Bacterial Mechanosensitive Channels as a Paradigm for Mechanosensory Transduction

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Key Words

Bacteria • *E. Coli* • MscL • MscS • Patch clamp • Liposomes • EPR spectroscopy • FRET spectroscopy

Abstract

Research on bacterial mechanosensitive (MS) channels has since their discovery been at the forefront of the MS channel field due to extensive studies of the structure and function of MscL and MscS, two of the several different types of MS channels found in bacteria. Just a few years after these two MS channels were cloned their 3D structure was solved by X-ray crystallography. Today, the repertoire of multidisciplinary approaches used in experimental and theoretical studies following the cloning and crystallographic determination of the MscL and MscS structure has expanded by including electronparamagnetic resonance (EPR) and Förster resonance energy transfer (FRET) spectroscopy aided by computational modelling employing molecular dynamics as well as Brownian dynamics simulations, which significantly advanced the understanding of structural determinants of the gating and conduction properties of these two MS channels. These extensive multidisciplinary studies of MscL and

MscS have greatly contributed to elucidation of the basic physical principles of MS channel gating by mechanical force. This review summarizes briefly the major experimental and conceptual advancements, which helped in establishing MscL and MscS as a major paradigm of mechanosensory transduction in living cells.

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A personal note in place of an introduction

The science I have been doing over the last 25 years has been like a good wine getting better with age. Since my very first patch-clamp recordings from bacterial mechanosensitive (MS) channels I have remained under their spell. Looking today at some of my first patch-clamp recordings of the MS channel currents and reading many recent publications from laboratories around the world describing the latest progress made towards better understanding of the workings of these channels I feel very lucky that my interest in these fascinating molecules is shared today by so many of my peers. It was to my pleasant surprise when I received the

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news that (cit.) “owing to the outstanding achievements in research on Mechanosensitive Ion Channels” I was selected as the “Peter Lauf Lecturer” at the International Congress on “Hydration & Cell Volume Regulation” in September, 2011 in Tübingen. The news added a joyous flavour to the wine I have been drinking for so long.

Ancient nature of mechanosensory transduction

In the *Creation of Adam* Michelangelo painted around 1511 God is stretching out his arm to touch Adam’s finger to spark life into him. By choosing the sense of touch as the origin of life in this painting the genius of the great Renaissance master summarizes it all.

For most of its age the Earth has been inhabited by a vast number of life forms of which microbes form the largest group (Fig. 1A) [1]. Their diversity, number, and adaptability to all kinds of environments found on the planet enabled them to populate all niches of life. Osmotic forces resulting from the essential role that water plays for the existence of life, present an important factor in everyday’s survival of microbial cells. Bacteria are protected by a cell envelope consisting of a cell wall and a cytoplasmic membrane (Fig. 1B) harbouring different types of mechanosensitive (MS) channels allowing these prokaryotic microbes to adjust to a large range of osmotic forces acting upon them [2-4]. Given that a difference in osmotic forces of only few milliosmols across the cell membrane of a bacterium is sufficient to fully activate MS channels [5-9] these membrane proteins may have from very early on served as cellular osmoregulators measuring small changes in the concentration of solutes and water across microbial cell membranes [10, 11]. These ancient sensors of mechanical stimuli impacting on cellular membranes of early microorganisms (Fig. 1C) could have during evolution transformed into regulators of cell size and volume [12-14]. With the appearance of multicellular organisms they could have further evolved into molecular sensors functioning in more specialized forms of mechanosensory transduction including gravitropism in plants [15], contractility of the heart [16-19] as well as senses of hearing and touch [7, 20]. By operating on a millisecond time scale and passing millions of ions and organic osmolytes per second MS ion channels must have been the most prevalent type of signalling macromolecules functioning as molecular transducers of mechanical force converting mechanical stimuli into

chemical and/or electrical signals. In the evolution of different life forms on Earth MS ion channels such as the bacterial MscL and MscS channels of large and small conductance [21-23], are likely descendants of the primordial sensors of mechanical force that evolved as primary signalling molecules supporting mechanosensory physiology of living organisms.

Structure and function of bacterial MS channels: A multidisciplinary study

Studies of MS ion channels conducted over the last twenty five years have greatly contributed to our understanding of molecular mechanisms underlying the physiology of mechanosensory transduction. Thanks to the invention of the patch clamp technique [24], which was instrumental for discovery of MS channels as molecular entities [25-27], direct measurements of single MS channel currents in bacteria were made possible [21]. These membrane proteins function as emergency valves by releasing excess osmolytes upon hypo-osmotic stress. Besides this well-known function of bacterial MS channels they have recently been shown to contribute also to the physiologic regulation of the compatible solute concentration in the cytoplasm of bacterial cells under conditions of hyperosmotic stress. Under these conditions both active uptake by membrane transporters and passive efflux via MS channels (*pump-and-leak mechanism*) contribute to the fine-tuning of the bacterial cell response to hyperosmotic conditions [9].

Bacterial MS channels have been the object of extensive multidisciplinary research, which was greatly advanced by the cloning [22, 23, 28] and determination of the 3D crystal structure [29, 30] of MscL and MscS. The 3D structures of these MS channel proteins have provided the basis for further experimental and theoretical studies investigating the mechanism of membrane tension sensing defined by lipid-protein interactions between the membrane bilayer and both of these structurally unrelated membrane proteins (Fig. 2) [31, 32].

The X-ray crystal structure of Tb-MscL, the MscL homologue from *Mycobacterium tuberculosis* (Fig. 2), revealed that the MscL channel is a homopentamer of monomers consisting of two transmembrane helices TM1 and TM2, N- and C-terminal domains located at the cytoplasmic side and a flexible loop connecting the two helices at the periplasmic side of the bacterial cell membrane (Fig. 2) [29]. The channel pore of MscL is lined by five TM1 helices. This structure is generally

A

Bacteria

Archaea

Eucarya

0.1 changes per site

B

Teichoic acid

Three-dimensional layer of peptidoglycan

Cytoplasmic membrane

Phospholipid

Protein

Fatty acids

Lipopolysaccharide

O antigen

Protein pore

Outer membrane

Fatty acids

Monolayer of peptidoglycan

Phospholipid

Lipoprotein

Cytoplasmic membrane

Protein

Phospholipid

C

Earth formed

Stabilization of crust

Microorganisms

Oxygen atmosphere forming

Trilobites

Dinosaurs

Horses

Humans

Billions of years ago

(Δ26 SaMscL) showed that this channel was organized as a homotetramer rather than a homopentamer [33] indicating an unusual plasticity in oligomerization of the

Fig. 2. Bacterial MS channels. The structure of the pentameric MscL channel (left) and a channel monomer (right) from *M. tuberculosis* according to the 3D structural model (top) [80, 81]. 3D structure of the MscS homoheptamer (left) and a channel monomer (right) from *E. coli* (bottom) [81, 82]. Physical and chemical stimuli affecting the gating of MscL include membrane stretch, amphipaths (e.g. lyso-phospholipids, chlorpromazine, trinitrophenol) and parabens. The channel activity is inhibited by Gd^{3+} and static magnetic fields (SMF) and is modulated by temperature and intracellular pH [5]. MscS is activated by membrane stretch, amphipaths and parabens and is modulated by voltage. The activity of the channel is inhibited by Gd^{3+} and high hydrostatic pressure (HHP) [5]. The arrows point at membrane structures (i.e. channel protein and/or lipid bilayer) affected by the specific stimuli. (Reproduced from [83]; with permission).

MscL channel family. Another study however, showed that the MscL channel was a pentamer *in vivo* and that detergents used to solubilize the protein could influence the channel monomers to arrange as pentamers as well as tetramers indicating that detergents could have a profound effect on the structure of membrane proteins [34]. The currently accepted oligomeric structure of MscS is that of a homoheptamer consisting of monomers having three transmembrane helices TM1, TM2 and TM3, and the N- and C-terminal domains located at the periplasmic and cytoplasmic end of the bacterial cell membrane, respectively (Fig. 2). This structure is based on the X-ray crystal structure of an intermediate conformation of Eco-MscS, the MscS channel from *E. coli* [30].

According to this structure the pore of the MscS channel is formed by seven TM3 helices. Future structural studies may reveal if the MscS family of MS channels possesses similar plasticity of oligomerization as shown for the MscL family [35].

Multidisciplinary studies of the MscL and MscS channels have employed a wide range of methods and techniques towards understanding their structure and function. Some of the various methods that have been used to study these unique types of channels are shown in Fig. 3.

Very small size of an *E. coli* cell is prohibitive for direct application of the patch-clamp recording to it. Therefore, a larger bacterial object is necessary for

Fig. 3. Methods and techniques employed in studies of the structure and function of MscL and MscS. The giant spheroplast preparation and liposome reconstitution are the main methods to study MscL and MscS function by the patch-clamp technique. X-ray crystallography was essential for determination of their 3D structure, whereas electron-paramagnetic (EPR) and Förster resonance energy transfer (FRET) spectroscopy have together with molecular dynamics simulations been used in studies of structural dynamics of both channel proteins.

recording ion channel activities from the bacterial cell membrane. This is achieved by generating the preparation of giant spheroplasts, which presented a major technical advancement allowing for the electrophysiological investigation of *E. coli* cell membranes that lead to discovery of bacterial MS channels [21, 36-38]. The second method employed in functional patch-clamp studies of MscL and MscS consists of reconstituting the purified MS channel proteins into liposomes [39-42]. Liposome reconstitution was essential for molecular identification of the MscL protein as the first MS channel identified at the molecular level [22, 28]. Cloning of the corresponding *mscL* gene enabled further detailed structure and function studies of these MS channels. The reconstitution method

used for this unorthodox approach in cloning of an ion channel was a refinement of the method reported by Criado and Keller [43]. The refinement consisted in exposing azolectin proteoliposomes to tens of millimols of MgCl₂, which led to formation of *blisters* (unilamellar or paucilamellar liposomes) arising from the multilamellar proteoliposomes allowing higher gigaohm seals between the patch pipette and liposome bilayer required for MS channel recording [39]. This method called *Dehydration/Rehydration (D/R)* method is used today as the standard for MscL reconstitution into liposomes (Fig. 3). Other liposome reconstitution methods developed in the meantime include the *sucrose method* [44] and an electroformation technique developed commercially by

Nanion technologies GmbH (München, Germany; <http://www.nanion.de/products/port-a-patch/port-a-patch-data.html>) [45]. Besides giving very good reconstitution efficiencies for both MscL and MscS, the sucrose method also offers significant time-savings over the D/R method with recordings of functional MscL and MscS channel activity being reported in 3 hours [46]. Furthermore, an optimized purification and liposome reconstitution method was used for functional patch-clamp analysis and spectroscopic studies of structural dynamics of MscS [47].

Studies focusing on dissecting the molecular mechanism of MscL and MscS channel function are often based on the use of amphipaths, such as chlorpromazine (CPZ), trinitrophenol (TNP), local anesthetics and lysophospholipids known to insert preferentially in one leaflet of the lipid bilayer [48-50], which strongly supports the bilayer model of MS channel activation by mechanical gating force resulting from membrane bilayer deformation [49]. The use of the amphipath lysophosphatidylcholine (LPC) was particularly important in deciphering the open channel form of MscL by SDSL EPR spectroscopy (Fig. 3) [50, 51], which to date could not be achieved by X-ray crystallography. Furthermore, MscS closed [52] and open channel structure [53] have also been determined using the same approach. The EPR spectroscopic studies have recently been complemented by FRET spectroscopic studies investigating structural dynamics of MscL (Fig. 3) [54, 55]. FRET spectroscopic studies of MscL, which also relied on the use of the amphipath LPC to open MscL in liposomes, have largely confirmed the findings of the EPR spectroscopic studies [54]. They also helped to improve the open channel model of MscL by showing that contrary to previous models [51, 56, 57] transition to the open state is less dramatic than previously proposed and that the N-terminus is able to directly translate membrane tension to the pore lining TM1 helix [55]. The structural studies of MscL and MscS were paralleled with molecular dynamics simulations of both channels embedded in the lipid bilayer (Fig. 3) [32]. The computer modelling provided a wealth of information on structural determinants of the gating and conduction properties of the two channels.

In addition to the techniques described above researchers have recently employed a range of other techniques, including NMR spectroscopy, small-angle neutron scattering (SANS), small-angle X-ray scattering (SAXS) and atomic force microscopy (AFM), to name a few [58, 59]. This was prompted by the great interest in understanding the structure and function relationship in

membrane proteins including MS ion channels, whose function is closely linked to the membrane lipid environment in which these proteins operate.

For interested reader a number of excellent reviews provide further information on the structure, function and gating mechanism of the MscL and MscS channels [5, 7, 31, 32, 38, 41, 60-70].

What Have Bacterial MS Channels Taught Us About Mechanosensory Transduction

The structure and function studies of MscL and MscS employing a multidisciplinary approach based on the patch-clamp recording, X-ray crystallography, computer simulations, and EPR and FRET spectroscopy, have provided researchers with much of what is known today as to how MS channels function and are regulated [68]. In particular, experiments employing chemical manipulation of the physical properties of the membrane lipid bilayer [49, 50] have caused a paradigm shift in understanding the activation of MS channels by membrane tension by demonstrating that mechanical force within the lipid bilayer was sufficient to gate an MS ion channel, i.e. that tethering to other proteins such as structural proteins was not needed for the channels to open and close [62, 63]. Although the bilayer mechanism of gating MscL and MscS by bilayer deformation forces is undisputed the specific lipid/protein interactions controlling the conformational changes have not been fully resolved. The ability of both MscL and MscS to be activated by amphipaths enabled researchers to use SDSL EPR spectroscopic studies to identify protein-lipid bilayer hydrophobic mismatch and bilayer curvature as two potential triggers of the MscL and MscS channel gating by the bilayer mechanism [50, 51, 53]. Spontaneous restructuring of the periplasmic loops and their interaction with the phospholipid headgroups suggested further that curving the bilayer may be sufficient to induce a conformational change in the MscL channel [71]. Another study however, indicated that lateral tension rather than membrane curvature could be the physical parameter gating the channel [41] in accordance with the results of a molecular dynamics study suggesting that the packing of lipid headgroups is determinant of pressure profile perturbation [72] and consequently membrane tension gating MscL. The same study further showed that the presence of phosphatidylethanolamine, the major endogenous lipid of the *E. coli* cell membrane, caused MscL to become less sensitive to membrane tension

requiring higher tension for activation suggesting that phosphatidylethanolamine altered channel activity through changes in the physical properties of the membrane bilayer, rather than through a specific lipid-MscL interaction [41]. In contrast, a separate study demonstrated that the function of MscL could be modulated by direct interaction with the surrounding, annular phospholipids, which are in direct contact with the channel protein [73]. A further independent study showed that replacement of hydrophobic residues near the periplasmic rim of MscL by hydrophilic ones caused MscL to lose apparently its mechanosensitivity, since it could not be opened by membrane tension before breaking the membrane patch [74]. Moreover, recent *in vivo* studies demonstrated that MscS concentrates at the poles of *E. coli* cells and co-localizes with the cardiolipin, the concentration of which increases with the osmolarity of the growth medium [75]. Cardiolipin (also referred to as diphosphatidylglycerol) is a doubly charged phospholipid that is most likely asymmetrically distributed between the two leaflets of the bacterial membrane bilayer. Obviously, more experimental insight is necessary to understand the precise molecular interactions between lipid molecules and MS channel components to fully understand the bilayer mechanism of gating MscL and MscS by mechanical force.

What makes MscL and MscS mechanosensitive? The answer lies in the fact that both channels undergo large conformational changes upon mechanically stressing the lipid bilayer resulting in the area of an open channel being bigger than the area of a closed channel. The free energy difference between these two conformational states is given by the following expression:

$$\Delta G = T (A_o - A_c) = T \Delta A \quad (1)$$

where T is the membrane tension opening the MS channel and A_o and A_c are areas of the open and closed channel, respectively. The larger the difference in area ΔA , the higher the mechanosensitivity of the channel. In those terms MscL is a prototypical MS channel since it undergoes a large in-plane protein expansion of 18-20 nm² upon opening under membrane tension [55], which accounts well with its large open channel pore of ~30 Å in diameter [76]. The open channel pore of MscS is with 16 Å in diameter twice as small compared to the MscL pore [68] but nevertheless it indicates a large conformational change between closed and open form of MscS. Generally, one may discuss mechanosensitivity of a channel if the radius of the channel protein increases by at least 0.2 nm, as in the case of TREK-1 MS channel [77].

In summary, MscL and MscS have taught us that:

- (i) Mechanosensory transduction is ancient and has most likely resulted from the crucial role that water plays for the existence of all living forms.
- (ii) Mechanical force gating MS channels can originate from the membrane bilayer deformations and does not require any other cellular components such as cytoskeleton or extracellular matrix.
- (iii) Hydrophobic mismatch is one of the global determinants of the MS channel conformation in the lipid bilayer favoring opening of MscL in thinner compared to thicker bilayers.
- (iv) Bilayer curvature and/or changes in transbilayer pressure profile are the second major global factor regulating MscL and MscS channel structural conformation between the closed and open states.
- (v) Specific interactions between particular lipids and structural components of MscL and MscS channels usually located at the bilayer water-lipid interface are local determinants initiating structural changes in both MS channels.
- (vi) MscL and MscS are prototypical MS channels due to the large difference in area occupied by the open compared to the closed form of these channels.

Remaining Challenges

Textbook descriptions of cell membranes tend to focus on the various proteins they contain by emphasizing their role in regulating cellular functions such as cell permeability and signalling. Less emphasis is given to the lipid bilayers and their importance for the functioning of membrane proteins. However, cell membrane is a very dynamic environment in which lipids play much more important role than that of a supporting matrix. MS ion channels present a classical example of ion channels for which the composition and properties of the surrounding lipid matrix are crucial for their function. Their leitmotif is *force from lipids* [61, 62]. Studies on prokaryotic (MS) channels over many years have strongly indicated that the lipid bilayer transmits mechanical force directly to this type of MS channels enabling them to detect changes in osmotic forces acting upon these microorganisms [7, 9]. Testing the key hypothesis that mechanical force is delivered through the lipid bilayer to MS ion channels of membranes of all types of living cells is central to elucidation of the general physical principles underlying the biophysics of MS channel mechanosensitivity. Understanding of how MS channels of different

transmembrane architectures transduce mechanical stimuli into protein structural changes presents the key challenge to establishing the general mechanism of mechanosensory transduction in living cells.

Conclusions

The bacterial MscL and MscS channels present today the best studied example of MS type of ion channels. The mechanisms that underlie how individual MscL and MscS channels interact with their lipid environments need further investigation if we are to comprehend fully the molecular mechanisms of mechanotransduction. A better knowledge of these mechanisms will contribute to a better understanding of the basic molecular principles underlying mechanosensory transduction and could also help in designing nano-valves in various biomedical and engineering applications. Whatever their practical use the intellectual satisfaction resulting from deciphering the secrets of these membrane proteins presents the best reward for a scientist studying these fascinating molecules.

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