IP3 Receptors

2655-Pos

Novel Insights into the Role of Junctate in Calcium Homeostasis: Identification of Binding Domain on the InsP3R and Cellular Localization as Determined by TIRF Microscopy

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Junctate is a 33 kDa calcium binding protein with a single ER/SR membrane spanning domain expressed in excitable and non-excitable tissues. It is generated by alternative splicing from the AßH-J-J locus, which also encodes the enzyme aspartyl-ß-hydroxylase, the sarcoplasimc reticulum structural protein junctin and humbug, a truncated version of aspartyl-ß-hydroxylase, lacking its catalytic domain, which shares with junctate the high capacity moderate affinity Ca2+ binding domain and is over-expressed in a variety of tumours. We have previously shown that junctate forms a macromolecular complex with the InsP3R and TRPC3 channels and when transiently over-expressed in HEK293 cells, it induces extensive proliferation of the ER resulting in significantly larger and more frequent couplings between the ER and the plasma membrane. In the present work we have mapped the binding domain of the cytoplasmic NH2 terminus of junctate on the InsP3R and show that it binds to the domain involved in InsP3 binding. Such a result is supported by the finding that in the presence of a peptide encompassing the NH2 terminal domain of junctate, the Bmax for InsP3 binding is significantly higher than that obtained in the presence of an unrelated peptide. Transmission electron microscopy revealed that clones stably transfected with junctate-YFP display a significant larger number of junctions between the ER and the plasma membrane compared to control HEK293 cells and this effect is enhanced in clones that also over-express TRCP3. The size and distribution of these punctae however, was not affected by the addition of agonists mobilizing calcium via InsP3R activation.

2656-Pos

Visualizing Alpha Helices in the Transmembrane Region of IP_3R1 Calcium Release Channel by Single Particle Electron Cryomicroscopy

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Type 1 inositol 1,4,5-trisphosphate receptor (IP_3R1), a 1.3 MDa tetrameric membrane protein regulates the release of Ca^{2+} from endoplasmic reticulum stores into the cytoplasm and plays an essential role in a wide variety of cellular processes. We determined the structure of IP_3R1 in the closed conformation at ~9-Å resolution by using single particle electron cryomicroscopy. The channel protein was solubilized with detergent from rat cerebellar microsomes and purified by immunoaffinity chromatography. The purified channels were reconstituted into Ca^{2+}-loaded lipid vesicles and released Ca^{2+} in response to nanomolar concentrations of IP_3, indicating their functionality. Isolated IP_3R1 particles were embedded in vitreous ice for cryo-EM in the presence of 1 mM EGTA to drive the channel protein into its closed conformation. The 3D density map of IP_3R1 was generated with 37,231 particles extracted from 929 CCD frames collected on a JEOL2010F electron cryomicroscope. The reconstruction was performed using EMAN software. This structure allows visualizing a number of alpha-helices in the membrane-spanning region of the IP_3R1 channel, including the inner alpha-helices lining the tapering ion conduction pathway. The molecular architecture of the closed pore is established based on the 9-Å cryo-EM density map of IP_3R1 and via computational and bioinformatics approaches.
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2657-Pos

Spatial Modeling of IP3 Channel Clusters and Calcium Puffs

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Using detailed numerical analysis as well as strongly reduced models we study release of calcium from clusters of IP3 receptor channels. In the first part we present hybrid stochastic-deterministic simulations of release from a cluster of nine channels. We adopt recent advances in imaging of calcium release, which showed a considerable spatial separation of channels. We find that, due to the separation of channels and the three-dimensional transport of calcium away from the source area, the calcium concentration is generally heterogeneous in the cluster area. Based on a Markovian description of channel gating and a fitting of ligand/channel reactions to single-channel data, we obtain puffs that strongly resemble recent recordings in neuroblastoma cells. We conclude that spatial heterogeneity is crucial part of the understanding of puffs. In a second part of this work we take up the issue of deriving a reduced model in terms of a discrete or continuous description of gating variables. We argue that lack of homogeneity in [Ca2+] obtained in the detailed simulations obliterates the assumption of mixing of reactants (here ligands and channels) and thus the validity of the law of mass action. Effective reaction kinetics can be derived, however, by distinguishing concentrations of self-feedback of channels and coupling to different channels, thus eliminating detailed balance. We infer a minimal Markovian model as well as a corresponding Langevin model. Importantly, only the Markovian description reproduces calcium puffs, while a Langevin model wrongly predicts a stationary regime of high inhibition. The analysis of the Markovian model allows further insight into the functioning of calcium puffs.

2658-Pos

Role of Agonist-Independent Conformational Transition (AICT) in I P3 R Cluster Behavior

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Local intracellular calcium (C a^{2+}) signals arise due to the release of C a^{2+} ions from internal stores into the cytosol through small clusters of inositol-1,4,5-triphosphate (I P_3) receptors. To explain single I P_3 receptor open probability data from nuclear patch clamp experiments, theoretical simulations have favored the existence of an agonist-independent conformational transition(AICT) from closed to an open state. We present results from a computational study wherein we explore the impact of the proposed agonist independent conformational transition on the collective release of calcium from I P_3 R clusters. A wealth of experimental data profiles collective cluster release. Our results show that consistency of cluster release between theory and experiments in fact constrains the kinetics of the agonist-independent conformational transition (AITC) to values which lead to small open probabilities for a single I P_3 receptor, inconsistent with nuclear patch clamp experimental data.

2659-Pos

Global Dynamic Conformational Changes in the Suppressor Domain of IP_3 Receptor by Stepwise Binding of the Two Lobes of Calmodulin

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The roles of calmodulin (CaM) has been a key point of controversy in the regulation of inositol-1,4,5-trisphosphate receptor (IP_3R). Current views differ in terms of the involvement of CaM as calcium sensors and calcium's functions in the process. To help resolve these issues, we studied the interaction between CaM and the suppressor domain of IP_3R, a key allosteric regulatory domain, in the absence and presence of calcium. Through NMR binding experiments, we observed dramatic peak disappearances of the suppressor domain upon interaction with apo-CaM. These data indicated that apo-CaM induces large-scale dynamic conformational changes in the suppressor domain, most probably involving partial unfolding and sub-domain rearrangement. Resonance assignments of CaM surprisingly revealed that its C-lobe alone can cause these changes. Subsequent NMR binding experiments showed that calcium allows the free N-lobe to additionally bind to the suppressor domain, which induces extra conformational changes in both of the proteins. Our data also suggest that the extra changes in the suppressor domain are secondary to those in calmodulin. Based on these results, we propose that apo-CaM, through its C-lobe, can prime the allosteric regulation by partially unfolding the suppressor domain, which could be propagated to distant sites to open inhibitory calcium binding sites. Calcium then could bind to the CaM's N-lobe and the inhibitory binding sites in IP_3R, eliciting additional conformational changes and actual inhibition of IP_3R. We believe that our results reconcile previous allosteric models and provide new insights in the mechanism of calcium/CaM-mediated regulation of IP_3R.

2660-Pos

An Analysis of the Activity of Two Disease Associated Mutations of the Inositol 1,4,5-Trisphosphate Receptor Type-1

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Inositol 1,4,5-trisphosphate receptor type 1 ($InsP_3R1$) is abundantly expressed in the central nervous system. $InsP_3R1$ functions to release Ca^{2+} from the endoplasmic reticulum (ER) upon stimulation by inositol 1,4,5- trisphosphate