has been observed in a variety of cancer cells and it exerts an anti-apoptotic action, promoting cell proliferation and inhibiting the apoptotic response to tumor suppressor genes such as p53 and Bax. Although its mechanism of action remains unclear, there is evidence to suggest that Sigma1R regulates inter-organelle Ca^{2+} signaling thereby controlling cellular bioenergetics, and is also a regulator of plasma membrane ion channels. We have investigated its role in the regulation of store operated Ca^{2+} entry (SOCE) triggered by ER Ca^{2+} store depletion. Increased expression of Sigma1R in HEK293 cells profoundly inhibited SOCE, whereas knockdown of endogenous Sigma1R in CHO cells increased SOCE. An agonist at Sigma1R, SKF10047, further inhibited SOCE whereas the antagonists, BD1047, relieved this inhibition.

Co-immunoprecipitation experiments showed a novel interaction between Sigma1R and STIM1 which is ligand dependent, SKF10047 promoted the interaction and BD1047 reduced it. Total internal reflection fluorescence (TIRF) microscopy showed the recruitment of Sigma1R to ER-plasma membrane junctions was STIM1 dependent, whereas elevated Sigma1R expression slowed STIM1 recruitment. Plasma membrane biotinylation experiments also showed that STIM1 promoted Sigma1R recruitment to a surface complex while Sigma1R reduced the levels of STIM1 in the complex. Increased expression of STIM1 but not Orai1 partially rescued the inhibition of SOCE in cells overexpressing Sigma1R. Imaging of purified plasma membrane Orai1 channels complexes by atomic force microscopy (AFM) showed hexameric Orai1 decorated by both Sigma1R and STIM1. Overall our results suggest that Sigma1R inhibits SOCE in two ways; first, by binding STIM1 and reducing its recruitment to surface Orai1 channels and second, by directly modulating the STIM1-Orai1 complex.

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Functional Interaction of an Orai1 Pore Residue with the Inactivation Domain of STIM1

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The Inactivation Domain of STIM1 (ID_{STIM}: aa 470-491) was described as necessary for Ca^{2+}-dependent inactivation (CDI) of CRAC channels in three early reports (Mullins et al. 2009, Derler et al. 2009, Lee et al. 2009). We revisited the role of ID_{STIM} in light of the observation that STIM1:Orai1 ratio is a critical determinant of CDI (Scrimgeour et al. 2009, Hoover et al. 2011). We measured CRAC currents activated by a truncated STIM1 (aa 1-469), which lacks ID_{STIM}, in HEK cells transfected at higher STIM1:Orai1 ratios than were used previously. We observed limited but significant inactivation for STIM1(1-469) + Orai1 under these conditions (~1/3 of the extent seen with WT STIM1 + Orai1). The inactivation supported by STIM1(1-469) was sensitive to extracellular $[Ca^{2+}]$ and to intracellular BAPTA but not EGTA.

How does ID_{STIM} triple the extent of CDI? We first considered the possibility that ID_{STIM} increases local $[Ca^{2+}]$. Open probability and single-channel conductance determined by analysis of monovalent current noise were identical for STIM1(1-469) and WT STIM1, arguing against a model in which ID_{STIM} enhances inactivation indirectly by increasing local $[Ca^{2+}]$ near the inner pore mouth.

We next looked for a functional interaction between ID_{STIM} and the Orai1 pore. Like STIM1(1-469), the Orai1 inner pore mutation W76A also supported inactivation to ~1/3 of the WT extent when studied at a high STIM1:Orai1 ratio. While the mutations STIM1(1-469) and W76A reduced CDI to similar extents when studied individually, no additional reduction of inactivation was seen when STIM1(1-469) was co-expressed with Orai1 W76A. Together, these data suggest that ID_{STIM} and Orai1 residue W76 act in concert to boost CDI to a maximal level for a given local $[Ca^{2+}]$.

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STIM1 Binding to Both the N' and C' Termini of Orai1 is Critical for Gating of CRAC Channels

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Calcium flux through store operated calcium entry (SOCE) is a major regulator of intracellular calcium homeostasis and various calcium signaling pathways. The two key components of the store operated calcium release activated calcium (CRAC) channel are the Ca^{2+} sensing protein stromal interaction molecule 1 (STIM1) and the channel pore forming protein Orai1. Following calcium depletion from the endoplasmic reticulum, STIM1 undergoes a series of conformational changes which unmask a minimal Orai1 activating domain called CAD. CAD binds to two sites in Orai1, one in the N' terminus and one in the C' terminus. The energetic coupling of binding of the STIM1 ligand to opening of the channel pore is poorly understood. In this study we show that both N' and C' terminal domains of Orai1 synergistically contribute to interaction with CAD to allosterically couple STIM1 binding with channel gating and modulation of ion selectivity.

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Clustering of Inward Rectifier Potassium Channels in a PIP_2-Containing Membrane: A Molecular Dynamics Simulation Study

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Inward rectifying potassium (Kir) channels are activated by PIP_2, an anionic phospholipid in the plasma membrane. The channel proteins have been shown to possess PIP_2 binding sites[1,2] located at the bilayer/water interface. Furthermore, Kir2.1 forms clusters *in vitro* in the presence of PSD-95, a membrane-associated protein[3]. However, little is known regarding Kir channel clustering *in vivo*, nor the possible effect that PIP_2 interactions will have on clustering and, more generally, on behaviour of Kir channels in the context of the complex, crowded plasma membrane.

Previous molecular dynamics simulations show that simple membrane proteins induce formation of lipid nanodomains[4,5], and that protein crowding in simple membranes affects protein and lipid diffusion rates[6,7]. But the effect of crowding and complexity on more sophisticated membrane proteins remains to be explored using such approaches.

Now, with methodological developments that allow us to create models of complex, asymmetric bilayers[8], and with ever-increasing computational power, we have the tools to study such systems in molecular detail and at biologically relevant time and length scales.

In order to explore clustering of Kir2.2 channels, and the interplay with Kir2.2 – PIP_2 interactions, we have performed simulations that include over 100 copies of Kir2.2 embedded in a PIP_2-containing model of the plasma membrane. Analysis shows formation of protein clusters consistent with experimental data, and we can now further characterise these clusters. Also evident is clustering of PIP_2 around Kir2.2 into lipid nanodomains.

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646-Pos Board B426

Direct Contacts of K^+ Ions in the Selectivity Filter Enable the High Conductance of K^+ Channels

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K^+ channels exist in virtually all living organisms and play fundamental biological roles by conducting K^+ ions in a rapid and selective way. Previous models proposed that K^+ ions and water molecules alternately occupy four binding sites of the selectivity filter and permeate collectively when the channel is open and activated. However, our latest computational electrophysiology molecular dynamics simulations [1] indicate that this model can not yield measurable conductance under physiologically relevant transmembrane potential. Instead, the rapid ion permeation only occurs when there are no water molecules in the selectivity filter and K^+ ions form direct contacts. This has been further confirmed by Brownian dynamics simulations in a simplified model. A revisit to the previous X-ray crystallography data has validated the direct contacts of K^+ ions in the selectivity filter, which is in good agreement with classical and QM/MM molecular dynamics simulations. Therefore, we propose a direct Column knock-on mechanism that can account for the high ion conductance of K^+ channels.

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Evolutionary Diversity of Protein Nanodomains within Mammalian Sperm

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