These results demonstrate that the TRPM8 protein is directly activated by cold, menthol and phosphoinositides.

1184-Plat
Activation of TRPML Channels in the Lysosome
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The mucolipin family of Transient Receptor Potential (TRPML) proteins is predicted to encode ion channels of intracellular endosomes and lysosomes. The physiological importance of TRPMLs has been established genetically. Mutations of human *TRPML1* cause type IV mucolipidosis (ML4), a devastating neurodegenerative disease; mutations in the mouse *TRPML3* result in the *varitint-waddler* (*Va*) phenotype with hearing and pigmentation defects. The broad-spectrum phenotypes of both ML4 and *Va* appear to result from certain aspects of endosomal/lysosomal dysfunction. Lysosomes, traditionally believed to be the terminal "recycle center" for biological "garbage", are now known to play indispensable roles in membrane traffic and multiple intracellular signaling pathways. The putative lysosomal function(s) of TRPML proteins, however, has been unclear until recently. Studies on animal models and cell lines in which TRPML genes have been disrupted or genetically depleted have discovered roles of TRPMLs in a variety of cellular functions including membrane traffic, signal transduction, and organellar homeostasis. Physiological assays on cells in which TRPMLs are heterologously over-expressed revealed the channel properties of TRPMLs, suggesting that TRPMLs mediate cation (Ca^{2+}/Fe^{2+}) efflux from endosomes and lysosomes in response to unidentified cellular cues. Using our recently developed lysosome patch-clamp technique, we screened a variety of cytosolic and luminal factors that are known to affect endolysosomal functions and have identified an endogenous agonist for TRPML channels. We are currently investigating the activation mechanism in detail.

1185-Plat
Structural Models of Two-Pore-Domain Potassium Channels Focus on TREK
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Two-pore-domain background potassium (K2P) channels comprise a distinct gene family of widely distributed, well modulated channels. K2P channels have two similar or identical subunits, each of which has four transmembrane (TM) and two pore-forming (P) segments. Here we focus on mechanosensitive TREK channels. Unfortunately, the only structures available to be used as templates belong to the 2TM channels superfamily. These are distantly related at sequence level with different structural features: four symmetrically arranged subunits, each having two TM segments flanking a P segment. Our model building strategy used two subunits of the template (KcsA) to build one subunit of the target (TREK-1). Our models of the closed channel differ substantially from those of the template, primarily because TM2 of the 2nd repeat is near the axis of the pore whereas TM2 of the 1st repeat is far from the axis. Segments linking the two subunits and immediately following the last TM segment were modeled *ab initio* as α-helices based on helical periodicities of hydrophobic and hydrophilic residues, highly conserved and poorly conserved residues, and correlated mutations in multiple sequence alignments. The N-terminus segment preceding residue 35, the long loop between first and second TM segments (residues 76-125), and C-terminus past residue 333 were not included in the model due to lack of template. Experimental analysis of the similarly-truncated channel with these loop and C-terminus residues deleted revealed near native-like behavior. The models were further refined by two-fold symmetry-constrained molecular dynamics simulations using a protocol we previously developed. We also built models of the open state and suggest a possible tension-activated gating mechanism in which the inner portion of the TM2 helix of the 2nd repeat swings radially outward. This mechanism will be tested experimentally.

1186-Plat
Electrostatic Interactions Between the Transmembrane and Cytoplasmic Domains Critically Stabilize Tension-Sensitive States in MSCS
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The small mechanosensitive channel MscS is a bacterial osmolyte release valve with homologs found in all walled organisms. E. coli MscS readily responds to abrupt steps of tension in the cytoplasmic membrane, but under sustained stimulation it enters the tension-insensitive inactivated state. Upon tension release, MscS recovers within 2 s. In the crystal structure of WT MscS, the gate region (end of TM3a) is the only connecting element between the transmembrane (TM) and the cytoplasmic (cage) domains. It has been predicted that the two domains can make additional contacts through salt bridges between D62 on the TM1-TM2 loop and the R128-R131 cluster on the cage. Our experiments show that disrupting this salt bridge with D62R(N) substitutions does not affect desensitization, but instead, it drastically speeds up the process of inactivation and decreases the rate of recovery. The mutations also open a path for silent inactivation at sub-threshold tensions bypassing channel opening. Swapping the charges (D62R/R131D) restores the normal inactivation phenotype. Our new models suggest that the D62-R128/131 bridge critically stabilizes the positions of the lipid-facing TM1-TM2 helices along central TM3s and their association through the F68-L111-L115 hydrophobic cluster which transmits force from the membrane to the gate, in both closed and open states. Simulations suggested that not only the G113 flexible region on TM3 is necessary for inactivation, but the G76 hinge on TM2 might be needed, too. Experiments confirmed that G76A substitution abolishes inactivation. Analyzing combined mutations with opposing effects on inactivation (D62N/G113A, D62N/G76A) reveals a strong contribution of the loop-cage interactions to the stability of tension-sensitive states. The predicted hinge action of G76 suggests that twisting of TM1-TM2 may be the inactivation mechanism that disrupts the bridges while disengaging these helices from the gate.

Platform AA: Unconventional Myosins

1187-Plat
News from the Myosin Tree: 1000 New Sequences, 100 New Species, 1 New Class
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The myosins constitute one of the largest and most divergent protein families in eukaryotes. They are characterized by a motor domain that binds to actin in an ATP-dependent manner, a neck domain consisting of varying numbers of IQ motifs, and amino-terminal and carboxy-terminal domains of various length and function. Myosins are involved in many cellular tasks like organelle trafficking, cytokinesis, maintenance of cell shape, and muscle contraction. They are typically classified based on the phylogenetic analysis of the motor domain. In 2007, we have published the analysis of over 2200 myosins from more than 320 species that resulted in 35 myosin classes of which 16 had not been proposed before. Here, we present an update on the myosin tree that is now based on 3246 myosins from 422 species. All sequences were manually annotated and verified. Most of the newly sequenced species belong to taxa that have already been covered in the earlier analysis. However, 1 new class has been determined that is specific to metazoans. These class-36 myosins do not contain an N-terminal SH3-like domain and their tail consists of more than 10 transmembrane domains and a chitin synthase domain. In addition, the genome sequences of the amoeba Acanthamoeba castellanii and the coccolithophore Emiliana huxleyi revealed many new orphan myosins. All sequence related data is available via CyMoBase at www.cymobase.org.

1188-Plat
Processive Non-Muscle Myosin IIB Takes Load-Independent Backward Steps
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Non-muscle myosin IIB (NMIIB) is a molecular motor involved in the regulation of cell polarity during cellular migration. NMIIB forms thick filaments like other members of the myosin II family. Recent studies have shown that a NMIIB dimer can bind both heads to actin simultaneously with different ADP release rates from leading and trailing heads. Gating of ADP release suggests that the two heads communicate with each other and may be capable of processive stepping. We performed single molecule optical trapping assays to examine the stepsize and dwelltime of NMIIB on actin filaments. Our results show that NMIIB is an unconventional myosin that walks processively by taking 5.5 nm backward and forward steps along the long-pitch helix of actin filaments. Forward steps and detachment are weakly force dependent, suggesting ADP release is the rate-limiting step in these transitions. Backward steps are independent of force, suggesting that backward steps occur before ADP release in the lead head. Nucleotide independent backstepping could be a common mechanism for back steps in myosin.