Biophysics of mechanosensitive channels in bacteria

○Martincis Boris (Sch Biomed Sci, The Univ Queensland, AUS)

Mechanosensitive (MS) ion channels are a special type of integral membrane proteins activated by membrane deformation caused by mechanical stimuli experienced by living cells. They convert mechanical stimuli into electrical and/or chemical intracellular signals. These channels have been found in all types of prokaryotic and eukaryotic cells. In animals and humans they play a role in hearing, touch, proprioception or regulation of blood pressure. MS channels in bacteria constitute a mechanism that prevents excessive water inflow and build-up of excessive turgor pressure. Bacterial channels have large conductance and mostly lack ionic specificity. They act as mechanoelectrical switches responding to cell membrane deformations caused by osmotic forces under hypotonic conditions. Without this response, the bacteria lyse. Among prokaryotic MS channels the best characterised are bacterial Mscl and MscS channels. Their 3D structure was determined by X-ray crystallography allowing for in-depth studies of the gating mechanism in these channels. Mscl and MscS as well as other prokaryotic MS channels are gated by bilayer deformation forces indicating that mechanism of mechanotransduction in prokaryotic MS channels is defined by both local and global asymmetries in the transbilayer pressure profile and/or bilayer curvature at the lipid protein interface. The implication of this finding is that the lipid bilayer is much more than a neutral solvent by actively modulating the specificity and fidelity of signalling by membrane proteins.

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Applications of FRET in Biophysics

○dos Remedios C G1, Chhabra D1, Dedova I1, Safer D2, De La Cruz E1 (1Institute for Biomedical Research, Univ of Sydney, Sydney, Australia, 2Department of Physiology, University of Pennsylvania, Philadelphia, USA, 3Department of Molecular Biochemistry & Bio-physics, Yale University, New Haven, USA)

Actin is the principal component of microfilaments whose assembly/disassembly is essential for cell motility. It is present in the nucleus where it may regulate gene expression. Collin can bind actin and translocate it into the nucleus during times of stress. We use fluorescence resonance energy transfer (FRET) and confocal microscopy to analyze the interactions of collin and G-actin in the nucleus and cytoplasm. By measuring the rate of photobleaching of fluorescein-labeled actin and Cy5-labeled collin, we show that most of the nuclear G-actin is bound to collin, but only half is bound in the cytoplasm. A significant proportion of collin in the nucleus and cytoplasm binds added TMR-labeled G-actin. These data suggest there is significantly more collin-G-actin complex and less free collin in the nucleus. FRET spectroscopy can detect structural changes proteins. Using FRET we recently demonstrated that thymosin b4 (Tb4) binding induces spatial rearrangements within subdomains 1 and 2 of G-actin. Tb4 binding increases the distance between Gna-41 and Cys-374 of actin by 2 Å and decreases the distance between bound ATP (εATP at the NUC site) and Lys-61 by 1.9 Å. The distance between Cys-374 and Lys-61 is minimally affected. Our results favour a model where Tb4 changes the orientation of actin subdomain 2. This conformational change presumably accounts for the reduced rate of nucleotide and amide hydrogen exchange from actin monomers.

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