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Mechanosensitive Channels: Stress Relief

Dispatch

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Bacterial responses to decreasing osmolality involve mechanosensitive channels. The crystal structure has been determined of the small conductance mechanosensitive channel (MscS) from *Escherichia coli*, providing new insights into mechanical and voltage sensing by this and other channel proteins.

Bacteria protect themselves from changes in the osmolality of the surrounding environment by using stretch-activated, or mechanosensitive, channels. As their name suggests, these channels respond to the change in membrane tension when a cell expands. Expansion reduces the lateral pressure exerted by the lipid bilayer upon the protein, causing the channel to open and allowing passage of water and ions. Bacterial mechanosensitive channels fall into two families: large-conductance channels (MscL) and small-conductance channels (MscS). The small conductance channel is also sensitive to changes in the transmembrane voltage — depolarizing a cell increases the probability that the MscS channel will open [1,2].

Bacterial mechanosensitive channels are becoming something of a focal point for the study of the relationship between membrane protein structure and function. In 1998, Rees and colleagues [3] solved the structure of MscL in a closed-channel state, and more recently Perozo *et al.* [4] characterized the open state of this channel by electron paramagnetic resonance spectroscopy. Now, Rees and colleagues [5] have solved the crystal structure of MscS in what appears to be an open-channel state. Despite the similar functions of these two proteins, there is no sequence homology between them and the MscS structure reveals a very different architecture from its larger-conductance cousin. The structure of MscS is also of more general interest in that it is the first X-ray structure of a channel containing a voltage-sensing element.

The crystal structure [5] shows that MscS channel forms a symmetrical homoheptamer. The transmembrane domain of each subunit contains three α-helices — TM1 to TM3 — plus a large cytoplasmic domain composed mainly of β-sheet (Figure 1). The central pore runs down the centre of the heptamer, parallel to the seven-fold rotational axis. The first half of TM3 and the loop between TM2 and TM3 lines the transmembrane region of the pore. The second half of TM3 adopts an orientation almost parallel to the membrane plane, as a result of a large helix kink at residue glycine 113.

The central pore of the MscS channel is about 10 Å in diameter in the region of the membrane, consistent with a channel conductance channel of approximately 1 nS. Somewhat surprisingly, the channel lining is very hydrophobic and so might not be expected to interact favourably with permeant ions. Interestingly, some older work [6] on channel-forming peptides had indicated that high-conductance channels could be formed by bundles of purely hydrophobic helices forming membrane-spanning pores. The transmembrane pore of MscS expands at the intracellular face of the membrane to form a large bulbous chamber of approximately 40 Å diameter formed by the cytoplasmic regions of the protein. Simple calculations based on Ohm's law suggest that a pore diameter of about 8 Å is sufficient to give a conductance of about 1 nS [7]. This supports the proposal that the conformation of MscS in the crystal is indeed that of an open-channel state.

Access to the cytoplasmic chamber of the MscS channel is through seven lateral portals plus one ventral hole coincident with the long axis of the pore. This arrangement is reminiscent of the intracellular regions

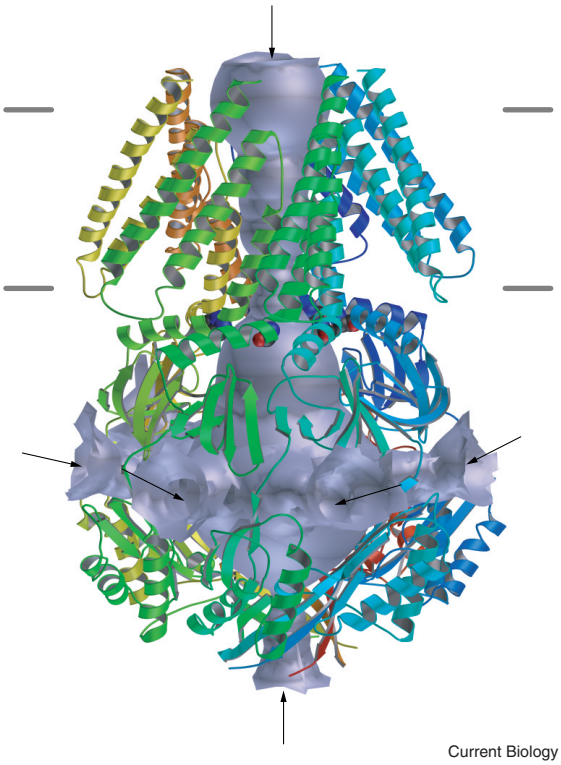

Figure 1. Structure of the MscS channel.

The structure is shown in cartoon format [15], with the gray surface indicating the openings that presumably allow ions to pass through the channel as calculated by HOLE [16]. The gray bars indicate the approximate location of the membrane. The highlighted region (spheres) indicates the location of glycine 113 which is postulated to play a role in the gating mechanism. Figure rendered with POV-Ray (www.povray.org).

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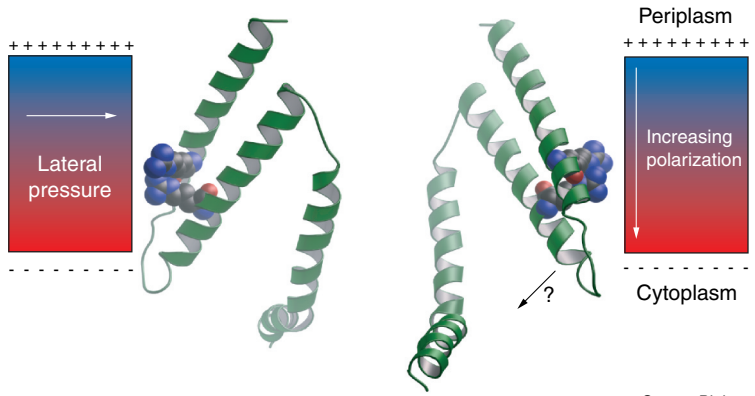

Figure 2. The three transmembrane helices of two of the subunits (chains A and D).

The arrows indicate what might happen upon channel closing. Reduction in membrane tension will increase the lateral pressure on the protein (horizontal white arrow). Increasing the degree of polarization — a more negative intracellular voltage, shown by the vertical white arrow — will also favour channel closing by pulling the arginines (indicated in space-fill) towards the cytoplasm. Both of these effects are proposed to move the transmembrane domains inwards in a similar fashion so as to close the channel (black arrow).

of some other channels such as the nicotinic acetylcholine receptor [8] and the voltage-gated potassium channel [9]. The large intracellular domain is thought to provide a docking site for regulatory proteins. It is unclear whether this is also the case for MscS.

The structure of MscS also provides some clues as to how the channel might switch between open and closed states. It has been difficult to establish a mechanosensory motif — there is little information from sequence comparisons — but Rees and colleagues [5] suggest that the presence of small amino acids (glycine and alanine) at every fourth position, in regions that participate in helix–helix packing, may underpin the mechanism that allows for large packing rearrangements under different lateral pressures from the surrounding membrane.

The mechanism for sensing a change in transmembrane voltage is a little clearer. Comparisons with mammalian voltage-gated channels have proved useful in this respect. It has long been established that the voltage-sensor in voltage-gated potassium, sodium and calcium channels is the S4 helix, a transmembrane helix that contains a conserved pattern of basic (arginine or lysine) residues [10]. Significantly, the TM1 and TM2 helices of MscS contain arginine residues that appear to be positioned such that they could detect a change in transmembrane voltage (Figure 2). Moreover, although TM1 and TM2 are closely packed relative to each other, they are displaced away from TM3 and the core of the protein. This and the fact that the electron density is less well defined in this region suggests that they may be more mobile, reinforcing the hypothesis these segments move upon gating of the channel.

It is tempting to infer the structure of the closed state of MscS from the open-channel crystal structure. For MscL, it had been proposed that two rings of hydrophobic sidechains may form a gate [11]. As the transmembrane pore of MscS is very hydrophobic, it is conceivable that, if the channel structure changes so as to narrow the pore, once the hydrophobic pore falls below a critical diameter the pore will become functionally closed, as has been suggested by recent calculations [12]. One possible model is that changes in membrane tension and/or voltage are sensed by TM1 and TM2, and that this results in a change in the packing of TM3 so as to narrow and/or lengthen the pore. One should note that the kink in TM3 is due to a glycine residue. It is perhaps relevant that a change in helix kink about a glycine residue has been implicated in the gating mechanism of the bacterial potassium channels KcsA and MthK [13].

Mechanosensitive channels are also found in mammalian cells [14]. It is not thought that they share any structural similarity with either MscS or MscL. Nevertheless, the general principles of channel gating emerging from studies of these bacterial channels may well be applicable to mammalian channels as well.

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