Chapter 1: Introduction to the Mechanosensitive Channel of Large Conductance

1.1 Using Bacterial Ion Channels as Models for Eukaryotic Ion Channels

Understanding how the human mind works remains a major challenge in many fields. At the chemical and biological level it has been said that, " The human brain is … the most complex structure, natural or artificial, on earth" (Green et al., 1998). Only in the past 50 years have we even begun to understand on the chemical level, what molecules are involved in neurobiology. Currently, the forefront of molecular neurobiology is busy mapping an enormous number of signaling and regulatory pathways.

As chemists, we look to understand the molecular mechanisms than underlie these complex pathways. The proteins of neurobiology provide complex and interesting targets to be understood at the chemical level. Understanding these molecules is an interesting and important feat within itself; however, it also allows us opportunities to develop new and specific pharmaceuticals.

Ion channels are one of the major classes of molecules involved in molecular neurobiology. These molecules are capable of converting an external stimulus into an electrochemical gradient. Ion channels open a pore in the cell membrane in response to large variety of stimuli, including external or internal application of agonist molecules, an electrical field, and mechanical stress. The pores that are opened are then capable of selectively letting molecules or ions in or out of the cell and also distinguishing between cations and anions.

Ion channels have received a great deal of attention over the past few years, since they have been implicated to play critical roles in many genetic and neurodegenerative diseases, including MS, epilepsy, Alzheimer's, Parkinson's, and schizophrenia (Barchi, 1998; Paterson and Nordberg, 2000). Much of the focus has been on finding pharmaceuticals that modulate the behavior of these channels.

In the process of trying to understand ion channels, good experimental systems have been developed to study ion channel proteins and many important ion channels have been cloned. Today, even the most complex ion channels can be routinely expressed in model systems, such as *Xenopus* oocytes, and their properties monitored electrophysiologically.

Understanding the structure and function of these channels is critical for developing an understanding of neurobiology and for development effective drugs. Traditionally, chemists have approached understanding structure-function relationships in these channels by developing pharmacological models, and molecular neurobiologist have approached understanding structure-function relationships in these channels through detailed electrophysiological analysis and mutational analysis. Recently, the Dougherty/Lester labs have developed more subtle ways to probe structure-function relationships in ion-channels using *in vivo* unnatural amino acid mutagenesis (Dougherty, 2000).

Ion-channel structure-function is hindered by the lack of high-resolution structures for these proteins. For many classes of proteins good atomic resolution structures exist, which are invaluable for understanding how these proteins function at the molecular level. However, for the ion-channels, which are important in mammalian neurobiology, no complete atomic resolution structures exist. Recently several extracellular domains of these channels and related proteins have been crystallized (Armstrong and Gouaux, 2000; Armstrong et al., 1998; Brejc et al., 2001), which have both confirmed many of the observations determined mutagenically and electophysiologically, and opened new doors for structure-function analysis.

In 1998, the first atomic resolution structures of bacterial ion-channels were determined (Chang et al., 1998; Doyle et al., 1998). Traditionally, these channels have received less attention than their eukaryotic counterparts; however, they are capable of serving as model systems for eukaryotic ion channels. In general, bacterial ion channels are physically smaller and considerably less complicated than eukaryotic ion channels. This makes them particularly amenable to biochemical and biophysical studies.

High-resolution structure of bacterial ion channels was possible because these channels can be easily overexpressed and purified in milligram quantities. Since 1998, four bacterial ion-channel crystal structures have been determined; three closed-state structures and one open state structure. The closed-state structures are for a potassium channel (Doyle et al., 1998), a chloride channel (Dutzler et al., 2002), and a mechanosensitive channel (Chang et al., 1998), and the open state structure is for a

potassium channel (Jiang et al., 2002a; Jiang et al., 2002b). These structures have provided new insights into how ion channels function.

These atomic resolution structures raise the question of whether structure is sufficient to understand ion-channel function, especially for the case of potassium channels, where both closed and open state structures are known. Clearly the answer to this is no, since function is a dynamic process and the obtained structures are static pictures. These structures, however, provide a platform from which biochemical and biophysical studies can be used gain a complete picture of ion-channel function.

With some structures in hand, it may be possible to gain a complete picture of how bacterial ion channels function. Although these channels are less complicated than their eukaryotic homologues, it is clear that some functional aspects are conserved between prokaryotic and eukaryotic ion channels. Understanding bacterial ion channel function will lead to a better functional understanding of the ion channels involved in mammalian neural biology.

In the remaining chapters of this section, detailed biochemical and biophysical studies on the mechanosensitive channel of large conductance (MscL) will be described. The *M. tuberculosis* homologue of this channel is one of the first bacterial ion channel high-resolution structures to be determined, and the studies described here are based on that structure. The remaining portion of this chapter provides a brief introduction to MscL and its structure.

1.2 The Mechanosensitive Channel of Large Conductance

1.2.1 Mechanosensation

Mechanosensation is a critical function of all organisms from single celled bacteria to humans (Hamill and McBride, 1993; Hamill and McBride, 1994; Hamill and McBride, 1996). Clearly the purpose of mechanosensation varies from organism to organism; however, some similarities exist in the mechanisms of mechanosensation. Mechanosensitive ion channels in all organisms are capable of converting a mechanical stimulus into an electrical signal. These channels work by sensing a force applied to a cellular membrane.

The actual roles of mechanosensitive ion channels vary from organism to organism. In humans, mechanosensitive ion channels play many critical roles. These channels are responsible for such varied tasks as blood pressure regulation and pain sensation (Hamill and McBride, 1994). Proprioception and gravitropism are major roles of mechanosenstive ion channels in plants (Hamill and Martinac, 2001). Bacteria utilize mechanosensitive channels to senses changes in their osmotic environments and prevent bursting upon osmotic downshock (Wood, 1999).

A major breakthrough in the field of mechanosensation occurred in 1994 when the bacterial mechanosensitive channel of large conductance was cloned (Sukharev et al., 1994). Although mechanosensitive ion channels from a large number of organisms had been observed by patch clamp analysis, no specific molecules of mechanosensation were

known. The first mammalian mechanosensitive ion-channel, TREK-1, was cloned in 1996 (Fink et al., 1996).

1.2.2 Pre-structural Analysis MscL

The mechanosensitive channel of large conductance is one of three mechanosensitive ion channels that have been identified electrophysiologically in bacteria. These channels were named according to their observed single channel conductances. Channels with a conductance greater than 1 nS were termed mechanosensitive channels of large conductance (MscL), channels with conductances between 0.3 nS and 0.5 nS were termed mechanosenstive channels of small conductance (MscS), and channels with conductances between 0.1 and 0.15 nS were termed mechanosenstive channels of mini conductance (MscM) (Martinac et al., 1987). MscS (YggB) was cloned by the Booth labs in 1999 and is the gene product of the yggb ORF in *E. coli* (Levina et al., 1999).

MscL was first identified by the Alder and Kung labs in 1987, using a modification of the patch clamp technique that allowed them to patch clamp the periplasmic membrane of *E. coli* (Martinac et al., 1987). The Kung labs subsequently cloned MscL by generating enriched active membrane fractions and obtaining a partial protein sequence that was then used to fish out the MscL gene (Sukharev et al., 1994). Since its initial cloning from *E. coli*, eight MscL homologues have been cloned and characterized by electrophysiology (Moe et al., 1998; Moe et al., 2000). Additionally, over thirty other homologues have been identified by sequence analysis (Maurer et al., 2000).

Even prior to a high-resolution structure, detailed electrophysiology and biochemical analysis provided useful information about the structure and function of MscL. Electrophysiology on isolated protein that was purified and reconstituted into lipid vesicles showed that MscL is necessary and sufficient to achieve channel activity in response to bilayer tension (Figure 1.1) (Sukharev et al., 1994). The tension required to open MscL has been quantitatively determined, and electrophysiology has predicted that there are several discreet steps on the opening pathway (Sukharev et al., 1999b).

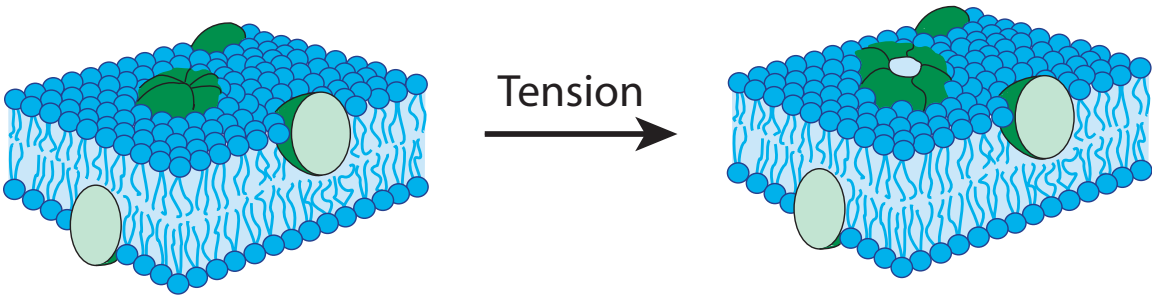

Figure 1.1: The mechanosensitive channel of lager conductance gates in response to bilayer tension.

The single channel conductance of MscL predicted the open pore of the channel to be around 40Å (Sukharev et al., 1997). This large pore was confirmed by molecular sieving experiments that suggested that molecules on the order of 30Å in diameter could pass through the pore of MscL, while molecules of on the order of 37Å in diameter were excluded (Cruickshank et al., 1997). Further evidence for this enormous open state comes from experiments in which small proteins, such as GFP and thioredoxin, have been shown to efflux through MscL upon osmotic downshock (Ajouz et al., 1998; Jones et al., 2000).

The ability of MscL alone to rescue bacteria from osmotic downshock was initially confirmed by introduction of *E. coli* MscL into *V. alginolyticus*, an osmotically sensitive bacterial strain (Nakamaru et al., 1999). This was later confirmed in *E. coli*, after the cloning of MscS and the production of MscS knockout *E. coli* strains (Levina et al., 1999). These experiments provided further evidence that the physiological role of MscL is to prevent bacteria from bursting upon osmotic downshock. This is a desirable attribute for bacteria, since it is likely that they will experience a variety of different osmotic conditions over their life cycle.

Mutagenesis of MscL produced channels with altered gating tensions (Blount et al., 1997; Blount et al., 1996b; Ou et al., 1998; Yoshimura et al., 2001; Yoshimura et al., 1999). Mutations that made MscL easier to gate than wild type MscL were termed gain of function mutations (GOF), while mutations that made MscL harder to gate than wild type MscL were termed loss of function mutations (LOF). Random mutagenesis of *E. coli* MscL revealed a series of gain of function mutations along one face of the helix making up the first transmembrane domain and identified the plug of the closed channel (Ou et al., 1998).

Initial biochemical analysis and two-dimensional crystallographical analysis suggested that MscL was a homohexamer (Blount et al., 1996a; Hase et al., 1997; Saint et al., 1998). The high-resolution crystal structure of MscL revealed the actual multimerization of the channel to pentameric (Chang et al., 1998). The multimerization state of MscL observed in the crystal structure was confirmed by subsequent biochemical analysis

(Maurer et al., 2000; Sukharev et al., 1999a). The multimerization state of the channel is the only biochemical or biophysical analysis of MscL that was proven to be inaccurate by the high-resolution structure.

1.2.3 The Crystal Structure of MscL

In late 1998, the Rees lab obtained a high-resolution crystal structure of the M. tuberculosis homologue of MscL (Tb-MscL) (Chang et al., 1998). Crystalization of Tb-MscL was obtained under acidic conditions (pH 3.0) and in dodecylmaltoside (DDM). The structure was refined to 3.5 Å by soaking the crystals in $Na_2Au(S_2O_3)_2$. Figure 1.2 shows side, top, and bottom views of the Tb-MscL crystal structure.

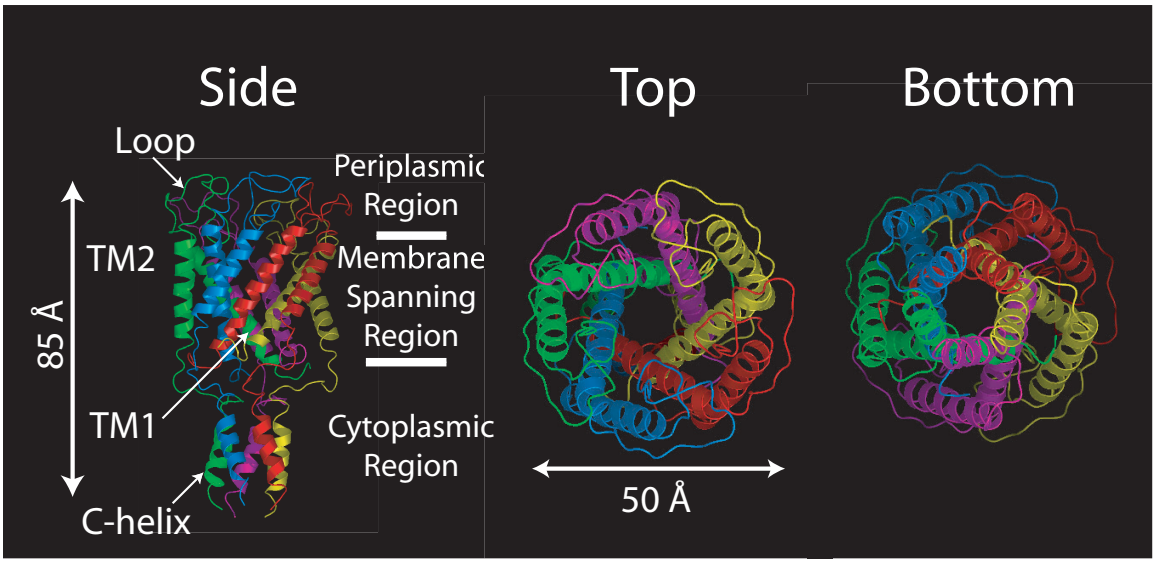

Figure 1.2: The Tb-MscL crystal structure viewed from side, top, and bottom.

As predicted, MscL consists of two transmembrane domains and a long carboxyl terminus. The proteib is 85 Å in length with hydrophobic membrane spanning region of

approximately 35 Å. The two transmembrane helices are tilted approximately 28˚ with respect to the membrane, and the cytoplasmic helix is tilted approximately 15˚ with respect to the membrane. The pore of the channel ranges in diameter from approximately 2 Å to 18 Å with a plug formed by V21.

Despite providing an impressive view of the MscL structure, significant portions of the structure could not be resolved. As a result the crystal structure is missing the first nine residues and the last thirty-three residues of Tb-MscL. Additionally there was insufficient electron density to build in the side chains for residues I56, L57, R58, I59, L97, K100, Q105, and R118.

1.2.4 Post-Structure Analysis of MscL

The high-resolution structure of Tb-MscL has opened up new and exciting opportunities to examine structure-function relationships using the crystal structure as a starting point. With the crystal structure in hand, a variety of techniques, which previously would have provided only minimal information or would not have been possible, are now applicable. These techniques are starting to explain the molecular function of MscL.

Computer modeling has been employed to gain insight into the molecular motions associated with channel function. Molecular dynamics has been applied to the Tb-MscL crystal structure (Bilston and Mylvaganam, 2002; Elmore and Dougherty, 2001; Gullingsrud et al., 2001; Kong et al., 2002). These simulations have provide some

interesting results; however, at present it is not possible to run molecular dynamics simulations long enough to see the transition from the closed to the open state.

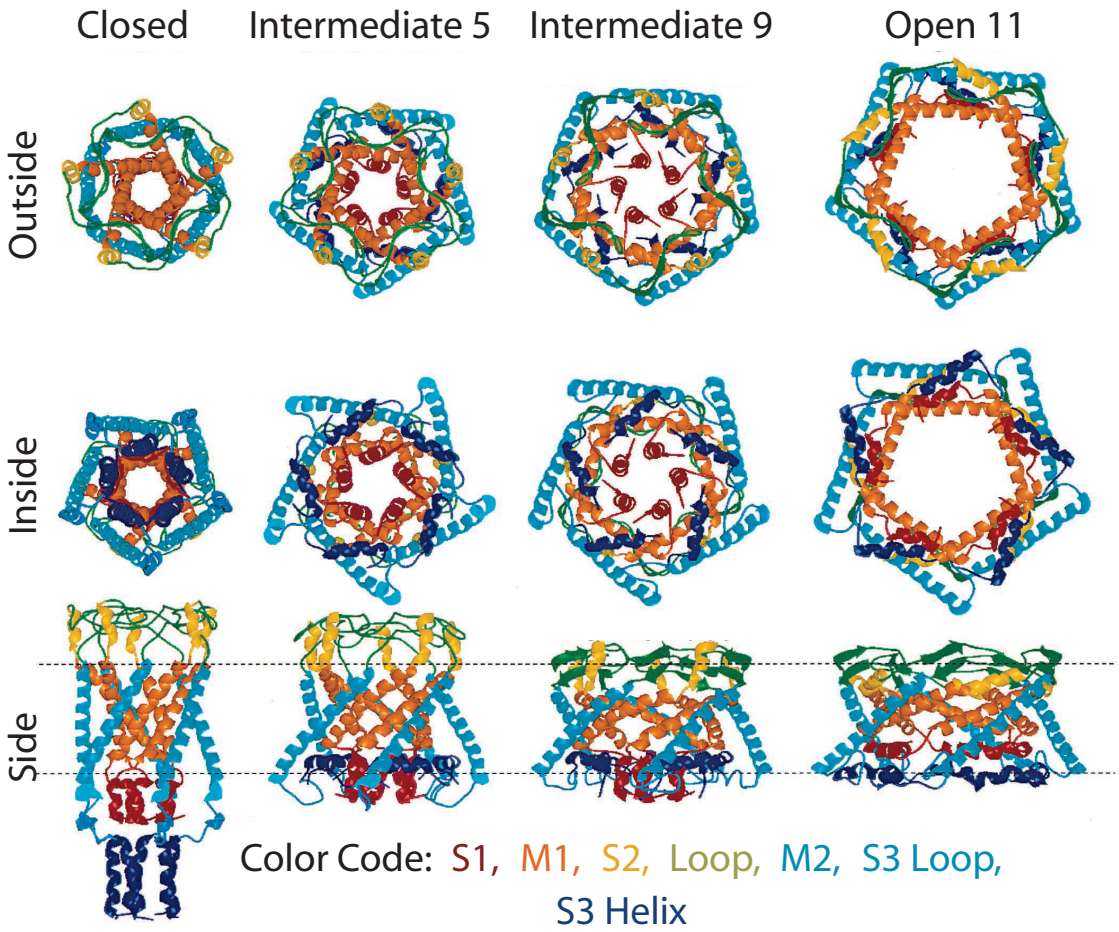

Figure 1.3: The de novo gating model for *E. coli* MscL developed by Sukharev, Guy, and co-workers.

Since the open state structure of MscL is unknown, de novo construction of molecular models for the open state and various intermediates on the opening pathway has been attempted (Sukharev et al., 2001a; Sukharev et al., 2001b). Figure 1.3 shows the atomic level gating model developed by Sukharev, Guy, and co-workers for the *E. coli*

homologue of MscL. Although the crystal structure is of the *M. tuberculosis* homologue of MscL, Sukharev, Guy, and co-workers chose to model the *E. coli* homologue so that they could make use of the large collection of experimental data that exists for *E. coli* MscL.

Recently, Perozo, Martinac, and co-workers have applied cysteine mutatagenesis and site specific spin labeling coupled with electron paramagnetic resonance spectroscopy to *E. coli* MscL (Perozo et al., 2002a; Perozo et al., 2001; Perozo et al., 2002b). Using these techniques, Perozo and co-workers have confirmed that the *E. coli* MscL homologue is structurally similar to the *M. tuberculosis* homologue. Additionally, they have developed a technique to image the open state and an intermediate state of *E. coli* MscL on the EPR time scale. Using this technique, they have been able to obtain a limited number of constraints for the open state of *E. coli* MscL. From these constraints they have developed a computational model for the transmembrane domains of E. coli MscL in the closed, intermediate, open state. Their model is shown if Figure 1.4.

Chapter 2 provides verification of the Tb-MscL crystal structure and a comparison of the *E. coli* and *M. tuberculosis* homologues of MscL. Here we show that the Tb-MscL crystal structure can be used as a starting point for structural design and that Tb-MscL is pentameric under physiological conditions. Additionally, we show that there are significant differences between the *E. coli* and *M. tuberculosis* homologues of MscL. These differences are shown by sequence analysis, circular dichroism studies, and mutational profiling.

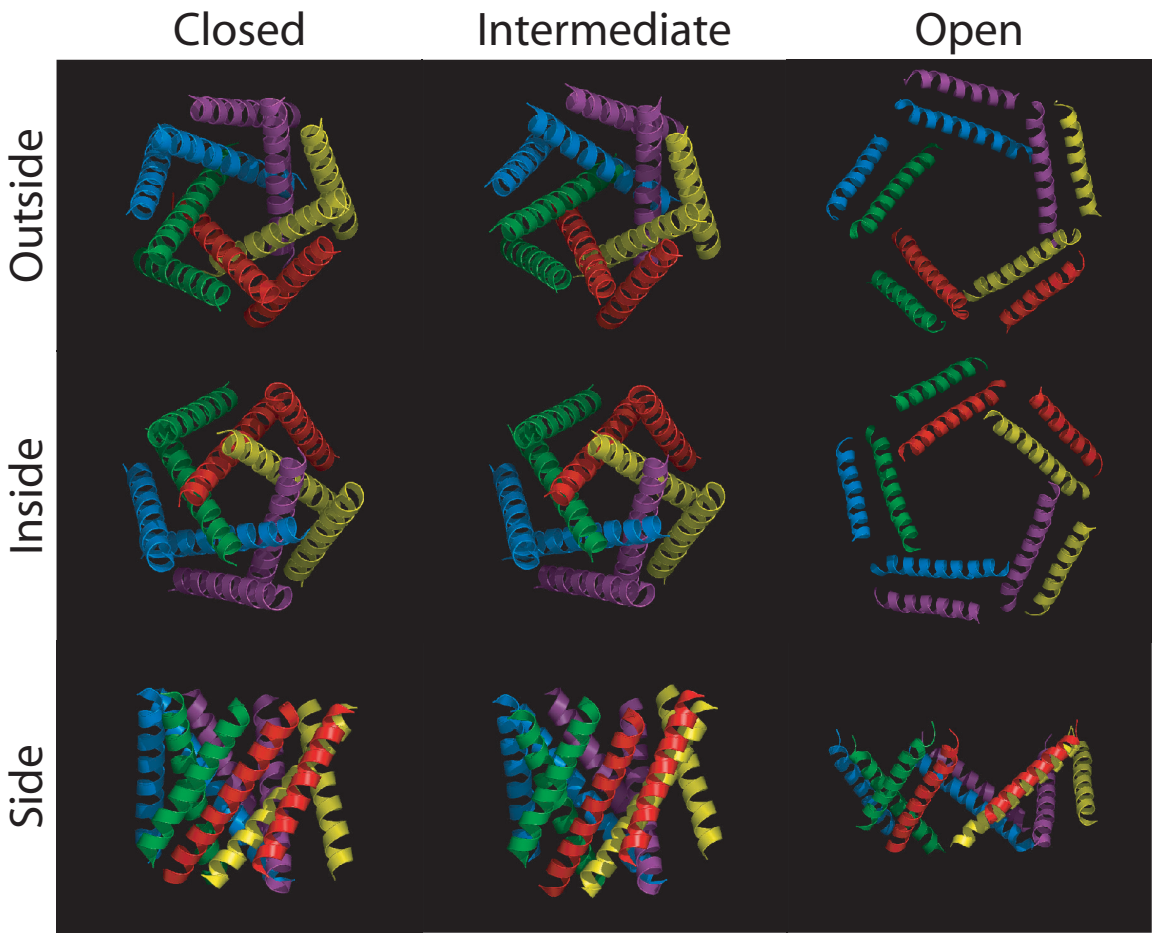

Figure 1.4: Computational model for the transmembrane domains of *E. coli* MscL developed by Perozo, Martinac, and co-workers using EPR constraints.

In Chapter 3, a high throughput fluorescent screen for mutagenic analysis of *E. coli* MscL is developed. This screen allows for rapid mutational phenotyping of mutant MscL channels. Application of this screen, in Chapter 4, to a large library of random MscL mutations provides data to evaluate the de novo *E. coli* MscL gating model. Although it is difficult to use point mutations to comment on the general mechanism of gating proposed by the model, these mutations highlight some regions of the model that need modification. Additionally, the clustering of loss of function mutations observed in the

library suggests that the tension sensor for MscL may be located in the transmembrane domains near the lipid headgroups.

Chapter 5 analyses the carboxyl terminal region of MscL. Once again significant differences are observed between *M. tuberculosis* and *E. coli* homologues of MscL. The carboxyl terminus of Tb-MscL has a significant function role, while the carboxyl terminus of *E. coli* MscL has no functional role. Strikingly, the thirty-three amino acid residues that could not be resolved in the Tb-MscL crystal structure dramatically affect channel function. Even a charge neutralization two residues from the end of the Tb-MscL protein is observed to have afunctional effect on the channel.

1.3 Literature Cited

Ajouz, B., Berrier, C., Garrigues, A., Besnard, M., and Ghazi, E. (1998). Release of thioredoxin via the mechanosensitive channel MscL during osmotic downshock of *Escherichia coli* cells. Journal of Biological Chemistry *273*, 26670-26674.

Armstrong, N., and Gouaux, E. (2000). Mechanisms for activation and antagonism of an AMPA-sensitive glutamate receptor: crystal structures of the GluR2 ligand binding core. Neuron *28*, 165-181.

Armstrong, N., Sun, Y., Chen, G. Q., and Gouaux, E. (1998). Structure of a glutamate-receptor ligand-binding core in complex with kainate. Nature *395*, 913-917.

Barchi, R. L. (1998). Ion channel mutations affecting muscle and brain. Current Opinion in Neurology *11*, 461-468.

Bilston, L. E., and Mylvaganam, K. (2002). Molecular simulations of the large conductance mechanosensitive (MscL) channel under mechanical loading. Febs Letters *512*, 185-190.

Blount, P., Schroeder, M. J., and Kung, C. (1997). Mutations in a bacterial mechanosensitive channel change the cellular response to osmotic stress. Journal of Biological Chemistry *272*, 32150-32157.

Blount, P., Sukharev, S. I., Moe, P. C., Schroeder, M. J., Guy, H. R., and Kung, C. (1996a). Membrane topology and multimeric structure of a mechanosensitive channel protein of *Escherichia coli*. Embo Journal *15*, 4798-4805.

Blount, P., Sukharev, S. I., Schroeder, M. J., Nagle, S. K., and Kung, C. (1996b). Single residue substitutions that change the gating properties of a mechanosensitive channel in *Escherichia coli*. Proceedings of the National Academy of Sciences of the United States of America *93*, 11652-11657.

Brejc, K., van Dijk, W. J., Klaassen, R. V., Schuurmans, M., van Der Oost, J., Smit, A. B., and Sixma, T. K. (2001). Crystal structure of an ACh-binding protein reveals the ligand-binding domain of nicotinic receptors. Nature *411*, 269-276.

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

Cruickshank, C. C., Minchin, R. F., LeDain, A. C., and Martinac, B. (1997). Estimation of the pore size of the large-conductance mechanosensitive ion channel of *Escherichia coli*. Biophysical Journal *73*, 1925-1931.

Dougherty, D. A. (2000). Unnatural amino acids as probes of protein structure and function. Current Opinion in Chemical Biology *4*, 645-652.

Doyle, D. A., Morais Cabral, J., Pfuetzner, R. A., Kuo, A., Gulbis, J. M., Cohen, S. L., Chait, B. T., and MacKinnon, R. (1998). The structure of the potassium channel: molecular basis of K+ conduction and selectivity. Science *280*, 69-77.

Dutzler, R., Campbell, E. B., Cadene, M., Chait, B. T., and MacKinnon, R. (2002). X-ray structure of a ClC chloride channel at 3.0 A reveals the molecular basis of anion selectivity. Nature *415*, 287-294.

Elmore, D. E., and Dougherty, D. A. (2001). Molecular dynamics simulations of wild-type and mutant forms of the *Mycobacterium tuberculosis* MscL channel. Biophysical Journal *81*, 1345-1359.

Fink, M., Duprat, F., Lesage, F., Reyes, R., Romey, G., Heurteaux, C., and Lazdunski, M. (1996). Cloning, functional expression and brain localization of a novel unconventional outward rectifier K+ channel. Embo J *15*, 6854-6862.

Green, T., Heinemann, S. F., and Gusella, J. F. (1998). Molecular neurobiology and genetics: investigation of neural function and dysfunction. Neuron *20*, 427-444.

Gullingsrud, J., Kosztin, D., and Schulten, K. (2001). Structural determinants of MscL gating studied by molecular dynamics simulations. Biophysical Journal *80*, 2074-2081.

Hamill, O., and McBride, D. (1993). Molecular clues to mechanosensitivity. Biophys J *65*, 17-18.

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

Hamill, O. P., and McBride, D. W., Jr. (1994). The cloning of a mechano-gated membrane ion channel. Trends Neurosci *17*, 439-443.

Hamill, O. P., and McBride, D. W., Jr. (1996). The pharmacology of mechanogated membrane ion channels. Pharmacol Rev *48*, 231-252.

Hase, C. C., Minchin, R. F., Kloda, A., and Martinac, B. (1997). Cross-linking studies and membrane localization and assembly of radiolabelled large mechanosensitive ion channel (MscL) of *Escherichia coli*. Biochemical and Biophysical Research Communications *232*, 777-782.

Jiang, Y., Lee, A., Chen, J., Cadene, M., Chait, B. T., and MacKinnon, R. (2002a). Crystal structure and mechanism of a calcium-gated potassium channel. Nature *417*, 515-522.

Jiang, Y., Lee, A., Chen, J., Cadene, M., Chait, B. T., and MacKinnon, R. (2002b). The open pore conformation of potassium channels. Nature *417*, 523-526.

Jones, S. E., Naik, R. R., and Stone, M. O. (2000). Use of small fluorescent molecules to monitor channel activity. Biochemical and Biophysical Research Communications *279*, 208-212.

Kong, Y. F., Shen, Y. F., Warth, T. E., and Ma, J. P. (2002). Conformational pathways in the gating of Escherichia coli mechanosensitive channel. Proceedings of the National Academy of Sciences of the United States of America *99*, 5999-6004.

Levina, N., Totemeyer, S., Stokes, N. R., Louis, P., Jones, M. A., and 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 Journal *18*, 1730-1737.

Martinac, B., Buechner, M., Delcour, A. H., Adler, J., and Kung, C. (1987). Pressure-sensitive ion channel in *Escherichia coli*. Proceedings of the National Academy of Science of the United States of America *84*, 2297-2301.

Maurer, J. A., Elmore, D. E., Lester, H. A., and Dougherty, D. A. (2000). Comparing and contrasting *Escherichia coli* and *Mycobacterium tuberculosis* mechanosensitive channels (MscL) - New gain of function mutations in the loop region. Journal of Biological Chemistry *275*, 22238-22244.

Moe, P. C., Blount, P., and Kung, C. (1998). Functional and structural conservation in the mechanosensitive channel MscL implicates elements crucial for mechanosensation. Molecular Microbiology *28*, 583-592.

Moe, P. C., Levin, G., and Blount, P. (2000). Correlating a protein structure with function of a bacterial mechanosensitive channel. J Biol Chem *275*, 31121-31127.

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

Ou, X. R., Blount, P., Hoffman, R. J., and Kung, C. (1998). One face of a transmembrane helix is crucial in mechanosensitive channel gating. Proceedings of the National Academy of Sciences of the United States of America *95*, 11471-11475.

Paterson, D., and Nordberg, A. (2000). Neuronal nicotinic receptors in the human brain. Progress in Neurobiology *61*, 75-111.

Perozo, E., Cortes, D. M., Sompornpisut, P., Kloda, A., and 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., and Martinac, B. (2001). Site-directed spin-labeling analysis of reconstituted Mscl in the closed state. J Gen Physiol *118*, 193-206.

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

Saint, N., Lacapere, J. J., Gu, L. Q., Ghazi, A., Martinac, B., and Rigaud, J. L. (1998). A hexameric transmembrane pore revealed by two-dimensional crystallization of the large mechanosensitive ion channel (MscL) of *Escherichia coli*. Journal of Biological Chemistry *273*, 14667-14670.

Sukharev, S., Betanzos, M., Chiang, C. S., and Guy, H. R. (2001a). The gating mechanism of the large mechanosensitive channel MscL. Nature *409*, 720-724.

Sukharev, S., Durell, S. R., and Guy, H. R. (2001b). Structural models of the MscL gating mechanism. Biophysical Journal *81*, 917-936.

Sukharev, S. I., Blount, P., Martinac, B., Blattner, F. R., and Kung, C. (1994). A Large-Conductance Mechanosensitive Channel in *E. Coli* Encoded by Mscl Alone. Nature *368*, 265-268.

Sukharev, S. I., Blount, P., Martinac, B., and Kung, C. (1997). Mechanosensitive channels of *Escherichia coli*: The MscL gene, protein, and activities. Annual Review of Physiology *59*, 633-657.

Sukharev, S. I., Schroeder, M. J., and McCaslin, D. R. (1999a). Stoichiometry of the large conductance bacterial mechanosensitive channel of *E. coli*. - A biochemical study. Journal of Membrane Biology *171*, 183-193.

Sukharev, S. I., Sigurdson, W. J., Kung, C., and Sachs, F. (1999b). Energetic and spatial parameters for gating of the bacterial large conductance mechanosensitive channel, MscL. Journal of General Physiology *113*, 525-539.

Wood, J. M. (1999). Osmosensing by bacteria: Signals and membrane-based sensors. Microbiology and Molecular Biology Reviews *63*, 230.

Yoshimura, K., Batiza, A., and Kung, C. (2001). Chemically charging the pore constriction opens the mechanosensitive channel MscL. Biophysical Journal *80*, 2198-2206.

Yoshimura, K., Batiza, A., Schroeder, M., Blount, P., and Kung, C. (1999). Hydrophilicity of a single residue within MscL correlates with increased channel mechanosensitivity. Biophysical Journal *77*, 1960-1972.