Platform AQ: TRP Channels & Intracellular Ca2+ Channels

2015-Plat

Two-pore Channels for Calcium Mobilization from Acidic Organelles and Cell Signaling by NAADP

Peter J. Calcraft[1], Abdelilah Arredouani[2], Zui Pan[3], Xiaotong Cheng[2], Jisen Tang[4], Margarida Ruas[2], Katja Rietdorf[2], Peihui Lin[3], John Parrington[2], Jianjie Ma[3], A. Mark Evans[1], Antony Galione[2], **Michael X. Zhu**[4].
[1]University of Edinburgh, Edinburgh, United Kingdom, [2]University of Oxford, Oxford, United Kingdom, [3]UMDNJ-Robert Wood Johnson Medical School, Piscataway, NJ, USA, [4]The Ohio State University, Columbus, OH, USA.

Two-pore channels (TPCs) are novel members of the superfamily of voltage-gated ion channels. Their predicted structures indicate 2-fold symmetry with a total of 12 putative transmembrane (TM) α-helices. Sequence homology and membrane topology analyses suggest that TPCs may represent evolutionary intermediates from the single domain 6-TM architecture K^+ and non-selective cation channels to the four-repeat 24-TM structure of voltage-gated Ca^{2+} and Na^+ channels. Three genes (TPCN1-3) exist in most vertebrates but their functions remain elusive. We now show that TPC1 and TPC3 are expressed on the membrane of different endosome populations while TPC2 is expressed on the membrane of lysosomes. We provide functional data showing that TPC2 is a target of nicotinic acid adenine dinucleotide phosphate (NAADP), a potent Ca^{2+} mobilizing messenger that evokes Ca^{2+} release from acidic organelles rather than from the sarco/endoplasmic reticulum. Thus, microsomal membranes enriched with TPC2 exhibit similar high affinity binding with NAADP as native NAADP receptors. In response to NAADP, cells overexpressing TPC2 exhibit enhanced intracellular Ca^{2+} release and more efficient coupling to IP3 receptors to evoke global Ca^{2+} transients. These effects were blocked by disrupting lysosomal H^+ gradient or RNAi-mediated silencing of TPC2 expression. Our findings provide for the first time a molecular basis for further detailed characterization of the regulatory mechanisms and physiological functions of NAADP-mediated signaling and, in addition, suggest a general role for TPCs in Ca^{2+} mobilization, Ca^{2+} homeostasis, and Ca^{2+} signaling from endosomal/lysosomal compartments of vertebrate cells, which are known to be important for diverse functions in many physiological systems.

2016-Plat

Bcl-xL Regulation of InsP3 Receptor Gating Mediated by Dual Ca2+ Release Channel BH3 Domains

J. Kevin Foskett, Jun Yang, King-Ho Cheung, Horia Vais.
University of Pennsylvania, Philadelphia, PA, USA.

Interaction of anti-apoptotic Bcl-xL with inositol trisphosphate receptor (InsP3R) Ca2+ release channels sensitize them to InsP3, enhancing low-level constitutive Ca2+ signaling that affords apoptosis resistance (White et al. Nat Cell Biol 7(2005); Li et al. PNAS 104(2007)). Here, we have identified two binding domains in the channel C-terminus that are both required for channel activation: the first is located at the bottom of TM6 immediately distal to the gate, and the second is near the extreme C-terminus. Each site binds to anti-apoptotic Bcl-2, Bcl-xL and Mcl-1 with similar affinities, but not to pro-apoptotic Bid or Bax. Bcl-xL binding to a construct containing both sites had apparent affinity several times higher than for either individual site or for Bax. Bcl-xL interaction with Bcl-2 proteins is mediated by pro-apoptotic protein BH3 domains. Several features suggest that each binding site in the InsP3R is similar to a BH3 domain. Sequence and secondary structural features are reminiscent of BH3 domains; mutations of key residues known to disrupt BH3 domain interactions disrupted Bcl-XL binding to either channel site; mutations in Bcl-xL that disrupt binding to BH3 domains inhibited binding to the channel sites; ABT-737 that binds in the Bcl-XL BH3 binding pocket inhibited binding to each channel site. In single-channel recordings, Bcl-xL activation of gating was abolished by mutations of either channel BH3-like domain or of the Bcl-xL BH3 binding pocket, or by ABT-737. These results suggest that dimeric Bcl-xL cross-links TM6 and the distal C-terminus through interactions involving channel BH3 domains. This interaction allosterically enhances the channel sensitivity to InsP3 by enabling the channel to open more easily.

2017-Plat

TRPC Channels Function Independently Of STIM1 And Orai1

Wayne I. DeHaven, Bertina Jones, John Petranka, Takuro Tomita, James Putney.
NIEHS, RTP, NC, USA.

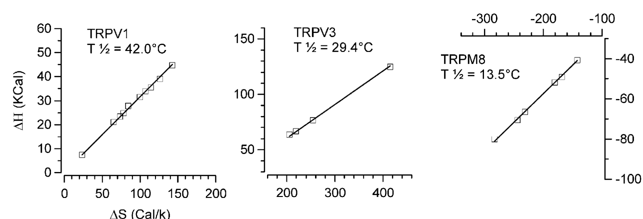
Recent studies have defined roles for STIM1 and Orai1 as calcium sensor, and calcium channel, respectively, for CRAC channels, channels underlying store-operated Ca^{2+} entry (SOCE). However, the roles of these proteins in signaling and constructing other channels with biophysical properties distinct from CRAC channels are not known. We examined the hypothesis that STIM1 or Orai1 can interact with and regulate a family of non-selective cation channels (TRPC) which have been suggested to also function in SOCE pathways under certain conditions. Our data reveal no role for either STIM1 or Orai1 in signaling of TRPC channels. Specifically, Ca^{2+} entry seen after carbachol treatment in cells expressing TRPC1, 3, 5, or 6 were not enhanced by the co-expression of STIM1. Further, knockdown of STIM1 in cells expressing TRPC5 did not reduce TRPC5 activity, in contrast to published reports. Disruption of lipid rafts significantly attenuated TRPC3 activity, while having no effect on STIM1 localization or the development of I_{CRAC}. This suggests that TRPC signaling and STIM1/Orai1 signaling occur in distinct plasma membrane domains. In vascular smooth muscle cells, arginine-vasopressin (AVP) activated non-selective cation currents, and single channel events recorded in cell-attached configuration from these cells detected a current with a slope conductance of 33.65 pS, similar to that published for TRPC6. Further, RT-PCR analysis of TRPC transcripts in A10 cells revealed the predominant expression of TRPC1 and TRPC6 mRNA. Using a membrane potential-sensitive dye as an assay, we determined that knockdown of either STIM1 or Orai1 had no effect on the function of this AVP-activated current, while store-operated entry was substantially reduced. Thus, both STIM1 and Orai1 appear to be specific molecular components of the I_{CRAC} pathway and in our studies did not influence the function of exogenously or endogenously expressed TRPC channels.

2018-Plat

Activation of Thermosensitive TRP Channels

Fan Yang, Jie Zheng.
University of California, Davis, CA, USA.

Recently identified thermosensitive transient receptor potential (ThermoTRP) channels are thought to be sensors for ambient temperature. How temperature changes drive activation conformational rearrangement remains unknown. We used electrophysiological methods to investigate temperature-dependent activation of thermoTRP channels as well as the highly temperature-sensitive CLC-0 chloride channel in culture cells. We developed a fast temperature switching technique (20-80°C/sec) to analyze current responses to temperature change.
We observed that temperature-driven activation rates of thermoTRP channels were different, while temperature-driven deactivation rates of all the thermoTRP channels were similarly fast. These results indicate that thermoTRP channels can be divided into two groups: fast-activation channels and slow-activation channels. Together with their diverse expression profiles, our results indicate that thermoTRP channels may serve multiple temperature-sensing functions.
We also observed that entropic and enthalpic changes associated with temperature-driven activation vary when thermoTRP channels permeate different ions. With previous mutagenesis studies showing that certain residuals in the pore region of TRPV1 and TRPV3 are critical for their temperature-dependent behaviors, our observation further suggests that the pore region is involved in temperature-sensing and gating of thermoTRP channels.



Half activation temperature of TRPV1, TRPV3 and TRPM8 channel derived from thermodynamic measurement.

2019-Plat

Oxidative Challenges Sensitize the Capsaicin Receptor by Covalent Cysteine Modification

Huai-hu Chuang, Stephanie Lin.
Cornell University, ITHACA, NY, USA.

The capsaicin receptor TRPV1, one of the major transduction channels in the pain pathway, integrates the information from extracellular milieu to control the excitability of primary nociceptive neurons. Sensitization of TRPV1 heightens our pain sensation by enhancing the responsiveness of sensory afferents to moderate noxious or even innocuous stimuli. We report here that oxidative stresses markedly potentiate the ligand-induced TRPV1 currents. This modulation mechanism is conserved in multiple species orthologs of TRPV1 but not among other homolog channels in TRPV family. The sensitization operates synergistically with kinase or receptor-mediated modulations in wild type receptors but still occurs in TRPV1 mutants lacking phospho-acceptor