Hofmann, M.W., K. Weise, J. Ollesch, A. Agrawal, H. Stalz, W. Stelzer, F. Hulsbergen, H. deGroot, K. Gerwert, J. Reed, and D. Langosch. 2004. De novo design of conformationally flexible transmembrane peptides driving membrane fusion. Proc. Natl. Acad. Sci. U S A 101:14776-14781.
Poschner, B.C., S. Quint, M. Hofmann, and D. Langosch. 2009. Sequence-specific conformational dynamics of model transmembrane domains determines their membrane fusogenic function. J. Mol. Biol. 386:733-741.

Platform Z: Mechanosensitive & TRP Channels

1179-Plat

Outer Pore Domain of TRPV1 Ion Channel is Required for Temperature-Independent Step During Temperature-Activation

Jorg Grandl[1], Sung Eun Kim[1], Valerie Uzzell[1], Badry Bursulaya[2], Matt Petrus[2], Michael Bandell[2], Ardem Patapoutian[1].
[1]The Scripps Research Institute, La Jolla, CA, USA, [2]Genomics Institute of the Novartis Research Foundation, La Jolla, CA, USA.

TRPV1 is the founding and best-studied member of the family of temperature-activated transient receptor potential ion channels (thermoTRPs). Voltage, chemicals, and heat allosterically gate TRPV1. Molecular determinants for TRPV1 activation by capsaicin, allicin, acid, ammonia, and voltage have been identified. However, many years after the discovery of TRPV1, the structures and mechanisms mediating temperature-sensitivity remain unclear. Recent studies of the related channel TRPV3 identified residues within the pore region required for heat activation. Here we describe both random and targeted mutagenesis screens of TRPV1 to identify single point-mutations that specifically affect temperature-activation. The mutations found are all located in the outer pore region, in close proximity to but distinct from residues previously implicated in acid-activation. Electrophysiological analysis shows that mutations affect a temperature-independent step that is part of the temperature-gating pathway. These results suggest that the outer pore plays a general role in heat-sensitivity of thermoTRPs.

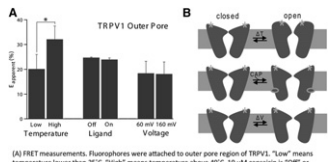
1180-Plat

Temperature-Driven Activation of Thermotrps: A Distinct Pathway Involved

Fan Yang[1], Yuanyuan Cui[1,2], Kewei Wang[2], Jie Zheng[1].
[1]University of California, Davis, Davis, CA, USA, [2]Peking University Health Science Center, Beijing, China.

A group of thermosensitive transient receptor potential (ThermoTRP) channels, with high temperature sensitivity of channel gating, are the cellular temperature sensors. ThermoTRPs are polymodal receptors. Besides temperature they are gated by voltage, ligand, extracellular pH and other stimuli. How temperature changes drive activation conformational rearrangement remains unknown. Here we combine functional, mutational, and site-directed fluorescence studies to demonstrate that temperature-dependent activation uses a pathway distinct from those for ligand- and voltage-dependent activation.

We observed that neither strong depolarization nor application of capsaicin could significantly alter thermodynamics of temperature-driven TRPV1 activation. In addition, voltage and ligand exhibited additive gating effects over temperature gating. Indeed, a TRPV1 mutant in which part of the outer pore region was replaced by an artificial sequence showed virtually no temperature sensitivity but maintained near normal capsaicin sensitivity. Furthermore, site-directed FRET measurements showed that conformational changes in outer pore can only be induced by heating, but not by voltage or ligand. Together these observations suggest that a distinct pathway for temperature to gate TRPV1 involves the outer pore region.



(A) FRET measurements. Fluorophores were attached to outer pore region of TRPV1. "Low" means temperature lower than 25°C, "High" means temperature above 40°C. 10 μM capsaicin is "Off" or "On". * indicates $P < 0.05$ (B) A schematic representation of the proposed gating conformational changes in TRPV1 induced by heat and capsaicin. The green stars represent fluorophores attached to the outer pore.

1181-Plat

Role Of Pip2 On Ca^{2+}-Dependent Desensitization of Trpv2

Jose Mercado, William N. Zagotta, Sharona E. Gordon.
University of Washington, Seattle, WA, USA.

TRPV2 is a member of the transient receptor potential superfamily of ion channels involved in chemical and thermal pain transduction. Unlike the related TRPV1 channel, TRPV2 does not appear to bind either calmodulin or ATP in its N-terminal ankyrin repeat domain. In addition, it does not contain a calmodulin-binding site in the distal C-terminal region, as has been proposed for TRPV1. Importantly, though, we have found that TRPV2 undergoes Ca^{2+}-dependent desensitization similar to TRPV1, suggesting that the mechanism of desensitization may be conserved in the two channels. To elucidate the molecular mechanism underlying Ca^{2+}-dependent desensitization in TRPV2 we used whole-cell recordings of F-11 cells transiently transfected with TRPV2. We found that prolonged applications of the TRPV2 agonist 2-APB led to nearly complete desensitization of the channel in the presence of extracellular Ca^{2+}. In contrast, no desensitization was observed in the absence of Ca^{2+}. TRPV2 desensitization was not altered in whole-cell recordings in the presence of calmodulin inhibitors or upon co-expression of mutant calmodulin, suggesting that CaM does not play a major role in Ca^{2+}-dependent desensitization of TRPV2. Interestingly, simultaneous confocal imaging and electrophysiological recording of whole cells expressing TRPV2 and a fluorescent PI(4,5)P2 binding probe showed a high degree of temporal correlation between the Ca^{2+} induced desensitization and depletion of PI(4,5)P2. Thus, Ca^{2+} influx through TRPV2 is sufficient to trigger a dramatic decrease in PI(4,5)P2 levels, presumably by activating PLC. We propose that the decrease in PI(4,5)P2 levels upon channel activation underlies at least a major component of Ca^{2+}-dependent desensitization of TRPV2.

1182-Plat

TRPM8 Cation Channel. Effects of Voltage, Cold and Menthol on Single-Channel Gating

Jose A. Fernandez, C. Norman Scholfield, J. Graham McGeown, Alexander V. Zholos.
Queen's University of Belfast, Belfast, United Kingdom.

Single-channel patch-clamp recording allows mechanistic insights into fundamental ion channel properties. Thus, a single kinetic model can formally describe the complex interactions during poly-modal activation of the channels. For TRPM8, activation depends on temperature, voltage and chemical signaling [1, 2], and such modeling provides the most comprehensive approach to the study of the channel. In this study, we examined the influence of voltage, cold and menthol on TRPM8 gating using patch-clamp recording techniques. In HEK293 cells stably expressing TRPM8, single-channel currents were measured (filtered at 2 kHz and sampled at 10 kHz) in cell-attached patches at different voltages (−100 to 140 mV), temperatures (20 or 30°C) and menthol concentrations (10 or 100 μM) (n = 7-11). As has been reported for whole-cell TRPM8 currents [3], shifts in the voltage-dependent single-channel open probability curve toward less positive potentials were induced by cold or menthol. Thus, the potential for half-maximal activation was reduced from 162.4 to 116.1 mV during cooling from 30 to 20°C, with a further shift to 52.8 mV with 100 μM menthol. To investigate the relationship between these modulators, we used different techniques - HJCFIT [4], QuB [5] and 2D fitting [6] - to develop a single-channel kinetic model aiming to identify the most likely potential-, menthol- and cold-regulated transitions. A model with 5 closed and 2 open states showing correlation between brief openings and long closings and between brief closings and long openings, was able to describe our macroscopic and single-channel data. Interestingly, temperature and menthol mimicked voltage-dependent activation of the channel at the model level by increasing the probability of transitions from long closed states to brief ones. This is the first complete kinetic model based on single-channel data for any of the TRP channels.

1183-Plat

Phosphoinositide Regulation of TRPM8 Channels in Planar Lipid Bilayers

Eleonora Zakharian, Tibor Rohacs.
UMDNJ New Jersey Medical School, Newark, NJ, USA.

The cold and menthol receptor TRPM8 is regulated by membrane phosphoinositides. To study the effects of lipids directly on the channel, we have reconstituted the purified TRPM8 in planar lipid bilayers. This system allows full control of the lipid composition in our experiments. The reconstituted channel was activated by menthol or cold, and its activity depended on the presence of specific phosphoinositides. In the presence of menthol, TRPM8 exhibited the highest probability of opening in the presence of phosphatidylinositol 4,5-bisphosphate [$PI(4,5)P_2$] with Po ~ 0.89 at +100 mV and Po ~ 0.4 at −100 mV. Less channel activity was induced by phosphatidylinositol 3,4-bisphosphate [$PI(3,4)P_2$] with Po ~ 0.53 at +100 mV and Po ~ 0.2 at −100 mV. Phosphatidylinositol 3,4,5-bisphosphate [$PI(3,4,5)P_2$] resulted in irregular TRPM8 channel currents and lower open probability with Po ~ 0.21 at +100 mV and Po ~ 0.087 at −100 mV. Among the tested lipids the lowest TRPM8 channel activity was induced by phosphatidylinositol 4-phosphate [PI(4)P] with Po ~ 0.12 at +100 mV and Po ~ 0.019 at −100 mV. The lipid specificity profile in lipid bilayers is very similar to that observed in excised patches. We have also studied the activation of TRPM8 channels in lipid bilayer with cold. Cooling the system with reconstituted TRPM8 channels also required the presence of $PI(4,5)P_2$. The main shift in the channel behavior was observed in the temperature range from 21°C to 18°C where the channel showed drastic changes in the open probability from 0.05 to 0.85 at +100 mV.

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

1184-Plat
Activation of TRPML Channels in the Lysosome
Haoxing Xu, Xian-ping Dong, Dongbiao Shen, Xiang Wang.
Univ Michigan, Ann Arbor, MI, USA.
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
Adina L. Milac[1], Andriy Anishkin[2], Sarosh N. Fatakia[3], Carson C. Chow[3], Sergei Sukharev[2], Robert H. Guy[1].
[1]National Cancer Institute, National Institutes of Health, Bethesda, MD, USA, [2]Department of Biology, University of Maryland, College Park, MD, USA, [3]National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD, USA.
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
Kenjiro Yoshimura, Kishore Kamaraju, Vladislav Belyy, Naili Liu, Andriy Anishkin, Sergei Sukharev.
University of Maryland, College park, MD, USA.
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
Martin Kollmar.
MPI for Biophysical Chemistry, Goettingen, Germany.
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
Melanie Norstrom, Ronald S. Rock.
University of Chicago, Chicago, IL, USA.
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.