Mechanosensitive Ion Channels, Part A

Edited by
Owen P. Hamill

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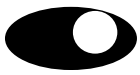

Current Topics in Membranes, Volume 58

Mechanosensitive Ion Channels, Part A

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Foreword

Mechanosensitive Ion Channels, Part A

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Mechanosensitive (MS) ion channels represent the third major class of gated membrane ion channels after voltage- and receptor-gated channels. MS channels are formed by membrane proteins that are able to sense and transduce mechanical force into electroosmotic/signaling events that increase cell survival in a dynamic and sometimes hostile mechanical environment. On the other hand, when specific MS channels operate inappropriately, the consequences can lead to reduced cell viability and the development of several clinically relevant human pathologies. Over the last two decades, research on MS channels has expanded and diversified from the early electrophysiological studies of specialized mechanosensory nerve endings to include studies of cell types across the full evolutionary spectrum and to involve the new disciplines of structural biology, molecular genetics, drug discovery, and biotechnology. As a result there has been flood of new information with the potential for even greater breakthroughs in the near future. To highlight the excitement of the field, *Current Topics in Membranes* has compiled two volumes on MS channels that include chapters written by many of the leading researchers studying MS channels.

Part A of this volume is organized into three sections. The first section covers topics on the atomic structure of two different bacterial MS channel proteins MscL and MscS, the physical and thermodynamic principles that underlie mechanical and electromechanical activation of membrane proteins, and the cellular aspects that determine how mechanical forces are conveyed to membrane proteins via lipid–protein and protein–protein interactions. The second section provides an update from several laboratories on the molecular gating dynamics and the structure–function relations of the channels MscL and MscS. The third section covers MS channels in fungi and plant cells describing the identification of a transient receptor potential

MS Ca^{2+} channel in yeast and an MscS-like channel in plant cells, as well as providing new insight into the special role of mechanical force and MS channels in growing plants.

I would like to thank Dale Benos for his invitation to submit the original proposal to Elsevier. I would also like to thank all those involved in the production of the volumes and, in particular, Phil Carpenter for his continual and patient efforts during the compilation phase. Finally, I would like to thank all the scientists for presenting their discoveries regarding MS channels.

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CHAPTER 1

Structures of the Prokaryotic Mechanosensitive Channels MscL and MscS

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I. OVERVIEW

The prokaryotic mechanosensitive channels of large (MscL) and small (MscS) conductance respond directly to tension applied to the bacterial membrane. Crystal structures of the *Mycobacterium tuberculosis* MscL and

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Escherichia coli MscS were initially reported at 3.5- and 3.9-Å resolutions, respectively. In subsequent refinements described in this chapter, sequence register errors have been corrected to produce improved models for both channels. The pentameric MscL and heptameric MscS are each organized into transmembrane and cytoplasmic domains, although their detailed architectures are distinct in terms of polypeptide folds and oligomeric states. The basic structural framework of the MscL and MscS transmembrane domains is provided by α-helices; each subunit of MscL has 2 helices for a total of 10, whereas MscS has 3 helices per subunit for a total of 21. In contrast to the common architectural theme of helix packing evident in the transmembrane domains, the cytoplasmic domains of MscS and MscL are markedly different in terms of both overall size and polypeptide fold. The permeation pathways in both structures are formed by the right-handed packing of helices that create funnel-shaped pores constricted near the cytoplasmic side by the side chains of hydrophobic residues. From considerations of the relationship between pore geometry and conductance, it is likely that both channel structures represent closed states.

II. INTRODUCTION

Membrane integrity is vital to cellular growth and survival. Among the insults that may be experienced by organisms are changes in external osmolarity; concentration differences of only 10 mM can generate osmotic pressure differences of $\sim$0.2 atm that may rupture membranes of radii $\sim$3 μm (Hamill and Martinac, 2001). Cells immersed in environments that can encounter even modest osmolarity changes must consequently be able to respond on a sufficiently rapid timescale to prevent lysis. Osmotic downshock conditions, such as the sudden exposure of a bacteria to rain or other source of freshwater, represent a particularly challenging situation (Booth and Louis, 1999; Poolman *et al.*, 2002). Without safety-value mechanisms to release cellular contents (Britten and McClure, 1962), cells would not be able to withstand the resultant turgor pressures of tens to hundreds of atmospheres associated with the influx of water. Through the pioneering efforts of C. Kung and coworkers (Martinac *et al.*, 1987; Sukharev *et al.*, 1994, 1997), the proteins in bacteria responsible for sensing the increase in membrane tension accompanying osmotic downshock have been identified. These proteins form high-conductance channels in the inner membrane that can open and close in direct response to tension applied to the bilayer. Such properties are consistent with a biological role for these channels in responding to sudden increases in turgor pressure to jettison water and other cellular contents to prevent cell lysis during hypoosmotic shock. To date, two

general families of these channels have been identified, the mechanosensitive channel of large conductance (MscL) (Sukharev *et al.*, 1994) and of small conductance (MscS) (Levina *et al.*, 1999). Reviews of these channels have appeared (Perozo and Rees, 2003; Strop *et al.*, 2003; Sukharev and Corey, 2004; Blount *et al.*, 2005; Booth *et al.*, 2005; Sukharev *et al.*, 2005) that emphasize different aspects of these channels. Although they are relatively simple, intrinsically stretch-activated systems, the basic principles of how MscL and MscS sense forces applied to the lipid bilayer likely reflect the mechanisms underlying such diverse cellular phenomena as touch, hearing, gravity, and pressure (Kung, 2005).

From a structural perspective, MscL and MscS represent fascinating targets as they provide an opportunity to explore the coupling between protein conformation and the membrane environment responsible for channel gating. Tension and pressure sensitive systems, such as MscL and MscS, have the added attraction that these environmental properties are energetically coupled to changes in protein area and volume, respectively, which may be directly quantitated from structural models. The crystallographic analyses of the *M. tuberculosis* MscL (Chang *et al.*, 1998) and the *E. coli* MscS (Bass *et al.*, 2002) described in this chapter were motivated by these considerations to provide the structural frameworks essential for a mechanistic understanding of mechanosensitive systems at the molecular level.

III. CONDUCTANCES OF MscL AND MscS: GENERAL CONSIDERATIONS

The conductance of a channel, g, describes the coupling between the current flow through the channel, I, and the driving force across the membrane, V, in the Ohm's Law expression:

$$gV = I \tag{1}$$

where g is the inverse of the channel resistance. When I and V are expressed in amperes and volts, respectively, the units of g are siemens (S) which are equivalent to reciprocal ohms. The conductances of MscL and MscS have been reported as $\sim$3 and 1 nS, respectively (Sukharev *et al.*, 1997, 1999; Levina *et al.*, 1999), when measured in solutions containing 200-mM KCl and 40- to 90-mM $MgCl_2$. With a potential difference of 100 mV, a conductance of 1.6 nS equals 160 pA, which is equivalent to the flow of $\sim 10^9$ ions/s across the membrane. These are quite high-conductance channels; for comparison, K^+ channels and the acetylcholine receptor have conductances that are $\sim$100 times smaller than MscL (Hille, 2001). While these conductances reflect the properties of the fully open state, subconductance states have been reported for both channels (Sukharev *et al.*, 1999; Shapovalov and