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Bacterial mechanosensitive channels: progress towards an understanding of their roles in cell physiology☆

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Bacterial mechanosensitive channels sense the changes in lateral tension in the bilayer of the cytoplasmic membrane generated by rapid water flow into the cell. Two major structural families are found widely distributed across bacteria and archaea: MscL and MscS. Our understanding of the mechanisms of gating has advanced rapidly through genetic analysis, structural biology and electrophysiology. It is only recently that the analysis of the physiological roles of the channels has kept pace with mechanistic studies. Recent advances have increased our understanding of the role of the channels in preventing structural perturbation during osmotic transitions and its relationship to water flow across the membrane. It is to these recent developments that this review is dedicated.

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Introduction

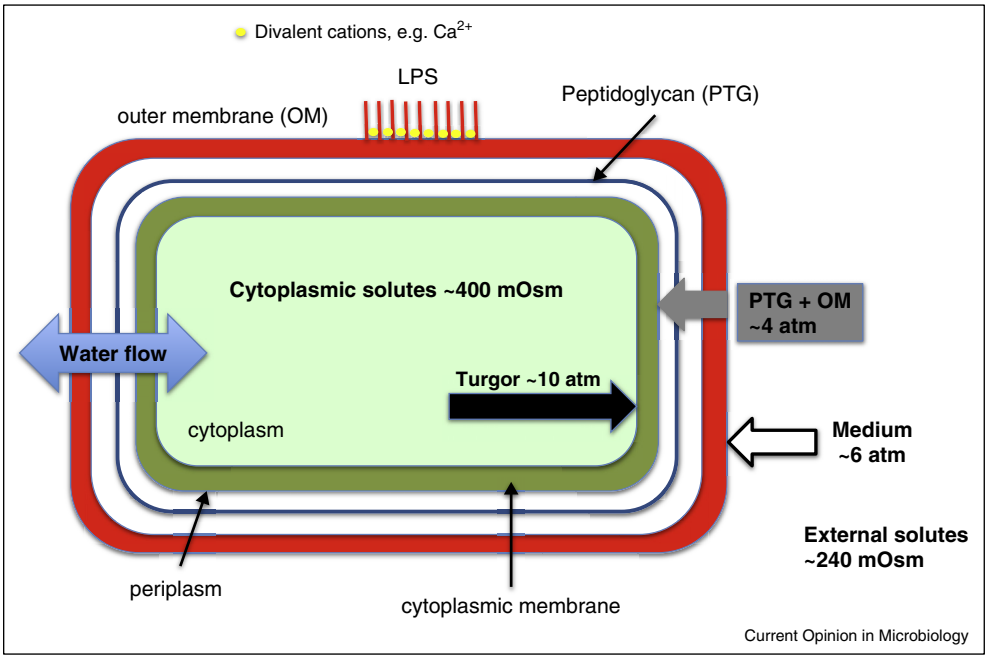
Mechanosensitive (MS) channels sense changes in the tension in the lipid bilayer of the cytoplasmic membrane [1•]. Bacterial channels have been well-studied in a range of organisms [2•] and they are considered to be useful models for mechanotransduction in higher organisms [3]. Mammalian channels are frequently ion-selective and thus generate specific signals that are integrated by the neuronal system leading to an altered behaviour. In contrast, bacterial mechanosensitive channels are generally non-specific in terms of the ions and molecules that pass through the open pore. Their transition from the closed to the open state creates a transient pore of quite large dimensions, minimally ≥6 Å diameter (the size of a hydrated K^+ ion) through to ~30 Å diameter for MscL [2•]. Their proposed major role in cell physiology is well-established, namely protection of the physical integrity of the cell during transitions from high osmolarity to low [4]. One of the most important questions remaining addresses channel abundance, structural diversity and plurality in bacterial species. This short article will review the timing of channel gating and its importance for the roles of the channels.

Osmoregulation and cytoplasmic solute concentrations

Bacterial cells accumulate solutes in their cytoplasm well beyond the concentrations that might be required for metabolism. In most bacteria there is a preference for the accumulation of potassium and glutamate [5]. However, diverse metabolic anions accumulate to millimolar levels, such that the cytoplasm may contain as much as 200 mM osmotically active anions even when grown at moderately low osmolarity (~240 mOsm) [6]. This would generate a net turgor pressure of ~4 atm (~40 mOsm solute ~ 1 atm [2•]; the osmolarity of the medium is equivalent to ~6 atm) directed outwards from the cell (Figure 1). Movement of water across the membrane into the cytoplasm generates the turgor pressure and provides the expansion space required for growth through biosynthesis of new polymers. Measurement of turgor pressure is extremely difficult and there is no certainty that this parameter does not vary with either growth conditions or with the identity of the organism. A net outward pressure of 4 atm in *E. coli* cells was suggested [7], but recent experiments have questioned this [8•].

When subjected to hyperosmotic stress, Gram negative bacteria exhibit a biphasic strategy to counter water loss. In the initial phase potassium and glutamate pools increase and subsequently these ions may be replaced by compatible solutes, such as trehalose, betaine and proline [5]. A cell adapted to high osmolarity is at risk when transferred to low osmolarity due to the osmotically driven water flow into the cytoplasm. A decrease in the external osmolarity of 800 mOsm (equivalent of transfer from growth medium containing 0.5 M NaCl into growth medium alone or approximating the transfer of cells from sea water to fresh water) could raise the turgor pressure by 20 atm [2•]. The actual increase experienced by the cells

Figure 1



The generation of turgor and resistance to the force. In *E. coli* cells growing in a medium of ~240 mOsm (a standard minimal medium or LB containing 5 g/L NaCl) one may confidently expect to find ~200 mM cytoplasmic anions and ~300 mM K^+. Approximately 100 mM of the K^+ matches fixed anions and is thus not considered for the calculation of the outward turgor of ~10 atm. Given the medium contributes ~6 atm the net turgor pressure is ~4 atm. MS channels will gate if there is a net outward pressure of ~0.1 atm and thus the cell wall and outer membrane, between them, contribute a resistance of ≥4 atm to maintain MS channels closed. There are at least two contributions to the strength of the cell wall — the first, already described, is the crosslinking of the peptidoglycan and the second is the outer membrane that can provide some resistive force through the binding together of the lipopolysaccharide chains by divalent cations [46].

depends on the rate of water penetration into the cytoplasm, the elasticity of the peptidoglycan (PTG) and the activity of mechanosensitive channels.

The centrality of water in life

Central to understanding the core physiology of bacterial MS channels is an appreciation of the rapidity of water fluxes across the lipid bilayer. The membrane bilayer is highly permeable to water and in some bacterial species this natural permeability is further augmented by expression of aquaporins [9]. In response to hyperosmotic shock [10,11] (J Mika, PhD Thesis, Groningen, 2012) and hypoosmotic shock [12••] the cell shrinks or expands, respectively, on the very rapid timescales (30% volume change in <1 s is typical). A bacterial cell of 10^{-15} L contains ~3–4 × 10^{10} water molecules. Considering an *E. coli* cell as a cylinder (~2 μm length and 1 μm diameter) that can expand along its length but not readily change its diameter (at least over the very short timescales associated with osmotically driven water movements), which is consistent with current theories of peptidoglycan structure [13•], an expansion of ~12% [14] would require ~4–5 × 10^9 water molecules to cross the membrane, which, in *E. coli*, can occur in 100 ms [10,11] (J Mika, PhD Thesis, Groningen, 2012). The capacity to withstand rapid water movements is dependent upon the operation of MS channels and on the strength of the cell wall.

Peptidoglycan, which gives the cell its physical integrity and shape [13•], is a dynamic, semi-elastic polymer constructed from oligosaccharides of varying lengths (N-acetylglucosamine and N-acetylmuramic acid pentapeptide units; NAG-NAM-p5) crosslinked by short peptides. Sugar chains are organised principally in the circumferential direction, while the peptides are oriented in the long direction of the cell [13•,15,16,17]. Peptidoglycan is not a continuous structure; the sugar chains are of variable length (a single circumference requiring many independent polysaccharide chains) and the peptide crosslinking is incomplete [18]. This variation creates a mesh in which there are holes (of varying sizes) that are bounded by the sugars and peptides [18]. Growth of *E. coli* cells is largely by extension in the long direction and this requires the peptidoglycan be a highly dynamic structure; increasing the length of the cell is principally achieved by breakage of the peptide bonds and the insertion of new wall material [13•]. Although the peptidoglycan is a unique structure between the cytoplasmic and the outer membranes, there are important connections to both membranes through synthetic complexes and lipoproteins,