REVIEW

Structure and function of the bacterial mechanosensitive channel of large conductance

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Abstract

Mechanosensation in bacteria involves transducing membrane stress into an electrochemical response. In *Escherichia coli* and other bacteria, this function is carried out by a number of proteins including MscL, the mechanosensitive channel of large conductance. MscL is the best characterized of all mechanosensitive channels. It has been the subject of numerous structural and functional investigations. The explosion in experimental data on MscL recently culminated in the solution of the three-dimensional structure of the MscL homologue from *Mycobacterium tuberculosis*. In this review, much of these data are united and interpreted in terms of the newly published *M. tuberculosis* MscL crystal structure.

Keywords: ion channel; mechanosensation; mechanosensitive channel of large conductance; membrane protein

Organisms are sensitive to physical stresses such as gravity, touch, and changes in osmolarity. Ion channels activated by mechanical tension are thought to mediate these effects in many systems. The first evidence for mechanosensitivity was found during patch-clamp experiments on animal cells (Brehm et al., 1984; Guharay & Sachs, 1984). Subsequently, patch-clamp experiments demonstrated the presence of mechanosensitive (MS) channels in a number of plant, animal, and bacterial cell types (reviewed by Sackin, 1995) first documented in *Escherichia coli* spheroplasts (Martinac et al., 1987).

In *E. coli*, three ion channel activities that are activated by membrane stretch, including MscM, MscS, and MscL (Mechanosensitive channels of Mini, Small, and Large conductance), have been identified (Berrier et al., 1996). These channels are located on the inner membrane of bacteria (Berrier et al., 1989; Blount et al., 1996a, 1996b; Häse et al., 1997). MscL and other stretch activated channels possibly act as safety valves upon sudden osmotic down-shock (Sukharev et al., 1997).

A 136 amino acid protein found to be sufficient and necessary for MscL activity in *E. coli* was sequenced and cloned (Sukharev et al., 1994). The C-terminal segment was observed to contain a cluster of charged residues Arg-Lys-Lys-Glu-Glu (residues 104–108 [98–102]³). Representative structural MscL homologues from a variety of different bacteria were shown to have MscL-like ac-

tivity (Moe et al., 1998), and MscL-like activities have been identified in *Archaea* (Le Dain et al., 1998). PhoA fusion experiments (Blount et al., 1996b) and secondary structure analysis (Arkin et al., 1998) led to a putative model of the protein (Fig. 1A) consisting of five consecutive structural domains, denoted S1, M1, S2, S3, M2, and C (Blount et al., 1996b; Sukharev et al., 1996). In this model, the N- and C-termini are on the cytoplasmic side of the membranes (as are the S1 and C domains). The S1 domain contains an α -helix, the M1 and M2 domains form transmembrane α -helices, with the S2–S3 domain forming a short loop and α -helix, respectively, on the periplasmic face of the membrane.

The three-dimensional structure of MscL

Until recently there was a large amount of functional but only limited structural data available on MscL. The determination of the crystal structure of the MscL homologue from *Mycobacterium tuberculosis* (Chang et al., 1998) (Fig. 1B) was therefore a major breakthrough. The channel was shown to be a homo-pentamer with two transmembrane α -helices (TM1 and TM2) and a C-terminal cytoplasmic helix. The pentamer has radial symmetry with the pore formed along the axis of symmetry that is perpendicular to the membrane. The major discrepancy between the crystal structure and previously obtained data was the multimeric state of MscL, which was proposed to be hexameric (Blount et al., 1996b; Saint et al., 1998). The N- and C-termini are found on the cytoplasmic side of the membrane. The TM1 helix runs from the cytoplasmic to the periplasmic space and consists of residues that line the inside of the channel. This helix is tilted with respect to the membrane perpendicular, and consequently the channel diameter increases

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³In this paper all residue numbers for the *Mycobacterium tuberculosis* MscL homologue appear in square brackets.

Fig. 1. Representations of the MscL models. **A:** Schematic representation of *E. coli* MscL proposed by Sukharev et al. (1996). The labels denote various structural domains. M1 and M2 are transmembrane α -helices, S1 and S3, are α -helices, and S2 and C are loops. **B:** Cartoon diagram of the MscL from *M. tuberculosis* by Chang et al. (1998) (PDB ID: 1MSL). The pentamer (with each subunit drawn in a different color) and a single monomer (drawn in white) is shown. The transmembrane helices TM1 and TM2, the periplasmic loop spanning them, and the C-terminal helix are labeled. The location of the membrane interfaces with the periplasmic and cytoplasmic spaces are denoted by horizontal bars. This figure was produced with MOLSCRIPT (Kraulis, 1991).

with increasing proximity to the periplasmic face of the membrane. Helix TM1 is followed by a loop that forms the periplasmic face of the channel. Next is helix TM2, running from the periplasmic to the cytoplasmic face. It contains most of the residues that are exposed to the lipid bilayer in which the channel sits. Following the second TM helix is another loop containing many basic and acidic residues and finally the C-terminal cytoplasmic helix.

Structure and function: Re-examining the data

Much data from the molecular dissection of *E. coli* MscL channels may be re-interpreted in terms of the recently solved structure. Many papers describe the effects of deletion and substitution mu-

tation and proteolysis on gating pressure and kinetics; however, in the absence of a three-dimensional structure, the interpretation of these data has been difficult. Further information may be drawn from alignments of MscL homologue sequences that have been deposited in the various databases. Using the *E. coli* MscL protein sequence, the GenBank, CDS translations, Protein Data Bank (PDB), SwissProt, Protein Information Resource, and Protein Research Foundation databases were searched using BLAST (Altschul et al., 1997) for MscL homologues. A sequence alignment based on the *M. tuberculosis* structure and conserved sequence characteristics was compiled using AMPS (Barton, 1990) and ALSCRIPT (Barton, 1993). This alignment was used to calculate a sequence identity matrix (Table 1). Homologues of *E. coli* MscL from *Staphylococcus aureus*, *Pseudomonas fluorescens*, *Erwinia carotovora* (Moe et al., 1998), *M. tuberculosis* (Cole et al., 1998), *Haemophilus influenzae* Rd. (Fleischmann et al., 1995), *Clostridium perfringens* (Matsushita et al., 1995), *Synechocystis* sp. (Kaneko et al., 1996), and *Bacillus subtilis* (Presecan et al., 1997) were retrieved from those databases. Intermonomer contacts (using CONTACT, CCP4, 1994) (Fig. 2), a sequence alignment (Fig. 2), and corresponding sequence identities (Table 1) were calculated. From these, several characteristics common to all MscL homologues were identifiable. Figure 3A shows residues conserved in all MscL homologues. The residues 2–41 in *E. coli* MscL [1–39] are the most highly conserved in all MscL homologues. In the *M. tuberculosis* structure, residues [15–42] (17–45 in *E. coli*) forming the TM1 helix are at the core of the channel. The TM1 helices are close to the central fivefold axis of symmetry at their bases and (due to the tilt of the helix relative to the membrane) further from the axis at their C-terminal ends (Fig. 1B). Most differences between the sequences in this region are conservative substitutions. These observations can be compared with the available mutation data. Ou et al. (1998) have identified a number of mutants of *E. coli* MscL that are more sensitive to mechanical stretch than the wild-type MscL. Mutations were identified by the hampered growth suffered by bacteria (due to leakage of cytoplasmic solutes) harboring these mutations. Blount et al. (1997) identified other similar mutants that were also more sensitive to mechanical stretch. Mutants were classed based on the severity of the impact on cell proliferation. The classification system used by Ou et al. (1998) was applied to all gain-of-function point mutants, and these are listed in Table 2 and indicated in the sequence alignment (Fig. 2). The mutations have been mapped

Table 1. Pairwise sequence comparison of aligned MscL homologues^a

	9	8	7	6	5	4	3	2	1
1. <i>B. subtilis</i>	32	46	47	31	44	49	46	46	100
2. <i>C. perfringens</i>	25	39	35	24	41	40	38	100	
3. <i>E. carotovora</i>	24	42	56	23	61	78	100		
4. <i>E. coli</i>	27	45	62	29	66	100			
5. <i>H. influenzae</i>	25	46	53	27	100				
6. <i>M. tuberculosis</i>	24	33	27	100					
7. <i>P. fluorescens</i>	25	43	100						
8. <i>S. aureus</i>	24	100							
9. <i>Synechocystis</i> sp.	100								

^aSequence identities were calculated based on the sequence alignment shown in Figure 1.

Fig. 2. Sequence alignment of bacterial MscL homologues. The location of α -helices found in the *M. tuberculosis* structure are represented as cylinders above the sequences. The numbering system is that for *E. coli* MscL. In the row labeled "mutation effect," Residues susceptible to gain-of-function mutation in *E. coli* MscL are labeled V, S, or M for very severe, severe, or mild, based on Table 1. Both V and S mutation effects are possible for V30 (cyan highlight). In the row labeled "contacts," those residues in the *M. tuberculosis* MscL structure that contact other monomers are indicated. Each letter represents the contacts of residues in monomer A with the B, C, D, and E monomers. Groups of conserved residues are boxed. Absolutely conserved residues are in bold. Residues are colored red for acidic, blue for basic, green for polar, and yellow for hydrophobic. (Glycines and prolines are not colored.)

Fig. 3. Stereo-images of the MscL homologue from *M. tuberculosis*. The backbone is represented in ribbon form with each monomer shown in a different color. A single monomer is shown with residue labeled. **A:** Conserved residues are shown in CPK form using standard atom colors. **B:** Gain-of-function mutants are drawn in CPK form and color-coded according to severity grey = very severe, blue = severe, cyan = mild. This figure was produced with MOLSCRIPT (Kraulis, 1991).

onto the *M. tuberculosis* crystal structure (Fig. 3B). Not surprisingly, most of the mutations occur at residues in the highly conserved region near the N-terminus of MscL.

The role of the cytoplasmic helix and the following sequence running through to the C-terminus is unclear. The region is poorly conserved between the MscL homologues, and deletion of C-terminal residues 110–136 in *E. coli* MscL [104–151] has little effect on mechanosensitivity, kinetics, or conductance (Blount et al., 1996c; Häse et al., 1997). C-terminal segment may therefore have either a regulatory or an undiscovered function unrelated to mechanosensing, or it may be an evolutionary artifact.

Where is the channel gate?

Interestingly, residues susceptible to “very severe” gain-of-function mutation in *E. coli* MscL (G22 [A20], V23 [V21], G26 [G24],

and G30 [T28]) are close to the narrowest part of the pore in the *M. tuberculosis* MscL structure (Fig. 3B). This part of the structure lies close to the cytoplasm/lipid interface and has a diameter of about 4 Å. Three of the mutation sites are glycine in the wild-type structure, suggesting that the mutations may interfere with the packing and hence destabilized the pentamer. Alternatively, high main-chain flexibility may be required for function. Another mutation with similar effect is V23 [V21] to Ala, Asp, or Gly. Residue [V21] in the *M. tuberculosis* MscL directly lines the narrowest part of the pore, and it would appear that this part of the MscL molecule represents the channel gate and is essential for maintaining the closed state. Importantly, mutation of V23 [V21] to Gly did not appear to result in a “leaky” channel, demonstrating that this residue is not absolutely required for maintaining the channel in its closed state. In contrast, two other mutants G22D and G22N in the highly conserved G22 [A20] residue next to V23 [V21] exhibited

Table 2. Classification of gain of function point mutations in *E. coli* and their equivalent residues in *M. tuberculosis*

Mutation	M.Tb residue	Classification	Author
G22D	A20	Very severe	Ou et al. (1998)
G22N	A20	Very severe	Ou et al. (1998)
G22S	A20	Very severe	Ou et al. (1998)
V23A	V21	Very severe	Ou et al. (1998)
V23D	V21	Very severe	Ou et al. (1998)
V23G	V21	Very severe	Ou et al. (1998)
G26S	G24	Very severe	Ou et al. (1998)
G30R	T28	Very severe	Ou et al. (1998)
N15D	N13	Severe	Ou et al. (1998)
L19Y	L17	Severe	Ou et al. (1998)
G46D	N44	Severe	Ou et al. (1998)
N100D	N95	Severe	Ou et al. (1998)
R13C	R11	Mild	Ou et al. (1998)
G14E	G12	Mild	Ou et al. (1998)
V21E	V19	Mild	Ou et al. (1998)
G30E	T28	Mild	Ou et al. (1997)
K31D	A29	Mild	Blount et al. (1997)
K31E	A29	Mild	Blount et al. (1997)
K55T	D53	Mild	Ou et al. (1998)
Q56P	V54	Mild	Blount et al. (1996)
Q56R	V54	Mild	Blount et al. (1996)
I92F	V86	Mild	Ou et al. (1998)

channel activity without any applied suction (Ou et al., 1998) suggesting that these mutant channels may be “leaky” since residual stretch resulting from the seal formation was sufficient to activate them. Thus G22 [A20] seems to be required to keep the channel closed. The residues are all clustered in a region that is in line with the cytoplasm/membrane. The observations of residue conservation in MscL homologues and susceptibility of residues in this region in the *E. coli* MscL homologue to gain-of-function mutation suggest that this feature of the pore is relatively inflexible with regard to function. Regions of the pore not involved in closing the channel gate can be inferred from the diameter and solvent accessibility of the pore. The pore diameter is largest just below the periplasmic face (~ 36 Å), narrowing slightly at the face itself due to surface loops between TM1 and TM2 dipping into the mouth of the pore. The *M. tuberculosis* MscL structure was solved at low resolution, and so water molecules were not built into the model; however, some idea of the solvent accessible surface of MscL can be gained with use of the program VOIDOO/FLOOD (Kleywegt & Jones, 1994). (VOIDOO identifies cavities in molecules and FLOOD fills them with solvent molecules.) These programs showed that the pore could be filled with water molecules to a point 24 Å below the periplasmic face of the channel. Thus, much of the space inside the membrane-spanning domain of the pore appears to be solvent accessible. Below this point is the channel gate, and below that is another cavity that FLOOD was able to fill with water molecules. The minimum distance between the water molecules (placed by FLOOD) on the periplasmic and cytoplasmic side of the occlusion is 8 Å. The channel gate thus appears to be thin compared to the thickness of the membrane (~ 35 Å).

How does MscL detect stretch?

Stretch detection is a fundamental property of MscL (Blount et al., 1996a). The structural and functional data for MscL provide some clues as to which parts of the protein are responsible for this sensing. As noted previously (Gustin et al., 1988; Sukharev et al., 1997), it is expected from Laplace's law that MS channels respond to stretch forces along the plane of the cell membrane and not pressure perpendicular to it. This is evidenced by the observation of the effect of amphipathic molecules (that partition differently into the membrane), which was to activate MS channels (Martinac et al., 1990). Thus stretch sensation would be expected to involve residues in proximity to the lipid bilayer.

Several mutations that result in a channel that can maintain the closed state, but have altered sensitivity to membrane stretch, have been identified. The existence of severe gain-of-function mutants of *E. coli* MscL shows that the energy required to open the pore (derived from stretch forces acting on the membrane) is not what limits sensitivity to pressure in wild-type MscL. Thus, residues susceptible to gain- or loss-of-function mutation must in some way be involved directly in pressure sensing or in balancing the pressure sensitivity of the pore. Gain-of-function mutations in the region of the channel gate, at the narrowest point in the pore, could interfere with the pore's ability to stay closed by affecting the responsiveness of the channel to membrane tension. This view is supported by the finding that substitutions of the highly conserved residue G22 (*E. coli* sequence) by hydrophobic amino acids made the mutant channels less sensitive to membrane tension (Batiza et al., 1999). More negative pressure applied to the patch pipette was necessary to activate these mutant channels compared to the wild-type MscL or the mutant channels having G22 replaced by hydrophilic residues. Other residues clustered around the gate that were also shown to be potential gain-of-function mutants are found toward the C-terminal end of helix TM2. These residues, I92 and N100, would be expected to be in contact with the membrane acyl groups and the membrane/cytoplasm interface, respectively.

Some of the strictly conserved residues are phenylalanine (F7 and F10 in *E. coli*) whose side chains would appear to interact intimately with the phospholipid bilayer. They are close to the aqueous/lipid interface on the cytoplasmic side of the membrane. These phenylalanine residues, which are highly conserved in the MscL homologues, may be directly involved in stretch detection. In accordance with this notion are the results of a site-directed mutagenesis study in which another highly conserved residue, G14, was replaced by hydrophobic or hydrophilic residues (Liu et al., 1999). Substitutions by hydrophobic amino acids abolished channel activity, whereas replacements by hydrophilic residues altered channel conductance, gating as well as pressure sensitivity. Moreover, deletion of the G14 residue resulted in a “leaky channel” that has lost its sensitivity to membrane tension. The channel flickers permanently at a reduced conductance of ~ 0.4 ns with an overall open probability of $P_o \approx 0.4$, irrespective of applied pressure or voltage (Liu et al., 1999). In *E. coli* MscL, the $\Delta 2-4$ truncation mutant has altered gating properties, and the $\Delta 2-12$ truncation mutant is not pressure sensitive (Blount et al., 1996c). These results suggest that not only phenylalanine residues but probably also the whole N-terminus comprising residues from M1 to G14 may be involved in stretch detection, as suggested by the electromechanical coupling (EMC) model (Gu et al., 1998). Although the EMC model is incorrect in its assumption about little change in the diameter of the pore between the closed and open state of the channel, the model assumption about

the N-terminal flexibility appears to be correct. This is because the N-termini are not visible in the Tb-MscL structure (Chang et al., 1998) suggesting that either they are highly mobile or they may possess multiple conformations. How stretch detection happens is not obvious and remains to be elucidated.

There are a number of gain-of-function point mutations that are not in proximity to the channel gate. These are Q56P, Q56R (Blount et al., 1996c), K55T, and G46D (Ou et al., 1998). Equivalent residues on the *M. tuberculosis* structure are to be found in the loop between TM1 and TM2 helices. They are found at the edge of the pore close to the membrane/periplasm interface. Indeed, the cleavage of the periplasmic loop of MscL of *E. coli* by the proteases trypsin or chymotrypsin led to a dramatic increase in the channel sensitivity to membrane tension (A. Ghazi, pers. comm.). This result indicates that the loop may act as a spring resisting the opening of the channel and thus possibly setting the level of the channel mechanosensitivity. Sequence-wise, the loop region is poorly conserved between MscL homologues, but this fact does not preclude a structurally and functionally conserved role for the region.

In all MscL homologues, just prior to the region corresponding to the cytoplasmic helix, there is a conserved motif in which between one and four basic residues is followed closely by one or two acidic ones (Arg-Lys-Lys-Glu-Glu in *E. coli*). Whereas the $\Delta 110$ –136 *E. coli* MscL mutant has almost identical function to the wild-type channel, the $\Delta 104$ –136 mutant, lacking the amino acids Arg-Lys-Lys-Glu-Glu, has no channel activity (Blount et al., 1996c; Häse et al., 1997). This evidence suggests that charged residues would appear to be vital for pressure sensing (Gu et al., 1998). In the *M. tuberculosis* structure, this motif is located on the cytoplasmic face of the channel. How this structural feature could detect stretch is unclear, since this part of the channel does not appear to be close to the membrane. With a cavity separating the two domains, the contacts made with the transmembrane domain appear to be quite limited. Apart from the polypeptide chain itself, there are no direct contacts between the two domains. The closest approach of the two domains is an ~ 6 Å gap between N13 and I14 at the base of TM-1 helix and R98 and K99 in the cytoplasmic domain. One interesting feature of this highly charged region is the position of residue E104. This acidic residue (side-chain $pK_a \sim 4.25$) faces the central axis of symmetry of the pore and is surrounded by copies of the same residue from other monomers. The crystallization experiments were performed at pH 3.6–3.8, well below the pK_a of the side chain of E104. At pH values higher than about 4.25, we would expect the glutamate residues to lose protons and become negatively charged. If this is the case, then it is difficult to see how this negatively charged cluster could be stable without some charge compensation such as the attractive movement of nearby basic residues or the binding of a cation. The function of wild-type *E. coli* MscL has been reported to be the same at pH 7.5 and 5.0 (Blount et al., 1996c). At these pHs the ionization states of the basic and acidic side chains ($pK_a \sim 10$ –12 and ~ 3 –4, respectively) would be expected to have the same charge state. However, at very low pH, *E. coli* MS channels become less sensitive to pressure (Blount et al., 1996a). Therefore, at physiological pH the *M. tuberculosis* structure could be somewhat different to the published structure. pH-dependent structural changes have been observed in several proteins. For example, low pH induces a conformational change in the influenza virus haemagglutinin, which then mediates fusion of the host cell and viral membranes (Bullough et al., 1994). Further work is needed to determine if such an effect is important in MscL.

How many monomers in the channel?

The issue of the exact multimeric state of MscL has been the subject of a number of investigations, but has not been settled. Studies by Blount et al. (1996b) showed that tandems of two MscL subunits expressed as a single polypeptide chain formed functional channels, providing evidence that the channels possess an even number of subunits. (This observation also showed that the N- and C-termini were on the same side of the membrane.) Cross-linking experiments by the same authors revealed the presence of homo-hexameric channels. Studies of two-dimensional crystals of *E. coli* MscL also provided evidence that the molecule forms homo-hexamers (Saint et al., 1998), although the resolution of 15 Å at which the two-dimensional crystal diffraction data were obtained is probably too low to exclude the pentameric organization of the channel as shown in the structure of *M. tuberculosis* MscL (Chang et al., 1998). Nevertheless, the multimeric state of the channel may be variable among MscL homologues. It is also conceivable that the conditions used to obtain crystals of the putative *E. coli* MscL hexamers in the study of Saint et al. (1998) and the *M. tuberculosis* MscL pentamers in the study of Chang et al. (1998) favored only these particular multimeric states and therefore do not reflect the structural heterogeneity of the channel.

What is the structure of the open MscL channel?

There are a number of lines of evidence showing that the open MscL channel is very different from the channel in the closed state. Estimates of the open pore size based on conductance were in the range of 34 to 46 Å (Cruickshank et al., 1997). Furthermore, the pore has been shown (Ajouz et al., 1998) to be permeable to thioredoxin (radius ~ 35 Å). For such molecules to pass through the pore, a substantial adjustment of the channel structure would be required. An open state model for *E. coli* MscL has been proposed in which six MscL monomers form a ring with two helices from each monomer at the periphery of the pore. Thus, the open pore would have 12 transmembrane helices making up its circumference (Cruickshank et al., 1997). This structure would allow molecules such as thioredoxin to pass through its center. The transition to this open state would require substantial re-arrangements of the channel molecules. Such a radical change in structure between the closed and proposed open state model of the channel would perhaps require a large input of energy unless some low energy transition pathway existed. The existence of gain- and loss-of-function mutants shows that tension sensitivity is the most important factor that determines the opening of the channel. One estimate of the energy required to keep the channel open 50% of the time is 5.0 kJ/mol (Gu et al., 1998). According to another estimate, the free energy of transition from the closed to the open channel state was calculated to be 46.3 kJ/mol (Sukharev et al., 1999). Both energies can be compared with an estimate of the folding free energy of 54 kJ/mol (using 0.4 kJ/mol/residue as an estimate of folding free energy). Further research will be required to confirm aspects of the model as well as the energetics of transition from closed to open state.

Conclusions

The newly available structural and functional data from MscL have provided substantial insights into this unique membrane protein. Based on the currently available data, the channel gate appears to be restricted to hydrophobic clusters that line the pore at its narrowest point. The actual pressure sensing mechanism appears to be com-

plex and involves many areas of the molecule. Parts of the protein involved in pressure sensing are the N-terminal residues up to about the start of TM1, residues around the channel gate, and the cluster of charged residues prior to the C-terminal helix. There appears to be a delicate balance in pressure sensing, since structurally diverse mutations alter the pressure-gating threshold. The TM1–TM2 spanning loop appears to function to dampen pressure sensitivity, as do the N- and C-terminal segments. Importantly, it has been shown that the pressure sensing and channel opening functions can be decoupled by deletion of G14, although the open state of this “leaky mutant” is probably different from that of the wild-type channel.

While the understanding of the structure and function of MscL has been greatly improved by recent experimental evidence, several mysteries remain. There are items of data that have not been correlated with function. The N-terminus was observed to be disordered and yet is known to participate with channel gating. Although those regions of the protein involved in pressure sensing have been delineated, the exact mechanism by which this occurs remains unknown. Further research is required to characterize the transition of MscL from the closed to open states. Such dynamic movements in proteins are difficult to analyze, and it would appear that the structure of the channel in its open state would be very difficult to solve, unless some way of obtaining the channel in a permanently open form could be found.

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