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Mechanisms of mechanosensing — mechanosensitive channels, function and re-engineering

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Sensing and responding to mechanical stimuli is an ancient behavior and ubiquitous to all forms of life. One of its players 'mechanosensitive ion channels' are involved in processes from osmosensing in bacteria to pain in humans. However, the mechanism of mechanosensing is yet to be elucidated.This review describes recent developments in the understanding of a bacterial mechanosensitive channel. Force from the lipid principle of mechanosensation, new methods to understand protein–lipid interactions, the role of water in the gating, the use of engineered mechanosensitive channels in the understanding of the gating mechanism and application of the accumulated knowledge in the field of drug delivery, drug design and sensor technologies are discussed.

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Introduction

Mechanosensation (MS) is the ability of cells to perceive mechanical stimuli and couple it to cellular processes. It is ubiquitous in all kingdoms of life and vital for sensing and responding to the ambient environment. In eukaryotes, mechanosensation plays key roles in, for instance, touch, proprioception, hearing, pain, and numerous cellular functions including gene expression, fluid homeostasis and vesicular transport [1–4]. Recent information on the physiological aspects of mechanosensing can be found in Pflugers Arch-Eur J Physiol [2].

In bacteria, mechanosensation protects cells from lysis upon osmotic downshock depending on the rate of the osmotic change [2,5]. One of the primary players in this process is mechanosensitive ion channel of large conductance, MscL (Figure 1a). It is a homopentameric protein with cytoplasmic N-terminus (S1) and C-terminus (CP) and has two transmembrane helixes (TM1 and TM2), which are connected *via* a periplasmic loop (PL) (Figure 1b). Under hypoosmotic conditions, water enters the cells and builds up the osmotic pressure. Once the pressure reaches ~10 mN/m, MscL senses the resulting tension in the membrane. Upon large conformational changes, it opens a large, water-filled pore and releases molecules up to ~7 kDa, to relieve the tension. Its ability to retain its functionality also in synthetic environments [6] with no additional cellular components, make MscL a 'simple' model to study the principles of mechanosensation. Even though the gating models have been developed, we still do not know how the channel senses the mechanical forces and respond to it.

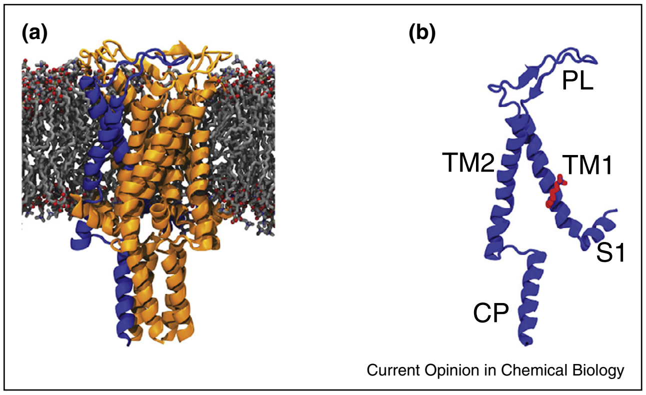
The scope of this review is to highlight recent developments in the last two years in understanding the functioning of MscL and application of this knowledge to nanomedicine and biosensor technologies.

Force-from-lipid principle: a fundamental and invariant physicochemical principle

Force-from-lipid (FFL) principle has been established more than two decades ago. Two recent articles [7••,8] states that from eukaryotes to prokaryotes all membrane proteins work by the physics of the lipid bilayer. Since the lipid bilayer is a self-assembled and stable structure, it inherent large and anisotropic forces that are balanced by counter forces. For being embedded in such an environment, the functional responses of mechanosensitive ion channels are also governed by bilayer force changes.

Unlike soluble proteins, which are isotropically pressed by the surrounding molecules, membrane proteins are subjected to large and anisotropic forces from the lipid bilayer. The surface tension at the lipid-water interface generates significant lateral pressures in the range of hundreds of mN/m at the two polar-nonpolar interfaces. Forces at the other levels within the lipid bilayer balance this lateral pressure. While a resting membrane encourages the ion channel to stay in a defined shape, any input to the system in the form of a stretching force or a chemical compound that partitions in the lipid bilayer can alter the force distribution and magnitude, causing embedded ion channel to adapt a different conformation. Furthermore, such changes in the lipid forces will also change the energy cost of a conformational change, defining the extent of the mechanosensitivity of an ion channel [7••,8].

Figure 1



Structure of MscL. **(a)** Viewed along the membrane plane. **(b)** MscL monomer with highlighted G22C residue. The structure is based on WT MscL from *Mycobacterium tuberculosis*.
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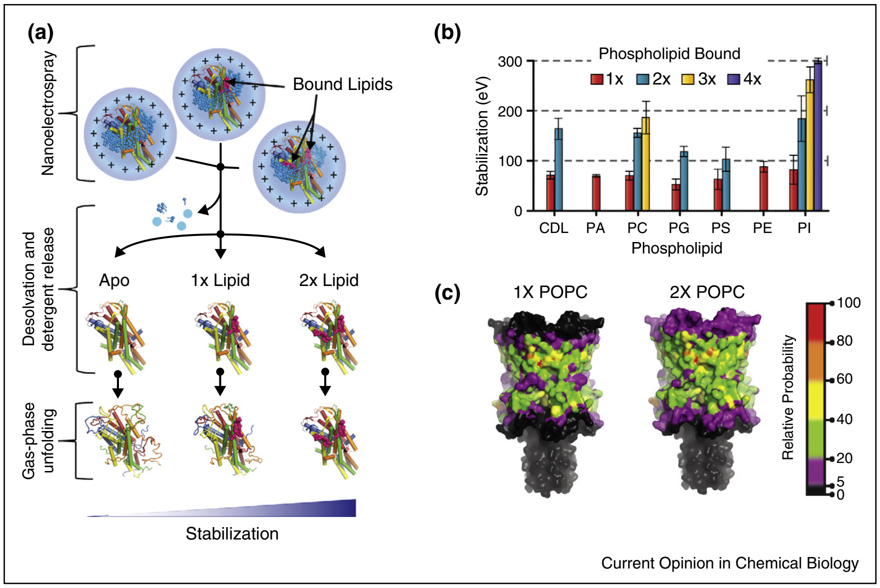
Indeed, in the last few years there has been increasing awareness of the role of lipids on the functioning of MS channels [9].

Protein–lipid interactions

One of the breakthroughs in studying the interaction of MscL with its lipid environment has been the application of mass spectrometry [10]. Even though mass spectrometry has been used to study soluble proteins for the last two decades, its application to membrane proteins is recent [11–13]. Employing electrospray ionization mass spectrometry allowed studying proteins while keeping the membrane subunits intact and retaining protein–lipid interactions [13]. Laganowsky *et al.* [14••] studied the interaction of MscL from *Mycobacterium tuberculosis* (Tb-MscL) with various lipids. It has been shown that detergent micelles protect membrane proteins in their transition from solutions to the vacuum of the mass spectrometer [12]. However, to study the protein–lipid interaction, the protein should stay intact in the gas phase upon the removal of the detergent from the micelles. Therefore, various detergents and experimental conditions were first screened based on the rotationally averaged collision cross sections (CCS) of proteins in the gas phase. If the conditions for removing the detergents perturbs the folded state of the protein, the CCS will be substantially greater than that calculated from the crystal structure. On the basis of this method, tetraethylene glycol monooctyl ether (C_8E_4) was identified as one of the suitable detergents [14••]. Therefore, to assess the protein–lipid binding events, various synthetic lipids were mixed with Tb-MscL in C_8E_4 micelles. Well-resolved mass spectra revealed populations of the channel protein in complex with up to five lipids (Figure 2a). Finally, the specificity of lipid binding to Tb-MscL was assessed on the capacity of the lipid to resist the applied collision-induced unfolding of Tb-MscL.

Figure 2



Stabilization of MscL by lipid binding. **(a)** Detergent-lipid-MscL in nanospray droplets undergoes desolvation and detergent release followed activation. **(b)** Stabilization of MscL with various lipids. **(c)** Probable lipid binding sites determined by molecular dynamics simulations. CDL: cardiolipin, PA: 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphate (16:0-18:1), PC: 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (16:0-18:1), PG: L-α-phosphatidylglycerol, PS: 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phospho-L-serine (16:0-18:1), PE: L-α-phosphatidylethanolamine, PI: 1,2-di-oleoyl-*sn*-glycero-3-phospho-(1′-myo-inositol-3′-phosphate) (18:1) and POPC: 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine.Adapted by permission from Macmillan Publisher Ltd: Nature (Ref. [14••]), copyright 2014.