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Review

Lipid–protein interactions: Lessons learned from stress☆

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ABSTRACT

Biological membranes are essential for normal function and regulation of cells, forming a physical barrier between extracellular and intracellular space and cellular compartments. These physical barriers are subject to mechanical stresses. As a consequence, nature has developed proteins that are able to transpose mechanical stimuli into meaningful intracellular signals. These proteins, termed Mechanosensitive (MS) proteins provide a variety of roles in response to these stimuli. In prokaryotes these proteins form transmembrane spanning channels that function as osmotically activated nanovalves to prevent cell lysis by hypoosmotic shock. In eukaryotes, the function of MS proteins is more diverse and includes physiological processes such as touch, pain and hearing. The transmembrane portion of these channels is influenced by the physical properties such as charge, shape, thickness and stiffness of the lipid bilayer surrounding it, as well as the bilayer pressure profile. In this review we provide an overview of the progress to date on advances in our understanding of the intimate biophysical and chemical interactions between the lipid bilayer and mechanosensitive membrane channels, focusing on current progress in both eukaryotic and prokaryotic systems. These advances are of importance due to the increasing evidence of the role the MS channels play in disease, such as xerocytosis, muscular dystrophy and cardiac hypertrophy. Moreover, insights gained from lipid–protein interactions of MS channels are likely relevant not only to this class of membrane proteins, but other bilayer embedded proteins as well. This article is part of a Special Issue entitled: Lipid–protein interactions.

Contents

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1. Introduction and scope of this review

Biological membranes are a crucial component of the structure of living cells by providing a physical functional barrier between extracellular and intracellular compartments as well as intracellular and organellar compartments. As originally formulated in the "fluid mosaic model" of Singer and Nicholson [1] the function of cellular membranes, as highly dynamic barriers composed of membrane proteins and (phospho)lipid bilayers, is to control the traffic of ions, water and nutrients between the compartments. Over the past decades this model has been refined by inclusion of lipid rafts and cytoskeletal "fences" and "pickets" [2–4] as further elements adding to the membrane complexity crucial for the function of membrane proteins. The lipid bilayer of cellular membranes is a composite of a very large variety of lipid molecules, held together by the "hydrophobic effect" resulting from the combined effect of hydrophilic and hydrophobic interactions regulated by the structure of water [5]. Depending on the cell type the lipid bilayer can either be supported by a cell wall as in bacterial, archaeal and plant cells or may form a composite structure with extracellular (EC) and cytoskeletal (CSK) layers as in animal and human cells [6]. In addition, membrane proteins, anchored firmly in the lipid bilayer of the plasma and organellar membranes, perform a range of functions essential to the survival of biological cells.

As physical barrier cell membranes are subjected to mechanical forces, which can stretch, compress, bend or break them. Given that water is the major component of living cells by constituting about 90% of their volume and weight living cells had from the very beginnings of life on Earth to cope with osmotic force as a result of the essential role that water plays for life [7,8]. Thus, all living cells have the ability to sense and transduce mechanical forces, which over 3.8 billion years of evolution have ultimately manifested itself in appearance of the senses of touch, pain and hearing [8]. A variety of membrane proteins can function as mechanical force sensors among which mechanosensitive (MS) ion channels present an important class of mechanosensor transducing mechanical stimuli into meaningful intracellular signals.

The discovery of MS channels in bacteria [9] pointed towards early evolutionary origins of this class of ion channels in living cells. Shortly after their discovery the two types of bacterial MS channels belonging to the MscL (Mechanosensitive channel of Large conductance) and MscS (Small conductance) families of membrane proteins were shown to serve as osmotically operated valves protecting bacterial cells from hypoosmotic shock [10,11].

Both MscL and MscS (Fig. 1) have been subjected to extensive biophysical, biochemical, genetic, and structural analyses, which helped to establish them as model systems for mechanosensory transduction. In more recent years and in particular after the discovery and molecular identification of the Piezo MS channels in mouse and drosophila [12,13], there has been a growing interest in the role that MS channels may play in cell physiology and in pathology of diseases. Nevertheless, except for MscL, MscS and TRAAK (Fig. 1) [14–16] there are no other 3D structures of the MS channel proteins currently available despite molecular identification of a large number of MS channels from many organisms of diverse phylogenetic provenience. In addition, although MS channels have extensively been studied in a great variety of cells and tissues by different experimental approaches it is, with exception of MscL- and MscS-like channels and 2P-type K^+ channels TREK-1 and TRAAK, very little known about how these channels sense mechanical force.

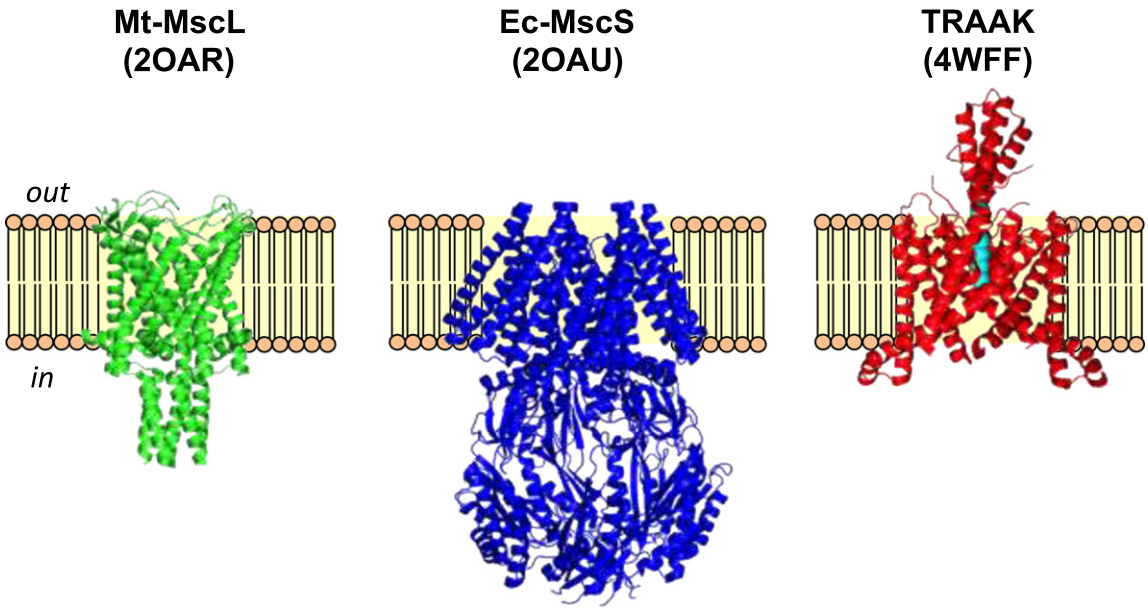

Fig. 1. Crystal structures of mechanosensitive channels. Crystal structure of *M. tuberculosis* MscL, closed/inactivated structure of *E. coli* MscS, and TRAAK viewed perpendicular to the plane of the membrane. MscL forms a homopentameric structure, while MscS and TRAAK form homoheptameric and homodimeric structures, respectively.

The aim of this article is to provide a brief overview of the lipid–protein interactions that have to date been characterized and found to play an important role for the gating and function of MS channels in biological membranes. The scope of the article is largely founded on the present knowledge and ideas about the basic biophysical principles of mechanosensitivity derived from the studies of bacterial MS channels. Our approach seems justified by the repeated occurrence of basic principles re-emerging throughout evolution showing how fundamental mechanisms resulting from the physics and chemistry of biological molecules that first evolved in bacteria-like protocells remained conserved and/or became refined to carry out more diverse and specialized functions in the variety of cells and organisms we know of today.

2. Molecular basis of mechanosensory transduction

During the evolution of different life forms on Earth cells and organisms have developed and adopted various mechanosensors not only to cope with environmental mechanical stimuli but also to use them for various biological functions and their own morphology design [17–19]. All these functions are fulfilled by proteins, which can be functionally classified into three groups comprising of (i) linkage proteins, among which integrins are the best known; they mechanically couple the extracellular matrix and actin cytoskeleton [20,21], (ii) structural elements, which include cytoskeletal and extracellular matrix proteins [22,23] and (iii) MS ion channels, which form a large and diverse group of membrane transport proteins [6,24,25]. Together with the cytoskeleton and, most notably muscle, MS channels have been firmly established as biological mechanosensors [25]. MS channels and their interactions with lipids in the surrounding membrane are the focus of this review.

3. Mechanosensitive ion channels

By operating on a millisecond time scale and passing millions of ions and organic osmolytes per second MS ion channels must have early on evolved as a prevalent type of molecular transducers of osmotic forces into meaningful biological signals. Bacterial MscL- and MscS-like channels [9,10,19,26–28], are thus likely descendants of the primordial sensors of mechanical force that evolved as primary osmosensors supporting mechanosensory physiology of prokaryotic cells. Later on, in plants, MscS-like channels continued to function in their primary role as osmosensors and evolved further as morphosensors [19]. In parallel novel types of MS channels, most notably Piezo1 and Piezo2 [29], Mec4/Mec10 [22], TRPV4 [30], TRPC6 [31], TMC1 and TMC2 [32], and TREK-1, TREK-2 and TRAAK [33,34] have evolved to serve as transducers of mechanical force in plants and animals as well as in specialized senses like touch and hearing [17,22,35,36]. Extending beyond what historically have been called MS channels, a number of studies showed that the canonical voltage-gated potassium channel Kv is in fact mechanosensitive [37,38] and its mechanosensitivity is of physiological consequence [39]. Nevertheless, it is important to mention that although many, if not most ion channels and other types of membrane proteins may be mechanosensitive (as well as thermosensitive), this is not necessarily true for all ion channels and membrane proteins. This is because it has been shown, for example, that the canonical MS channel MscL loses the ability to open in response to membrane tension when a hydrophilic amino acid replaces one of the hydrophobic residues that make contact with the membrane lipid near the periplasmic end of the TM1 or TM2 transmembrane domain [40]. Furthermore, theoretical considerations indicate that the sensitivity of an ion channel to membrane tension could be tuned by changing the size of the hydrophobic interface between the channel and the bilayer [41]. To express it in terms of the relevance of protein mechanosensitivity to cell physiology, a membrane protein can be considered as being *physiologically mechanosensitive* if its activity is modulated by membrane tension of ≤20 mN/m, which is roughly the lytic tension of the lipid bilayer [42]. This is the range of membrane tensions within which all today known mechanosensitive channels are gated. Consequently, as ligand-gated channels can be voltage dependent as well as mechanosensitive [43] and voltage-gated channels can be mechanosensitive [38], whether this sensitivity to the bilayer's chemistry and physics of a membrane protein such as an ion channel is of physiological consequence will have to be examined case by case.

3.1. Molecular diversity of MS channels

In terms of their structural and functional diversity MS channels can roughly be divided into two main groups: (i) prokaryotic (bacterial and archaeal) and (ii) eukaryotic (fungal, plant, animal and human) mechanosensors. However, we would like to stress here that this is a very simplified and historical way of grouping these channels because there is significant overlap between the two groups with MscS- and MscL-like channels being found also in the cells of cell-walled eukaryotes. In Prokaryotes members of the MscL or the MscS family of membrane proteins are the only types of MS channels found [18,44]. Additional types of MS channels have evolved with the appearance of Eukaryotes to account for the complexity of eukaryotic organisms. Currently, there are four types of MS channels found in Eukaryotes unrelated to MscS- and MscL-like channels and they include members of DEG/ENaC, 2P-type K^+, TRP and Piezo channel families [45]. These MS channels could have evolved separately as molecular sensors functioning in more specialized forms of mechanosensory transduction including gravitropism in plants [46], contractility of the heart [47,48] as well as senses of hearing and touch [23,32,49,50].

3.1.1. Prokaryotic MS channels

A large number of genome sequences of Bacteria and Archaea available in genomic databases have enabled the analysis of the phylogenetic distribution of MS channels in these microbes. Given that MscL- and MscS-like channels form separate families of prokaryotic MS channels *mscL* and *mscS* genes have most likely followed separate evolutionary pathways [18,51–53]. Homologues of both types of channels have been found in numerous bacteria and archaea [18,52,53] with one significant difference with regard to their spread among different species. MscL is not ubiquitous across bacteria and is notably absent from many (but not all) bacteria that are native to marine environments [18]. MscL-like homologues have also been identified in numerous fungi [54] showing that examples of MscL-like membrane proteins exist in all three domains of life [55]. However, to date neither MscL nor MscS homologues have been identified in animal and human cells [18,54].

There are over 2000 members of the family of MscL-like proteins according to the UniProt data base, which are widely spread including phytoplasma and mycoplasma, specialized cell-wall deficient bacteria [18,54]. Only a single copy of the *mscL*-like genes is found in most of these organisms and multiple MscL homologues have been identified in very few organisms (e.g. *Prevotella dentalis*, *Mesorhizobium loti*), which possibly resulted from relatively recent gene duplication events [18,54]. Compared to MscL, the MscS subfamily is represented not only in prokaryotic and fungal cells, but also in plant cells. Also in contrast to MscL multiple MscS homologues are present within an organism so that the variety and number of MscS-like proteins supersede those of the MscL-like ones. In addition to being represented in Bacteria and Archaea MscS-like genes have been abundant in higher plants including Arabidopsis and Oryza, in single-celled alga Chlamydomonas, and in fission yeast *Schizosaccharomyces pombe* [18,54]. Furthermore, many bacterial strains possess multiple genes of this highly diverse family [19]. *Escherichia coli*, for example, expresses six different MscS-like proteins. In addition to the canonical MscS channel the other five homologues include MscK, a potassium-dependent MS channel, MscM (yjeP), the MS channel of mini conductance and a further three

being products of ybdG displaying MscM-like activity, ybiO and ynal [18,56,57].

The tertiary and quaternary structure of the currently accepted oligomeric structure of MscL and MscS is that of a homopentamer and homoheptamer, respectively [7]. MscL monomer consists of two transmembrane helices TM1 and TM2, N-terminal and C-terminal domains located at the cytoplasmic end of the bacterial cell membrane and a periplasmic loop. Three transmembrane helices TM1, TM2 and TM3, form the backbone of the MscS monomer with its N- and C-terminal domains being located at the periplasmic and cytoplasmic end of the bacterial cell membrane, respectively. The conservation of amino acid residues in MscL homologues is high for the TM1 and TM2 transmembrane domains in all three domains of life, whereas in MscS homologues the conservation is high only for TM3 helices of bacterial and archaeal channels [44].

Both MscL and MscS as well as their homologues that have to date been investigated by the patch-clamp technique, are gated purely by the tension in the lipid bilayer, which has been referred to as the "bilayer mechanism" [42,58–61]. This paradigm shift (referred today to as the "force-from-lipids" principle (62)) in understanding the activation of MS channels by membrane tension demonstrated that mechanical force within the lipid bilayer was sufficient to gate an MS channel indicating that tethering to cytoskeleton and/or extracellular matrix was not required for these channels to open and close.

3.1.2. "Force from lipids" (FFL) principle

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 anaesthetics and lysophospholipids, whose insertion in a single leaflet of the lipid bilayer was shown to activate both channels [25,59]. This strongly supports the bilayer model of MS channel activation by mechanical force resulting from membrane bilayer deformation [7,58,61]. The use of lysophosphatidylcholine (LPC) was particularly important in deciphering the open channel structure of MscL by SDSL EPR spectroscopy [59,63] and later SDFL FRET spectroscopy [64,65], which to date could not be achieved by X-ray crystallography.

It has been well established that the force gating the MscS- and MscL-like channels requires the lipid bilayer alone without requirement for other cellular components [7]. This mechanism of the channel gating by membrane tension is today referred to as the "force-from-lipids paradigm" or FFL principle [62]. Similar to MscL and MscS other ion channels found in animal and human cells, most notably TREK-1 and TRAAK, have also recently been shown to be inherently mechanosensitive and thus gated according to the FFL principle [34,66,67]. Furthermore, recent studies have demonstrated that the activity of MscS and MscL channels can be differentially modulated by various types of lipids present in the membrane bilayer surrounding the channels [42]. The mechanical properties vary significantly with the composition of the lipid bilayer [68], which was shown to affect the force required for gating of MS channels [42]. Consequently, MS ion channels present an excellent model for examination of the coupling of molecular dynamics of membrane proteins to the mechanics of the surrounding membrane.

3.1.3. Eukaryotic MS channels

One of the key studies, which indicated that MS channels of animal cells may also be gated by the bilayer mechanism according to the FFL principle, was reported by Hamill and coworkers [69]. This study provided the motivation for subsequent successful studies showing that MS channels in animal cells can be reconstituted into liposomes and activated by the bilayer tension similar to the MscS and MscL channels. To date several different types of animal ion channels including NMDA receptors [43], TREK-1 and TRAAK 2P-type potassium channels [34,66] and TRPC1 ion channels [70] have been reconstituted into liposomes and shown to be mechanically gated by the bilayer mechanism, although the mechanosensitivity of TRPC1 seems currently controversial [71]. In addition, there are strong indications that Piezo1 [72,73] channels may also be inherently mechanosensitive and thus gated according to the FFL principle. These channels are conserved among many plants and animal species, but no homologues have been identified in yeast or bacteria. Piezo1 is required for mechanically activated (MA) currents in Neuro2A cells, and Piezo2 is required for MA currents in dorsal root ganglion (DRG) neurons. Further studies by the Patapoutian group [12] identified that Piezo1 channels from *Drosophila melanogaster* also induced currents in cells, but were selectively blocked by the pore blocker ruthenium red, as was shown through whole cell recordings. An unusual feature of these channels is their large size, expected to consist of 120–160 transmembrane segments [12]. Patch-clamp recordings of purified mouse Piezo1 (reconstituted into azolectin liposomes and planar bilayers) have shown the channel to be functionally active, and, in tandem with the whole cell recordings can also be blocked by ruthenium red [12].

Piezo2 has also been very recently identified as being involved in touch sensation in mice [74]. Electrophysiological recordings of Piezo2 knockout (KO) mice compared to Piezo wild-type (WT) models showed a much lower response to pressure ramps. Furthermore Piezo2 KO mice exhibited profound difference to physical mechanical stimuli compared to the Piezo WT mice. With regard to their gating mechanism the literature seems divided with some authors suggesting that stretching the bilayer could be sufficient for Piezo1 activation [72], whereas others provide the evidence of cytoskeleton, such as stomatin-like protein 3 (STOM3), being required for tuning the sensitivity of sensory neurons to mechanical stimuli [75]. Nevertheless, the two activation mechanisms may not be mutually exclusive but rather be combined into a universal one, as suggested by several recent reviews [22,76,77].

In humans, mutations in Piezo1 have been shown to be the cause for the disease xerocytosis [78], a hereditary genetic disorder characterized by abnormalities in the mechanisms controlling water and cation content in red blood cells resulting in haemolytic anaemia. Using patch-clamp studies on mutations at residues 2225 and 2456, Gottlieb and co-workers [72] identified that these mutations in the channel resulted in altered channel kinetics by both slowing inactivation of the channel and introducing a pronounced latency for activation, which affect cation fluxes in red blood cells. Extracellular protonation of Piezo1 inhibits channel activity by almost 90% [79], however, the double mutant channel responsible for xerocytosis was found to be insensitive to pH.

Gain-of-function mutations in Piezo2 have been linked to distal arthrogryposis, a congenital neuromuscular and connective tissue channelopathy characterized by developmental malformations and joint contractures [80]. In their study Patapoutian and colleagues identified E2727del and I802F as two de novo Piezo2 variants underlying DA5, a subtype of autosomal dominant form of distal arthrogryposis, which restricts respiratory function in DA5 patients and causes also the generalized contractures, ptosis and ophthalmoplegia. Both mutant channels are characterized by a fast recovery from inactivation compared to the wild-type channels indicating that the DA5 could result from altered behaviour of body mechanoreceptors such as muscle spindles and Golgi tendon organs important for proprioception and muscle tone.

4. Interactions of MS channels with lipids

The unique chemical structure of lipids as amphipathic molecules and their interaction with water results in the "hydrophobic effect" as the organizational force of living matter [81]. Fig. 2A shows a selection of various lipids, with particular reference to those relevant to MS channels, which is the focus of this section of the review. For more detailed information on structure and mechanics of the lipid bilayer, as well as lipid–protein interactions with other integral

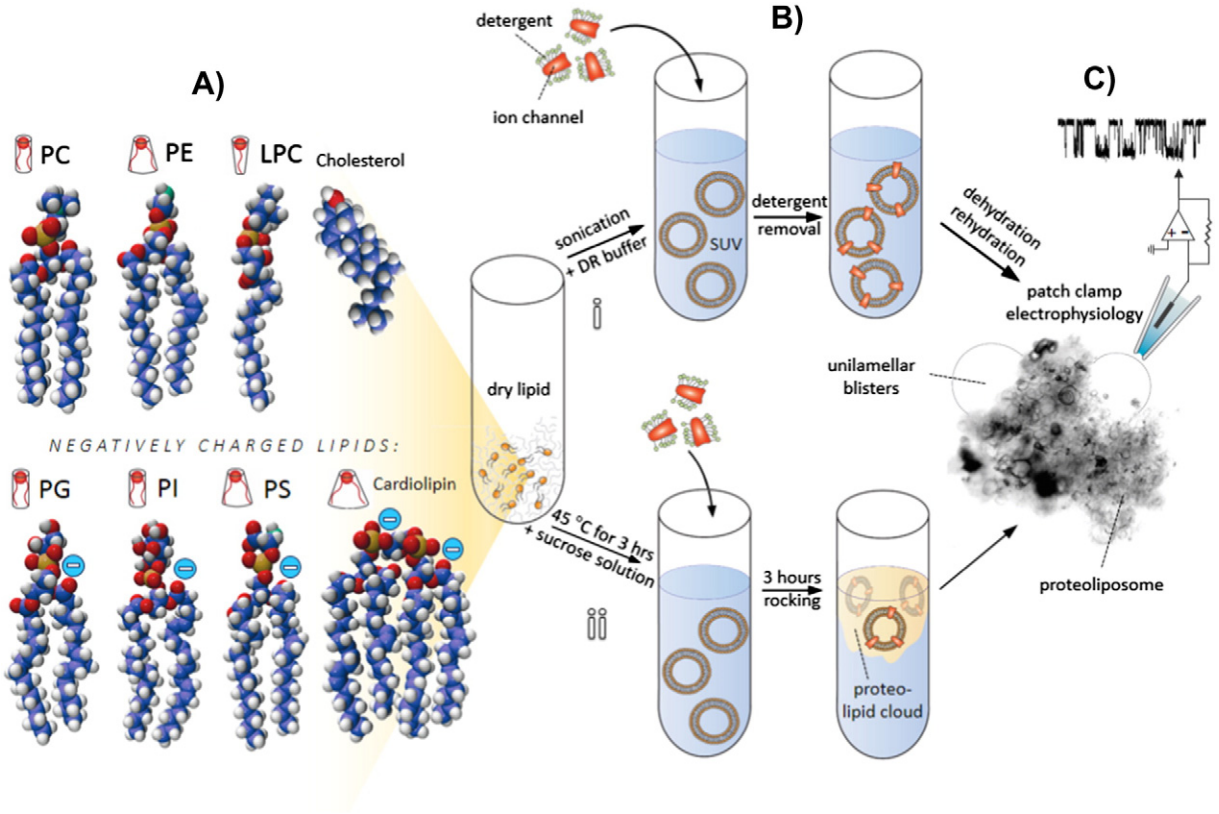

Fig. 2. Study of MS channels in liposomes. (A) Ball and stick representation of different types of phospholipids. The lipid head and tail group differences induce changes in the thickness and shape of the lipid bilayer membrane that is formed on contact of the lipid with water. (B) Reconstitution of proteo-liposomes containing ion channel proteins for patch-clamp recording (C), where a membrane seal is formed across a small opening in a patch pipette containing a suitable ionic medium resulting in a gigaohm resistance (termed a seal). A silver wire electrode is in the pipette and a circuit is made with a silver chloride pellet in the "bath" solution containing the cells/liposomes. By application of suction ion channel currents (and hence openings) can be recorded. The dried lipid can be rehydrated using either (i) the D/R method or (ii) the sucrose method (see text for description).

membrane proteins, the reader is directed to several excellent reviews describing wide ranging lipid–protein effects [62,82–87].

4.1. Global lipid interactions with MS channels

Lipid–membrane protein interactions are central to the function of cellular membranes. The lipid composition of cellular membranes is highly heterogeneous consisting of a large variety of lipid molecules organized frequently as lipid rafts in association with cholesterol and sphingolipids [88]. This heterogeneity contributes to separation and clustering of membrane proteins into functional modules due to tight coupling of specific lipid molecules to some types of membrane proteins. Some of these membrane proteins are rendered non-functional by removing such tightly coupled lipids during a detergent solubilization and purification process [89].

MS channels were first discovered through patch-clamp recordings of single channels recorded from red blood cells [90] and chicken and frog muscle [91,92]. Three years later, MS channels were discovered in *E. coli* giant spheroplasts [9]. This discovery has led onto the cloning, purification, and liposome reconstitution of MscL as the first MS channel identified at the molecular level [27]. In *E. coli*, the major lipid present in the inner membrane is phosphatidylethanolamine (PE), while in human cells phosphatidylcholine (PC) is the major lipid found. Other lipids, such as phosphatidylserine (PS), phosphatidylinositol (PI), phosphatidylglycerol (PG) and cardiolipin may be present in varying ratios depending on the system studied. For example, Gram-positive and Gram-negative bacteria have quite different lipid components in the membrane. As a typical Gram-negative bacterium, *E. coli* has 75–85% PE, 10–20% of the anionic lipid PG and about 5–15% of cardiolipin (CL) [93] On the other hand, Gram-positive bacteria have in general much negatively charged lipids, such as PG and phosphatidylinositol (PI). Each of these lipids has different spatial arrangements (Fig. 2A), as well as charge, both of which may interact directly with single protein residues within the bilayer. The physical shape of the lipid itself and the hydrophobic thickness of the bilayer are the main factors playing a significant role in global effects that lipids have on membrane proteins. For example, possessing two acyl chains and a large choline head groups determines a cylindrical shape of PC (Fig. 2A), while PE, containing the much smaller ethanolamine head group (Fig. 2A) is conical and promotes hexagonal phase formation. Lysophosphatidylcholine (LPC) (Fig. 2A) which has only one acyl chain has an inverse conical shape to PE, as its head group is broader than the acyl chain.

In order to energetically match the hydrophobic domains of the protein [94], the lipid can either expand or thin around the hydrophobic surface of the protein, resulting in the *hydrophobic mismatch*. Studies on MscL using a variety of monounsaturated PC lipids of chain length between 14 and 20 revealed that shorter chain lengths (and hence thinner membrane) favoured opening of MscL [59]. Using the patch-clamp method, lower tension was required to activate MscL with shorter chain lipid. Indeed, spontaneous partial openings can occur with PC16, while longer chain PC's including PC20 require significantly more tension to open the channel. When examining effects of bilayer thickness and stiffness on MS channels using cholesterol differential effects are observed. The puckered ring structure of cholesterol means that it will preferentially interact with saturated acyl lipid chains through more efficient packing of the tail with the cholesterol surface. In azolectin liposomes containing 0–30% cholesterol, both channels required, as expected, higher pressure to open [42]. MscS showed a higher *relative* increase in activation threshold in the azolectin/cholesterol mixture than MscL, which may be a result of its decreased fluidity [95]. Therefore, hydrophobic mismatch may be playing a more significant

role in the activation of MscL than MscS, where bilayer stiffness appears to have a more significant role.

Negatively charged lipids are known to change the gating behaviour of bacterial membrane proteins. For example, *Corynebacterium glutamicum*, an industrial amino acid-producing microorganism, has more than 70% PG as a major lipid component. Recently, MscCG, an MscS-like protein from *C. glutamicum* has been identified as a major glutamate exporter and understanding the gating mechanism of this channel is required to improve the productivity of glutamate. Compared to *E. coli* MscS, MscCG has positively charged transmembrane domain, the region which possibly interacts with negatively charged lipids in a specific manner. A betaine transporter in *C. glutamicum*, BetP, which together with MscCG forms a "pump-and-leak" mechanism regulating cellular turgor pressure under hyperosmotic conditions [96], has a positively charged helix in the C-terminal domain and it restricts movement of the loops connecting the bundle helices by the interaction with the lipid membrane [97]. The lipid–protein interactions are also important for the proper function of BetP, which depends on the presence of negatively charged lipids.

Membrane tension plays a significant role in other channels as well. Martinac and Hamill showed that the small peptide gramicidin, which is not membrane spanning, forms ion-conducting pores upon application of tension to the membrane allowing two gramicidin monomers to meet [98]. Another example of membrane tension resulting in a global lipid bilayer effect is illustrated by the clustering of MscL channels reconstituted into liposomes [99]. Evaluation of the spatial distribution of MscL channels in a lipid bilayer using patch-clamp recording combined with fluorescence and atomic force microscopy, as well as neutron scattering and reflection techniques in addition to mathematical modelling of the mechanics of a bilayer crowded with proteins, indicated that MscL forms clusters under a wide range of conditions as a result of bilayer-mediated protein–protein interactions. Within each cluster MscL remained active and mechanosensitive. However the channel activity was modulated by the presence of neighbouring proteins such that opening of one channel led to either opening or closing of the second one resulting in segregation of channels into domains of open and closed channel clusters. This segregation occurs due to hydrophobic mismatch between the open and closed channels and spatial constraint within the channel cluster, which indicates that segregation into domains is energetically favourable.

Due to the fact that many bacterial and eukaryotic MS channels such as TRAAK (Fig. 1) and TREK-1 have been proven to sense force directly transmitted from the bilayer, it is therefore essential to understand the contribution of lipid composition in gating of these channels. With regard to the gating mechanism of MS channels, force-from-lipid studies can be categorized into two broad groups, lipid bilayer composition and direct lipid–channel interactions. The first category involves understanding the effect of lipid perturbations on the protein shape in the resting and active states, and latter one focuses on the specific chemical interactions between some lipid molecular components and protein residues at the protein–lipid interface.

Change in the lipid composition alters membrane pressure profile as well as the membrane thickness. It is noteworthy to mention that there are a few other important mechanical properties that are directly influenced by the pressure profile and the thickness of the lipid bilayer. These properties include first and second moment of pressure profile of lipid bilayer, spontaneous curvature, Gaussian curvature, bending stiffness and areal elasticity modulus which are well defined in the literature [100–103].

Pressure profile of different lipid bilayers has been mainly characterized by different computational approaches including Monte Carlo, mean-field theory (MFT) [104–108] and molecular dynamics (MD) simulations [109–111]. This has enabled interesting analytical studies on the effect of different lipid compositions on modulation of MS channels using MD [110,112,113] and continuum mechanics approaches [41,68,114–119], which will be discussed further in the text. In addition to the global properties of the lipid bilayer affecting the structural dynamics and function of membrane proteins, specific interactions seem to reflect a distinct pairing of lipid molecules with some membrane proteins, which can either facilitate or inhibit their function.

4.2. Specific interactions

4.2.1. Bacterial MS channels

It is clear that global lipid interactions play a major role in MS channel behaviour, however the exact mechanism by which MS channels sense membrane tension is not known. Several studies of MscS and in particular MscL reconstituted into artificial membrane consisting of pure (or mixtures of) lipids have revealed that lipid tail length, saturation and head-group all influence the channel gating properties. Furthermore, amphipathic molecules that are able to insert into the lipid membrane, also influence the gating behaviour. These particular lipid interactions with MS channels are detailed below.

The first reconstitution experiments of cloned *E. coli* MscL into artificial membrane were performed in azolectin liposomes [27,120] (Fig. 2B). Compared to opening thresholds in spheroplasts, MscL opened at a lower pressure threshold in azolectin [54]. MscL was also shown to gate in pure lipids, such as PC18 and PC16, where the channel may also exhibit spontaneous gating [59]. Purification of *E. coli* MscS and functional activity in azolectin liposomes was demonstrated by Sukharev [121], and, like MscL, MscS opens at a lower threshold than in spheroplasts [122]. The absence of membrane cytoskeleton indicated that both MscS and MscL require contact with the membrane lipid and not the cytoskeleton to activate. Moe and Blount [60] investigated the effect of lipid headgroups on the gating behaviour of *E. coli* MscL. Using a mixture of lipids, the authors determined that negatively charged lipids (such as PS) did not affect tension sensing, but that increasing amount of PE, which is the major component of the inner membrane of *E. coli*, caused increased pressure to be required to open the channel through a change in the thickness and lateral pressure profile within the membrane, in agreement with the previously reported results of the MscL EPR study [59].

Conversely, in another study Lee and co-workers [123] showed that the rate of flux of the fluorescent dye calcein was dependent on the percentage of anionic lipids. In pure PC lipids, the rate of flux was small, but increased upon increasing addition of anionic lipids to the PC lipid/protein mixture. Replacement of the anionic lipids with PE caused a marked decrease in efflux of calcein, corresponding to earlier patch-clamp observations by others [59], showing that increasing amounts of tension were required as the percentage of PE was increased [42].

The crucial role of PI in gating of *Mycobacterium tuberculosis* MscL (Mt-MscL) was shown in recent work by Blount and colleagues [124]. In bilayers consisting of neutral lipids Mt-MscL was much more difficult to open then *E. coli* MscL (Ec-MscL). Upon reconstitution into lipid containing PI the gating threshold of Mt-MscL was greatly reduced, indicating that PI (present in native *M. tuberculosis* cytoplasmic membrane but *not* in *E. coli* cytoplasmic membrane) increased the sensitivity of Mt-MscL enabling it to function within the physiological range of membrane tension. In another recent study Robinson and co-workers [125,126] confirmed the strong interaction between Mt-MscL and PI lipid by using ion mobility mass spectrometry [127]. While both neutral and anionic lipids were all shown to stabilise Mt-MscL, maximum stabilisation occurs with PI. In contrast, aquaporin Z was most stabilised by cardiolipin, which also modulates its function [125].

E. coli MscS has been used as a standard to examine changes in pressure sensitivity of mutant MscL channels [128], where the ratio of the first full opening of the MscS channel is compared to the opening of the second channel (WT or mutant MscL). This method has also been applied to these channels when co-reconstituted into azolectin

liposomes [42,129]. In azolectin, the threshold ratio for opening of MscL to MscS is larger compared to giant spheroplasts containing both WT MscS and MscL. This method thus enables not only comparison between channels, but also reveals the effect of lipid composition on the gating of the channels. Differences in lipid environment affect the co-reconstituted channels individually. When both channels were reconstituted into mixtures of 100% azolectin and 70/30% PC18/PE18 and thinner PC16/PE16 respectively, the activation threshold of MscL decreased significantly in the pure lipid mixtures, while that of MscS was largely unchanged, resulting in sharp decrease in the ratio of opening. This seems to indicate that hydrophobic mismatch has a much larger effect on MscL than MscS.

Cardiolipin is one of the major lipid constituents of *E. coli* cells, or about 5% of *E. coli* total lipid [93]. It localises mainly at the cell poles [130], presumably because of its intrinsic negative curvature [114], where it may represent up to 30% of the total lipid at the pole domains. MscS localization at the poles [131] and expression [132,133] are correlated to the presence of cardiolipin and the latter was shown to modulate the activity of the channel in patch-clamp experiments on proteoliposomes by abolishing the hysteresis gating of MscS [134]. This effect is thought to arise from a change in the mechanical properties of the lipid bilayer with respect to cardiolipin-deficient bilayers, which are overall less stiff [135]. It was also experimentally shown to cause the opposite effect of the spider toxin GsmTx4 on the channel gating, which for cardiolipin was characterized by an increase in activation threshold along with abolishment of gating hysteresis [136–138]. The lipid-ordering effect of cardiolipin can be compared to the properties of cholesterol in liposomal systems [139]. The two phosphate groups of a single cardiolipin molecule have been shown to be motionally restricted and therefore behave as a planar entity perpendicular to the bilayer axis. Furthermore, the bulky quadruple acyl chains are thought to exert an increased lateral pressure from the hydrophobic section of the bilayer onto the hydrophobic core of the embedded MscS, hence favouring the closed state of the channel as gating occurs [140]. It is possible that the head group of cardiolipin, relatively large and negatively charged, also plays a role through some specific interaction with positive Lys–Arg clusters at the lipid–protein interface. The effect of cardiolipin on MscL was on the other hand only detected in pure lipid liposomes (70% DOPE–20% DOPC–10% CL) where it increased the activation threshold by ~20 mm Hg with respect to 70% DOPE–30% DOPC. A similar effect was reported for MscS in the same pure lipid system as above, thus highlighting the cholesterol-like properties of cardiolipin on bilayer rheology [42].

4.2.2. Eukaryotic MS channels

Two mechanisms of activation have been reported for TRAAK. Although both report importance of lipid interaction with the channel for the channel gating, the proposed mechanisms differ greatly. MacKinnon and colleagues [141] described a physical mechanism for the conductance of ions through the TRAAK pore by examining crystal structures of both conductive and non-conductive states of the channel. In the non-conductive state, an acyl chain of a lipid accesses the channel pore and physically blocks ion conductance. Conformational changes in TM4 upon changes in membrane tension prevented lipid access thereby allowing ion conductance. Importantly, physical changes in the channel three-dimensional structure induced by TM2–TM3 and TM4 conformations affect the conductance of the channel: the non-conductive channel being wedge shaped, while the conductive channel is more cylindrical. Furthermore, the wedge shape forces the lipid to curve near the channel, resulting in a higher energy cost. Therefore with little or no applied tension the channel remains in the closed state, but increasing tension results in the energetically more favoured cylindrical shape [142].

Minor and co-workers [25] examined conformational changes in the crystal structures of two mutant (G124I and W262S) K_{2P}4.1 TRAAK channels, and compared them to the structure of the WT channel. As a consequence of the large structural changes involving tilting and straightening of the TM4 inner helix and a buckling of the TM2 helix, a passage is opened lateral to the pore that faces the inner leaflet of the lipid bilayer. The authors postulated that this would allow for facile lipid binding of modulators of TRAAK.

TREK1 and TRAAK have also been reconstituted into liposomes. Both channels were expressed in the yeast *Pichia pastoris*, followed by reconstitution into azolectin lipid [34]. By applying stretch to membrane patches a mechanosensitive response of these channels was seen demonstrating that both channels are inherently mechanosensitive. This study found that TREK1 was activated by both positive and negative pressure. A separate study also showed that TREK1 exhibits mechanosensitivity in azolectin liposomes [66]. However, in this study TREK1 was inhibited by positive pressure applied to the patch pipette. The reason for this discrepancy between the two studies is unclear.

TRPV4, a member of the vanilloid subfamily of TRP-type ion channels, presents another example of a mechanosensitive ion channel for which direct interaction with phospholipids is essential for its gating by mechanical force. As a homologue of TRPV1, its founding family member, TRPV4 is also polymodally regulated by various physical and chemical stimuli including cell swelling and mild heat [143,144]. In their recent study Kung and co-workers [30] proposed a model of TRPV4 gating in which the invariant TRP box tryptophan and the carbonyl oxygen at the pivot beginning at the S4–S5 linker would act as a latch to keep the channel gate closed. Bilayer tension or heat could disrupt the hydrogen bond between L596–W733 in TRPV4, thus allowing the displacement of the S4–S5 linker and the outward motion of the TRP-domain helix leading to opening of the channel gate. The L596P mutation in TRPV4 that causes skeletal-dysplasia in humans, distorts the TRP helix backbone and weakens the L596–W733 bond, favouring opening, which thus explains the gain-of-function phenotype of this mutation.

5. Techniques used for studies of MS channel–lipid interactions

Multidisciplinary studies of the MscL and MscS channels have employed a wide range of methods and techniques that have led to better understanding of their structure and function (Figs. 2B, C and 3). Some of the methods that have been used to study these unique types of channels are discussed here.

5.1. Patch-clamp technique

Patch-clamp recording technique is used as the gold standard for ion channel characterization. Since the first single channel recordings from Neher and Sakmann [145], the technique has caused a paradigm shift in electrophysiology allowing ion channels and transporters from all sorts of excitable and non-excitable tissues and single celled organisms to be identified and studied at the single molecule level (Fig. 2C). The adherence of phospholipid membranes to the glass surface of the patch-clamp pipette is very tight and modulated by cation concentration such as Ca^{2+}, Gd^{3+} and $NMDG^{+}$. During development of the technique it was found that gentle suction improves the resistance of the seal, allowing giga-seal patches to be formed and pico-ampere currents to be detected, effectively improving the signal to noise ratio [146]. Possible explanations to why the giga-seal has such high electric resistance and the membrane patch adheres so strongly to the glass has recently been explored and examined in a study proposing two physical models of the structure of the seal zone and the membrane-glass adhesion forces [147].

The easy application of pressure (mouth/syringe suction, high-speed pressure clamp) to isolated patches is crucial to the study of MS channels through the patch-clamp technique. Such pressure, usually negative, causes a dome-shaped inflation of the patch area and an increase of the in-plane membrane tension which is ultimately sensed by membrane proteins. Mechanosensitive channels are able to transduce

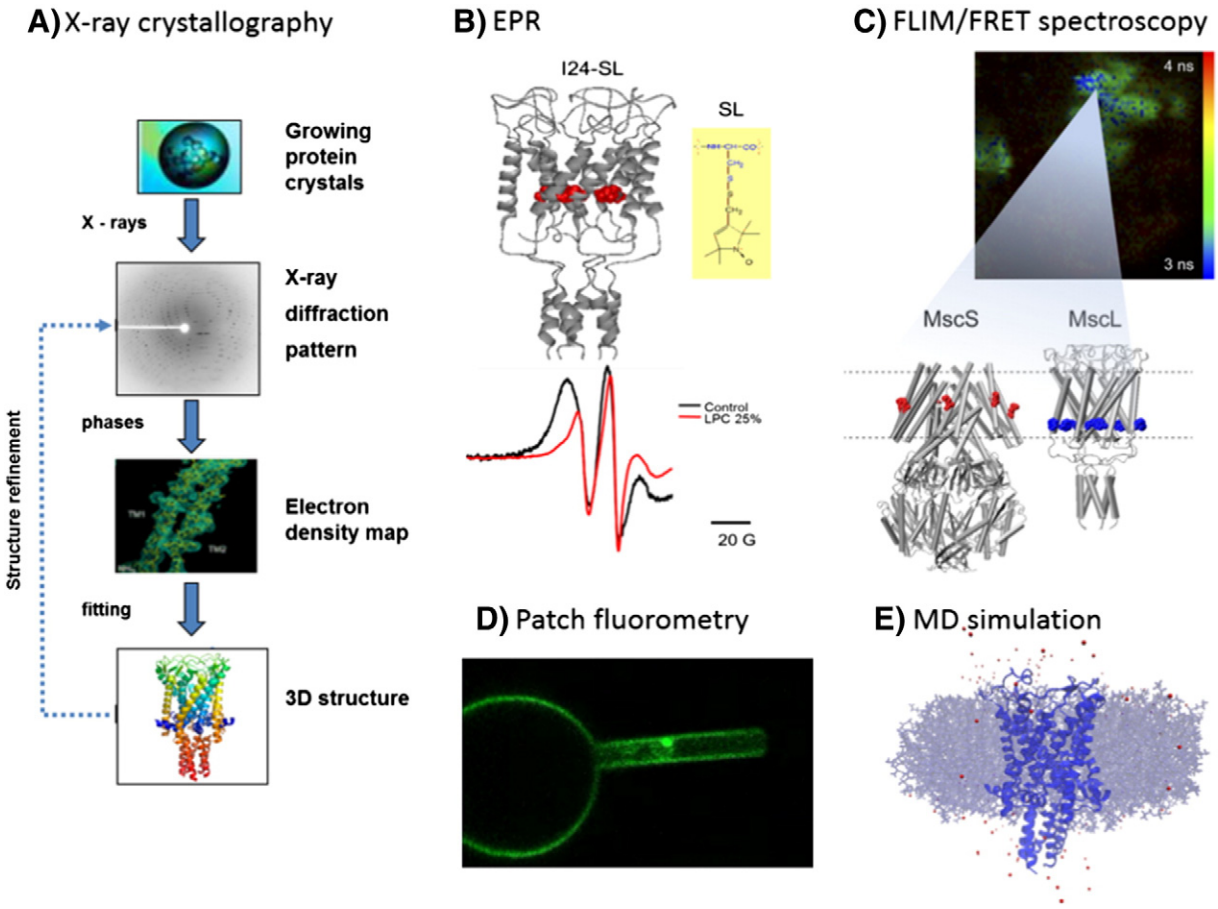

Fig. 3. Various methods used for studying mechanosensitive channel properties. (A) X-ray crystallography: Suitable protein crystals are grown for diffraction under an X-ray source, the phases calculated from the electron density/difference map, to structurally refine the 3D crystal structure. (B) Cysteine labelled sites (red balls), which is paired with a nitroxide spin-label (SL) at pertinent residues on MscL for EPR studies of channel gating properties to measure an EPR signal resulting from the interaction of the spin label with an externally imposed electromagnetic field. (C) Clustering studies of *E. coli* MscL and MscS. Specific residues are labelled with fluorophores for FLIM/FRET studies to examine both clustering behaviour of the channels and pore sizes (see text). (D) Channel currents and shape changes in membrane measured concurrently using Patch fluorometry. Membrane and/or channels of interest are fluorescently labelled. (E) MD simulations of ion channels in liposomes. A 3D matrix consisting of liposomes (typically PC lipid) and water molecules is constructed around a 3D structure of an ion channel.

such stimulus into ionic currents. The first observation of these channels was a serendipitous consequence of applying negative pressure inside the patch pipette. Guharay and Sachs [91] observed an increase in nicotinic ion channel activity upon application of negative pressure onto a chick skeletal muscle cell in culture, and soon realized the effect was caused by a different cation selective channel in the cell membrane, whose identity is currently still unknown.

The mechanosensitive ion channels MscS/MscK were the first bacterial MSC to be discovered during patch-clamp recordings of *E. coli* spheroplast currents [9]. The subsequent activation of these channels by amphipaths [58] followed by cloning of the gene of MscL in 1994 and reconstitution of the functional protein in artificial bilayers [27] set a new gold standard for the field of Mechanotransduction introducing the FFL principle to the scientific community [62]. Together with MscS, MscL represents the best studied model of mechanotransduction because of the ease of incorporation into chemically well-defined lipid bilayers. The next section discusses the methodologies used for liposome reconstitution of MS channels.

5.2. Liposome reconstitution

5.2.1. Prokaryotic MS channels

The cloning of *E. coli* MscL [28] and subsequent functional reconstitution into artificial membrane was a major advance in the field, as it revealed mechanosensitivity to be independent of the cellular matrix, but largely dependent on the lipid membrane itself. In the above study, reconstitution of a liposomal extract of purified MscL with soy azolectin liposomes [148] was done using a modified procedure described by Criado and Keller [149] (Fig. 2B). Functional activity was demonstrated by the patch-clamp method (Fig. 2C). Further refinement of the method was reported by Häse and co-workers, where a purified recombinant MscL protein, dissolved in detergent, was incorporated into liposomes using a process of several dehydration and rehydration (D/R) steps. [120]. This method enabled efficient reconstitution of the recombinant MscL into soy azolectin liposomes and pure lipid systems [42,59,60,150,151]. This method has also been used to reconstitute *E. coli* MscS [121,152,153].

Although the D/R method results in not only efficient incorporation of *E. coli* MscS and MscL, but other prokaryotic MS channels [53, 154–156], it is a time consuming procedure that takes a minimum of two days before experimental recordings can take place. A rapid method, termed the "sucrose method" was further developed [129], where the time required for recording of incorporated channels has been reduced to as little as three hours. Both D/R and sucrose method enabled efficient reconstitution of MscL and MscS into soy azolectin and pure lipid systems. Other bacterial channels that have been reconstituted using these methods include KirBac3.1 [157] and KcsA [158].

5.2.2. Eukaryotic channels

Although less functionally studied by liposome reconstitution due to difficulties in protein expression and purification, some eukaryotic

channels have also been reconstituted into liposomes using the D/R method. Examples include TREK-1 and TRAAK [33,34,66] as well as TRPC1 [70] and NMDA [43,159] receptor channels.

5.2.3. Crystallography

X-ray crystallography (Fig. 3A) was used to determine the 3D structure of both MscL and MscS. The structure of Tb-MscL, the MscL homologue from *M. tuberculosis*, was determined at 3.8 Å resolution (Fig. 1) [14]. The oligomeric structure shows that five TM1 helices line the channel pore of MscL. Another crystallographic study of a truncated form of the MscL channel from *Staphylococcus aureus* (Δ26 Sa-MscL) showed that the oligomeric structure of Sa-MscL is that of a homotetramer rather than a homopentamer [160]. This unusual plasticity in oligomerization of the MscL channel however, was later found to be incorrect. A different study demonstrated that Sa-MscL channel is a pentamer in vivo indicating that detergents used to solubilize the protein could influence its oligomeric structure [161]. Together these results show that detergents could have a profound effect on the structure of membrane proteins.

Oligomeric structure of MscS from *E. coli* is that of a homoheptamer consisting of subunits consisting of three transmembrane helices (Fig. 1) [15]. In addition to the 3D structure of Ec-MscS there are other two different MscS-like crystal structures available currently from bacterial homologues of *Helicobacter pylori* [162] and the extremophile *Thermoanaerobacter tencongensis* [163]. All these structures show that the pore of the MscS channel is formed by seven TM3 helices. MscS-like channels are part of a large and highly diverse superfamily of membrane proteins found in cell-walled organisms of Bacteria, Archaea, fungi and plants, which largely vary in their monomer structure consisting in some family members of up to 12 transmembrane helices [19,164].

The first 3D crystal structure of a mammalian MS channel that has been resolved by X-ray crystallography is that of the human TRAAK, a 2P-type potassium channel, whose structure has recently been determined at 3.8 Å resolution [16]. The oligomeric structure shows a dimer comprising two protomers with each of them containing two distinct pore ([2]P) domains, which together create the pore of this channel. The characteristic feature of TRAAK is a helical cap of 35 Å in height. This cap forms an entryway to the channel pore, whereas two diagonally opposed inner transmembrane helices lining the pore of the channel form structures interacting with the surrounding lipid bilayer of the cell membrane and thus may underlie TRAAK mechanosensitivity [33,34]. The availability of the 3D structure of TRAAK presents a significant advancement for the mechanobiology field given that the 2P-type family of potassium channels play an important role in regulation of the noxious input threshold for pressure and temperature sensitivity [165].

5.3. SDSL EPR and SDFL FRET spectroscopy

The structure, structural dynamics and membrane localization of secondary structural domains of both MscL and MscS channels were investigated by site-directed spin-labelling (SDSL) EPR spectroscopy (Fig. 3B). In combination with the patch-clamp technique SDSL EPR helped to elucidate how the global physical properties of the lipid bilayer (i.e. bilayer thickness, curvature and related changes in the bilayer pressure profile) affected the channel gating [59,63,152,166]. For SDSL EPR spectroscopy, a single cysteine residue introduced into the channel structure using site-directed mutagenesis is used for spin labelling with a nitroxide spin label (i.e. SDSL) characterized by an unpaired paramagnetic electron within its N–O group, which is required for measuring an EPR signal resulting from the interaction of the spin label with an externally imposed electromagnetic field [166,167]. The continuous-wave (CW) EPR signals were used to measure distances between the multiple spin labels in the MscL pentamer and MscS heptamer, which gave an estimate of spatial orientation of transmembrane helices during the opening of MscL [63] and MscS [152]. To determine localization (i.e. inside or outside the membrane) of individual MscL channel domains Perozo, Martinac, and co-workers used [121–123] micro-environment paramagnetic collisional probes. Molecular oxygen was used to determine the localization of the transmembrane domains TM1 and TM2, whereas the nickel-chelated complex Ni(II)-ethylenediaminediacetate (NiEdda) enabled determination of the extracellular location of the N- and C-terminal domain as well as the periplasmic loop. Using the same approach Perozo and co-workers were able to determine the location of transmembrane and extracellular domains of MscS [152].

Site-directed fluorophore labelling (SDFL) FRET spectroscopy has also been used to study the open state of both MscL [64,65,168] and MscS [169] as well as the interaction of the C-terminal domain of MscS with its transmembrane domains during the channel gating [170]. Similar to the SDSL EPR fluorophores are attached to residues mutated to cysteines for SDFL FRET studies [171]. The advantage of SDFL FRET compared to CW SDSL EPR is that distance changes determined during the channel opening can be precisely determined as a result of an inverse 6th power relation between FRET efficiency (i.e. the fraction of energy transferred per donor excitation event) and the donor-to-acceptor separation distance [172]. Recently, the SDFL FRET technique at the single molecule level (smFRET) [173] combined with total internal reflection fluorescence (TIRF) microscopy was used to determine the size of the MscL open channel pore to 28 Å [65] in good agreement with the EPR results [63] and ensemble FRET experiments [64]. Similar to the SDSL EPR study the conformational changes during the MscL opening were determined in a liposome bilayer by applying the conically shaped lysophosphatidylcholine (LPC) to one side of the bilayer, which by inserting into one bilayer leaflet opened the channel. The improved open-state MscL model suggests a much smaller rotational movement and tilt of the transmembrane helices from the closed to the open state with the TM2 helix lying closer to the channel pore than in previously suggested models [63,174]. Machiyama et al. [169] also used FRET spectroscopy to monitor liposome-reconstituted MscS channel opening and closing in response to the addition of LPC. They showed that the cytoplasmic domain of MscS underwent a structural change when the channel opens. More recently SDFL FRET was used in conjunction with fluorescence lifetime imaging (FLIM) to study MscL and MscS clustering upon reconstitution into liposomes [42]. This study confirmed previous results showing that MscL self-assembles into clusters in lipid membranes [99]. It demonstrated furthermore, that MscS channels do not form clusters with themselves but do cluster with MscL [42] (Fig. 3C). Importantly, the EPR and FRET experiments have enabled computational modelling of the MscL and MscS channel structure with a spatial resolution at the level of the protein backbone fold [63–65,152].

5.4. Patch fluorometry

Patch fluorometry is the combination of the patch-clamp technique and confocal microscopy, and powerful technique for measuring channel currents and shape changes of patch membrane [175] (Fig. 3D). The activation of MscS and MscL depends on membrane tension, not pressure applied to the membrane. Based on Laplace's law [176,177] membrane tension is calculated by the radius of patch membrane curvature during application of pressure. MscS and MscL are reconstituted into azolectin liposomes labelled with rhodamine-conjugated phosphatidyl-ethanolamine (0.1%) and rhodamine fluorescence is used to visualize patch membrane curvature. Nomura and co-workers measured channel current and membrane curvature simultaneously using patch fluorometry and determined the gating kinetics of MscS and MscL for membrane tension in the real time [42].

Recently, patch fluorometry combined with computer FE modelling was also used to study mechanical properties of liposome bilayers [68]. The study compared the results of the traditional

micropipette aspiration (MA) method with the results obtained from excised liposome patches under the usual patch-clamp recording conditions. Surprisingly, this study indicated that the excised liposome patch fluorometry was superior to traditional MA method, which failed to accurately describe the mechanical properties of lipid bilayers.

5.5. Mass spectrometry

An important discovery, which has opened up the application of mass spectrometry as the most recent technique for studies of the higher order structure of membrane proteins, their complexes and interactions with ligands and lipids was the idea that the vacuum inside the mass spectrometer and the hydrophobic interior of the lipid bilayer are both low dielectric constant environments [178]. Consequently, membrane protein structures should be largely preserved inside the mass spectrometer. This idea was realized by the development of soft desorption ionization methods of John B. Fenn [179] and Koichi Tanaka [180], whose Nobel-prize winning work enabled mass spectrometry to be accepted as a technique for analyses of biological macromolecules. This paved the way for the development of the new field of structural biology in the gas phase [67]. A considerable amount of fine-tuning has been required in terms of optimization of detergent micelles or lipid bilayer nanodiscs that protect the transition of membrane proteins from solutions to vacuum and can serve as shuttles to transport membrane proteins into the collision cell, where atoms of argon collide with the proteins to desolvate them from their shuttles [125,178]. The collisions between argon atoms and membrane proteins required to remove lipids from the proteins have very recently been used to study the structure of MscL [181]. This approach has also been used to identify the specific lipids involved in stabilising membrane protein structures including those of ion and water channels [125].

5.6. Computer modelling

There is a large number of computational studies on gating of MS channels situated in a lipid bilayer [64,182–188]. Herein we review those studies that have examined the effect of different lipid compositions on the channel function. Using all-atom MD simulation of MscL gating in lipid bilayers (Fig. 3E) Gullingsrud and Schulten [110] measured the lateral pressure profile for different lipid compositions and concluded that gating of Tb-MscL depends on the second moment of the bilayer pressure profile in a tension dependant manner. For example they showed that changing lipid composition from DOPC to DOPE lowers the activation threshold by 2–4 k_BT, which is a very small share of the total free gating energy (50 k_BT). Using the same approach (all-atom MD simulation), Elmore and Dougherty reported the effect of chain length on the gating of MscL function. They showed that MscL adjusts to membrane thinning [113] in agreement with experimental data [63]. Using all-atom MD simulation, Meyer et al. [189] embedded *E. coli*-MscL in a curved bilayer composed of single and double tailed lipids in the absence of any external force. Although the stress redistribution due to the initial curvature and the single tail lipid did not fully gate the channel in their relatively short simulation, they showed a rearrangement of the periplasmic loop as a result. They suggested that depending on the geometry and composition of the bilayer the protein structure could be affected even on short timescales.

Macroscopically, lateral pressure profile and geometry of the lipid bilayer determine the mechanical properties of lipid bilayer such as bending stiffness and areal elastic compressibility. Bavi and colleagues [68] used recently a framework, which combines finite element (FE) modelling with patch-clamp fluorometry for the assessment of bilayer properties and its implications for studying the behaviour of cohort of MS channels in a membrane patch. This work also includes results describing stress distribution in the lipid bilayer in two widely used experimental paradigms for the study of MS channels, namely cell-attached and excised configurations. They demonstrated in both configurations that due to the application of suction to a liposome patch, stress is distributed heterogeneously with the maximum being in the middle of the dome and minimum close to the pipette wall. Moreover, in contrast to the cell-attached configuration, they showed that there was a significant difference between the stress developed in the outer and the inner monolayer of the liposome patch in the excised patch configuration. Thus, these results caution against the extrapolation of MS channel behaviour from one experimental paradigm to another.

6. Conclusions

The cell membrane is subject to a variety of natural stressors. Both prokaryotic and eukaryotic organisms have developed various methods for controlling and sensing changes in mechanical membrane stress, from such diverse physiological processes as emergency relief valves response in bacteria to hypoosmotic shock to sensing touch in mice and humans. There is increasing evidence showing that the interaction between the (phospho)lipids and membrane proteins in living cells plays an intricate role in not only cellular homeostasis, but also in several disease states. The chemical structure and biophysical properties of the lipid membrane profoundly influences both channel insertion and behaviour in lipid. Global effects such as membrane tension, lipid tail length and head group (hydrophobic mismatch) all contribute to gating behaviour. The demonstration of functional activity after successful incorporation of some of these channels into purely artificial liposomal membrane has revealed that specific contact with the lipid bilayer can activate or inhibit these channels. Such specific interactions have included charge on lipid head groups and composition of the lipid bilayer (e.g. PI interaction with Mt-MscL and cardiolipin interaction with Ec-MscS). Using cutting edge methods including mass spectroscopy, molecular modelling and spectroscopic methods including FRET, FLIM and patch fluorometry, the past several years have seen significant advances in our understanding of eukaryotic MS channels, not least the recent discovery of the Piezo1 and Piezo2 channels, which are conserved among many plant, animal and eukaryotic species. Exciting developments with these channels have shown their role in touch sensation, as well as diseases such as xerocytosis and arthrogryposis. Since their discovery over two decades ago, much has been learned from the research on bacterial MscS and MscL channels about basic biophysical principles underlying mechanosensory transduction and lipid–protein interactions that as of recently have been shown to apply also to eukaryotic MS channels from different evolutionary provenience. Thus it seems justified to expect this journey in the upcoming decades to bring even deeper insights into functioning of eukaryotic MS channels and their interaction with the lipids of cellular membranes and make the research in future decades just as fruitful as during the previous two. Information gleaned from these discoveries may be equally important to not only MS channels, but voltage- and ligand-gated channels, as well as other non-channel bilayer-spanning membrane proteins.

Transparency document

The Transparency document associated with this article can be found, in the version.

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