Mechanosensitive Channels of Bacteria:

Structure and Function.

Electrophysiology As a High Resolution Technique of Ion Channel Study.

Thesis by

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Abstract

Mechanosensitive (MS) ion channels commonly play a role of transducers converting mechanical stimuli into electrical or chemical signaling, thus allowing the cell to regulate its behavior in response to changing environment conditions. MS channels participate in sensation of sound and orientation in inner ear (hair cells), in touch sensation and in osmoregulation of bacteria. Structure of bacterial MS channels of large (MscL) and small (MscS) conductance has been recently solved at atomic resolution, stimulating various structural and functional studies. In this work author presents series of experiments enhancing an understanding of mechanosensation in bacteria. In Chapter 2 author performs cysteine cross-linking experiments suggesting asymmetric gating pattern of Tb-MscL ion channel. Chapters 3 and 4 establish a possibility of successful synthesis of fully functional Tb- and Ec-MscL proteins displaying a phenotype identical to recombinant channels. Studies in Chapter 5 and Appendix 1 extend the resolution of single-channel patch clamping technique, and describe a fine structure of MS channel gating by collecting and characterizing intersubstate transitions.

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Abbreviations

CPS chemical protein synthesis

EPR electron paramagnetic resonance

FFT fast Fourier transform

GOF gain of function

LOF loss of function

LRET luminescence resonance energy transfer

MD Molecular Dynamics

MS mechanosensitive

MscL mechanosensitive channel of large conductance

MscM mechanosensitive channel of mini conductance

MscS mechanosensitive channel of small conductance

POPC phosphatidylcholine

POPE phosphatidylethanoalamine

TM transmembrane domain

WT wild type