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Review

Bacterial mechanosensitive channels: Experiment and theory

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Abstract

Since their discovery in *Escherichia coli* some 20 years ago, studies of bacterial mechanosensitive (MS) ion channels have been at the forefront of the MS channel research field. Two major events greatly advanced the research on bacterial MS channels: (i) cloning of MscL and MscS, the MS channels of Large and Small conductance, and (ii) solving their 3D crystal structure. These events enabled further experimental studies employing EPR and FRET spectroscopy in addition to patch clamp and molecular biological techniques that have successfully been used in characterization of the structure and function of bacterial MS channels. In parallel with the experimental studies computational modelling has been applied to elucidate the molecular dynamics of MscL and MscS, which has significantly contributed to our understanding of basic physical principles of the mechanosensory transduction in living organisms.

Keywords: MS channels; Patch clamp; Bilayer model; Mechanosensory transduction; EPR spectroscopy; FRET; molecular dynamics; Brownian dynamics

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1. Introduction

Studies of MS channels in bacteria [1–4] were facilitated by the advent of the patch-clamp technique [5], which has removed the constraints of being able to study electrophysiologically only cells that are large enough to be impaled with glass microelectrodes. MS channels are indispensable for survival of a bacterial cell when the external environment becomes hypo-osmotic relative to the cell interior causing an increase in cellular turgor sufficient to lyse the microbe [6]. MscL and MscS, two of the several types of MS channels existing in

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bacteria, have been well characterized at the structural and functional level in particular. The 3D structure of both channels has been solved by X-ray crystallography [7,8] just a few years after they have been cloned [6,9]. Their structural dynamics has recently been studied by electron paramagnetic resonance (EPR) [10,11] as well as fluorescence resonance energy transfer (FRET) spectroscopy [12]. These experimental studies have been aided by computational modelling studies employing molecular dynamics [13–16] as well as Brownian dynamics [17] providing a wealth of information on structural determinants of the gating and conduction properties of the two channels. MS channels are also starting to find applications in nanotechnology, with recent reports of a modified MscL protein acting as a nanovalve that could be opened and closed upon illumination of ultraviolet and visible light and/or pH [18,19]. In this review we have summarized the key experimental and theoretical findings on the structure and function of the MscL and MscS channels. Interested readers may find information on bacterial MS channels not covered here in a number of recently published reviews [20–27].

2. Experiment

2.1. Discovery, cloning and structure

Despite the fact that the patch-clamp technique [5] has allowed electrophysiologists to examine cells of almost any size, patch clamping bacterial cells presented a difficult technical challenge. The examination of a bacterial cell membrane by the patch clamp technique became possible some 20 years ago with development of a "giant spheroplast" preparation in *Escherichia coli* [28], which has led to a discovery of MS channels in these microbes (Fig. 1) [29]. This technical advance opened a window of opportunities for structure and function studies of this class of membrane proteins. Given that bacteria can be grown in large quantities delivering the milligram amounts of channel proteins required for structural studies, the advantage of using these microorganisms for structure and function studies in MS channels is obvious. In fact, 3-dimensional (3D) structures of MscL from *Mycobacterium tuberculosis* [7] and MscS from *E. coli* [8] were solved by X-ray crystallography just a few years after their identification and cloning in *E. coli* (Fig. 2) [6,9].

Different strategies were applied for the cloning of MscL and MscS. Kung and coworkers [9,30] used an unusual strategy for cloning of the *mscL* gene. The strategy involved detergent solubilization and fractionation of *E. coli* membrane constituents by column chromatography in combination with functional examination of the individual fractions for MS channel activity by patch clamp [31]. This approach took advantage of the fact that mechanosensitivity of MscL is fully preserved upon the reconstitution of the channel into artificial liposomes. Once the protein fraction containing MscL activity was identified the cloning of the *mscL* gene encoding the *E. coli* MscL protein (Eco-MscL) comprising 136 amino acid residues became possible [9,30]. The expression of the *mscL* gene in a heterologous as well as in vitro transcription/translation system has demonstrated that this gene alone is necessary and sufficient for the MscL activity.

Although functionally described first, MscS was cloned several years after MscL [6,32]. Booth and coworkers identified two genes, *yggB* and *kefA*, on the *E. coli* chromosome, whose

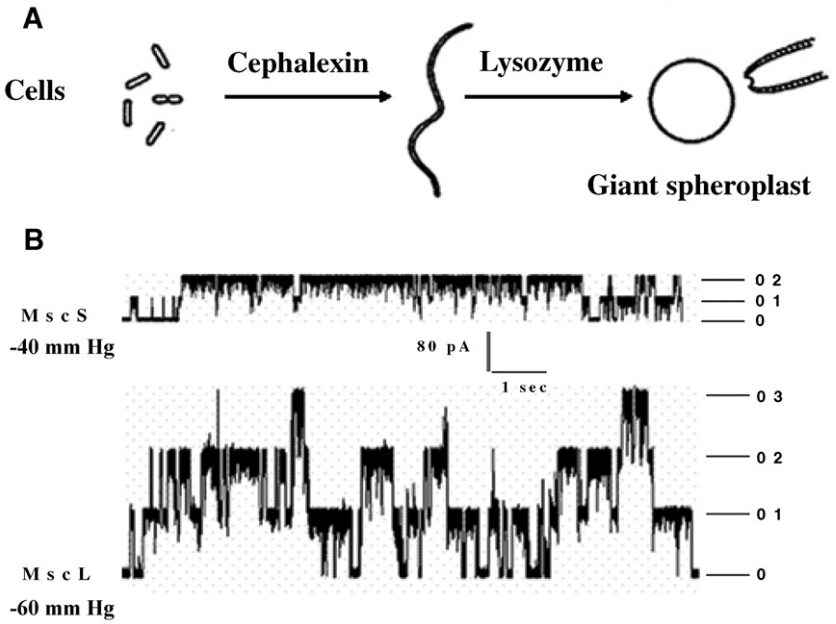

Fig. 1. MS channels of *E. coli*. (A) A bacterial cell in the presence of the antibiotic cephalexin fails to septate and grows into a long filament. When treated with EDTA and lysozyme the filament transforms into a giant spheroplast of 5–10 (m in diameter, which can be examined for channel activity by the patch clamp (reproduced from [97]). (B) Current traces of MscS and MscL recorded at +40 mV pipette voltage. Channels opened upon suction (indicated in mm Hg on the left of each current trace) applied to the patch-clamp pipette (adapted from [98]). Note: 1 mm Hg=133 Pa.

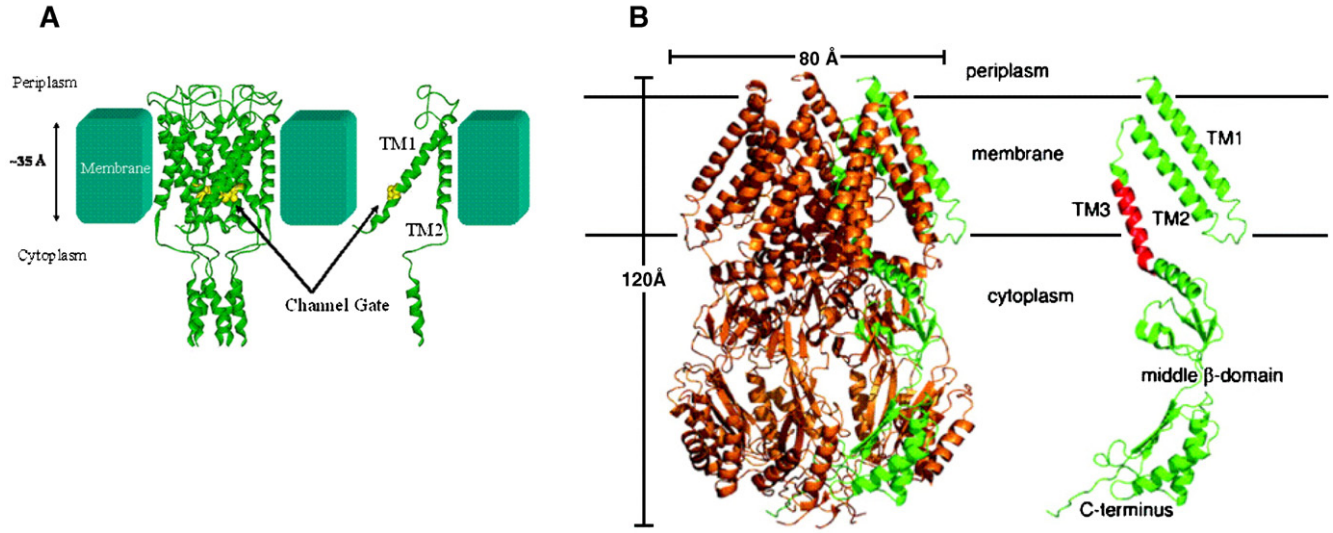

Fig. 2. 3D crystal structure of MscL and MscS. (A) MscL channel homopentamer (left) and a channel monomer (right) from *M. tuberculosis* [7]. The crystal structure is most likely representing a closed channel. The thickness of the membrane bilayer (shown as solid blocks) is approximately 3.5 nm. The channel gate is formed by a group of amino acids at the cytoplasmic end of the TM1 transmembrane domain (modified from [99]). Figure based on model 1MSL in Protein Data Bank (http://www.rcsb.org/pdb). (B) The crystal structure at 3.9 Å resolution of MscS from *E. coli* showing the channel homoheptamer (left) and a monomer (right) [8] viewed by PyMol19. Residues 27 to 280 were resolved. Secondary structural domains and the position of the TM3 transmembrane helix are indicated in the diagram of the monomer. A conserved structural motif of glycine and alanine residues in the pore-lining transmembrane helix TM3 essential for MscS gating is highlighted in red (reproduced from [100]).

deletion led to the abolishment of the activity of the MS channels of small conductance [6]. Mutants that lacked both proteins, KefA and YggB, showed no activity of the small conductance MS channel but retained MscL activity. Mutants lacking MscS and MscL are severely compromised in their survival of osmotic downshock. Levina et al. [6] thus demonstrated for the first time a physiologically important cell phenotype associated with loss of mechanosensitive channel activity. Originally indistinguishable in the patch clamp experiments [29], the MS channels of small conductance were thus identified as two different channels: MscS, encoded by the *yggB* gene and MscK (a potassium dependent MS channel) [33] encoded by the *kefA* gene. A functional property that distinguishes MscS from MscK is a rapid inactivation of MscS upon sustained application of pressure [34]. MscS is a small membrane protein of 286 amino acids whereas MscK is a much larger, multi-domain membrane protein comprised of 1120 amino acid residues. A common structural feature shared between the two channels is the primary amino acid sequence of MscS, which closely resembles the sequence of the last two domains of the MscK protein.

Rees and coworkers determined the 3D crystal structure of MscL by X-ray crystallography [7] just 4 years after the cloning of this MS channel [9]. A few years later they also determined the 3D structure of MscS [8], which clearly demonstrated the advantage of using bacteria for structure and function studies of membrane proteins. 3D oligomeric structure of the MscL homologue from *M. tuberculosis* (Tb-MscL) was obtained at 3.5 Å resolution showing a homopentameric channel in a closed state. The channel monomer is composed of two α-helical transmembrane (TM) domains, TM1 and TM2, cytoplasmic N- and C-terminal domains and a central periplasmic domain (Fig. 2A). The five transmembrane TM1 helices of the channel form a tightly packed bundle funnelling to a hydrophobic constriction 2 Å wide at the cytoplasmic side of the pore functioning as the channel gate. During gating the diameter of the hydrophobic gate varies between 2 and 30 Å (Fig. 3A) [11,35]. The overall change in diameter of the channel pentamer between its closed and open configurations was determined to be approximately 16 Å based on a FRET spectroscopic analysis (Fig. 3B) [12]. Thus, during opening the MscL channel is undergoing one of the largest conformational changes known in membrane proteins. The short N-terminal domain comprising approximately a dozen of amino acids has been proposed to function as a second channel gate working in accord with the hydrophobic gate formed by the TM1 helix bundle [36,37]. From N-terminal amino acid deletion and substitution studies it is known that structural changes in the N-terminus severely affect MscL function [38,39]. Since the secondary structure of the N-terminal domain remains unresolved at present the precise role of the N-terminus for the MscL gating still awaits to be determined experimentally.

The TM1 and TM2 helices of MscL are connected by a periplasmic loop, which like the N-terminus is structurally not well defined. The periplasmic loop (shown at the top of Fig 2A) has been envisaged to function as a spring resisting the channel opening [40]. This notion has been supported by a molecular dynamics study of MscL embedded in a curved bilayer [41]. According to this study the periplasmic loop is the first domain of MscL to change its structure leading to channel opening. The C -terminal domain forms an α-helical bundle [7] protruding into the cytoplasm. Its physiological role is unclear at present given the unorthodox crystallographic conditions (pH 3.5) at which the structure was obtained. Nevertheless, an

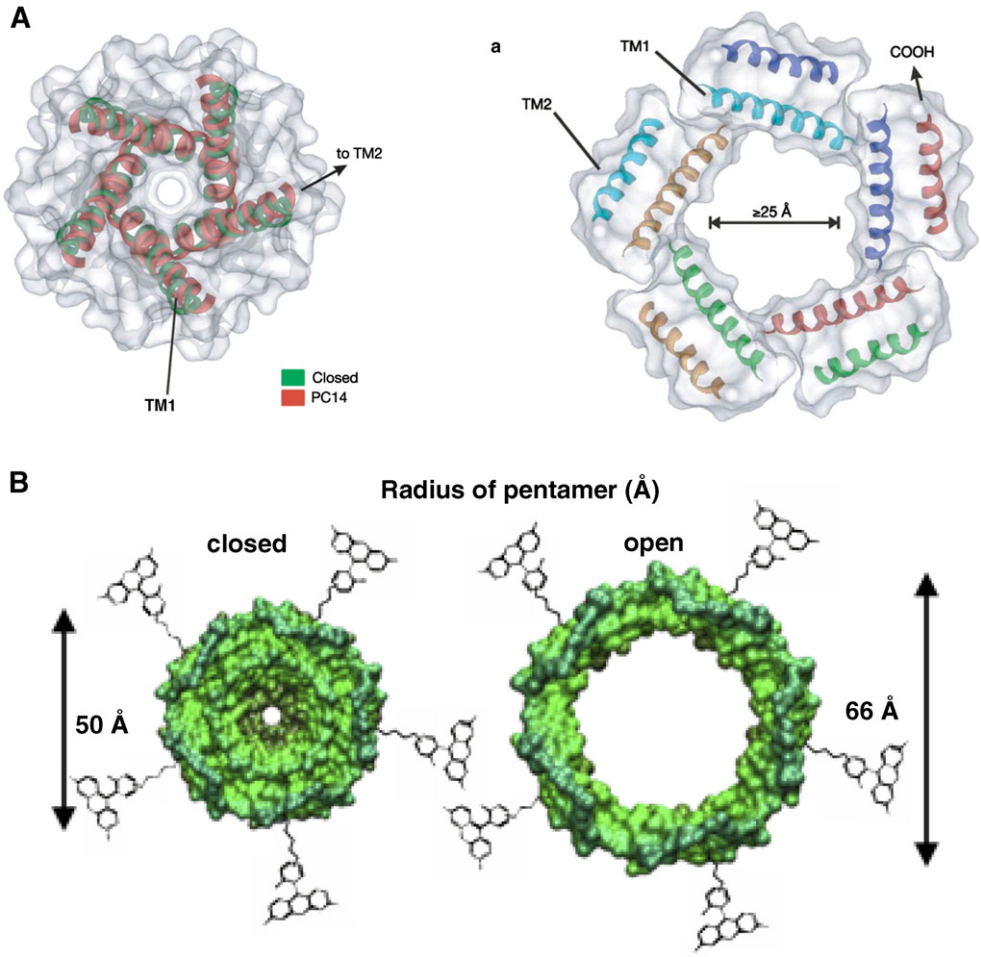

Fig. 3. Open channel structure of MscL. A diagram of a closed and open MscL channel indicating the scale of conformational change involved in channel gating based on (A) EPR spectroscopy and (B) a FRET spectroscopic study (adapted from [11,12]).

EPR spectroscopic study demonstrated that the C-terminus could also form an α-helical bundle under physiological conditions (pH 7.0) [42]. This result was further supported by two independent molecular dynamics studies [43,44]. Functionally, the C-terminal bundle seems to be the least important structural domain for channel gating, since a deletion of 27 C-terminal residues constituting a large portion of the C-terminus was shown not to significantly affect the mechanosensitivity of the channel [38–40]. A current view of the role of the C-terminus is that of a size-exclusion filter, which should prevent a loss of essential metabolites at the cytoplasmic side of the MscL channel pore [44]. To function as a filter the C-terminal domain is stably associated in both closed and open channel conformations. Based on a recent functional study, however, this model may have to be revised since the stability of the domain was shown to be pH dependent [45]. Consequently, the C-terminal domain may not only function as a size-exclusion filter, but may also influence the channel gating in a pH-dependent manner.

A 3D crystal structure of MscS of *E. coli* was obtained at 3.9 Å resolution [8]. The structure shows a channel folding as a homoheptamer having a large, cytoplasmic region (Fig. 2B). Each of the seven MscS subunits contains three transmembrane domains with N-termini facing the periplasm and C-termini facing the cytoplasm. TM3 helices line the channel pore whereas the TM1 and TM2 helices are thought to constitute the sensors for membrane tension and voltage [8,46]. Initially, the 3D structure of MscS was thought to depict an open channel [8]. However, as described in Section 3, simulation studies have cast doubt on this assertion.

The TM1 and TM2 transmembrane domains, which are in a direct contact with the surrounding lipids are thought to constitute the sensor for membrane tension as well as a voltage sensor because of the presence of three arginine residues in their structure [8,46]. Since the precise contribution of these charged residues to the channel voltage dependence is not known their role in the channel gating awaits to be established experimentally. A large C-terminal cytoplasmic domain is in contact with the cytoplasm through multiple openings. It is characterized by a large interior chamber of 40 Å in diameter. Not unlike the C-terminal domain of MscL the cytoplasmic domain of MscS may also function as a molecular sieve designed to exclude essential solutes from leaving bacterial cells during a hypo-osmotic shock.

2.2. Conductance, selectivity and voltage dependence

MscL and MscS differ not only in the pressure threshold required for the channel activation by membrane tension, which is approximately 1.5 higher for MscL compared to MscS, but also in terms of their conductance and ion selectivity. MscL is non-selective for both anions and cations. Its very large conductance of ~3 nS results from a very large open pore of ≥30 Å diameter [31,35]. The conductance of MscS of ~1 nS is about three times smaller than MscL. MscS shows a preference for anions over cations with a permeability ratio P_{Cl}/P_K 1.5–3.0 [29,31,47]. In addition, MscS rectifies such that in a symmetric solution its conductance is approximately one third bigger at positive (corresponding to hyperpolarizing membrane potentials) compared to that at negative pipette voltages (corresponding to depolarizing membrane potentials) [29,31]. MscK, the second MS channel of small conductance, seems to also have a preference for anions [33], although mutational analysis has suggested that the channel could be cation specific [48]. Another property that distinguishes MscK from MscS is its sensitivity to the extracellular ionic environment [33]. In addition, different from MscK, MscS exhibits inactivation upon sustained membrane tension [6].

MscS is voltage dependent, whereas MscL is not. The activity of MscS increases with membrane depolarization [29,47]. Voltage alone however, is not sufficient to activate the channel. It has been reported to remove the channel inactivation [49], which means that it is acting in synergy with the channel mechanosensitivity by exerting a modulatory effect on the response to membrane tension. Although one could imagine possible cellular scenarios during which changes in membrane potential would favour opening or closing of MscS for the benefit of the bacterial cell homeostasis the physiological reasons for the voltage sensitivity of MscS remain unknown at present. A summary of major functional characteristics of MscL and MscS is given in Table 1.

2.3. Gating of MscL and MscS by bilayer mechanism

Bacterial MS channels directly sense membrane tension developed in the lipid bilayer alone [50,51]. This mechanism of the MS channel gating has since been named the bilayer mechanism [52]. Further studies on MscL and MscS gating by mechanical force have shown that bacterial MS channels preserved their mechanosensitivity after reconstitution into artificial liposomes [10,53–55], which has since been well documented for MS channels of prokaryotes, bacteria and archaea [9,31,53–63].

Activation of MscL and MscS by membrane tension follows a Boltzmann distribution function of the form:

$$\mathrm{Po} = \exp[\alpha(p_{1/2} - p)]^{-1} = \exp\,[(\Delta G_0 - t\cdot\Delta A)kT]^{-1} \quad (1)$$

where Po is the single channel open probability, α is the slope of ln [Po/(1−Po)] plotted against negative pressure, $p_{1/2}$ is the negative pressure (suction) applied to the patch pipette at which the MS channel is open 50% of the time (i.e. Po=0.5), ΔGo is the difference in free energy between the closed and open conformations of the channel in the absence of externally applied membrane tension, ΔA is the difference in membrane area occupied by an open and closed channel at a given membrane tension, and $t\ \Delta A$ is the work required to keep an MS channel open by external mechanical force at the open probability 0<Po<1. The conversion from negative pressure (suction) p applied to a patch pipette to membrane tension t is obtained using the Laplace's law:

$$t = p\ (r/2) \quad (2)$$

where r is the radius of curvature of the membrane patch. This conversion is based on the experimental evidence showing that MS channels respond to mechanical forces along the plane of the cell membrane (membrane tension), and not pressure perpendicular to it [64,65]. The membrane tension required for half activation of MS channels is in the order of several dynes/cm (10^{-3} N/m) [66].

The estimates of ΔGo obtained for MscL and MscS are ~17.0 and ~7 kT respectively [4], which is in a good agreement with the patch-clamp results showing that approximately two times less negative pressure is required for activation of MscS compared to MscL in giant spheroplasts of *E. coli* [38,67] (Table 1).

The fact that prokaryotic MS channels can be activated by amphipaths known to insert preferentially in one leaflet of the bilayer [50] provided a basis for a recent spectroscopic and patch-clamp study in which two potential triggers of MS channel gating by the bilayer mechanism were evaluated: (i) protein–lipid-bilayer hydrophobic mismatch and (ii) bilayer curvature [10] (Fig. 4). In this study structural changes in MscL induced by either hydrophobic mismatch or curving the bilayer by insertion of the amphipath lysophosphatidylcholine (LPC) were examined by combining cysteine-scanning mutagenesis with site-directed spin labelling (SDSL), EPR spectroscopy and patch-clamp functional analysis of MscL reconstituted into liposomes. The study demonstrated that hydrophobic surface match could stabilize intermediate conformations of MscL requiring less tension to open the

Table 1
Summary of basic properties of bacterial MscL and MscS channels

MS channel	Source	Conductance (ns)	d_{pore} (Å)	Selectivity	Amphipaths	ΔG (kT)	References
MscL	Bacteria	3.3–3.8	~30	Non-selective	CPZ, TNP, LPC	14–19	55, 35, 56, 61, 10.
MscS	Bacteria	0.97 (+ve) 0.65 (−ve)	18	P_{Cl}/P_K ~1.5–3.0	CPZ, TNP, LPC, local anaesthetics	7	27, 29, 31, 47, 49.

Abbreviations: CPZ (chlorpromazine), TNP (trinitrophenol), LPC (lysophosphatidylcholine).

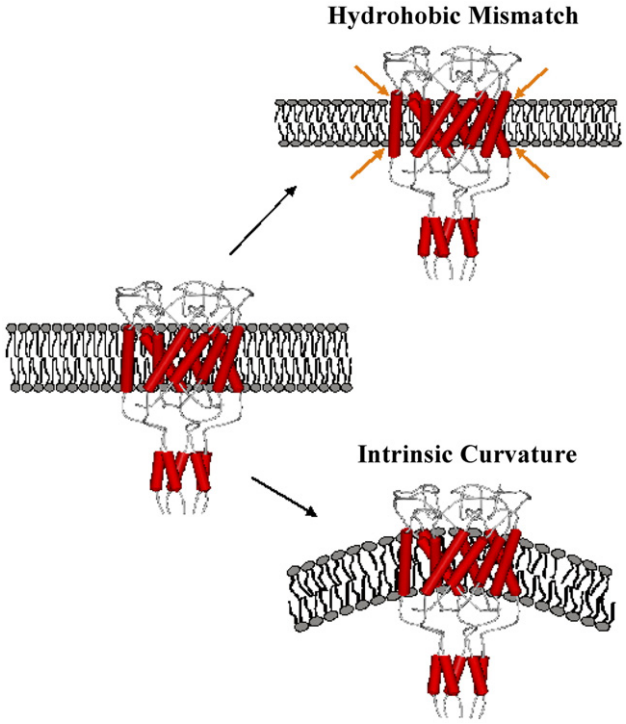

Fig. 4. Schematic diagram of two possible mechanisms of MscL activation by bilayer deformation forces. Hydrophobic mismatch and bilayer curvature are considered as deformation forces of pressure-induced changes in the lipid bilayer causing conformational changes in MS channels. These changes were studied experimentally by reconstituting purified MscL proteins in liposome bilayers prepared from synthetic phosphatidylcholine lipids of well-defined composition [10] (reproduced from [101]).

channel in thin bilayers (<18 hydrocarbons per acyl chain) compared to thick bilayers (>18 hydrocarbons per acyl chain), but was insufficient to fully open the channel. However, curving the bilayer by asymmetric insertion of LPC opened MscL without applying membrane tension [10], which indicated that the mechanism of mechanotransduction in MS channels is defined by both local and global asymmetries in the transbilayer tension profile at the lipid protein interface. The SDSL EPR approach allowed details of the open state of MscL to be determined showing a water-filled pore of >25 Å in diameter which is lined by the TM1 helices from the five subunits [11]. This result is in agreement with several studies showing that MscL undergoes a large conformational change when opening and closing [13,14,36,37,68,69]. Conformational changes involved in MscL gating have also very recently been measured using FRET spectroscopy [12] showing that, similar to the SDSL EPR study, the diameter of the MscL protein increased by 16 Å upon channel activation by LPC (Fig. 3B).

The evidence for bilayer-controlled functional properties of MscL suggests a general mechanism underlying the gating of MS channels by mechanical force. It also emphasizes the important role that the lipid bilayer plays in actively modulating the specificity and fidelity of signaling not only by MS ion channels, but possibly also by other types of membrane proteins [70,71].

3. Theory

3.1. Molecular dynamics of MscL

In order to conduct molecular simulations of an ion channel, it is crucial to have atomic resolution structures of the pore. Thus, a large number of simulation studies aimed primarily at unravelling the gating pathway of the channel and how the protein detects changes in membrane tension or composition have emerged since the publication of the Tb-MscL crystal structure. The crystal structure of the channel contains a narrow constriction only ~2 Å wide in the centre of the pore flanked by a number of hydrophobic residues that have been hypothesised to 'lock' together [7]. The open channel conductance of the pore suggests a much wider channel in the order of 30 Å in diameter which implies a very large conformational change upon channel gating.

When the protein is placed in a bilayer with no applied tension or force, the extramembrane regions of the protein (C-terminal domain and periplasmic loop) display significant structural alterations in molecular dynamics (MD) simulations, but the transmembrane helices all remain relatively stable and the pore remains occluded [69,13,72]. However, large conformational changes are observed when the protein is influenced by an external force. Many simulations have been carried out to investigate the nature and sequence of these conformational changes, differing most notably in the way that the external force is applied to the protein. Gullingsrud and Schulten applied surface tension to the bare Tb-MscL protein (i.e. not in a lipid bilayer) [13] as well as direct, predominantly radial forces to selected regions of the Eco-MscL protein that would contact the lipid to mimic the membrane pressure profiles [73]. Bilston and Mylvaganam [74] examined direct force application to the outer TM2 helix and anisotropic pressure coupling also to a bare Tb-MscL protein. Colombo et al. [69] applied pressure to the membrane around Tb-MscL, and finally Kong et al. [75] used targeted molecular dynamics to force a conformational change of Eco-MscL from the crystal structure to a pre-determined end point. Not surprisingly, the different methods of force application resulted in differing conformational changes, and the limited simulation times mean that these studies cannot be expected to sample the channel opening event to different degrees, however, some common features are evident.

In all cases the application of force resulted in a flattening of the protein in the direction of the pore axis due to an increased tilt in the TM1 and TM2 helices, an increase in the in-plane area of the protein, as well as a shortening of the pore all of which are in agreement with EPR studies [11] and results obtained by engineering disulfide bonds between subunits [37] all of which suggest an iris like opening of the pore. In most cases opening of the major pore constriction around residues 14 to 23 was observed when the strength of the applied force or pressure was above a critical point, and water flooded into the entire length of the pore. The force or pressure required to observe this was always higher than that used experimentally to open the pore otherwise conformational changes would not

occur during the short length of the simulations (typically <10 ns).

The exact channel opening events and their ordering did differ among the simulations. The first study of Gullingsrud et al. [13] showed two distinct conformational changes: a widening of the extracellular end of the pore created by a retraction of the periplasmic loop, followed by a simultaneous shift in the angle of the TM1 and TM2 helices. Their second study, however, did not show much movement by the periplasmic loop [14]. Instead, the initial motion was a widening of the cytoplasmic end caused by a tilting of the transmembrane helices, followed by a simultaneous expansion of both the TM1 and TM2 helices that forced the hydrophobic constriction apart (Fig. 5A). Colombo et al. [69] saw similar tilting, but noted that the TM1 helix only moved after the TM2 had made space for it and also that the tilt of the TM2 helix was greater than that of the TM1 helix unlike in the simulations of Gullingsrud et al. [13]. Due to the length of the simulations employed, none of these studies claim to witness the entire gating process. Kong et al. on the other hand [74] do reach a final open state due to the targeted nature of their simulations. Some caution must be used, however, in interpreting the sequence of opening events seen in this study. As the driving force in this simulation is the distance of the atoms from their final location, the resulting order of events need not follow that seen in reality and is likely to favour large changes before small ones.

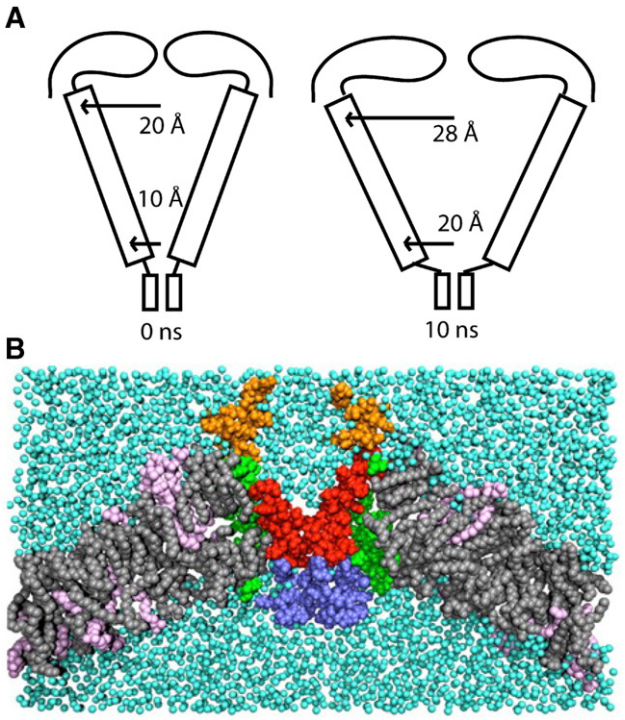

Fig. 5. Conformational changes of MscL studied by molecular dynamics simulation. (A) Schematic representation of the structural changes witnessed in a 10-ns simulation in which forces were applied to the protein to reproduce bilayer pressure gradients of a membrane under tension (reproduced from [14]). (B) Snapshot of a simulation system in which MscL is placed in a curved bilayer to initiate conformational changes (reproduced from [41]).

The radial forces applied in all these studies are used to speed up conformational changes that may be associated with gating, but it is possible that such forces bias protein motions. For example, radial movements may take place sufficiently quickly such that movements in other directions do not have time to take place. To avoid these difficulties, Meyer et al. [41] examined the conformational changes displayed in MD simulations of Eco-MscL embedded in a curved bilayer composed of single and double tailed lipids without the application of any external force (Fig. 5B). Although the conformational changes seen in this 9.5-ns simulation were not as large as in some of those using external forces and channel opening was not seen, some interesting motions possibly associated with channel gating were observed. In particular, the periplasmic loop was seen to be particularly mobile, something that may have been overlooked in previous simulations. There is evidence that this loop is important in channel gating as cutting it with proteases [40] or reconstituting protein without this connection between the C- and N-terminal halves of the protein [76] resulted in functional channels with increased pressure sensitivity. This loop may possibly have to move first to allow the transmembrane helices to adjust to pressure.

Two alternatives to direct simulations of the protein to witness channel openings have also been utilised: normal mode analysis in which local fluctuations of the protein about a minimum energy conformation are extrapolated to show large scale conformational changes, and continuum models in which a non-atomistic description of the system is used. Some common motions were extracted from a normal mode analysis starting from a number of different closed and open structures [77]. Most notable was a twist and tilt of the helices, in particular TM1, as suggested previously. Notably the top half of the TM2 helix was seen to be less mobile than the bottom which resulted in a kinking of this helix, a property that had also been suggested in some of the MD studies [41,74]. Another result in common with previous studies included the lack of interaction between the C-terminus and the remainder of the protein and the lack of motion seen in the central gating residues. In order to witness large scale motions in a short time span, Tang et al. [78] developed a continuum modelling approach in which the lipid bilayer and protein are represented as elastic sheets and rods rather than as atomistic structures. Using this simplified model a widening of the pore is observed upon membrane stretching created by helix tilting and radial movement caused by a thinning of the membrane, but no significant opening was seen with membrane bending.

A second focus of attention has been on the interactions between the membrane and the protein, and the influence that different conditions within the membrane have upon the protein. Is the coupling between the membrane and the protein simply a result of van der Waals interactions and hydrophobic matching, or are hydrogen bonds with the lipid headgroups important? Simulations have also been used to help understand why changing the nature of the lipid headgroups or the length of the lipid chains alters the pressures required to initiate channel gating. Elmore and Dougherty [72] examined the Tb-MscL in a POPE bilayer using MD simulations. A very large number of

hydrogen bonds formed between the lipid headgroups and the protein, primarily with either the C-terminal domain or a localised region of the periplasmic loop suggesting that these portions of the protein may be important for sensing changes in membrane tension. Exchanging the POPE bilayer for POPC resulted in many less hydrogen bonds forming which provides a possible mechanism for explaining the different gating tensions observed in membranes of different lipid composition [79]. An alternative explanation for the influence of lipid composition on MscL gating has also been presented, in which the differing pressure profiles of the lipids could influence the forces exerted upon the MscL protein. Gullingsrud and Schulten used MD simulations to determine the lipid bilayer pressure profiles in POPE and POPC membranes [77]. A non-uniform pressure profile exists across the membrane as the hydrophilic headgroups are squeezed together to avoid water contacting the hydrophobic tails while maintaining a nearly constant volume in the lipid. In POPC bilayers the lateral pressure is concentrated near the headgroups, but stretching the membrane concentrates the pressure closer to the start of the aliphatic chain, a phenomenon that also arises when switching to POPE. Shortening the length of the lipid tails in MD simulations results in a thinning of the protein created by pore constriction and helix kinking to avoid a hydrophobic mismatch [79]. Membrane thinning has also been suggested to cause a direct tilting of the helices as noted earlier [78].

In an analytic study of the energetics of channel opening, Wiggins and Phillips [80] proposed the competition between the hydrophobic mismatch of the protein and the applied membrane tension result in a bistable system representing the open and closed states of the channel. This model suggests that hydrophobic matching may be all that is required to induce gating within the protein and also predicts the effects altering the hydrophobic thickness of either the lipid or the protein as well as the curvature of the membrane have on channel gating.

3.2. Computer modelling of MscS

Most of the computational studies that have appeared since the publication of the crystal structure of the MscS channel have addressed a surprisingly simple question: what is the conduction state of the crystallised structure. Although simulation studies discussed below appear to suggest the crystal structure most likely does not represent the fully open state of the pore, it is still not clear exactly what state the pore is in. Finding a satisfactory answer to this problem is of particular importance as it will have a large impact on our understanding of the gating mechanism of the channel and the conformational changes required to move between conducting and non-conducting states.

It is obvious from the crystal structure of the protein that there is a large non-occluded pore passing across the membrane (see Fig. 6A). Noting this, Bass et al. suggested that the protein has most likely been imaged in an open state [8]. This is somewhat surprising, as the closed state of MscS is usually favoured by ~7 kcal/mol in membranes in the absence of lateral tension, but as the protein was crystallised from detergent micelles rather than in lipid this may have altered the resting state of the channel.

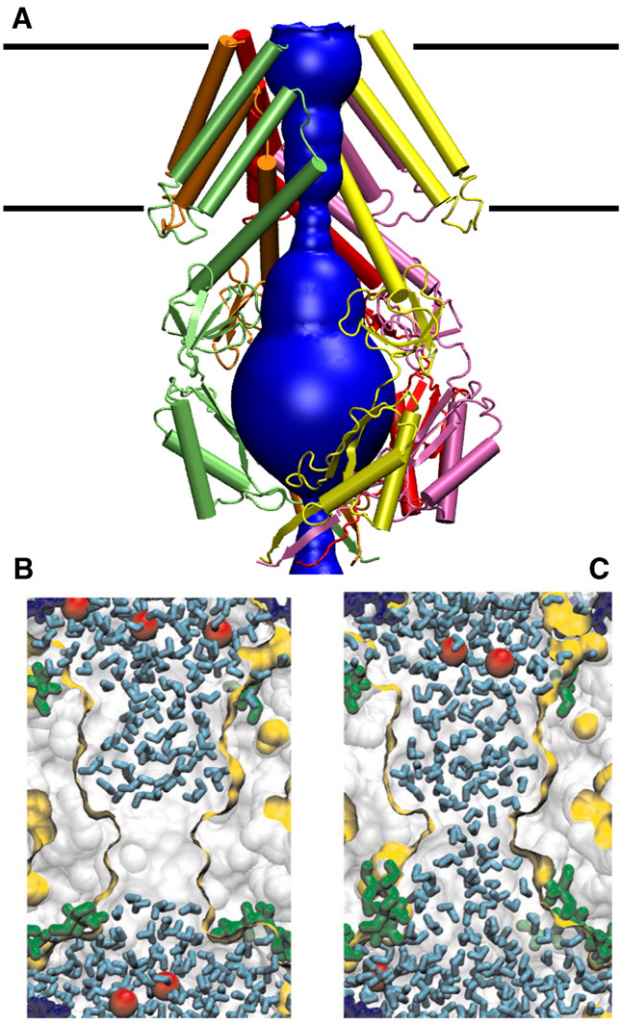

Fig. 6. Structure of the MscS pore. (A) Dimensions of the crystal structure of the pore are shown by the blue surface (traced using HOLE [102]) indicating the pore constriction and the cytoplasmic chamber. MscS monomers are each differently colored, and two are removed to show the pore more clearly. (B) Molecular simulations of the pore held near the crystal structure lead to an evacuation of the hydrophobic constriction. (C) The gain of function L109S mutant leads to the pore remaining fully hydrated. The edge of the pore is represented by the yellow lines and water in blue in (B and C) (reproduced from [88]).

Closer inspection of the structure, however, indicates that the narrowest region of the pore has a diameter of only ~3.5 Å, and is surrounded by two rings of non-polar luecine residues (L105 and L109). It has been suggested that such narrow hydrophobic pores can prevent the passage of ions without presenting a physical occlusion. Indeed, a number of molecular dynamics investigations of model pores [81–83], carbon nanotubes [84,85], and the nicotinic acetylcholine receptor [86,87] have shown that water tends to evacuate the hydrophobic regions of such pores under certain conditions. In particular if the radius of the pore is under a certain critical value that depends on its particular geometry and surface character, then the pore is often empty. But, if the radius is increased or a more polar surface is

introduced then water will fully hydrate the channel. This critical radius is around 4–4.5 Å for the model pores [83] and nicotinic receptor [86], roughly the radius required to allow three shells of water at which stage the average number of hydrogen bonds per water molecule can approximate that found in the bulk [88]. If water evacuates a region of a pore it is unlikely that an ion will traverse through it. In these conditions the ion will have to be stripped of most of its hydration shell (some waters may be pulled through the channel with the ion) without being able to compensate this energy with coordination to any polar groups in the protein as is the case for example in the selectivity filter of potassium channels.

As the radius of the constriction in the MscS pore lies below the critical radius for hydration seen for other pores, it is possible that a similar hydrophobic gating mechanism is at play in MscS, in which case the crystal structure could represent a closed or non-conductive state rather than an open conformation as originally proposed. Initial molecular dynamics studies of this pore indicated that water does indeed evacuate the constricted region of the MscS pore when it is held constrained near the crystal structure (Fig. 6B) [15,88] and provided some of the first evidence for a hydrophobic gating mechanism in a real rather than model channel. Adding a polar gain of function mutation L109S at this constriction lead to stable hydration of the pore (Fig. 6C) and significantly reduced the barrier to ion permeation [88], demonstrating that a change in the surface character of the pore can significantly alter its characteristics. When no harmonic constraint was applied to the protein holding it near the crystal structure, the pore was seen to constrict and become physically occluded, but when a large tension (20 dyn/cm) was applied to the membrane, the pore was seen to widen and remain fully hydrated [15] suggesting that the crystal structure may neither be fully open nor fully closed.

If an evacuated pore can be equated with a non-conducting state of the channel, then these results suggest that the crystal structure of the protein most likely represents a non-conducting state. The fact that the protein can actually present a physical occlusion in the absence of constraints, and only remains in this crystal conformation in simulations under the right conditions, however, complicates the decision as to whether the crystal structure truly represents a closed state of the pore or not. A further complication has also emerged as a more recent molecular dynamics study has shown that the evacuation of the narrow region of the pore seen in earlier simulations is voltage dependent [89]. Although this study witnessed evacuation of the pore when held restrained near the crystal structure or a constriction when unrestrained as in previous studies, when a large electric field was applied to mimic the transmembrane potential the pore remained open and hydrated. This remarkable result has significant implications, suggesting that application of a large enough membrane potential may result in leakage currents in hydrophobically gated pores. It should be noted, however, that a large electric field was required to observe these results. When a more modest fields of 20–50 mV/nm (tansmembrane potential ~220–550 mV) were applied the pore did not remain fully hydrated suggesting that a hydrophobic gating mechanism may be used to prevent conduction through MscS.

One of the difficulties in determining the conductance state of the MscS crystal structure arises from the difficulties involved in relating the 3 dimensional protein structure to ionic currents. Although there are now studies in which the single channel current has been determined in physiological conditions in large pores using molecular dynamics [90], it is still too computationally demanding to be done routinely. Spronk et al. [89] did witness ionic currents in their MD simulations of the MscS pore under large applied potentials, however relating currents measured under these conditions to those likely under smaller potentials is difficult since most channel I–V curves become non-linear under large potentials. It can be hypothesised that a pore evacuated by water will be non-conductive and rough estimations of the current can be made from the size of energy barriers determined in MD. As an alternative, mesoscopic simulation techniques have often been employed in which some of the atoms in the system are not treated explicitly in order to reduce the computational demand and allow the current be determined explicitly. In two such studies of the MscS channel [16,17], the protein, lipid and water are treated as continuous dielectric media. Ions are treated explicitly and move under the electrostatic force induced by the membrane potential, partial charges of the protein atoms and other ions, as well as experiencing scattering and frictional forces to replicate the collisions with water molecules or the protein boundary. Both studies showed that when the protein is held in its crystal structure the current (carried almost exclusively by anions) is much below recorded single open channel currents, supporting the notion that this does not represent the open state of the pore. The use of bulk like dielectric constants (80 [16] or 60 [17]) for the water in the pore may lead to overestimations of the current if the pore is often evacuated as evidenced in MD simulations suggesting an even lower current through the crystallised pore. Indeed there has been some debate as to the most appropriate dielectric and diffusion constants to use in continuum simulations even in non-evacuated pores [91], although good results have been obtained with well informed choices [92]. Simulations using widened structures demonstrate that only minimal structural changes are required to create a highly conductive pore [16,17].

An oddity of the MscS protein is the large cytoplasmic domain apparent in the crystal structure (Figs. 2B, 6A). This extends a long way from the membrane and contains a large central chamber accessible through seven side openings and one distal pore. This domain has been suggested to play a role in channel gating [93], desensitization and stability [94,95] as well as in ion transport and selectivity [96]. Although the seven side entries to the chamber are surrounded by a number of basic amino acid residues that could play a role in valence selectivity, both K^+ and Cl^- ions have been seen to pass through them and enter the cytoplasmic chamber in MD [15] and mesoscopic simulations [16,17], but no ions pass through the distal opening. The distribution of ions within the chamber, however, is such that more Cl^- than K^+ ions reside near the transmembrane pore mouth, thus it is possible that this domain does play an important role in ionic selectivity. It has also been suggested that this domain may act as a molecular sieve [93], preventing large

solutes from passing through the channel where they may either block it, or be expelled undesirably from the cell. Further molecular simulations including solutes may shed further light on this issue.

As the nature of the crystal structure has not been clearly determined, and due to the short length of molecular studies, it has been difficult to infer much about the gating pathway of MscS from simulation studies. Interactions between the lipid headgroups with a number of charged and polar residues in the TM1 and TM2 helices have been noted [15] that may provide a mechanism of linking membrane tension to channel opening. In the simulations in which the pore became physically constricted a possible location of an occlusion gate has been suggested. The pore lining TM3 helix in one of the seven subunits buckles slightly, moving Leu105 toward the center of the pore [15,89]. Salt bridges have also been noted between Asp62 in the TM1–TM2 linker and Arg128 at the cytoplasmic end of the TM3 helix on the adjacent subunit [15]. It has been suggested that this link between the lipid contacting TM1–TM2, cytoplasmic domain and pore lining TM3 may be important for gating or stability although no clear conclusions have been drawn.

4. Conclusions

This review provides a brief summary of an area of MS channel research that over the last 20 years has made a significant progress from its beginnings marked by a discovery of the MS channels in *E. coli*. Experiments and theory that are briefly outlined have significantly contributed to our understanding of basic principles and evolutionary origins of mechanosensory transduction in living cells. We may expect further exciting developments of this research area in the future.

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