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Spectator no more, the role of the membrane in regulating ion channel function

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A pressure gradient across a curved lipid bilayer leads to a lateral force within the bilayer. Following ground breaking work on eukaryotic ion channels, it is now known that many proteins sense this change in the lateral tension and alter their functions in response. It has been proposed that responding to pressure differentials may be one of the oldest signaling mechanisms in biology. The most well characterized mechanosensing ion channels are the bacterial ones which open when the pressure differential hits a threshold. Recent studies on one of these channels, MscS, have developed a simple molecular model for how they sense and adapt to pressure. Biochemical and structural studies on mechanosensitive channels from eukaryotes have disclosed pressure sensing mechanisms. In this review, we highlight these findings and discuss the potential for a general model for pressure sensing.

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Introduction

Membrane proteins, by definition, function in combination with the lipid bilayer. The degree to which they are embedded within the lipid membrane ranges from fully embedded (spanning the entire bilayer) to associated (usually by an anchor such as a short peptide sequence or fatty acid modification). The region in contact with the bilayer is called transmembrane domain and the structure of this region must have co-evolved alongside the lipid bilayer, a very different environment from water. Ion channels are a key class of integral membrane proteins, which connect the cell's inner world with the wider world outside. Cell survival requires that nutrients enter but toxins are blocked and waste exits, whilst metabolites are retained. This in turn requires that the cell is able to control when channels are open or closed and what kind of molecules they allow to pass. The opening and closing of ion channels are known to be regulated by defined stimuli such as ions, ligands, peptides and pH. Often the membrane was treated like water to be little more than milieu in which the ion channel functioned, its role being to stabilize and localize the protein structure, with the lipid molecules often considered as bystanders. Although true in general, there were examples of lipid binding modifying protein behaviour, for example phosphatidylinositol 4,5-bisphosphate binds K^+ channels and alters their function [1].

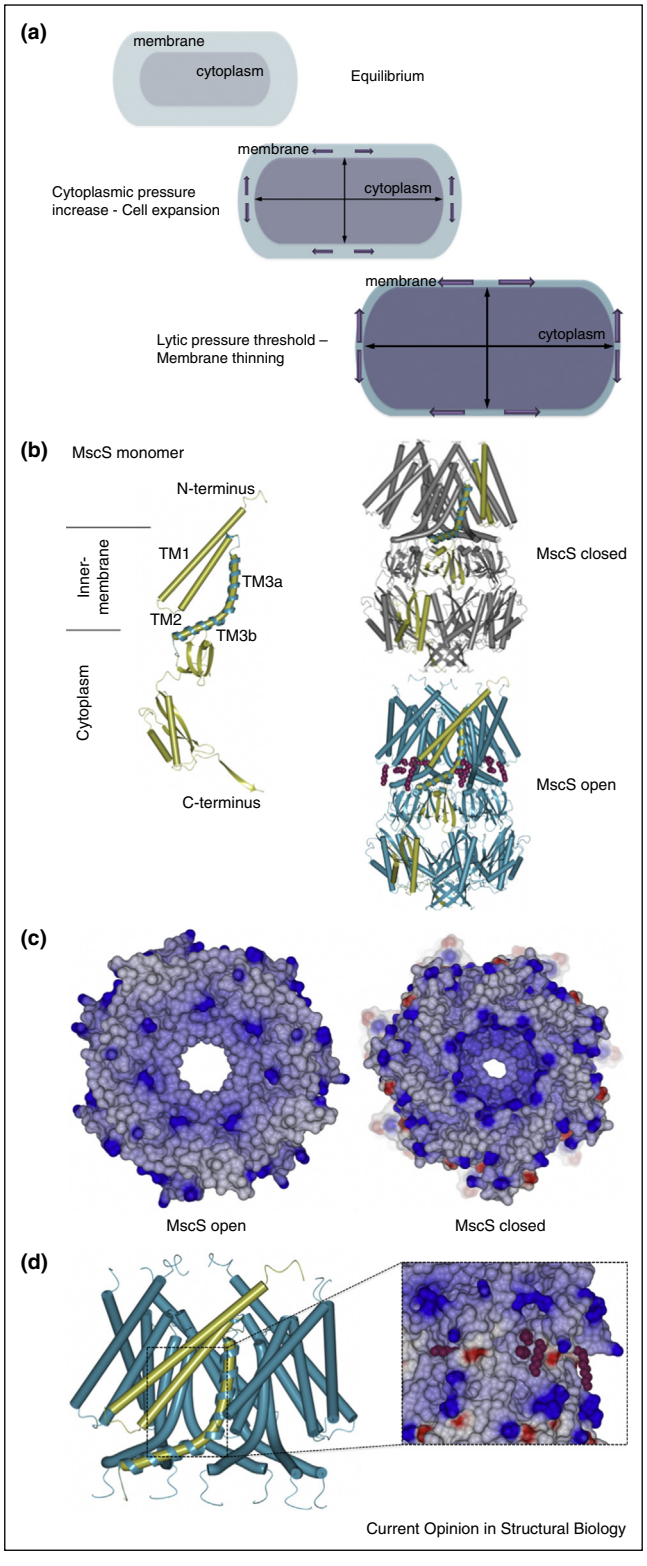
A change in the perception of lipids as actively regulating protein function came from the study of a pair of bacterial mechanosensitive channels, the heptameric mechanosensing channel of small conductance (MscS) [2,3] and the pentameric mechanosensing channel of large conductance (MscL) [4,5]. These proteins are controlled by changes in the lipid bilayer that arise from increased pressure inside the cell (known as turgor). In this review, we discuss recent progress in the structural understanding of the role of lipids play, in regulating a wide range of ion channels. We highlight the differing molecular models that have emerged to rationalize this behaviour and develop the suggestion by Kung and co-workers, that sensing the pressure differential across lipid bilayer is amongst the earliest evolutionary events in channel regulation [6].

Bacterial mechanosensors MscS and MScL: open and shut stories

Mechanosensitive channels open and close in response to changes in the cell turgor pressure. The family of mechanosensing channels are not confined to bacteria there are many examples from higher organisms, which do not share any sequence homology with the bacterial ones but fill important roles including K^+ transport [7,8••] and mechanotransduction [9,10•]. However, the bacterial systems are the most well studied.

In bacteria, the opening of the channels occurs at defined pressures and allows the rapid efflux of ions, solute and small molecules reducing the turgor pressure, thus protecting the cell against hypo-osmotic shock [11]. The increased turgor pressure manifests itself as lateral tension within the bilayer and it is for this reason the proteins are also known as stretch channels (Figure 1a). The gating

Figure 1



Pressure sensing and mechanosensitive protein of small conductance (MscS). **(a)** A pressure gradient across a lipid bilayer will lead to a lateral stretching force within the bilayer. Mechanosensitive channels open in response to a pressure differential across the lipid bilayer allowing ions and solvent to flow through them. **(b)** MscS has three TM helices and these are identified in a cartoon diagram. TM1 and TM2 undergo a large rotational movement upon gating, TM3b moves outwards from the central axis and thereby creating an open channel. One monomer is shown in order to illustrate the movements that occur. All figures which contain structural models have been created using QtMG (CCP4 routine). **(c)** A surface view of the closed and open states of MscS viewed from the periplasm. **(d)** The TM helices of MscS are not close packed and creating pockets between them. In a recent study these pockets were found to bind *E. coli* lipids. The figure shows the alkyl chains identified in the crystal structure.

of mechanosensitive channels is controlled by the lipid bilayer itself. Interestingly, both MscS and MscL proteins can also be opened by addition of *lyso*-phospholipids (*lyso-PC*). The original open [12] and closed [2] crystal structures of the heptameric MscS protein revealed that the protein undergoes a very significant re-organisation of the transmembrane helices (Figure 1b) when it opens. Upon gating of MscS, the first two helices (TM1/2) pivot by a 1/7 of revolution and a central pore of over 14 Å diameter is created by movement of the seven TM3a (central pore) helices (Figure 1c). The pore helix is connected by a kinked region of structure to final transmembrane region, TM3b, which sits almost parallel to the membrane bilayer unlike TM1, 2 and 3a, which are approximately perpendicular to the membrane. Recent studies on MscS have shown that mutations on highly conserved residues on TM3b altered gating kinetics [13]. A cryo-EM structure of the presumed MscS homologue YnaI confirms structural conservation of the TM helices within the MscS family [14].

MscL gates at a higher pressure than MscS but as its name implies (large conductance) it creates a larger pore. Interstingly, increased expression of MscL was recently found to increase steptomycin effectiveness against bacterial cells [15] suggesting the protein maybe a future drug target. Indeed, it is now known, that streptomycin binds, opens and passes through MscL [16]. The activation of MscL in droplet interface bilayers [17] may herald novel nano-technological applications of the protein. The closed crystal structure of pentameric *Mycobacterium tuberculosis* MscL [4] has been known for several years. Native mass spectrometry of MscL suggested the *in vitro* oligomerisation state (pentamer versus tetramer) is influenced by temperature, detergent and amino acid variability as well as by the precise construct used [18•]. Protein stability has been shown to be influenced by the presence of particular lipids [19••]. The variety of oligomeric states and their sensitivity to lipidation may explain the challenges in obtaing a structure of a fully open form. Consequently a variety of biophysical and spectroscopical tools were used to probe gating. FRET [20] measurements of *Escherichia coli* MscL opened by addition of *lyso-PC* estimated a pore with over 25 Å in diameter and favoured a helix-tilt gating model [20]. A combination of cwEPR, electrophysiology measurements and computational studies highlighted the importance of the N-terminal