results suggest that the divergent selectivities observed for endogenous store-operated channels might involve a heteromeric Orai1-Orai3 channel complex. Supported by FWF P18169.

2880-Plat

Fast Ca^{2+} Dependent Inactivation Of CRAC Channels Requires A Cytosolic Region Of STIM1

Franklin M. Mullins, Chan Young Park, Ricardo Dolmetsch, Richard S. Lewis.
Stanford University, Stanford, CA, USA.

The distinguishing biophysical features of mammalian Ca^{2+}-release activated Ca^{2+} (CRAC) channels include high Ca^{2+} selectivity, small unitary conductance, and fast Ca^{2+}-dependent inactivation on a millisecond time scale. Our previous studies of fast inactivation in Jurkat T cells suggested that Ca^{2+} binds to sites several nanometers from the intracellular mouth of the CRAC channel pore, possibly on the channel itself. The identification of STIM1 as the ER Ca^{2+} sensor and Orai1 as the pore-forming subunit of the CRAC channel has enabled studies of the molecular basis of activation and inactivation. We have recently identified a 107-residue cytosolic STIM1 fragment corresponding to the minimal STIM1 domain required for activation of the CRAC channel. The CRAC activation domain, or CAD, binds directly to Orai1 to activate CRAC current to the same mean level as wild-type STIM1, but while bypassing store depletion. CRAC currents were measured by whole-cell patch-clamp electrophysiology in HEK 293 cells coexpressing human Orai1 with a range of constructs derived from the cytosolic region of human STIM1. CAD-induced CRAC currents retain high Ca^{2+} selectivity, but surprisingly lack fast Ca^{2+}-dependent inactivation, revealing a critical role for STIM1 in the inactivation gating process. Truncating STIM1 at the C-terminal end of CAD also yielded currents without fast inactivation in store-depleted cells. Extending CAD in the C-terminal direction partially reconstituted fast inactivation, but full reconstitution required both C- and N-terminal extensions of CAD. We conclude that a domain of STIM1 C-terminal to CAD is absolutely required for fast Ca^{2+}-dependent inactivation of the CRAC channel. Elements of STIM1 N-terminal to CAD may enhance fast inactivation, possibly by increasing the local density of CRAC channels and Ca^{2+} influx, or by concentrating critical STIM1 domains near the inner pore mouth.

2881-Plat

Differential Modulation of Type-1, Type-2 and Type-3 Inositol (1,4,5)-Trisphosphate Receptors by ATP

Matthew J. Betzenhauser[1], Larry E. Wagner[1], Hyung Seo Park[2], Lyndee Knowlton[1], David I. Yule[1].
[1]University of Rochester, Rochester, NY, USA, [2]Konyang University, Daejeon, Republic of Korea.

Inositol (1,4,5)-trisphosphate receptors ($InsP_3R$) are the predominant route of Ca^{2+} release in non-excitable cells and they play a vital role in regulating intracellular Ca^{2+} signals. There are three isoforms ($InsP_3R$-1, $InsP_3R$-2 and $InsP_3R$-3) of $InsP_3R$ expressed in mammalian cells. This sequence diversity along with varied tissue distributions suggests that there are isoform-specific regulatory mechanisms. One such regulatory mechanism is the modulation of Ca^{2+} release from $InsP_3R$ by cytosolic ATP. ATP positively regulates all three $InsP_3R$ isoforms, but with distinct functional characteristics. We found that ATP was required for maximal $InsP_3$-induced Ca^{2+} release from $InsP_3R$-1 and $InsP_3R$-3 while $InsP_3R$-2 attained maximal activity in the absence of ATP. Furthermore, $InsP_3R$-2 was more sensitive to ATP modulation than either $InsP_3R$-1 or $InsP_3R$-3. All three isoforms contain putative ATP binding domains, but the contributions of these sites to ATP modulation of $InsP_3R$ are poorly understood. $InsP_3R$-1 contains two predicted ATP binding domains (ATPA, and ATPB) while $InsP_3R$-2 and $InsP_3R$-3 each express a single ATPB site. We examined the contributions of these ATP binding sites to the subtype-specific effects of ATP on $InsP_3R$ isoforms. ER Ca^{2+} measurements from permeabilized DT40 cells and single channel recordings of $InsP_3R$ were used to measure the effects of ATP on wild-type and mutated $InsP_3R$. We found that ablation of the ATPB site in $InsP_3R$-2 eliminated the enhancing effects of ATP on this isoform. Surprisingly, the positive effects of ATP were retained in $InsP_3R$-1 and $InsP_3R$-3 devoid of their respective ATP binding sites. ATP, therefore, differentially regulates the three $InsP_3R$ isoforms and likely regulates $InsP_3R$-1 and $InsP_3R$-3 via novel ATP binding sites. The implications of this differential regulation on Ca^{2+} signals would likely be determined by the relative ratios of the three isoforms expressed in a given cell.

2882-Plat

Functional Stoichiometry Of The Unitary Calcium-release-activated Calcium Channel Revealed By Single-molecule Imaging

Wei Ji[1], Pingyong Xu[1], Zhengzheng Li[1], Jingze Lu[1], Lin Liu[2], Yi Zhan[2], Yu Chen[1], Bertil Hille[3], Tao Xu[1], **Liangyi Chen**[1].
[1]Institution of Biophysics, Beijing, China, [2]Institute of Biophysics and Biochemistry, Huazhong University of Science and Technology, Wuhan, China, [3]Department of Physiology and Biophysics, University of Washington, Seattle, WA, USA.

Two proteins, STIM1 in the endoplasmic reticulum and Orai1 in the plasma membrane, are required for the activation of Ca^{2+} releaseactivated Ca^{2+} (CRAC) channels at the cell surface. How these proteins interact to assemble functional CRAC channels has remained uncertain. Here, we determine how many Orai1 and STIM1 molecules are required to form a functional CRAC channel.

We engineered several genetically expressed fluorescent Orai1 tandem multimers and a fluorescent, constitutively active STIM1 mutant. The tandem multimers assembled into CRAC channels, as seen by rectifying inward currents and by cytoplasmic calcium elevations. CRAC channels were visualized as fluorescent puncta in total internal reflection microscopy. With single-molecule imaging techniques, it was possible to observe photo-bleaching of individual fluorophores and to count the steps of bleaching as a measure of the stoichiometry of each CRAC channel complex. We conclude that the subunit stoichiometry in an active CRAC channel is four Orai1 molecules and two STIM1 molecules. Fluorescence resonance energy transfer experiments also showed that four Orai1 subunits form the assembled channel. From the fluorescence intensity of single fluorophores, we could estimate that our transfected HEK293 cells had almost 400,000 CRAC channels and that, when intracellular Ca^{2+} stores were depleted, the channels clustered in aggregates containing ~1,300 channels, amplifying the local Ca^{2+} entry.

Platform BB: Channel Regulation & Modulation

2883-Plat

Disruption Of Interactions Between AKAP79/150 And KCNQ K^+ Channels By Ca^{2+}/Calmodulin Observed Using TIRF/FRET

Manjot Bal, Jie Zhang, Ciria C. Hernandez, Oleg Zaika, **Mark S. Shapiro**.
UT Health Science Center, San Antonio, TX, USA.

KCNQ2-5 subunits encode the M-current, a K^+ conductance that controls neuronal excitability. Stimulation of $G_{q/11}$-coupled receptors depresses M current via multiple intracellular signals, including depletion of PIP_2, generation of Ca^{2+}/calmodulin, and phosphorylation of KCNQ2 by PKC, recruited to the channels by AKAP79/150. We examined the interplay between these signals via FRET measurements performed under total internal reflection fluorescence (TIRF) microscopy, in which mostly membrane events are isolated. CHO cells were transfected with CFP-tagged KCNQ2-4, and a YFP-tagged construct containing the first 153 residues of AKAP79 ($AKAP79_{1-153}$) shown to be sufficient for binding to KCNQ2, PKC and receptors (Hoshi et al., *Nat Cell Biol*. **7**:1066-73). We found significant FRET between all KCNQ2-4 subunits and $AKAP79_{1-153}$ (13 $\pm$ 1.3%, 7.1 $\pm$ 2.0% and 10.1 $\pm$ 2.0% for KCNQ2-4, respectively). Since Ca^{2+}/calmodulin binding not only inhibits M channels (Gamper and Shapiro, *JGP* 122:17-31), but also acts on AKAP79/150 (Faux and Scott, *JBC* 272:17038-17044), we asked whether calmodulin alters KCNQ channel-AKAP79/150 interactions. Indeed, FRET between all CFP-KCNQ2-4 subunits and YFP-$AKAP79_{1-153}$ was much less in cells also co-transfected with wild-type calmodulin, but not when dominant-negative calmodulin was used that cannot bind Ca^{2+}. FRET was also substantial between the CFP-KCNQ2 (R345E) mutant that cannot bind calmodulin and YFP-$AKAP79_{1-153}$, which was however not reduced by calmodulin co-expression. Furthermore, the FRET between KCNQ2-4 and $AKAP79_{1-153}$ was also not reduced when the cells were depleted of PIP_2 by co-expression of a PIP_2 phosphatase. We conclude that calcified, but not apo, calmodulin interferes with KCNQ subunit-AKP79/150 interactions by binding to the channels, likely reducing their affinity for AKAP79/150, and that M channel-AKAP79/150 interactions may serve to anchor PKC to M-channel containing microdomains, where it stands poised to phosphorylate the channels upon stimulation of appropriate $G_{q/11}$-coupled receptors.

2884-Plat

Are Voltage-gated Potassium (Kv) Channels Recruited Into Lipid Rafts In Mammalian Brain Neurons?

Andrew J. Shepherd, D.P. Mohapatra.
University of Iowa Carver College of Medicine, Iowa City, IA, USA.

Differential sub-cellular distribution and voltage-dependent gating properties of Kv channels are crucial for the regulation of neuronal excitability. In mammalian central neurons, the majority of delayed-rectifier K+ currents (IK) are contributed by Kv2.1 channels, and Kv4.2 and Kv4.3 channels constitute most of the A-type K+ currents (IA) in the soma and dendrites. Kv2.1 channels are localized in distinct cell surface clusters in the soma and proximal dendrites,