Mechanosensitivity of Ion Channels

Chapter · January 2018

DOI: 10.1007/978-3-642-35943-9_376-1

M

Mechanosensitivity of Ion Channels

Charles G. Cranfield[1,2], Anna Kloda[3], Yury A. Nikolaev[2], Adam D. Martinac[4], Pietro Ridone[2,5], Navid Bavi[6,7], Omid Bavi[8], Evgeny Petrov[9], Andrew R. Battle[10], Takeshi Nomura[11], Paul R. Rohde[2], Yoshitaka Nakayama[2], Kadla R. Rosholm[12], Charles D. Cox[2,5], Matthew A. Baker[13] and Boris Martinac[2,5]

[1]School of Life Science, University of Technology Sydney, Ultimo, NSW, Australia
[2]Molecular Cardiology and Biophysics Division, Victor Chang Cardiac Research Institute, Darlinghurst (Sydney), NSW, Australia
[3]Department of Physiology and Pharmacology, School of Biomedical Sciences, University of Queensland, Brisbane, QLD, Australia
[4]Neuroscience Research Australia, Randwick, NSW, Australia
[5]St Vincent's Clinical School, The University of New South Wales, Sydney, NSW, Australia
[6]Institute for Biophysical Dynamics, University of Chicago, Chicago, IL, USA
[7]Department of Physiology and EMBL Australia Node for Single Molecule Science, University of New South Wales, Sydney, NSW, Australia
[8]Department of Mechanical and Aerospace Engineering, Shiraz University of Technology, Shiraz, Iran
[9]Laboratory of Biochemistry and Molecular Biology, Tomsk State University, Tomsk, Russia
[10]School of Biomedical Sciences, Queensland University of Technology, Brisbane, QLD, Australia
[11]Department of Physical Therapy, Faculty of Rehabilitation, Kyushu Nutrition Welfare University, Kitakyushu, Japan
[12]Sophion Bioscience A/S, Ballerup, Denmark
[13]School of Biotechnology and Biomolecular Science, University of New South Wales, Sydney, NSW, Australia

Synonyms

Mechanically-activated ion channels; Mechano-gated ion channels; Mechanosensitive ion channels; MS channels; Stretch-activated ion channels

Definition

Mechanosensitive ion channels are molecular transducers of physical forces. They respond to mechanical stresses including shear, compressive, and tensile forces. These forces are either transmitted to the channel via the surrounding lipid bilayer or via molecular tethers. Mechanosensitive ion channels are essential components of numerous mechanosensory systems including hearing, touch, and blood pressure control. Multidisciplinary studies on mechanosensitive ion channel proteins have contributed considerably to

G. C. K. Roberts, A. Watts (eds.), *Encyclopedia of Biophysics*,
https://doi.org/10.1007/978-3-642-35943-9_376-1

understanding of their structure, function, and mechanisms of mechanosensory transduction.

Introduction

The first mechanosensitive (MS) channels were reported in embryonic chick skeletal muscle (Guharay and Sachs 1984) and frog muscle (Brehm et al. 1984). Over the last 30 years, this type of ion channel has been identified in a variety of living cells from the three domains of life: Archaea, Bacteria, and Eukarya. Today, many channels have been recognized as being mechanosensitive and include the prokaryotic MS channels of large and small conductance (MscL and MscS, respectively), the eukaryotic transient receptor potential (TRP) type cation-selective channels, two-pore domain (K2P) potassium channels (TREK-1, TREK-2, TRAAK), sodium channels from the MEC/ENaC degenerin family (ENaC, MEC4/MEC10), and Piezo channels (Piezo1, Piezo2) (Martinac and Cox 2017; Ranade et al. 2015). Please see Table 1 for a nonexhaustive list of MS channels that have been cloned to date. See Martinac and Cox (2017) and Ranade et al. (2015) for a broad review of each channel.

The structure and function of the prokaryotic MS channels, MscL and MscS, have provided researchers with much of what is known today as to how MS channels function and are regulated. In bacteria, these channels serve to protect the cells from osmotic shock (Martinac and Cox 2017). In conditions of hypotonicity, a bacterial cell will typically expand or swell. The swelling is a result of increased turgor pressure, which in turn causes the cell membrane to stretch. Stretching of the bacterial cell membrane is what is responsible for the opening of these MS channels leading to a release of ions and other small osmolytes from within the cell cytoplasm. This discharge of small osmolytes relieves the built-up turgor pressure preventing cell lysis. Here, an outline of the various methods and techniques that have been employed to probe the function, and in particular the molecular mechanisms of these unique types of channels, is given.

Patch-Clamp Recording

The patch-clamp technique is widely used to study MS channels. This technique allows single channel activity to be recorded with millisecond time resolution (Hamill et al. 1981). Bacterial MS ion channels were the first of this class to be cloned. These experiments showed that they required nothing more than their surrounding lipid bilayer to function. Here we discuss the application of the patch-clamp technique to bacterial cells such as *Escherichia coli*.

An *E. coli* cell is approximately 0.8 μm wide and 2 μm long, which is almost the same size as the diameter of a patch pipette. Therefore, a larger bacterial object (with cell wall removed) is necessary for patching the inner membrane. This is accomplished by generating giant spheroplasts formed from bacterial cells (Martinac and Cox 2017) (Fig. 1a). The presence of MS channels in a spheroplast membrane patch is determined by stretching the patch. This is achieved by applying a negative or positive pressure to the membrane patch by using a pressure clamp connected to the pipette holder of the recording apparatus (Fig. 1a).

A new MS channel family Piezo, including Piezo1 and Piezo2, was recently discovered in eukaryotes (Ranade et al. 2015). These are non-selective cationic MS channels with sensitivity to generic MS channel blockers (streptomycin, Gd^{3+}, ruthenium red, and GsMTx4) (Martinac and Cox 2017). Piezo1 and Piezo2 are expressed in multiple tissues within the human body, and they contribute to sensing light touch and proprioception, cell differentiation, regulation of erythrocyte cell volume, vascular development, and homeostasis (Ranade et al. 2015). The biophysical approach to studying eukaryotic MS channels usually starts by expression of these channels in heterologous systems, e.g., HEK293 cells. Such an approach allows multiple opening and closing channels' events to be recorded in response to mechanical force (Fig. 1b). In Cox et al. (2016), Piezo1 was shown to be mechanosensitive in cytoskeletal-free membrane blebs of HEK293 cells which suggest that the channel is gated by the lipid bilayer (Fig. 1b). Investigating ion channel

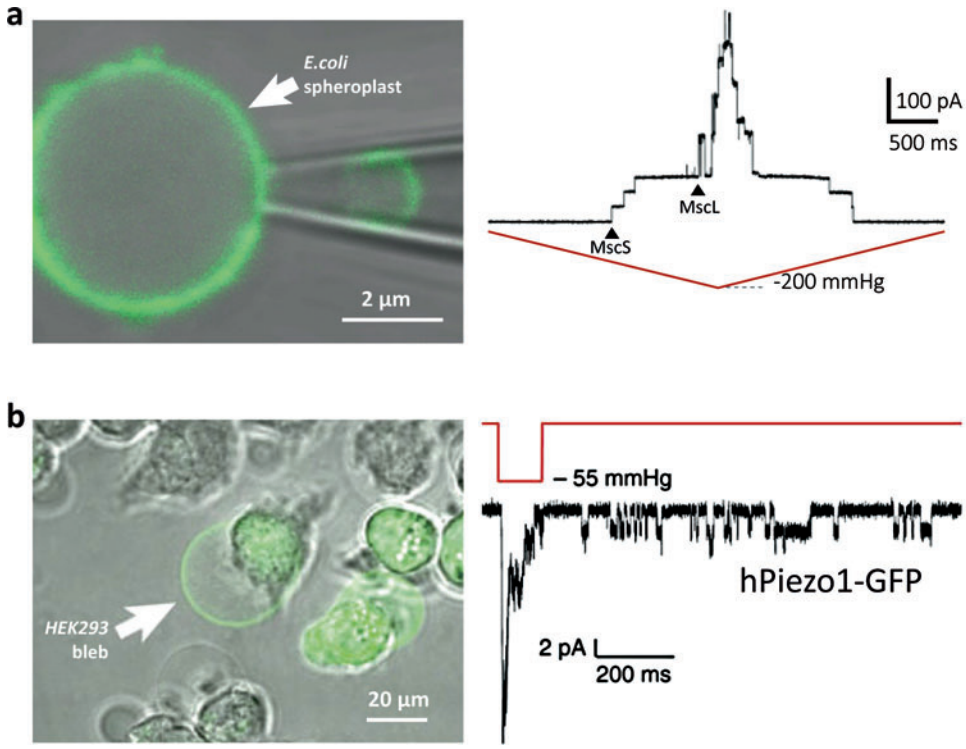
Mechanosensitivity of Ion Channels, Table 1 Diversity of prokaryotic and eukaryotic mechanosensitive ion channels

Domain	Family	Channel	Gating mechanism
Prokaryote (Archaea)	MscS-like	**MscA1** (*H. volcanii*)	Bilayer tension
		MscA2 (*H. volcanii*)	Bilayer tension
		MscMJ (*M. jannashii*)	Bilayer tension/Amphipaths
		MscMJLR (*M. jannashii*)	Bilayer tension/Amphipaths
Prokaryote (Bacteria)	MscL-like	**MscL** (*E. coli*)	Bilayer tension/Amphipaths
	MscS-like	**MscS** (*E. coli*)	Bilayer tension/Amphipaths
		MscK (*E. coli*)	Bilayer tension
		MscCG (*C. glutamicum*)	Bilayer tension
		MscSP (*S. pomeroyi*)	Bilayer tension
	MscM-like	**YbdG** (*E. coli*)	Bilayer tension
	MscM	**YjeP** (*E. coli*)	Bilayer tension
Eukaryote (Green algae)	MscS-like	**MSC1** (*C. reinhardtii*)	Bilayer tension
Eukaryote (Yeast)	TRPY	**TRPY1** (*S. cerevisiae*)	Bilayer tension
Eukaryote (Land plant)	MscS-like	**MSL8** (*A. thaliana*)	Bilayer tension
		MSL9 (*A. thaliana*)	Bilayer tension
		MSL10 (*A. thaliana*)	Bilayer tension
Eukaryote	Degenerins	**MEC** (*C. elegans*)	Tether?
	TRPN	**NOMPC** (*D. melanogaster*)	Tether/Bilayer tension
	Sodium channel	**ENaC**	Tether/Bilayer tension?
	Glutamate receptor	**NMDAR**	Bilayer tension/Amphipaths
	TRPC	**TRPC1**	Bilayer tension?
		TRPC6	Amphipaths/Bilayer tension
	TRPV	**TRPV1**	Tether?/Bilayer tension?
		TRPV2	Tether?/Bilayer tension?
		TRPV4	Amphipaths/Tether/Bilayer tension?
	TRPM	**TRPM7**	Bilayer tension?
	Polycystin complex	**PKD2** (TRPP1)	Tether?
		PKD2L1 (TRPP2)	Tether?
	TRPA	**TRPA1**	Tether?
	K2P	**TREK-1** (K2P2.1)	Bilayer tension/Amphipaths
		TREK-2 (K2P10.1)	Bilayer tension/Amphipaths
		TRAAK (K2P4.1)	Bilayer tension/Amphipaths
	Piezo-type	**Piezo1**	Bilayer tension/Amphipaths
		Piezo2	Bilayer tension?/Tether

mechanosensitivity in cellular blebs is very similar to recording MS channels in liposomes.

Liposome Reconstitution

Although studying mechanosensitivity of channels in cellular systems could point to their gating mechanism, multiple cellular components could influence channel mechanosensitivity such as the cytoskeleton/extracellular matrix or other transmembrane proteins such as GPCRs (Martinac and Cox 2017). To investigate the "inherent" gating mechanisms of MS channels, the "gold standard" technique is to reconstitute these channels into lipid bilayers of defined composition.

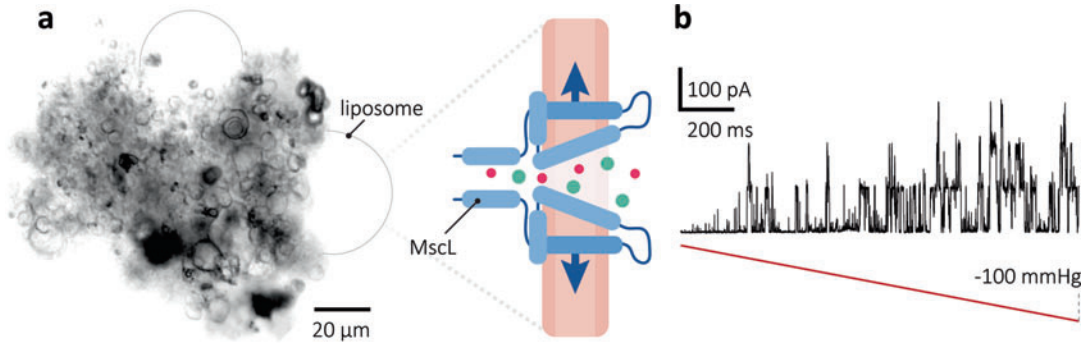


Mechanosensitivity of Ion Channels, Fig. 1 Patch-clamp recording of MS channels from cellular systems (**a**) *E. coli* giant spheroplast stained with *green*-fluorescent SYTO 9 dye. The cell is shown at the tip of the patch pipette. A fluorescence of the membrane patch can be seen inside the pipette. Channel activities of wild-type (strain AW737) MscS and MscL and corresponding negative pressure applied to the inside-out membrane patch of giant spheroplasts are shown. Pipette potential was held at +20 mV. (**b**) Piezo1-GFP expression in HEK293 blebs. The diameters of the blebs reach 15 µm. Mechanical activation of Piezo1-GFP in bleb-attached patches with a pressure pulse of −55 mmHg. Pipette potential was held at −75 mV

Given that they are the most widely studied MS channels, both MscL and MscS have been reconstituted into a range of lipid systems. A dehydration/rehydration (D/R) method (Delcour et al. 1989) for channel incorporation into liposomes was the first method developed that gave good reconstitution efficiencies when using the patch-clamp technique. These are the principal stages of the D/R method: drying the lipid after dissolving in chloroform, dissolving the dried lipids in D/R buffer, creation of small unilamellar vesicles using sonication, mixing the lipid solution with the MS protein solubilized in detergent, detergent removal, and finally dehydration/rehydration to form proteoliposomes. The D/R method has also been used to successfully incorporate other bacterial MS channels including MscK (MS channel dependent on external K^+) and those from *H. volcanii* and *M. jannaschii* (Martinac 2007). Also, eukaryotic channels have been purified and shown to be mechanosensitive in liposomes. These MS channels include TREK-1/2, TRAAK, and the NMDA receptor (Martinac and Cox 2017).

Another reconstitution approach occasionally used is the sucrose method (Martinac et al. 2010), which yields good reconstitution efficiencies for both MscS and MscL. Both methods have been successful using wild-type (WT) and mutant channels (Fig. 2).

One of the limitations of the liposome approach is that both leaflets of the liposome bilayer have the same lipid composition unlike biological cell membranes. In the plasma



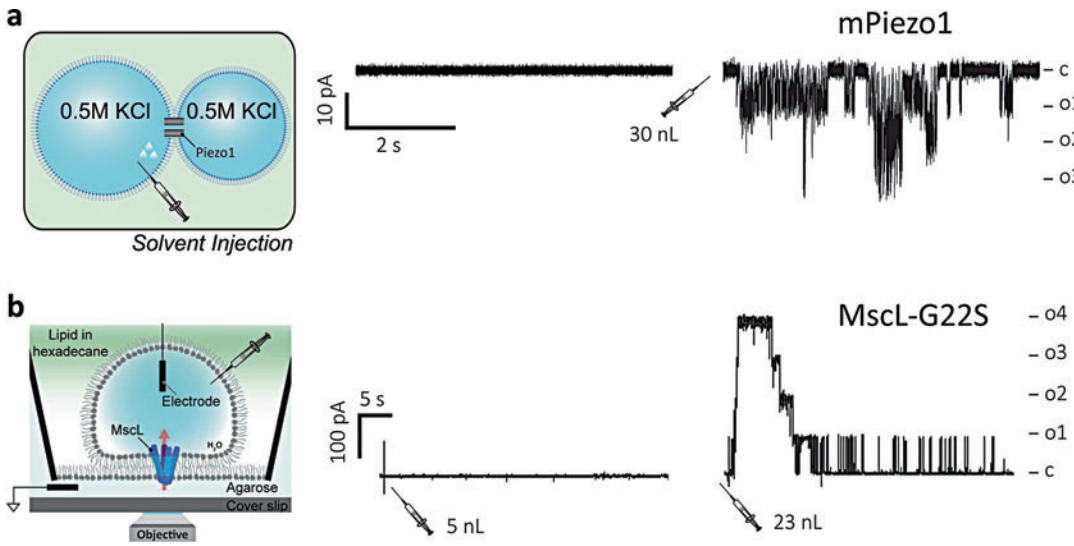
Mechanosensitivity of Ion Channels, Fig. 2 Mechanosensitivity of ion channels reconstituted into liposomes (**a**) Giant unilamellar liposomes (GUVs) containing MscL prepared using the dehydration/rehydration method. A cartoon of the MscL channel incorporated into the bilayer is shown. Red and green dots represent cations and anions, respectively. (**b**) Patch-clamp recording from the MscL ion channel reconstituted into liposomes. The pipette voltage was +30 mV

membrane of any cell type, there is a significant heterogeneity in lipid composition between the two monolayers. Both phosphatidylethanolamine (PE) and phosphatidylserine (PS) tend to accumulate in the inner leaflet of the plasma membrane. Thus, the unequal lipid composition is essential for proper folding, oligomerization, function, and gating of ion channels. Similarly, PIP_2 accumulates in the inner leaflet of the bilayer and is crucial for TRP-type channel activity.

Droplet-Interface Bilayers

The droplet-interface bilayer technique is also used to record MS channel activity in artificial bilayers and enables mechanical stimulation of the protein. This technique exists in two forms: (i) droplet-droplet bilayers and (ii) droplet-hydrogel bilayers (Leptihn et al. 2013). One of the main advantages of the droplet-interface bilayer technique, compared to liposome reconstitution, is the ability to control lipid composition in each monolayer. The bilayer tension is generated by injecting a buffer into the droplet, which results in stretching of the monolayer surrounding the aqueous droplet in oil (Fig. 3).

The droplet-droplet technique was used to demonstrate the inherent mechanosensitivity of the mouse homolog of Piezo1 (Fig. 3a) (Syeda et al. 2016). The authors demonstrated that Piezo1 is sensitive to changes in bilayer tension caused by either droplet inflation or incorporation of the channel into LPA/PC asymmetric bilayers. In the latter case Piezo1 was spontaneously active without requiring a buffer to be injected into the droplet. The droplet-droplet method requires minimal setup but does not allow for visualization of the bilayer area with a microscope, a limitation which has been overcome by the development of the droplet-on-hydrogel (DHB) planar bilayer (Leptihn et al. 2013). DHB consists of a planar lipid bilayer formed between a planar lipid monolayer on a hydrophilic agarose surface and a lipid monolayer bordering an aqueous droplet (Fig. 3b). This technique was successfully adopted to activate MscL-G22S, a low activation threshold mutant (Fig. 3b), by inflation of the droplet as shown in Fig. 3a (Rosholm et al. 2017). The horizontal orientation of the bilayer provides experimental versatility suitable for combining electrophysiology with microscopy techniques such as Förster resonance energy transfer (FRET) or total internal reflection fluorescence (TIRF) microscopy that can be used for single-molecule studies of ion channels (Neu and Genin 2014). Furthermore, DHBs allow electrophysiological investigation of biological membrane fractions inaccessible to patch-clamp such as the erythrocyte plasma membrane and organelles such as the mitochondrion, which have been reported to host MS channels (Martinac and Cox 2017).



Mechanosensitivity of Ion Channels, Fig. 3 Activation of MS channels in droplet interface bilayers. (**a**) Schematic description of the droplet-droplet bilayer setup. Injection of 30 nL of a buffer into one single droplet generates sufficient bilayer tension in the surrounding monolayer to drive the activation of purified mouse Piezo1 incorporated in the bilayer. (**b**) Schematic description of a DHB setup. Injection of a buffer into the aqueous droplet generates bilayer tension in the surrounding monolayer and activates the purified MscL G22S (low threshold mutant) when the bilayer tension in that monolayer exceeds the activation threshold of the channel

Another way to generate asymmetry in artificial lipid bilayers for studies of MS channel function is to insert amphipathic molecules into one of the bilayer leaflets (Martinac and Cox 2017). The use of amphipathic drugs to activate MS channels in liposomes and planar bilayers strongly supports the bilayer model of MS channel activation in which the mechanical gating force comes from the surrounding lipids. MscL and MscS, for example, are activated by amphipaths such as chlorpromazine (CPZ), trinitrophenol (TNP), local anesthetics, and lysophospholipids (Martinac and Cox 2017). Similarly, the activity of eukaryotic MS ion channels can be dramatically modulated by amphipathic lipid molecules such as phosphatidyl inositol-bisphosphate (PIP2), diacylglycerol (DAG), arachidonic acid, and lysophospholipids.

"Flying-Patch" Patch Clamp Recording

Life does exist under high hydrostatic pressure (HHP). Animals of varying organizational levels have been discovered near the bottom of the Mariana Trench (>80 MPa). How have they adapted to such harsh conditions? MS channels, reconstituted into artificial membranes (such as Azolectin) or present in native *E. coli*, can be used as a biophysical tool to study the effects of HHP on membrane bound proteins. Technically this is done by using a patch-clamp setup equipped with a "flying-patch" device. This device provides conditions for the safe transfer of a patch pipette to a recording chamber under high hydrostatic pressure. This then allows the currents of MS channels to be recorded while various levels of HHP are applied (Petrov et al. 2011). The key element of the device is a cylindrical plastic protecting chamber. Once a giga-Ohm seal patch has been formed inside the patch pipette, the experimenter can slide down the cylindrical protection chamber toward the tip of the pipette. A few microliters of solution from the recording chamber will be captured inside and held in place by capillary forces. Thus, the most fragile part – the electrode tip with membrane patch – is protected, surrounded by bath solution,

and can be positioned inside a high pressure chamber.

The design of the high pressure setup makes it impossible to apply negative pressure to the patch membrane in order to stretch it. This restricts its use to MS channels which have a lower activation threshold (such as MscS and MscK), or to using specifically mutated MS channels, which are able to spontaneously open. Both approaches have successfully been utilized. Recently, the spontaneously active gain-of-function (GOF) mutant of MscL, G22E-MscL, reconstituted into azolectin liposomes, was used as a simple biophysical model of xenon anesthesia under HHP. The "anesthetized" G22E MS channels were "awakened" by exposing them to high hydrostatic pressure of up to 100 MPa, resulting in partial recovery of their spontaneous activity (Petrov et al. 2018).

Electron Paramagnetic Resonance Spectroscopy

Site-directed spin labelling (SDSL) and electron paramagnetic resonance (EPR) spectroscopy presents an attractive approach for the study of the structure and molecular dynamics of MS channels. In SDSL, a single cysteine is introduced into a protein with all nondisulfide bonded cysteines removed by site-directed mutagenesis. This unique cysteine is modified with a nitroxide spin label, characterized by an N-O group containing the unpaired electron necessary to produce the EPR signal. Measurement of the distance between the spin label probes in multimeric MS channels, and the proteins in general, allows for determination of the spatial orientation of the protein's secondary structural elements. This enables modelling of the MS channel structure with a spatial resolution at the level of the backbone fold. Solvent accessibility (aqueous vs membrane environment) and the polarity of the spin label microenvironment contrast transmembrane versus extracellular channel domains, as well as their movements between the two environments. The distances between the structural channel domains can be measured in the range ~0.5–8 nm via the combination of continuous wave (CW) and pulsed EPR methods. Moreover, the structural dynamics of the channel domains can, by EPR spectroscopy, be detected on the millisecond timescale (Mchaourab and Perozo 2002).

SDSL EPR spectroscopy was instrumental to unravel the nature of the conformational transition from the closed form of MscL of *E. coli* to the open form that X-ray crystallography has, thus far, not been able to characterize. Changes in intrinsic membrane curvature induced by the asymmetric addition of the amphipath lysophosphatidylcholine (LPC) can create large EPR spectroscopic changes. In the open state, the residues forming the channel gate are further apart, resulting in an EPR spectrum that appears sharper and exhibits an overall change in line shape relative to the closed channel state. MscS of *E. coli* is another MS channel whose structural dynamics has been determined in a similar way using CW-EPR spectroscopy. More recently, EPR spectroscopy has been employed to study the role of the N-terminus in MscL gating (Bavi et al. 2016), the gating of MscS (Vasquez et al. 2008), and reversible gating of MscL in situ (Dimitrova et al. 2016).

Förster Resonance Energy Transfer Spectroscopy

To better understand the structural properties and mechanisms of MS ion channels, fluorescence spectroscopy methods are used. FRET can be employed to measure a change in inter- or intra-MS channel distances. MS channels can be fused to fluorescent proteins (e.g., green fluorescent protein [GFP]) usually via N- or C-terminal linkers. Many MS channels contain multiple identical subunits (i.e., they are homomeric); thus, each channel oligomer could be fused with multiple fluorescent proteins. This means that in order to produce a protein with an appropriate FRET pair, a cell might need to be co-transfected with two plasmids. How the various subunits combine together to form a functional MS channel is then entirely up to chance.

In certain cases, genetic addition of fluorophores to MS channels may interfere with oligomerization. An alternative approach is to label the purified functional multimer with fluorophores such as Alexa Fluor maleimides, but this introduces the problem of an unknown labelling efficiency with respect to the number of donors and acceptors bound to the protein. To determine intra-channel distances by measuring FRET efficiency then would require computational modelling to take into account the various possible combinations of donor/acceptor labelling arrangements. Fluorophore labelling (e.g., Alexa Fluor maleimides), similarly to spin labelling used for EPR spectroscopy, requires cysteine mutagenesis of single residues within the channel protein to attach the fluorophores. The selection of such residues needs careful consideration if the function of the MS channel is not to be affected. Endogenous reactive residues might also need to be mutated out to prevent labelling in unwanted regions of the protein.

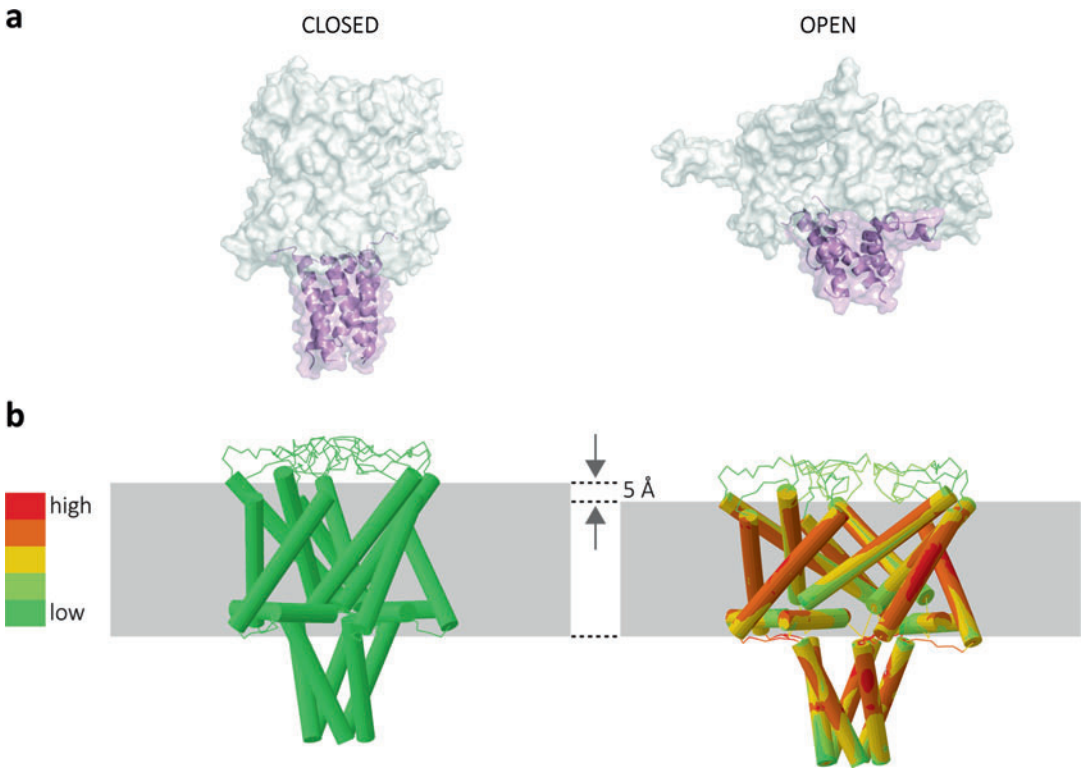
Keeping in mind all these considerations, it might be more advantageous to create a FRET pair where the donor fluorophore is itself an endogenous amino acid. Many studies use tryptophan for this purpose. Using tryptophan as the donor means that only an acceptor fluorophore would need to be attached to the protein under study.

Another consideration when undertaking FRET-based distance measurements of homomeric proteins concerns the orientation factor referred to in the literature as k^2. Determining this value accurately is essential to interpreting distances from FRET efficiency measurements. If multiple donors and multiple acceptors are attached to the protein, calculating the orientation factor accurately is challenging, and computational modelling might again be required. Nomura et al. (2012) used fluorescence lifetime imaging (FLIM) with FRET to demonstrate that the MS channels MscL and MscS cluster in lipid bilayers and that this clustering affects their function. The advantage of the FLIM-FRET method used in this study is that the quenching of the donor fluorophore could be measured independently from the relative concentrations of the donor and acceptor fluorophores and the fluorescence intensity.

Molecular Dynamic Simulations

Today, MD simulation methodology forms an integral part of molecular biophysics. In particular, MD adds valuable information on the structural dynamics of the system studied, so that it is considered as a "computational microscope," which provides structural views beyond what is achievable with current imaging methods. Nevertheless, to perform MD simulations of a protein, such as an ion channel, knowledge of its 3D structure at an atomic resolution is required, usually obtained by X-ray crystallography or cryo-electron microscopy (cryo-EM). MD simulations have become an important tool to study the gating cycle of MS channels embedded in various bilayer systems. Over the past few decades, meaningful MD simulations of MS channels in lipid membranes have enabled better understanding of their function and physical properties (Martinac and Cox 2017). Simulations have also been employed to address the question as to why changes of the lipid head-groups, or the length of the acyl chains, alter the membrane bilayer tension required to gate MS channels (Aryal et al. 2017). The hydrophobic lock of MscL and MscS has also been the focus of molecular dynamic simulations, particularly with regard to the hydration properties of the channel (Anishkin and Sukharev 2004). MscL simulations have been carried out to investigate the nature and sequence of channel structural changes under the influence of a force applied externally, an example is given in Fig. 4a. More recently, MD simulations were used as one of the main approaches to show that the short amphipathic N-terminus in MscL plays a crucial role during bilayer tension-induced gating of this channel (Bavi et al. 2016).

In the case of MscS, most of the computational simulations have dealt with the question about the channel conduction state represented by its crystal structure (Martinac and Cox 2017). All MD simulation studies suggest that the "open" crystal structure does not represent the fully open state of the pore, and the "closed" state structure may



Mechanosensitivity of Ion Channels, Fig. 4 MD and FE models of MscL highlighting C-terminal bundle dynamics. (**a**) MD model of EcMscL structure showing the C-terminal helical bundle in the closed and open state. The helical bundle is colored in rose pink. There is outward bending at the top of the bundle in the open state. (**b**) FE model of EcMscL in a membrane (grey) showing closed and open states. In the open state, the membrane thins from 35 Å to ~30 Å and there is outward bending in the upper part of the helical bundle compared to the resting (equilibrated) state, while the rest of the bundle does not change during the channel opening. The distribution of stress along each helix is shown ranging from green (low) to red (high)

represent an inactive state of the channel. Assigning a state to structures generated by x-ray crystallography or Cryo-EM is difficult as they represent a snapshot in time. Recent efforts have been made to use all atom MD simulations to assign the state (i.e., open or closed) of ion channel structures (Trick et al. 2016).

Finite Element Modelling

The idea of using Finite Element Modelling (FEM) for the study of MS channels is to employ an efficient framework of multiscale modelling, which can capture the conformational changes on the protein scale rather than in the atomistic details. It is because of this, excessive computation costs and simulation time can be avoided. Therefore, compared to techniques such as all-atom MD simulations, continuum-mechanics approaches, including FEM, offer a unique alternative to bridge detailed intermolecular interactions and biological processes occurring at large spatial scales and long timescales. The FEM framework also allows for systems with large deformations (geometric non-linearities) to be taken into consideration; thus, systems with complex geometries can be analyzed. FEM has widely been used for studying different biological phenomena at the molecular level, for example, modelling micropipette aspiration (a method to measure material properties of cells and lipid bilayers) of various cells (Bidhendi

and Korhonen 2012) and the gating mechanism of MscL (Bavi et al. 2016).

Two recent projects highlight the benefit of using FEM for exploring structural dynamics of *E. coli* MscL. In the first study, the molecular role of polar and electrostatic interactions, which maintain the stability of the cytoplasmic MscL C-terminal domain, was investigated (Fig. 4b). The second study examined van der Waals interactions between MscL pore residues to test an unusual approach to opening the channel by pulling on its N-terminus together with the TM1 pore helix without stretching the membrane bilayer (Martinac et al. 2017).

Summary

This entry presents an introduction into the special scientific techniques used to identify and characterize MS ion channels, both in vitro and in vivo. Since the first MS channel was identified over 30 years ago, many research hours have been devoted to better understanding these channels and their mechanisms of action. There is much, however, that is still unknown about these channels in general, and it is hoped that the brief descriptions presented here will encourage further research into the mechanosensitivity paradigm.

Cross-References

- ▶ EPR
- ▶ Fluorescence Techniques for Studying Ion Channel Gating: VCF, FRET, and LRET
- ▶ Förster Resonance Energy Transfer (FRET)
- ▶ Macromolecular Crystallography: Overview
- ▶ Molecular Dynamics Simulations of Lipids
- ▶ Patch-Clamp Recording of Single Channel Activity: Acquisition and Analysis

References

Anishkin A, Sukharev S (2004) Water dynamics and dewetting transitions in the small mechanosensitive channel MscS. Biophys J 86:2883–2895

Aryal P, Jarerattanachat V, Clausen MV, Schewe M, Mcclenaghan C, Argent L, Conrad LJ, Dong YY, Pike AC, Carpenter EP (2017) Bilayer-mediated structural transitions control mechanosensitivity of the TREK-2 K2P channel. Structure 25:708–718.e2

Bavi N, Cortes DM, Cox CD, Rohde PR, Liu W, Deitmer JW, Bavi O, Strop P, Hill AP, Rees D, Corry B, Perozo E, Martinac B (2016) The role of MscL amphipathic N terminus indicates a blueprint for bilayer-mediated gating of mechanosensitive channels. Nat Commun 7:11984

Bidhendi AJ, Korhonen RK (2012) A finite element study of micropipette aspiration of single cells: effect of compressibility. Comput Math Methods Med 2012:192618

Brehm P, Kullberg R, Moody-Corbett F (1984) Properties of non-junctional acetylcholine receptor channels on innervated muscle of *Xenopus laevis*. J Physiol 350:631–648

Cox CD, Bae C, Ziegler L, Hartley S, Nikolova-Krstevski-V, Rohde PR, Ng CA, Sachs F, Gottlieb PA, Martinac B (2016) Removal of the mechanoprotective influence of the cytoskeleton reveals PIEZO1 is gated by bilayer tension. Nat Commun 7:10366

Delcour AH, Martinac B, Adler J, Kung C (1989) Modified reconstitution method used in patch-clamp studies of *Escherichia coli* ion channels. Biophys J 56:631–636

Dimitrova A, Walko M, Hashemi Shabestari M, Kumar P, Huber M, Kocer A (2016) In situ, reversible gating of a mechanosensitive ion channel through protein-lipid interactions. Front Physiol 7:409

Guharay F, Sachs F (1984) Stretch-activated single ion channel currents in tissue-cultured embryonic chick skeletal muscle. J Physiol 352:685–701

Hamill OP, Marty A, Neher E, Sakmann B, Sigworth F (1981) Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches. Pflügers Arch 391:85–100

Leptihn S, Castell OK, Cronin B, Lee E-H, Gross LC, Marshall DP, Thompson JR, Holden M, Wallace MI (2013) Constructing droplet interface bilayers from the contact of aqueous droplets in oil. Nat Protoc 8:1048

Martinac B (2007) 3.5 billion years of mechanosensory transduction: structure and function of mechanosensitive channels in prokaryotes. Curr Top Membr 58:25–57

Martinac B, Cox CD (2017) Mechanosensory transduction: focus on ion channels. In: Comprehensive biophysics. Elsevier, New York

Martinac B, Rohde PR, Battle AR, Petrov E, Pal P, Foo AF, Vasquez V, Huynh T, Kloda A (2010) Studying mechanosensitive ion channels using liposomes. Methods Mol Biol 606:31–53

Martinac AD, Bavi N, Bavi O, Martinac B (2017) Pulling MscL open via N-terminal and TM1 helices: a computational study towards engineering an MscL nanovalve. PLoS One 12:e0183822

Mchaourab HS, Perozo E (2002) Determination of protein folds and conformational dynamics using spin-labeling EPR spectroscopy. In: Berliner LJ, Eaton GR, Eaton SS (eds) Distance measurements in biological systems by EPR. Springer US, Boston

Neu CP, Genin GM (2014) Handbook of imaging in biological mechanics. CRC Press Boca Raton, Florida

Nomura T, Cranfield CG, Deplazes E, Owen DM, Macmillan A, Battle AR, Constantine M, Sokabe M, Martinac B (2012) Differential effects of lipids and lyso-lipids on the mechanosensitivity of the mechanosensitive channels MscL and MscS. Proc Natl Acad Sci U S A 109:8770–8775

Petrov E, Rohde PR, Martinac B (2011) Flying-patch patch-clamp study of G22E-MscL mutant under high hydrostatic pressure. Biophys J 100:1635–1641

Petrov E, Menon G, Rohde PR, Battle AR, Martinac B, Solioz M (2018) Xenon-inhibition of the MscL mechano-sensitive channel and the CopB copper ATPase under different conditions suggests direct effects on these proteins. PLoS ONE 13(6): e0198110

Ranade SS, Syeda R, Patapoutian A (2015) Mechanically activated ion channels. Neuron 87:1162–1179

Rosholm KR, Baker MA, Ridone P, Nakayama Y, Rohde PR, Cuello LG, Lee LK, Martinac B (2017) Activation of the mechanosensitive ion channel MscL by mechanical stimulation of supported Droplet-Hydrogel bilayers. Sci Rep 7:45180

Syeda R, Florendo MN, Cox CD, Kefauver JM, Santos JS, Martinac B, Patapoutian A (2016) Piezo1 channels are inherently mechanosensitive. Cell Rep 17:1739–1746

Trick JL, Chelvaniththilan S, Klesse G, Aryal P, Wallace EJ, Tucker SJ, Sansom MSP (2016) Functional annotation of ion channel structures by molecular simulation. Structure 24:2207–2216

Vasquez V, Sotomayor M, Cordero-Morales J, Schulten K, Perozo E (2008) A structural mechanism for MscS gating in lipid bilayers. Science 321:1210–1214