Platform AQ: TRP Channels & Intracellular Ca2+ Channels

2015-Plat

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

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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

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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

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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

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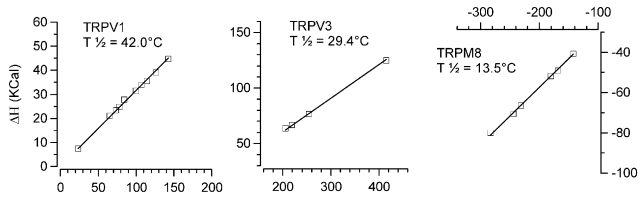
Recently identified thermosensitive transient receptor potential (ThermoTRP) channels are thought to be sensors for ambient temperature. How temperature changes drive activation conformational rearrangement remains unknown. We used electrophysiological methods to investigate temperature-dependent activation 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

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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

sites or domains required for either modulation. Robust oxidative modulation is also observed in receptors that enter the desensitization state after prolonged exposure to capsaicin in the presence of extracellular Ca2+. We show that this modulation can be fully recapitulated in the excised inside-out membrane patches under the divalent cation free condition, ruling out the involvement of major protein or lipid phosphorylation pathways. Pretreatment with the cysteine-reactive alkylating agent maleimide blocks the modulation, while cysteine oxidizing chemicals produce pronounced sensitization and occlude each other's effects. The effect of oxidation on channel activity can be reversed by application of strong reducing agents. By constructing receptor chimeras and point cysteine mutations, we identified multiple cysteines required for full modulation of TRPV1 by oxidative challenges. We conclude that the oxidative modulation is a robust mechanism to tune TRPV1 activity via covalent modification of conserved cysteine residues across different species and may play a role in pain sensing process during inflammation, infection or tissue injury.

2020-Plat

PKCßII-Specific Phosphorylation Counteracts Regulation Of Trpv6 By ATP And Points Towards A Functional Difference Between Its Polymorphic Alleles

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The calcium selective ion channel TRPV6 shows a highly unusual evolution of one of its polymorphic alleles which might have conferred a selective advantage during migration of humans out of Africa. Because the ancestral allele contains an additional PKC consensus site, we analyzed regulation by intracellular ATP and phosphorylation. We found that ATP prevents run-down of TRPV6-mediated currents and have mapped a relevant site for regulation by ATP to the finger loop between ankyrin repeat domains (ARD) 3 and 4. Stimulation of PKC preserves run-down even in the presence of ATP and uncovers a difference between the alleles. Using different inhibitors and isoforms of PKC, we show that regulation requires $PKC_{\beta II}$, which is able to phosphorylate the channel. Site-directed mutagenesis shows that phosphorylation sites within the ARD and also within the C-terminus are necessary to confer the effect. We propose a model where ATP stabilizes the channel by tethering the C-and N-termini of the subunits together. $PKC_{\beta II}$ disrupts this interaction and yields channels susceptible to inactivation and run-down.

2021-Plat

Ca^{2+} Activates TRPM2 Channels By Binding In Deep Crevices Near The Pore, But Intracellularly Of The Gate

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TRPM2 is a tetrameric Ca^{2+} permeable channel involved in immunocyte respiratory burst and postischaemic neuronal death. In whole cells TRPM2 activity requires intracellular ADP ribose (ADPR) and intra- or extracellular Ca^{2+}, but the mechanism and the binding site(s) for Ca^{2+} activation remain unknown. Here we study TRPM2 gating in inside-out patches while directly controlling intracellular ligand concentrations. Concentration jump experiments at various voltages, and Ca^{2+} dependence of steady-state single-channel gating kinetics, provide unprecedented insight into the molecular mechanism of Ca^{2+} activation. In patches excised from *Xenopus* oocytes expressing human TRPM2, co-application of intracellular ADPR and Ca^{2+} activated ~50-pS non-selective cation channels; $K_{1/2}$ for ADPR was ~1μM at saturating Ca^{2+}. Intracellular Ca^{2+} dependence of TRPM2 steady-state opening and closing rates (at saturating [ADPR] and low extracellular Ca^{2+}) reveals that Ca^{2+} activation is a consequence of tighter binding of Ca^{2+} in the open- than in the closed-channel conformation. Four Ca^{2+} ions activate TRPM2 with a Monod-Wymann-Changeux mechanism: each binding event increases the open-closed equilibrium constant ~33-fold, producing altogether 10^6-fold activation. Experiments in the presence of 1mM free Ca^{2+} on the extracellular side clearly show that closed channels do not sense extracellular Ca^{2+}, but once channels have opened Ca^{2+} entering passively through the pore slows channel closure by keeping the ''activating sites'' saturated, despite rapid continuous Ca^{2+}-free wash of the intracellular channel surface. This effect of extracellular Ca^{2+} on gating is gradually lost at progressively depolarized membrane potentials, where the driving force for Ca^{2+} influx is diminished. Thus, the activating sites lie intracellularly from the gate, but in a shielded crevice near the pore entrance. Our results suggest that in intact cells which contain micromolar ADPR even brief Ca^{2+} spikes likely trigger prolonged, self-sustained TRPM2 activity.

2022-Plat

X-ray Crystal Structure Of A Trpm Assembly Domain Reveals An Antiparallel Four-stranded Coiled-coil

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Transient receptor potential (TRP) channels comprise a large family of tetrameric cation-selective ion channels that respond to diverse forms of sensory input. Previous studies have shown that members of the TRPM subclass possess a self-assembling tetrameric C-terminal cytoplasmic coiled-coil domain that underlies channel assembly and trafficking. Here, we present the high-resolution crystal structure of the coiled-coil domain of the channel enzyme TRPM7. The crystal structure, together with biochemical experiments, reveals an unexpected four-stranded antiparallel coiled-coil architecture that bears unique features relative to other antiparallel coiled-coils. Structural analysis indicates that a limited set of interactions encode assembly specificity determinants and uncovers a previously unnoticed segregation of TRPM assembly domains into two families that correspond with the phylogenetic divisions seen for the complete subunits. Together, the data provide a framework for understanding the mechanism of the TRPM channel assembly and highlight the diversity of forms found in the coiled-coil fold.

Platform AR: Membrane Protein Function

2023-Plat

Simultaneous Monitoring The Two Rotary Motors Of A Single F_OF_1-ATP Synthase

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Synthesis of ATP from ADP and phosphate is performed by a stepwise internal rotation of subunits of the enzyme F_oF_1-ATP synthase. The bacterial enzyme also catalyzes ATP hydrolysis. The opposite direction of rotation during ATP synthesis and hydrolysis was confirmed by single-molecule fluorescence resonance energy transfer, FRET, using specific labeling of the rotary subunits γ or ϵ in the F_1 motor and the stator subunits [1-3]. The step size in the F_1 motor was 120°. In contrast the step size during proton-driven rotation of the c subunits in the F_o motor was 36° using single-molecule FRET. FRET artifacts could be minimized by 'duty cycle optimized alternating laser excitation'. As the two coupled motors of F_oF_1-ATP synthase showed apparently different step sizes, this mismatch has to be unraveled by mapping the contributions of rotor and stator subunits for transient energy storage. We present the simultaneous observations of F_1 and F_o motor rotations using a single-molecule triple FRET approach, which indicate elastic deformations of the rotor between ϵ and c subunits during ATP hydrolysis as well as synthesis.

References:
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2024-Plat

Tracking Single Protein Translocation Complexes In The Membranes Of Living Bacteria

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The Twin Arginine Translocation (Tat) system transports fully folded en sometimes even oligomeric proteins across the inner membrane of bacteria. Its mechanism is largely unknown. Remarkably, a stable translocation complex has not been observed. Instead, the three components of the system, i.e., TatA, TatB and TatC, are isolated from the membrane of Escherichia coli in various complexes of different sizes, which suggests that a complete and active Tat complex is formed only transiently. We have used single particle tracking in living bacteria to gain more insight into the dynamics of the Tat proteins. TatA has been genetically fused tot enhanced Green Fluorescent Protein (eGFP). Living bacteria expressing low levels of TatA-eGFP have been immobilized on glass slides and imaged with a sensitive wide-field fluorescence