SYMPOSIUM 1: Ca^{2+} Regulation of Channels

34-Symp
Calcium Regulated Channels in the Tmem16 Family
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35-Symp
Frontiers in the Ca^{2+} Regulation of the BK_{ca} Channel
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Large conductance Ca^{2+}-activated K^+ channels (BK_{Ca} channels) sense and respond to near-membrane Ca^{2+} in the micromolar range, and in so doing provide feedback control over such processes as smooth muscle contraction and Ca^{2+}-dependent exocytosis. They are unique among ion channels in that they are both ligand and voltage dependent, and they are unique among Ca^{2+}-binding proteins in that they contain no canonical Ca^{2+}-binding motifs and their apparent affinity for Ca^{2+} is strongly voltage dependent. In my laboratory we have been interested in determining the number of Ca^{2+} binding sites the BK_{Ca} channel contains, their affinities when the channel is open and closed, and to what extent these affinities are affected by membrane voltage and the expression of the BK_{Ca} β1 subunit. I will discuss the results of experiments we have performed to address these issues and place them in the context of the exciting crystal structures published this year that give us the first structural view of the BK_{Ca} channel's Ca^{2+} sensing mechanism.

36-Symp
Recognition of Voltage-Dependent Sodium Channels by Calmodulin
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Voltage-dependent or voltage-gated sodium channels (VDSC, VGSC, $Na_V1.x$) control essential processes in muscle cells and neurons. Associated channelopathies include Dravet syndrome, epilepsy, Long QT syndrome 3, ventricular fibrillation, familial autism, pain insensitivity, and defects in the generation and propagation of action potentials. Calmodulin (CaM), a eukaryotic calcium sensor, regulates sodium channels by binding to the intracellular C-terminal region of the pore-forming alpha subunit. Thermodynamic analysis and high resolution structural (NMR) studies focusing on how apo (calcium-depleted) and calcium-saturated ^{13}C-^{15}N-CaM recognize an IQ-motif (IQxxxBGxxxB, B=K,R) located in the tail of $Na_V1.2$ will be presented. These will be compared to studies of how CaM recognizes other sodium channels, unconventional myosin V (2IX7.pdb), and the small conductance potassium channel (1G4Y.pdb). NIH R01 GM57001.

37-Symp
Calmodulation of Voltage-Gated Calcium Channels: Frontiers of Biological Impact and Mechanistic Elegance
David T. Yue.
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Calmodulin (CaM) regulation of mammalian Ca_V channels is both biologically critical and mechanistically rich, rendering this system a central prototype for the decoding of Ca^{2+} signals and the modulation of channels. This system showcases the remarkable ability of the N- and C-terminal lobes of CaM to function as semiautonomous Ca^{2+} sensors and effectors, a theme initially recognized in *Paramecium* (Cell **62**:165). In mammalian Ca_V channels, Ca^{2+}-free CaM (apoCaM) starts off already preassociated with a host channel, and then the C-lobe turns out to respond preferentially to the strong Ca^{2+} influx through the host channel to induce one form of channel modulation (local selectivity), whereas the N-lobe frequently prefers the far weaker Ca^{2+} signal emanating from Ca^{2+} sources at a distance (global selectivity). These striking contrasts in spatial Ca^{2+} selectivity, crucial to biological Ca^{2+} signaling, can be simply explained by mechanisms involving the translocation of CaM among an apoCaM preassociation locus and multiple Ca^{2+}/CaM effector sites (Cell **133**:1228). However, beyond apoCaM preassociation at an IQ domain on the carboxy-terminus of channels, little has been definitively established regarding the structural identity of Ca^{2+}/CaM effector sites, outside of an *NSCaTE* element in the channel amino terminus (Nature **451**:830). Here, we outline new evidence identifying dominant Ca^{2+}/CaM effector sites outside of the IQ and *NSCaTE* regions (Ben Johnny *et al*, Bazzazi *et al*, this meeting), thereby bolstering a modulatory mechanism wherein CaM departs from an initial IQ preassociation locus, then interacts with structurally distinct effector sites. This intricate translocating dance of CaM complexed with a target molecule may be a general scheme enabling high-order Ca^{2+} decoding in many signaling complexes throughout biology; indeed, an analogous hypothesis of migratory CaM has been proposed for Na_V channels (J. Biol. Chem. 284:6436).

SYMPOSIUM 2: Noise and Fluctuations in Biology: Where is it Important?

38-Symp
On Noise and Filtering in Adaptive Signaling Networks
Yuhai Tu.
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Two different noise filtering strategies are identified and studied in a class of adaptive sensory systems. The high frequency noise is filtered by the Berg-Purcell time averaging scheme with the filtering carried out by the output decay process independent of the slow adaptation dynamics. The low frequency noise is reduced by adaptation and decreases as the feedback time shortens. Both filtering mechanisms introduce noises that are unfiltered and thus contribute to a significant internal noise floor. We show that both noise filtering mechanisms are utilized in the E. Coli chemotaxis pathway: the ligand binding noise is averaged by the response time and remains small due to its fast time scale, while the dominant signal noise, caused by the random cell motion in a gradient, is controlled by adaptation. We conclude that the chemotaxis pathway optimizes gradient sensing, strong response, and noise control in different time scales.

39-Symp
Bistability in the EnvZ/OmpR Operon Controls Osmotic Signaling in E. coli
Linda J. Kenney[1,2], Jeesun Lim[2], Danny van Noort[2].
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In bacteria, the paradigm for signal transduction is the two-component regulatory system. The first component is a sensor kinase and the second component is a response regulator (usually a DNA binding protein). The EnvZ/OmpR two-component system regulates expression of outer membrane proteins in response to osmotic stress. At low osmolality, the major porin is OmpF, at high osmolality, ompF is repressed and OmpC becomes the major porin. EnvZ is an inner membrane protein that transduces the osmotic signal, although the signal is presently unknown. EnvZ is autophosphorylated by ATP on a conserved histidine residue and then transfers the phosphoryl group to the response regulator OmpR on an aspartic acid residue. OmpR~P binds to the regulatory regions of the porin genes to differentially control their expression. Although high salt or 20% sucrose have been used interchangeably as osmotic stimuli, it is apparent that they cause distinct morphological effects as well as differentially affecting EnvZ. Using an ompC-GFP transcriptional fusion, we examined transcription in single E. coli cells. Approximately 25% of the cells exhibited high fluorescence at high osmolality, but the remainder of the cells were less responsive or unresponsive. In order to understand the underlying basis for this bistability, we constructed photoactivatable chimeras to EnvZ and OmpR and used photoactivatable localization microscopy (PALM) to examine their abundance and localization at low and high osmolality. Surprisingly, EnvZ levels varied dramatically; some cells had low levels, but others had very high levels of EnvZ. Additional experiments are in progress to further characterize this bistability of EnvZ and will be discussed. Supported by NIH GM-058746 and the Mechanobiology Institute (MBI), National University of Singapore.

40-Symp
Measurement Noise Limitations in Eukaryotic Chemotaxis
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Many types of eukaryotic cells are able to detect chemical gradients and move accordingly. Unlike the case for bacteria, these cells are large enough for the gradient detection to rely on differential receptor binding probabilities on the cell membrane. This talk will focus on recent experimental and theoretical work using the amoeba Dictyostelium discoideum as a model system to investigate this process. The main focus is on how the noisy input data from the cAMP receptors is processed by the cell to make the motion decision and on under what conditions response is limited by measurement accuracy.