IP3 Receptors

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Structural Insights into the Assembly and Function of the IP_3 Receptor Ligand-Binding Module
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Inositol 1,4,5-trisphosphate receptors (IP_3Rs) are calcium-releasing channels that are involved in numerous physiological functions such as learning and memory, muscle contraction and cell proliferation. Dysfunction of IP_3Rs and IP_3R-interacting proteins has been implicated in several diseases including neurodegenerative diseases and arrhythmia. The activation of IP_3Rs requires the binding of both IP_3 and Ca^{2+}. The IP_3-binding module is composed of two sub-regions: the suppressor domain and the IP_3-binding core. Previous studies showed that the suppressor domain reduces the affinity of the IP_3-binding core for IP_3 and that the isoform-specific IP_3-binding affinity of IP_3R is due to the different interactions between these two domains.
We crystallized and solved the structure of the whole N-terminal ligand-binding module of IP_3R isoform 3 by X-ray crystallography to 3.4 Å. This structure, which represents the closed state of the channel, shows a clear conformational change in the IP_3-binding core compared to previous open state structures. The suppressor domain interacts with IP_3-binding core mainly through a hydrophilic interface. Comparing this interface with the one from IP_3R isoform 1, we identified the structural element that is crucial for the isoform-specific IP_3-binding affinity. Docking into the IP_3R electron microscopy map places the module on the cytoplasmic part of the channel around the four-fold symmetry axis. Four ligand-binding modules form a cluster at the apex of the mushroom-shaped IP_3R protein. The crystal structure and the localization of IP_3R ligand-binding module resemble another type of intracellular Ca^{2+}-release channel, ryanodine receptors. We propose a model showing that these two channels share a common allosteric gating mechanism.

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Electron Cryo-Microscopic Study of the Type 1 IP3R
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Inositol 1,4,5-trisphosphate receptors (IP3Rs) play important roles in a battery of cellular activities. Structural study of the receptors is therefore very important for understanding how they are gated by their natural ligands and are modulated by their intracellular partners. In past several years, multiple groups have generated very disparate reconstructions of the type 1 IP3R since the first low-resolution cryoEM structure was published (Jiang, et al EMBO J. 2002). Striking structural variations have been reported for receptors in different detergents and for receptors prepared from native and sf9 cells by the same research groups, suggesting that the biochemical preparations of the receptors have significant variations and that the heterogeneity in the samples could be a limiting factor in reaching accordant results. To help resolve such discrepancies, we are conducting single particle reconstruction of the type 1 IP3Rs from both native tissues and heterogeneous expression systems. We worked out conditions that maintained the stability and biochemical homogeneity of the purified receptors, and introduced site-specific mutations and prepared accessory binding partners to facilitate the mapping of specific locations in the 3D reconstruction. We are currently collecting datasets from the receptors in different conditions in order to examine the consistency among the reconstructed structures.

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A Park/Drive Model for the Inositol-Trisphosphate Receptor (IPR)
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The inositol-trisphosphate receptor (IPR) is an ion channel that plays a crucial role in the generation of calcium (Ca^{2+}) oscillations; it regulates the release of Ca^{2+} ions from internal stores, such as the endoplasmic reticulum (ER), to the cytosol. Recently, it was discovered that the IPR spontaneously jumps between two levels of activity - the nearly inactive "park mode" and the highly active "drive mode" - even at constant concentrations of its ligands inositol-trisphosphate (IP_3), adenosine trisphosphate (ATP) and Ca^{2+}.
A new model for the IPR will be presented that takes into account mode regulation and which is based upon a large data set collected from type I and type II of the IPR at various ligand concentrations. It will also be shown how models for ion channels can be generated directly from single channel data sets by Markov chain Monte-Carlo (MCMC).

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A Data Driven Approach to Learning the Kinetics of Single Molecules: A Case Study of Single Inositol 1,4,5-Trisphosphate Receptor Channel
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The inositol 1,4,5-trisphosphate (IP_3) receptor (IP_3R) channel plays a central role in the generation and modulation of intracellular Ca^{2+} signals in animal cells. To gain insight into the crucial intricate ligand regulation of this ubiquitous channel, we constructed the simplest quantitative continuous-time Markov-chain model using a transparent iterative approach, which can be adopted to Markov chain models in general. Our model accounts for all experimentally observed gating behaviors of single native IP_3R channels from insect Sf9 cells. Ligand (Ca^{2+} and IP_3) dependencies of channel open probability (P_O) established six main ligand-bound channel complexes, where a complex consists of one or more states with the same ligand stoichiometry and open or closed conformation. Channel gating in three distinct modes added one additional complex and indicated that three of the complexes can gate in two different modes. This also restricted the connectivity between channel complexes. Finally, channel responses to abrupt ligand concentration changes defined a model with 9 closed states and 3 open states, and its network topology. The model with 24 parameters can closely reproduce the equilibrium P_O and channel gating statistics for all three gating modes for a broad range of ligand concentrations. It also captures the major features of channel response latency distributions. The model can generate falsifiable predictions of IP_3R channel gating behaviors not yet explored, and provide insights to both guide future experiment development and improve IP_3R channel gating analysis.

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Calcium-Flux-Mediated Modulation of $InsP_3R$ Channel Activity by ER Luminal Calcium Concentration
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The ubiquitous, mostly endoplasmic reticulum (ER)-localized $InsP_3R$ channel modulates cytoplasmic free $[Ca^{2+}]$ ($[Ca^{2+}]_i$) to generate complex Ca^{2+} signals by releasing Ca^{2+} stored in the ER lumen. Whereas cytoplasmic $InsP_3$ activation and biphasic $[Ca^{2+}]_i$ regulation of the channel have been well studied, its regulation by ER luminal free $[Ca^{2+}]$ ($[Ca^{2+}]_{ER}$) is poorly understood and controversial. Here, we used excised luminal-side-out nuclear patch clamping with perfusion solution exchange to study the effects of $[Ca^{2+}]_{ER}$ on homotetrameric rat type 3 $InsP_3R$ ($InsP_3R$-3) channel activity. In optimal 2 μM $[Ca^{2+}]_i$ (buffered by 0.5 mM diBrBAPTA) and sub-saturating 3 μM $InsP_3$, jumps of $[Ca^{2+}]_{ER}$ from 70 nM (no Ca^{2+} flux through channel) to 300 μM (substantial Ca^{2+} flux) reduced channel open probability (P_o) significantly. This inhibition was abrogated when saturating 10 μM $InsP_3$ was used, but was restored when $[Ca^{2+}]_{ER}$ was raised to 2 mM. This $[Ca^{2+}]_{ER}$ effect exhibited a biphasic dependence on $[Ca^{2+}]_i$ because jumps of $[Ca^{2+}]_{ER}$ (70 nM to 300 μM) in 3 μM $InsP_3$ *increased* P_o in sub-optimal 70 nM $Ca^{2+}{}_i$. These effects of $[Ca^{2+}]_{ER}$ were attenuated when an electrical potential was applied to oppose Ca^{2+} flux through the channel. Importantly, the effects of $[Ca^{2+}]_{ER}$ on channel activity depended on cytoplasmic Ca^{2+} buffering conditions: it was stronger when $[Ca^{2+}]_i$ was weakly buffered by 0.1 mM HEDTA (a slow Ca^{2+} chelator) but was *completely abolished* with 5 mM diBrBAPTA buffering. These observations are completely accounted for by Ca^{2+} flux driven by $[Ca^{2+}]_{ER}$ through the open $InsP_3R$ channel raising local $[Ca^{2+}]_i$ around the channel to regulate its P_o through its cytoplasmic activating and inhibitory Ca^{2+}-binding sites. These results provide no evidence for a role of $[Ca^{2+}]_{ER}$ in regulating $InsP_3R$-3 activity by effects mediated directly on the luminal aspect of the channel.

Ligand-gated Channels I

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Characterization the GABAA Channels in Various Cell Expression Formats using a Microfluidic Patch Clamp System
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Ensemble recording and microfluidic perfusion in IonFlux platform are designed to remove the laborious nature of manual patch clamp and improve the recording success rates. Here we present assay characteristics of an important class of ligand gated ion channels, $GABA_A$ channels study. A variety methods and backgrounds expressing $GABA_A$ channels were successfully studied on IonFlux (defined as $I_{GABA} > 500pA$) including stably transfected HEK cells expressing $\alpha1\beta3\gamma2$ $GABA_A$ channels, frozen "ready to assay" HEK cells expressing $\alpha1\beta3\gamma2$ or $\alpha3\beta3\gamma2$ $GABA_A$ channels, transiently transfected HEK cells expressing $\alpha1\beta3\gamma2$ $GABA_A$ channels and primary and immortalized