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.

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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