

Channel gate! Tension, leak and disclosure

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The crystal structure of a bacterial MscL shows how this homopentameric channel protein is held tightly shut to prevent leakage whilst at rest. By inference, the structure also shows how a stretch force in the lipid bilayer causes the channel to open. We now have a concrete picture as to how a stimulus 'gates' an ion channel.

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Gated pores

Membrane channels close their pores completely when at rest, but open them in response to specific physicochemical signals. When open, the channels allow particles, such as ions, to diffuse down gradients established through the work of other molecules. Thus, channels are like the mass media: they spread information but waste energy. In the parlance of physiology, membrane channels are said to be gated by the stimulus they transduce. Channels can be gated by a range of stimuli: ligand-gated channels open upon the binding of agonists from the outside or second messengers from within; voltage-gated channels open when the charge difference across the membrane rises or falls; and mechanically gated channels open in response to mechanical forces. The long and distinguished history of electrophysiology reflects the ingenious application of biophysical methods to ion-channel studies [1,2]. With the advent of the patch clamp, it has even been possible to follow the activities of single-channel molecules. These advances, together with the application of genetics and molecular biology, have resulted in a huge increase in our knowledge of this class of molecules over the past 15 years.

Many of the genes encoding channel proteins have been cloned and functionally expressed, but the crystallization of channel proteins remains a challenge. Because they cause leakage, cells do not need and cannot afford to contain too many channel proteins. Thus, besides the usual difficulties in handling membrane proteins, collecting enough protein of one channel species has proved difficult. Because animal channels expressed in *Escherichia coli* are often found in denatured forms, sequestered in inclusion bodies, the discovery of channels native to bacteria has circumvented many problems. The crystal structures of two bacterial ion

channels have recently been solved: KcsA, a K⁺ channel from *Streptomyces lividans* [3]; and Tb-MscL, a mechanosensitive channel from *Mycobacterium tuberculosis* [4]. KcsA is equipped with a narrow filter and the crystal structure elegantly explains how this filter allows the passage of K⁺ and not, for example, Na⁺. The filter is largely a fixed structure located towards the outside of the pore. The gate, the part that opens and closes, is located towards the cytoplasm, but is not resolved for KcsA; however, the gate is clearly seen in the crystal structure of Tb-MscL.

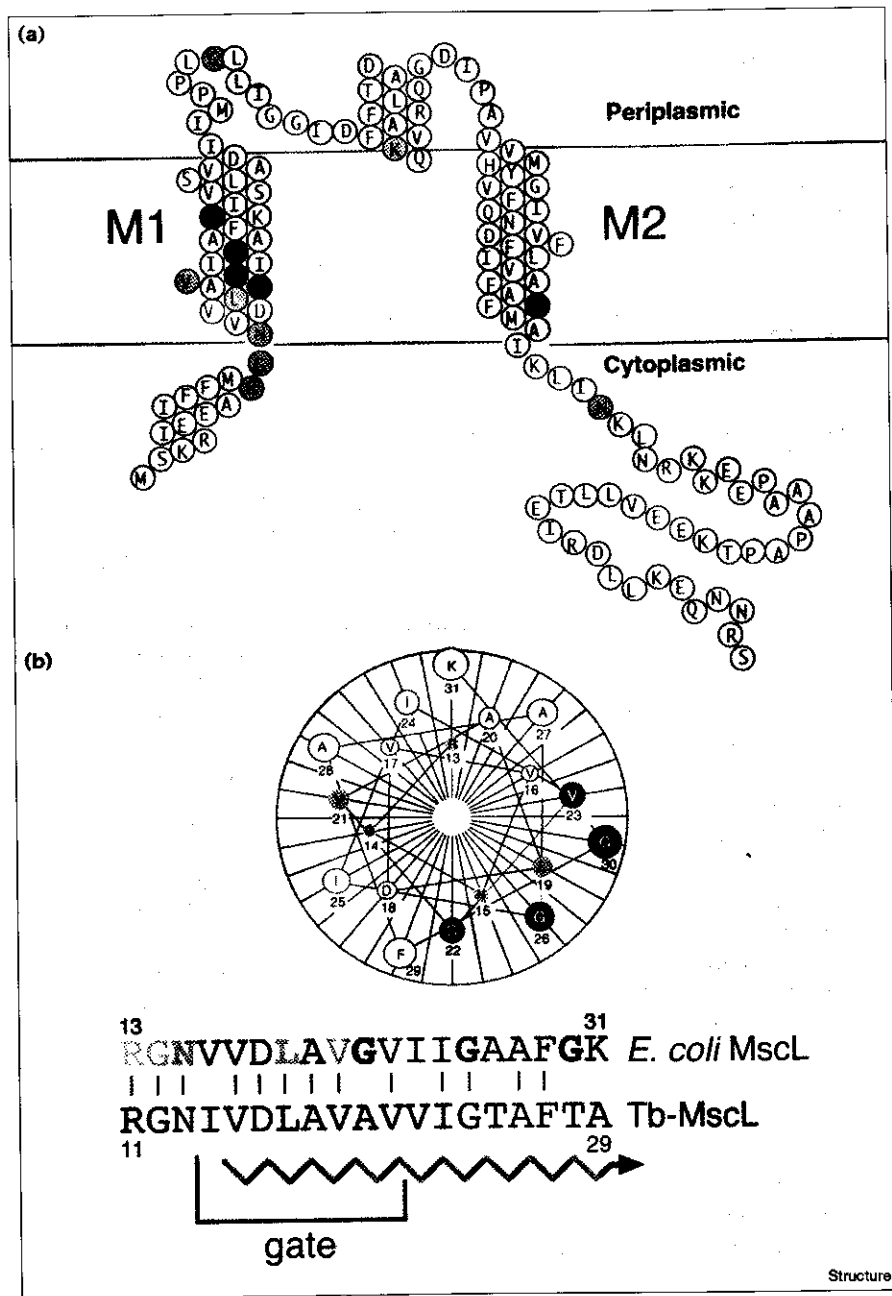
The MscL channel

Unitary currents in bacteria that were induced as a result of membrane stretch were first detected in a patch-clamp study of the *E. coli* membrane surface [5]. Such channel activities were found to survive the process of reconstituting membrane material onto artificial liposomes [6–8]. Fractionation of this material [8] led to the enrichment of a protein and subsequent cloning of the gene for this mechanosensitive channel of large conductance, MscL [9]. The gene product comprises only 136 residues, most of which are nonpolar. A hydropathy plot and PhoA fusion analysis, as well as Fourier transform, infrared and circular dichroism (CD) studies all implied a similar topology [10,11]: the MscL peptide traverses the inner membrane [7,10,12,13] twice with the loop between the M1 and M2 helices facing outwards and the N and C termini located in the cytoplasm (Figure 1). Purified MscL channels alone are necessary and sufficient for responding to stretch forces in the lipid bilayer, as shown with recombinant MscL. [10,14]. As a single MscL peptide provides insufficient material to enclose a pore, homomultimerization is expected. Early cross-linking experiments, expression of tandem subunits, and an electron-microscopic study of the *E. coli* MscL channel suggested a hexameric structure [10,13,15]. A re-examination of cross-linking now supports a pentameric model [16] compatible with the crystal structure of the Tb-MscL homolog [4].

A tour de force

In a magnificent effort by Chang *et al.*, researchers from Doug Rees's laboratory [4] screened and refined some 24,000 crystallization conditions, using nine different MscL homologs and 20 detergents, and obtained crystals of Tb-MscL that diffracted to a resolution of ~7 Å. They further tested, as additives, various detergents, organic molecules and heavy-metal compounds, and found Na₃Au(S₂O₃)₂ to improve the quality of the Tb-MscL crystal to a limiting resolution of 3.5 Å. This compound was found to bind at a set of crystal-lattice contacts. Although not unprecedented, this is an extreme example

Figure 1



The functional significance of altered residues within *E. coli* MscL correlates with the structural significance of equivalent amino acids within Tb-MscL. (a) Topology of an *E. coli* MscL subunit with respect to the membrane [10] highlighting the residues mutated in a random mutagenesis study [24]. M1 and M2 are the first and second transmembrane α helices. (In Tb-MscL, M1 is slightly longer and M2 slightly shorter. Similarly the Tb-MscL helices would exclude one (M2) or two (M1) residues near the cytoplasmic end.) Mutations that make the mutant channel open more easily and inhibit cell growth are colored red, blue and green with respect to the severity of the mutation, red being the most severe [24]. The reduced sidechain length of residue 23 causes easy opening, and this residue corresponds to the top of the presumed constriction [24]. (b) Partial helical wheel of M1 (and two preceding residues) showing the predicted locations of the mutations described above. Identity with the Tb-MscL sequence in this region is noted in the sequence alignment below. The gray helix below the sequence highlights residues contributing to the lower end of the TM1 helix, and sequences contributing to the constricted Tb-MscL gate are indicated. The most severe mutations fall on one side of the lower half of the M1 helix, which would by analogy generate the structure of the presumed MscL gate and the region just above it. (The figure was adapted from [10] and [24] with permission.)

of an improved crystalline lattice due to the presence of heavy atoms.

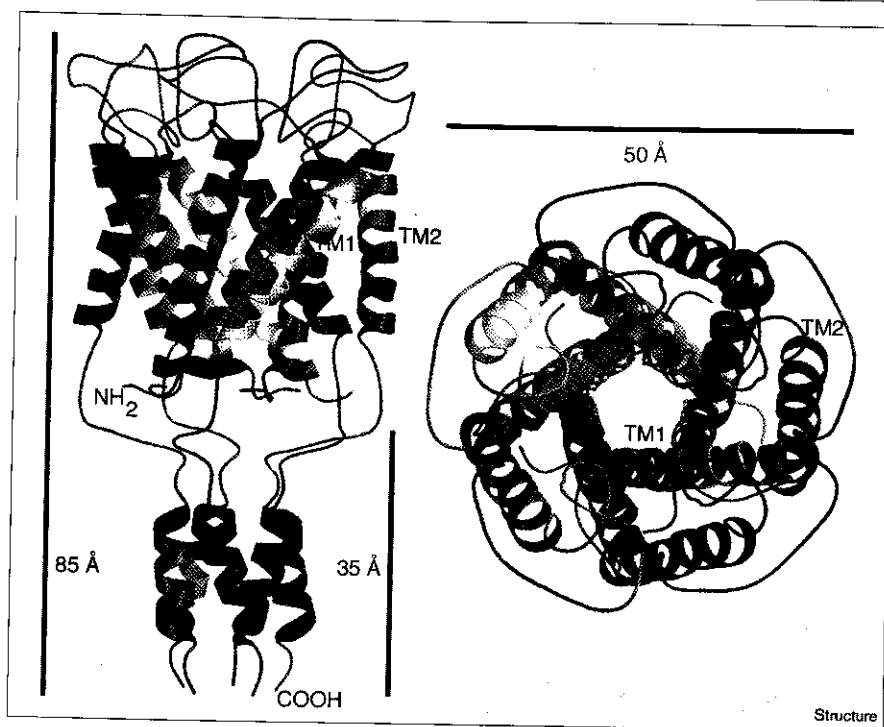
The Tb-MscL structure is a pentamer in a closed conformation (Figure 2). *In vivo*, the channel must be membrane-embedded with portions extending both above and below the lipid bilayer. Although the cytoplasmic domain may have stabilized pentamerization of the Tb-MscL channel under acidic crystallization conditions [4], the significance of this domain in the multimerization of both Tb-MscL and *E. coli* MscL is not clear. Of the 42

residues that follow the second transmembrane helix, the last 27 residues of *E. coli* MscL (based on the similarity to the Tb-MscL sequence [4]) can be deleted without dramatically affecting channel function [17,18]. However, a greater C-terminal deletion including six more residues suggests that these adjacent and largely conserved charges must be retained [17,18].

The membrane topology [10,11] of the *E. coli* MscL subunit (Figure 1) is supported by the Tb-MscL structure. The Tb-MscL pentamer protrudes outwards above the

Figure 2

Side and top view of the homopentameric Tb-MscL channel. Each of the five monomers is shown in a different color. The TM1 and TM2 helices and N and C termini of the cyan monomer are labeled. The overall dimensions of the molecule are given. Residues 1–9 and 119–151 of each subunit are not included. (The figure was generated using MOLSCRIPT and rendered using RASTER-3D [26,27].)



lipid plane with the loop between the transmembrane helices of each subunit (TM1 and TM2) gracefully capping the surface and then dipping into the external vestibule. All transmembrane helices are slanted with the five TM1 helices coming together at their cytoplasmic ends (Figure 2). The closed channel thus approximates a funnel extending above the membrane, but narrowing to only ~ 2 Å at the cytoplasmic end (Figure 3). Above the constriction, this funnel is lined with hydrophilic residues of TM1. A narrow channel at the bottom of the funnel precedes a bell-shaped cavity. This cavity (the gate), lined with hydrophobic residues, is closed by the convergence of five Val21 and five Ile14 residues (Figure 3). Thus closure is achieved by two sidechain rings 'pinching off' a bell-shaped cavity presumably devoid of water. This cavity is lined by the five, closely packed TM1 helices that span approximately two helical turns [4] (see Figure 3). The packing of the TM2 helices between the TM1 helices and the lipid bilayer buries hydrophobic surfaces and further stabilizes the closed configuration. Thus, the Tb-MscL crystal structure shows that upon closure water is squeezed out of the cytoplasmic end of the pore and the pore is held closed largely by the hydrophobic interactions of neighboring helices [4].

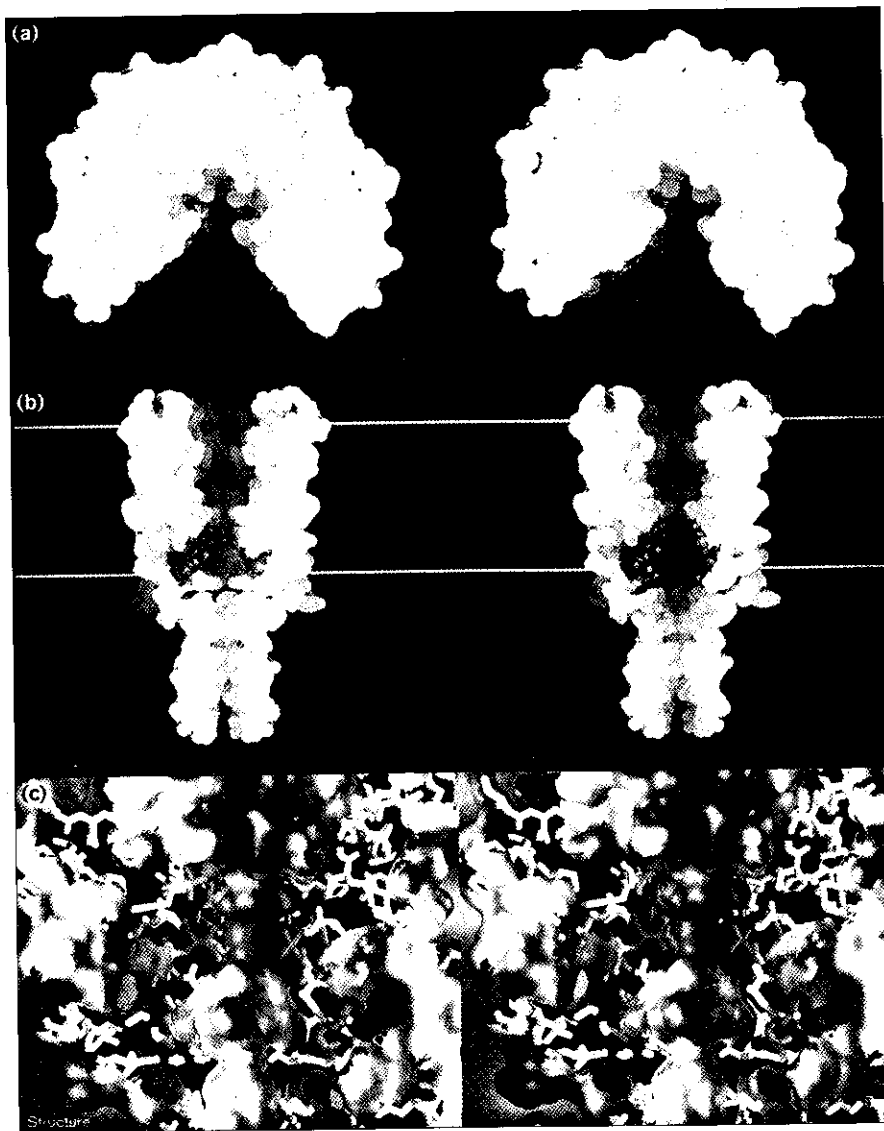
How does the channel open?

The number of ions transferred through the channel per unit time is enormous, conductance being ~ 3 nS for *E. coli* MscL. This conductance is huge, approximately tens to

hundreds of times larger than those of animal channels. The channel also has almost no selectivity, passing water, small solutes and even small proteins, such as the 12 kDa thioredoxin, into the periplasm [19]. Sieving [20] and conductivity measurements [20,21] both indicate an open MscL pore of some 30 to 40 Å in diameter. To contain such a large open barrel all ten helices need to be used as staves. Judging from the closed Tb-MscL structure, and assuming a similar large conductance to that of *E. coli* MscL, opening these large channels would require the cytoplasmic ends of all five inner helices to swing out from the center to the periphery. Even then, all ten transmembrane domains would have to move outwards to form a large pore [4] (see Figure 4). Presumably, this opening mechanism would also necessitate helix rotations to maximize buried hydrophobic surfaces and exposed hydrophilic surfaces. Nonetheless, it is expected that the net hydrophobic surface area exposed to a hydrophilic environment would be increased, a costly proposition in terms of energy requirements.

Mechanical work transmitted by stretch through the lipid bilayer allows the channel to overcome the tremendous energy barrier inherent in exposing its hydrophobic residues to the aqueous lumen. In fact, the tension required to open these channels reaches the limit of cell integrity; that is, opening 50% of the channels in a patch of membrane requires 12 dyne/cm, a tension which breaks many phospholipid bilayers [21]. The limits of resolution preclude a

Figure 3



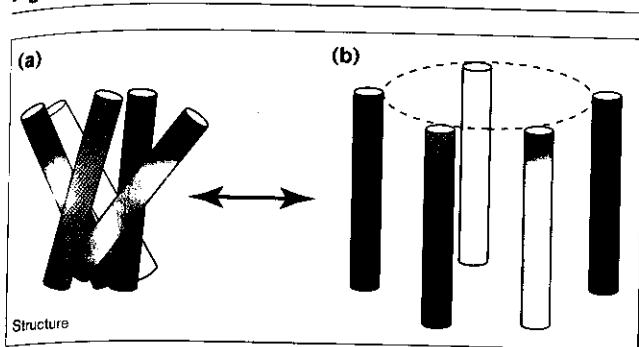
Stereoviews of Tb-MscL. Residues 1–9 and 119–151 in each of the five subunits are not included. (a) Top and (b) side mesh molecular surfaces (except a 90° wedge) of Tb-MscL. Helix residues generating the structure of the closed pore (Val15 to Ala20) highlight the contribution made by each TM1 helix, each being colored as in Figure 2. Residues forming the bottom and top constrictions (Ile14 and Val21) are shown in green. (c) Close-up side view (slice through the middle) of Tb-MscL highlighting the inside surface of the constricted pore, the narrow region above the pore and the lower part of the adjacent funnel, all of which are generated largely by the lower half of the five TM1 helices. The residues involved are color-coded: Ile14 (green), Val15 (cyan), Asp16 (pink), Leu17 (red), Ala18 (yellow), Val19 (purple), Ala20 (orange), Val21 (blue), Val22 (green), Ile23 (red), Gly24 (brown), Thr25 (pink), Ala26 (orange), Phe27 (yellow) and Thr28 (cyan). Residues visible in the inner pore regions include Ile14, Val15, Leu17, Ala18 and Val21 (lining the gate), Thr25 in the narrow region above, and Thr28 in the adjacent funnel. The corresponding M1 residues in *E. coli* are Val16, Val17, Leu19, Ala20, Val23, Ala27 and Gly30 (see Figure 1). (The figures were generated using the program GRASP [28].)

detailed description of hydrogen bonding in Tb-MscL; nonetheless, there appear to be very few bonds between subunits or between helices within subunits in the closed configuration of Tb-MscL, an unusual situation for a multimeric protein. It is possible that newly formed hydrogen bonds could contribute to the stabilization of the open states of these channels when these surfaces are exposed to the lumen. Although another model has been proposed [22], one can view gating as the tension-dependent exposure to the lumen of a sizeable previously buried hydrophobic surface.

These channels are considered to be the safety valves that release solutes upon osmotic downshock, such as that produced by rain, so that turgor pressure cannot build up to lyse the bacterium [19,23]. Indeed, genetically deleting both MscL and MscS activity (MscS is a

second mechanosensitive channel of more diminutive conductance), results in a mutant that lyses upon such downshocks [23]. *E. coli mscL* mutants with ultrasensitive channels that open at little or no stretch force have also been identified. When such 'loose-cannon' channels are produced, the host cells, which presumably leak solutes into the periplasm, cannot grow normally [24,25]. Most of the loose cannon mutations resulting from a random mutagenesis study mapped to a region that by analogy to Tb-MscL would contribute to the MscL gate, the narrow region above it, and the lowest part of the larger funnel [24] (compare Figures 1 and 3c). In fact only one face of the lower half of the highly conserved M1 helix was highlighted by 'very severe' and 'severe' mutations, suggesting a dramatically different environment for that portion of the helix during channel functioning [24] (Figure 1b).

Figure 4



The open and closed forms of Tb-MscL. The five TM1 helices in (a) the observed closed form of Tb-MscL and (b) the postulated open channel form. The helices are depicted using the same color scheme as in Figure 2; the TM2 helices (see Figure 2) are not pictured. Upon channel opening, the cytoplasmic ends of the TM1 helices must swing outwards. All helices (including the TM2 helices) are likely to move outwards slightly to form the pore. It is likely that helix rotation accompanies the TM1 trajectories [4].

A recent study examined the bacteriological and biophysical consequences of changing Gly22, an M1 residue which by analogy with Tb-MscL would surround the *E. coli* MscL gate, to all 19 other amino acids residues. (The analogous Tb-MscL residue Ala20 is buried below each Val21 [green] in Figure 3b and is in van der Waals contact with each Ala18 in an adjacent TM1 helix in the wall of the gate; Figure 3c.) The results of this study indicate that the hydrophilicity of residue 22 promotes easy gating, which is correlated with growth inhibition, and that this residue is exposed to the aqueous lumen upon channel opening [25].

Though much work is still needed to understand the dynamic reorganization during gating, we are grateful that we finally have a concrete image of this process after a century of electrical studies of channel activities.

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