Molecules in Focus

Mechanosensitive Channel of Large Conductance

Anna Kloda*, Evgeny Petrov*, Grisha R. Meyer*, Thom Nguyen#, Annette Hurst*, Livia Hool# and Boris Martinac*.

*School of Biomedical Sciences, University of Queensland, Brisbane, Queensland 4072 Australia; # School of Biomedical, Biomolecular and Chemical Sciences, University of Western Australia, Crawley, Western Australia 6009, Australia

E-mail addresses: a.kloda@uq.edu.au (A. Kloda), petrov67@yahoo.com (E. Petrov) grischa@uq.edu.au (G.R.Mayer), thom.nmn@gmail.com (T. Nguyen), a.hurst@uq.edu.au (A. Hurst), lhool@cyllene.uwa.edu.au (L. Hool), b.martinac@uq.edu.au (B. Martinac)

Word count: 1918

Keyword: MscL, mechanosensitive ion channels, lipid bilayer, hydrophobic mismatch

Correspondence to:

Dr. Boris Martinac,

School of Biomedical Sciences,

University of Queensland,

Brisbane, QLD 4072, Australia

Tel.: +61-7-3365-3113; Fax: +61-7-3365-1766

Email: b.martinac@uq.edu.au

Abstract

Microbial cells constitutively express the Large Conductance Mechanosensitive Channel which opens in response to stretch forces in the lipid bilayer. The channel protein forms a homopentamer with each subunit containing two transmembrane regions and gates via the bilayer mechanism evoked by hydrophobic mismatch and changes in the membrane curvature and/or transbilayer pressure profile. During the stationary phase and during osmotic shock the channel protein is up-regulated to prevent cell lysis. Pharmacological potential of MscL may involve discovery of new age antibiotics to combat multiple drug-resistant bacterial strains.

Introduction

Mechanical forces produced by pressure, touch, sound, gravity, or osmotic stress, are sensed by cells through mechanosensitive (MS) ion channels. These integral membrane proteins open a conductance pore and generate an ionic current in response to membrane tension. Bacterial MS channels most likely evolved to protect the micro-organisms from sudden osmotic shock. The Large Mechanosensitive Channel of Escherichia coli (Eco-MscL) was the first MS channel to be cloned and sequenced (Sukharev et al., 1994a). The MscL protein was identified by following its channel activity in liposome-reconstituted chromatographically separated protein fractions isolated from detergent-solubilized E. coli membrane by the patch clamp technique (Sukharev et al., 1994b). Determination of the crystal structure of MscL from Mycobacterium tuberculosis (Tb-MscL) (Chang et al., 1998) presented a major breakthrough that greatly advanced the research on bacterial MS channels after their

initial discovery some twenty years ago (Martinac et al., 1987). Since its molecular identification MscL has served as a model molecule for functional and structural studies of the MS class of ion channels.

Structure

The determination of the structure of the MscL homologue from Mycobacterium tuberculosis by X-ray crystallography demonstrated that Tb-MscL exists as a homopentamer (Chang et al., 1998). Each pentameric subunit consists of two transmembrane α-helices TM1 and TM2 and a third cytoplasmic α-helix. The TM1 (residues 15-43) is connected to TM2 (residues 69-89) by a loop which extends into the pore region and lines the periplasmic face of the channel. The TM2 is followed by a second loop (residues 90-101) leading to the cytoplasmic helix (residues 102-115) at the C-terminus. Each TM1 helix is in contact with two TM1 helices from adjacent subunits and two TM2 helices, one from the same subunit and the other from the neighboring subunit. The helices are slanted and five TM1 helices join together as they face the cytoplasm (Fig. 1). The ion conduction pathway of the MscL channel is formed by the TM1 transmembrane helices with their hydrophilic residues lining the channel pore. The pore of the closed channel is approximately 18 Å in diameter at the periplasmic site but narrows to the occluded apex at the cytoplasmic site (approximately 2Å). The tight constriction, which is believed to act as the channel gate, is formed by hydrophobic residues (5 Val and 5 Ile) that are highly conserved among bacterial members of the MscL family.

Expression and activation

The MS channels including MscL are up-regulated by osmotic stress and upon entry into the stationary growth phase. It has been demonstrated that the synthesis of stress sigma factor (RpoS) regulates the expression of MS channels and the rpoS knock-out cells are more sensitive to hypo-osmotic shock and cell lysis during the stationary phase compared to the wild-type cells (Stokes et al., 2003). The RpoS factor also controls the synthesis of cyclopropane fatty acids (CFAs) and their formation modifies the phospholipid bilayer of microbial cells during the stationary growth phase. Since the lipid bilayer is the tension-bearing element transmitting the mechanical force which gates the MS channels (Hamill and Martinac, 2001) it is possible that their increased expression is required to compensate for altered membrane fluidity. Additionally, the modification of the lipid bilayer by CFAs may further regulate the activity of MS channels during the stationary phase to enhance cell survival.

In electrophysiological experiments the MscL channel is activated by direct application of negative hydrostatic pressure to the lipid bilayer of either giant spheroplasts or proteoliposomes (Fig. 2A). The stretching of the bilayer is accompanied by a proportional change in the bilayer thickness. Therefore, the bilayer thinning should contribute to the stability of the open conformation of the channel due to a better hydrophobic match with the open channel (Hamill and Martinac, 2001; Perozo et al., 2002a). This is well supported by research studies confirming that the thinner bilayer matches better the open conformation compared to the closed conformation of MscL (Perozo 2002a, 2002b). Furthermore, studies based on the use

of amphipathic drugs indicate that the MscL channel could gate in response to intrinsic curvature created by asymmetries in the lipid bilayer pressure profile at the lipid-protein junction. Both cationic and anionic amphipaths affect gating properties of bacterial and eukaryotic MS ion channels (Martinac et al., 1990; Hamill and Martinac, 2001). It has been suggested that the amphipaths generate the gating force for MS ion channels by differential insertion into the two leaflets of the lipid bilayer (Martinac et al., 1990). Indeed, addition of lysophosphatidylcholine (LPC) to the external leaflet of the lipid bilayer trapped the channel in the fully open state (Perozo et al., 2002a, 2002b) and increased the channel diameter by 16 Å as determined by FRET spectroscopy (Corry, et al., 2005).

Recent molecular dynamic simulation studies indicate that the MscL protein structure can be affected through the composition and geometry of the surrounding lipid bilayer (Fig. 2). In the lipid bilayer modelled as a dome structure the simulation even on very short time-scales resulted in spontaneous re-structuring of the periplasmic loops leading to interactions between one of the loop residues and phospholipid head groups. Furthermore there was a rotation of TM helices which induced breakdown of structure of the TM1 helix and bending in the TM2 helix (Meyer et al., 2006).

Lipid bilayers have superdiamagnetic properties enabling moderate-strength magnetic fields to exert an effect upon membranes of living cells. Static magnetic fields (SMFs) of moderate intensity have also been shown to exert an effect on the activity of MscL reconstituted into artificial liposomes (Petrov and Martinac, 2006). However, SMFs of moderate intensity are too weak to affect directly conformational changes of ion channels due to Lorenz forces (St Pierre and Dobson, 2000). Therefore, the

functioning of mechanosensitive ion channels, which is closely dependent on the properties of surrounding lipids (Hamill and Martinac, 2001), could be a consequence of the SMFs effects on the physical properties of the lipid bilayer.

Biological function

Experimental studies showed that the exposure of E. coli cells to distilled water causes a rapid release of cellular osmoprotectants including proline, potassium glutamate, threhalose, ATP and small proteins such as 12 kDa thioredoxin in response to osmotic downshock (Ajouz et al., 1998), although it seems unlikely that this protein fluxes as a globular protein (Ewis and Lu, 2005). This ability prevents the cells from lysis by decreasing the turgor pressure upon the challenge of sudden shift in osmolarity. The efflux of metabolites in osmotically challenged E. coli can be blocked by gadolinium, the well known inhibitor of MS channels (Berrier et al., 1992).

The involvement of MS channels in osmoregulation was also documented by Levina and colleagues (1999), who demonstrated that the double mutant of E. coli lacking MscL and MscS was severely compromised in its survival when exposed to hypo-osmotic shock although loss of only one of the genes had no effect on cellular integrity. This indicates that the absence of one of the channels can be compensated with the other. Direct evidence of the involvement of MS channels, including MscL, in osmoregulation comes from studies of giant protoplasts examined by whole cell patch clamp, which demonstrated the opening of MS channels in vivo by differences in osmotic pressure (Cui et al., 1995). Furthermore, the expression studies of E. coli

MscL in marine bacterium Vibrio alginolyticus showed that cells harboring the *mscL* gene were protected from lysis upon osmotic downshock (Nakamaru et al., 1999). Identification of osmolarity dependent phenotypes further allowed the study of cellular responses to osmotic stress. The expression studies of MscL mutants with substitutions of charged residues showed dramatic changes in channel gating properties when examined by the patch clamp technique (Ou et al., 1998). Cells expressing the mutations with changed charges showed slow growth phenotype and leakage of cytoplasmic solutes, which was partially rescued by increasing the osmolarity of the media.

MscL may also be essential to maintain homeostasis of intracellular pH (Kloda and Martinac, 2006). In neutrophilic bacteria such as E. coli, the internal pH ranges between 7.5 and 8.0 but cells grow poorly at pH 6.0. Protonation of either positively or negatively charged residues within the C-terminal charge cluster RKKEE, achieved by decreasing the experimental pH or residue substitution within the cluster, significantly increased the free energy of activation for the MscL channel due to an increase in activation pressure. Changes in pH may cause ionization of charged residues or lipid head groups leading to conformational changes of the MscL protein and create protein-lipid hydrophobic mismatch. Therefore, the RKKEE cluster, which is highly conserved in various prokaryotic and eukaryotic MS channels, may function as a proton sensor enabling the MscL channel to regulate influx of protons.

Pharmacological potential of MscL – new age antibiotics

Multi-drug-resistance in pathogenic strains of bacteria has, in the last decade, presented an increasing problem in the treatment of bacterial infections and diseases. The re-emergence of tuberculosis (TB), for instance, is one of the serious threats that are spreading rapidly throughout the world. Furthermore, many strains of Enterococci have acquired resistance to vancomycin, the last antibiotic that was still able to fight them successfully. What is urgently needed is not only to find out how bacteria such as Mycobacterium tuberculosis and Enterococci species become resistant to drugs, but also to develop new antibiotics to combat the rapidly emerging strains of multiple drug-resistant bacterial strains.

Bacterial MS channels have recently been shown to have potential as a selective target for novel types of antibiotics (Nguyen et al., 2005). In particular, MscL, which to date has been identified in over 120 prokaryotic species, including many bacterial pathogens, appears as an ideal target for novel antibacterial agents. This is because the MscL type of MS channels is highly conserved in all studied prokaryotes and their homologues have not been found in animal and human cells (Hamill and Martinac, 2001). As a result, an antibiotic targeting MscL could be broad-spectrum and selective thus, potentially targeting a range of pathogenic bacteria with minimal side-effects to infected patients. Of particular interest are parabens, the alkyl esters of p-hydroxybenzoic acid, methyl-, ethyl-, propyl- and butyl-paraben, which were found to have similar effects to amphipaths (Martinac et al., 1990) however, parabens exert most likely their effect by directly binding to the channel gate (Nguyen et al., 2005). In the presence of parabens, MscL was observed to spontaneously open (Nguyen et

al., 2005). Parabens have been used as preservatives in the food and cosmetic industry for many years because of their antibacterial properties. Since the accumulation of parabens in bacterial cells occurs by both hydrophobic and hydrophilic interactions (Fukahori et al., 1996), it has been suggested that they may enter the cell via the aqueous pores of ion channels, which is consistent with the finding that parabens affect gating and opening of MscL. In addition to perturbing the homeostasis of a bacterial cell, the opening of the large pores (≥25 Å in diameter; Perozo et al., 2002a) of the MscL channels by paraben-like antibacterial agents could also provide a way of enhanced drug delivery to the inside of a bacterial cell.

References

Ajouz, B., Berrier, C., Garrigues, A., Besnard, M., & Ghazi, A. (1998) Release of thioredoxin via mechanosensitive channel MscL during osmotic downshock of Escherichia coli cells. *J. Biol. Chem.,* 273, 26670-26674.

Berrier, C., Coulombe, A., Szabo, I., Zoratti, M., & Ghazi, A. (1992) Gadolinium ion inhibits loss of metabolites induced by osmotic shock and large stretch-activated channels in bacteria. *Eur. J. Biochem.,* 206, 559-565.

Chang, G., Spencer, R., Lee, A., Barclay, M., & Rees, C. (1998) Structure of the MscL homologue from Mycobacterium tuberculosis: A gated mechanosensitive ion channel. *Science,* 282, 2220-2226.

Corry, B., Rigby, P. & Martinac, B. (2005) Conformational changes involved in MscL channel gating measured using FRET spectroscopy. *Biophys. J.,* 89: L49-L51.

Cui, C., Smith, D.O., Adler, J. (1995) Characterization of mechanosensitive channels in *Escherichia coli* cytoplasmic membranes by whole cell patch clamp recording. J. Membr. Biol. 144: 31-42.

Ewis, H. E., and Lu, C. D. (2005) Osmotic shock: a mechanosensitive channel blocker can prevent release of cytoplasmic but not periplasmic proteins. FEMS Microbiol. Lett. 253:295-301.

Fukahori, M., Akatsu, S., Sato, H., & Yotsuyanagi,T. (1996) Relationship between uptake of p-hydroxybenzoic acid esters by Escherichia coli and antibacterial activity. *Chem. Pharmaceut. Bull.,* 44, 1567-1570.

Hamill, O.P. & Martinac, B. (2001) Molecular basis of mechanotransduction in living cells. *Physiological Reviews*, 81, 685-740.

Kloda, A. Ghazi, A. & Martinac, B. (2006) C-terminal charged cluster of MscL, RKKEE, functions as a pH sensor. *Biophys. J.,* 90, 1992-1998.

Levina, N., Totemeyer, S., Stokes, N.R., Louis, P., Jones, M. A. & Booth, I. R. (1999) Protection of *Escherichia coli* cells against extreme turgor by activation of MscS and MscL mechanosensitive channels: identification of genes required for MscS activity. *EMBO J.,* 18**,** 1730-1737.

Martinac, B., Buechner, M., Delcour, A.H., Adler, J., & Kung C. (1987).Pressure-sensitive ion channel in Escherichia coli. PNAS, 84, 2297–2301.

Martinac, B., Adler, J. & Kung, C. (1990) Mechanosensitive ion channels of *E. coli* activated by amphipaths. *Nature,* 348, 261-263.

Meyer, G.R., Gullingsrud, J. Schulten, K. & Martinac, B. (2006) Molecular dynamics study of MscL interactions with a curved lipid bilayer. *Biophys. J.*, 91, 1630-1637.

Nakamaru, Y.N., Takahashi, Y., Unemoto, T. & Nakamura, T. (1999) Mechanosensitive channel functions to alleviate the cell lysis of marine bacterium, *Vibrio alginolyticus*, by osmotic shock. *FEBS Letters*, 444, 170-172.

Nguyen, T., Clare, B. & Martinac, B. (2005) The Effects of Parabens on the Mechanosensitive Channels. *Eur. Biophys. J.,* 34, 389-396, 2005.

Oakley, A. J., Martinac, B. and Wilce, M. C. J. (1999) Structure and function of the bacterial mechanosensitive channel of large conductance. Protein Science 8: 1915-1921.

Ou, X., Blount, P., Hoffman, R.J. & Kung, C. (1998) One face of a transmembrane helix is crucial for mechanosensitive channel gating. *Proc. Natl. Acad. Sci. USA*, 95, 11471-11475.

Perozo, E., Cortes, D.M., Sompornpisut, P., Kloda, A & Martinac, B. (2002a) Open channel structure of MscL and the gating mechanism of mechanosensitive channels. *Nature*, 418, 942-948.

Perozo, E., Kloda, A., Cortes, D.M. & Martinac, B. (2002b) Physical principles underlying the transduction of bilayer deformation forces during mechanosensitive channel gating. *Nat. Struct. Biol.*, 9, 696-703.

Petrov, E. & Martinac, B. (2006) Modulation of channel activity and gadolinium block of MscL by static magnetic fields. *Eur. Biophys. J.,* (DOI 10.1007/s00249-006-0109-z).

St Pierre T.G. & Dobson J. (2000) Theoretical evaluation of cell membrane ion channel activation by applied magnetic fields. *Eu.r Biophys. J.,* 29, 455-456.

Sukharev, S.I., Blount, P., Martinac, B., Blattner, F.R. & Kung, C. (1994a) A large mechanosensitive channel in *E. coli* encoded by *mscL* alone. *Nature,* 368, 265-268.

Sukharev, S.I., Martinac, B., Blount, P., & Kung, C. (1994b) Functional reconstitution as an assay for biochemical isolation of channel proteins. Application towards the molecular identification of a bacterial mechanosensitive channel. In: Methods: A Companion to Methods in Enzymology, M. Montal (ed.), Academic press, New York, 6:51-59.

Stokes, N.R., Murray, H.D., Subramaniam, C. et al. (2003) Theoretical evaluation of activation by applied magnetic fields. *Eur Biophys .J.,* 29, 455-456.

Figure captions

Figure 1. (A) The structure of the MscL channel pentamer (left) and a channel monomer (right) from *M. tuberculosis* according to the 3D structural model of a closed channel (Chang et al., 1998). The thickness of the membrane bilayer (shown as solid blocks) is approx. 35 Å. The channel gate is formed by a group of amino acids at the cytoplasmic end of the TM1 transmembrane domain (reproduced from Oakley et al., 1999) **(B)** A diagram of a closed and open MscL channel indicating the extent of the conformational change involved in the channel gating based on a FRET spectroscopic study (adapted from Corry et al., 2005).

Figure 2. (A) Representative current trace and the corresponding pressure trace of the liposome reconstituted *E. coli* MscL activated by suction applied to the patch pipette at a pipette potential of +30 mV (adapted from Kloda et al., 2006). **(B)** Stereo view of curved lipid bilayer with embedded MscL protein. Water molecules are not shown. Single-tailed lipids are presented in pink, double-tailed lipids in grey. The MscL periplasmic loop is shown in yellow, the first transmembrane helix is shown in red, the second one in green. **(C)** MscL at the beginning (left) and end (right) of the 9.5ns simulation. Shown are the N-terminal helices S1 (blue), the trans-membrane helices TM1 (red), the periplasmic helices S2 (orange), the periplasmic loops S2 (yellow), and the transmembrane helices TM2 (green). The C-terminus (S3) has been cut-off in the simulation and is not shown. (Reproduced from Meyer et al. 2006).