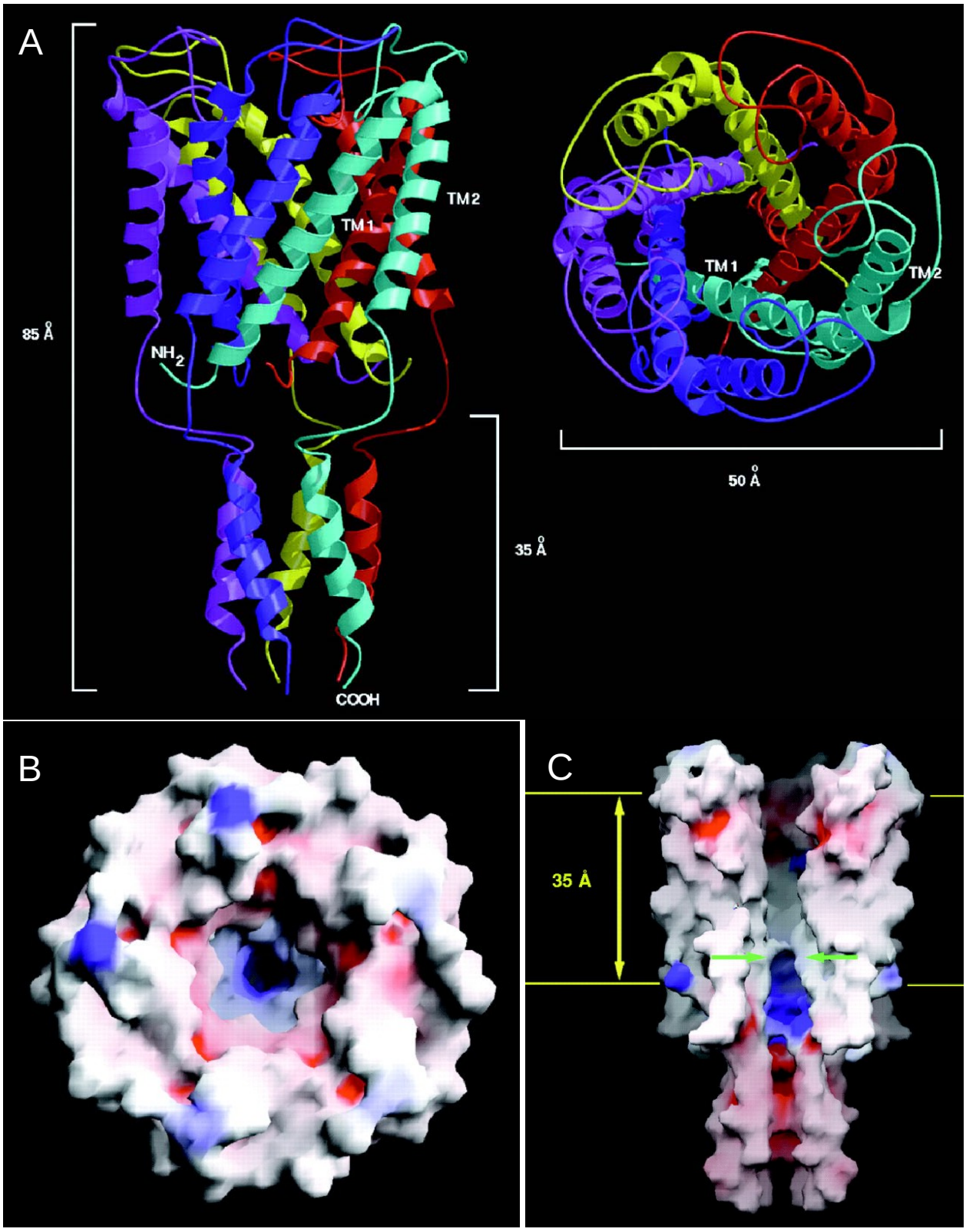

Figure 1 Structure of *M. tuberculosis* MscL ion channel (adapted from (Chang et al., 1998)). **A**. Side (left) and top (extracellular, right) view of a ribbon representation of the structure. The subunits in the channel are individually colored. **B**. and **C**. Molecular surface rendering of the channel viewed (B) from the extracellular side of the membrane and (C) a cut-away side view. The surface coloring varies continuously from blue in more basic regions to red in more acidic areas.

Multiple mutagenesis studies of *M. tuberculosis* and *E. coli* MscL proteins were performed. In particular the lack of endogenous cysteine residues allowed experimenters to apply one of the conventional manipulations in sampling protein structure, cysteine cross-linking experiments. Some of these studies (Sukharev et al., 2001a) suggest that the N-terminal region of ~ 10 residues, not resolved in the crystal structure (further on denoted S1), comprises a short α-helical structure and that these S1 domains bundle together to form an additional cytoplasmic gate (Sukharev et al., 2001b). However others (Maurer and Dougherty, 2003) report that the S1 region is largely insensitive to mutations. Additional cross-linking data (Betanzos et al., 2002) suggests that the transmembrane helices do not change their relative positions substantially during gating, although some rotations and 'scissoring' motions have been predicted by mutagenesis (Ou et al., 1998), molecular dynamics (Gullingsrud and Schulten, 2003) and electron paramagnetic resonance (EPR) studies (Perozo et al., 2002a).

Screening of a large number of generated mutants required developing an assay that would surpass a relatively low throughput of electrophysiological recordings. Maurer and Dougherty, 2001 developed a fluorescence-based screen for bacterial mechanosensitive ion-channel activity. This technique was then used to generate and characterize a large library of mutations of *E. coli* MscL protein (Maurer and Dougherty, 2003). Evaluation of this library has, somewhat unexpectedly, shown that loss of function (LOF) mutants are more common than gain of function (GOF) mutants, at 29% LOF vs. 7% GOF and 64% wild type (WT) of the total of 408 characterized. Other features emphasized by this study are the unimportance of salt bridges, elsewhere postulated necessary to stabilize ion channel structure (Sukharev et al., 2001b), identification of additional LOF and GOF mutants in the loop region, supporting the hypothesis of a spring-like action of the loop (Blount et al., 1996; Ajouz et al., 2000) and, finally, clustering of LOF mutations near the ends of TM helices in the regions facing lipid headgroups of the native lipid environment of the channel.

An alternative approach to studying MscL channel gating was employed in (Perozo et al., 2002b; Perozo et al., 2002c). A combination of patch clamping and EPR spectroscopy was used in order to investigate the effect of various lipid compositions on the gating of MscL ion channel. Wild type *E. coli* MscL was reconstituted in synthetic phosphatidylcholine (POPC) containing monosaturated chains of 14, 16, 18, 20 and 22 carbons. The authors found that POPC22 inhibited the channel, while shorter acyl sidechains lead to correspondingly reduced activation thresholds, supporting the models that propose a significant tilt of TM regions towards the plane of the bilayer in open state of the channel (Gullingsrud et al., 2001; Sukharev et al., 2001a; Sukharev et al., 2001b). A hydrophobic mismatch caused by such acyl sidechain shortening could not, by itself, induce spontaneous channel gating. However changes in membrane intrinsic curvature induced by the addition of lysophosphatidylcholine generated massive spectroscopic changes in the narrow constriction that forms the channel gate, trapping the channel in the open state.

Various molecular dynamics (MD) simulation studies have also addressed MscL channel dynamics. Thus Elmore and Dougherty (2003) confirm the importance of hydrophobic matching between MscL protein and the lipid membrane. In addition, varying the headgroups of the lipid box surrounding the channel (switching between POPC and POPE) leads to conformational differences in the C-terminal region of MscL. Furthermore Elmore and Dougherty (2001) point out that while the C-terminal helix bundle structure is unstable at physiological pH, it may have been stabilized under low pH conditions during the crystallization of the channel. Also comparision of trjectories of WT and Q51E GOF mutant led to observable changes in intersubunit interactions in the mutant. The initial phases of MscL opening have been investigated as well (Colombo et al., 2003). However, in order to observe the effect on 1 ns timescale, authors had to resort to application of unphysiologically large forces, corresponding to the pressures of 100 to 1000 bar.

MscS channel family

The activity of MscS channels, originally identified in *E. coli* spheroplasts (Martinac et al., 1987), is the result of gating of two similar proteins that originate from related gene products, *YggB* and *KefA* (Levina et al., 1999; McLaggan et al., 2002). Knock-out experiments have shown that *YggB* is responsible for most of the observed MscS activity. The *KefA* gene, on the other hand, encodes a potassium-regulated mechanosensitive channel (MscK) that does not appear to inactivate, but tends to be observed less frequently (thus null *KefA* mutants have no obvious phenotype, (Li et al., 2002)). The sequence of the last three TM segments of *KefA* is similar to that of *YggB* gene, suggesting that this is the most likely pore-forming region in both proteins.

Recently, the crystal structure of *E. coli* MscS channel was solved with a resolution of 3.9 Å (Bass et al., 2002). The channel is a homoheptamer with a large cytoplasmic region (Figure 2). Compared to MscL channels, MscS is a much bulkier protein. In overall dimensions, MscS extends for ~120 Å parallel to the sevenfold axis, with the membrane and extramembrane domains accounting for ~50 Å and ~70 Å, respectively, while the width in the perpendicular direction spans ~80 Å. The pore is lined by the TM3 helices of each subunit which are packed together with crossing angles of 22° to form a right-handed bundle. Common to *M. tuberculosis* MscL is the presence of conserved glycines in the pore-constriction region (at positions 101, 104, and 108), which allows adjacent TM3 helices to interact tightly, with an interhelical distance of ~8 Å. As a consequence, the permeation pathway resembles a tapered cylinder, with a diameter of ~11 Å at the constriction point.

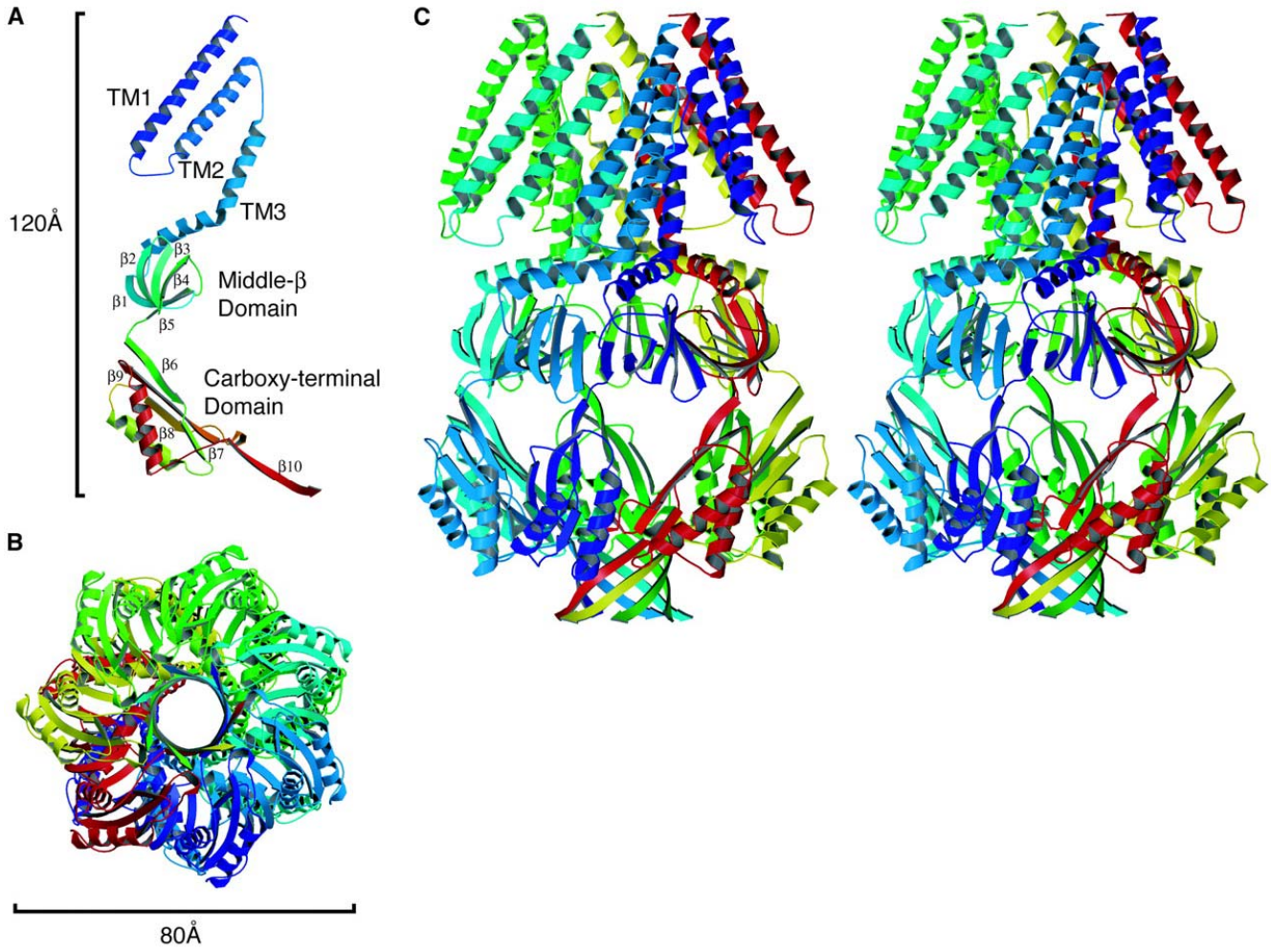

Figure 2. Ribbon diagrams of *E. coli* MscS (adapted from (Bass et al., 2002)). **A** The polypeptide fold of a MscS subunit, viewed perpendicular to the sevenfold axis, with a rainbow gradient coloring scheme from the NH_2- terminus (blue) to the COOH-terminus (red). **B** The MscS heptamer viewed down the sevenfold axis, looking into the permeation pathway from outside the cell. **C** Stereo side view of the MscS heptamer, viewed from the same direction as in A, with each subunit represented in a separate color.

The activity of the MscS ion channel, in addition to tension sensitivity, is regulated by voltage (Martinac et al., 1987). The channel also shows slight anionic selectivity and rectification (Martinac et al., 1987; Sukharev, 2002), corresponding to a range of single channel conductaces of 0.9 – 1.2 pS. *E. coli* MscS has higher sensitivity to membrane tension than the MscL channel. Combining electrophysiology with optical measurements of patch curvature in the pipette made it possible to establish activaton threshold at 5.5 ± 0.1 dyne/cm and gave estimates for the energy of opening $\Delta G = 11.4 \pm 0.5\ kT$, and the transition-related area change $\Delta A = 8.4 \pm 0.4\ \text{nm}^2$ (Sukharev, 2002).

The large pore diameter observed reported in the crystal structure poses an interesting question of whether the solved structure represents closed, open or intermediary state of the channel. According to recent simulations, the hydrophobic pore with such diameter should be partially water permeable (Sansom et al., 2002; Beckstein et al., 2003), supporting the claim in (Bass et al., 2002) that crystal structure represents an open state of the channel. On the other hand, (Anishkin and Sukharev, 2004) report that MD simulations of MscS filled with flexible TIP3P water shows that the narrow hydrophobic constriction site remains predominantly empty, showing only intermittent vapor-liquid transitions. Substitution of the polar residue in the GOF L109S mutation in the constriction results in stable hydration of the pore.

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