compounds and inflammatory mediators. Recent mutagenesis and chimeric studies have suggested that N-terminal cysteine residues (C622, C642, C666) located on the flexible linker region of the channel are involved in channel activation and desensitization, but how conformational changes in the flexible linker region lead to activation and desensitization is unresolved.
Capitalizing on our ability to isolate pure and functional TRPA1 channel protein, we recently provided the first insight into the channel architecture by reconstructing a TRPA1 structure to 16 Å resolution in resting state using electron microscopy (EM). Fitting TRPA1 homology model into the EM density lead us to hypothesize that the critical cysteines on the flexible linker region (C622, C642, C666) are in close proximity to one another and that covalent modification of cysteines within this pocket could promote conformational changes leading to channel gating and desensitization. Furthermore, we performed a mass spectrometry analysis of the in vivo TRPA1 thiol status and discovered that, when treated with a thiol-reactive TRPA1 agonist, C622 and C666 could form a disulfide bond with each other or with two other cysteine residues.
Our current structural and biophysical analyses using EM, mass spectrometry and homology modeling are elucidating ligand-induced conformational changes in the channel's flexible linker region that occur during activation and desensitization. These insights bring us a greater understanding of the structural rearrangements involved in the gating and desensitization mechanisms of the TRPA1 ion channel.

1936-Plat
Neural Growth Factor Increases the Cationic Non-Selective Currents in Neonatal Rat Pancreatic Beta-Cells
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Neural growth factor (NGF) is secreted by pancreatic beta-cells, which also present NGF auto-receptors with high (TrkA) and low (p75) affinities. NGF promotes beta cell survival and enhances glucose-stimulated insulin-secretion. The latter has been associated to an increase in the number of voltage-gated Na^+ and Ca^{2+} channels at the plasma membrane. However, further research is necessary to understand if other ion channels are implicated in NGF signaling.
In the present study, the long-term effect of NGF on the cationic non-selective (CAN) currents of beta cells from neonate rats was characterized, and compared to that of mature animals.
Pancreatic islets from adult and neonatal male Wistar rats were isolated and separated from acinar tissue by collagenase digestion and a density Ficoll gradient. Isolated cells were obtained from islets after an enzymatic digestion with trypsin. Whole-cell patch clamp recordings were obtained from beta cells with membrane capacitances of 6.0 ± 0.2 pF (N=32) and 6.3 ± 0.3 pF (N=28), for neonatal and adult rats, respectively.
Control adult beta cells showed a higher CAN density current compared to control neonatal cells. When the latter were incubated with NGF (50 ng/ml) during 48 h, CAN currents significantly increased in more than 2-fold at two test potentials, −120 and 80 mV, reaching a similar level to adult cells. Moreover, the effect of NGF was reverted by the incubation with K252a (200 nM), a blocker of high affinity TrkA receptors. Taken together, our results suggest that NGF could contribute to beta cell maturation by a gain of function in CAN currents.
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1937-Plat
TRPV1 Channels are Intrinsically Heat Sensitive and Negatively Regulated by Phosphoinositide Lipids
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The capsaicin receptor, TRPV1, is regulated by phosphatidylinositol-4,5-bisphosphate (PIP_2), although the precise nature of this effect (i.e., positive or negative) remains controversial. Here, we reconstitute purified TRPV1 into artificial liposomes, where it retains sensitivity to capsaicin, protons, spider toxins, and heat. Moreover, TRPV1 is fully functional in the absence of any phosphoinositides, arguing against an obligatory role in channel activation. Introduction of various phosphoinositides, including PIP2, PI4P and PI, inhibits chemical and thermal sensitivity of the channel, consistent with a model in which phosphoinositide metabolism by pro-algesic agents enhances TRPV1 sensitivity and contributes to thermal hyperalgesia. using an orthogonal chemical modification strategy, we further show that association of the TRPV1 C-terminus with the bilayer modulates channel gating, consistent with phylogenetic data implicating this region of the channel as a regulatory site for thermal and chemical sensitivity. Beyond TRPV1, these findings are relevant to understanding how membrane lipids modulate a diverse family of ''receptor-operated'' TRP channels.

1938-Plat
Calcium-Dependent Regulations of TRPP3 Channels
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TRPP3 channels or PKD2L1 channels, with high homology to another member of TRPP family, PKD2 (named after polycystic kidney disease), play potential roles in sour taste. TRPP3 channels are highly regulated by calcium, complicated with both calcium dependent activation and inactivation (Chen XZ, *et al.* 1999). However, the essential mechanisms of these calcium regulations are lacking (Li Q, *et al.*, 2002). In this study, we took advantage of the unique off-response (I_{off}) evoked by acid stimuli onto TRPP3 channels expressed in HEK 293 cells, to dissect the calcium dependent inactivation (CDI) from the complex calcium responses evidenced in oocyte preparations. By changing extracellular calcium concentrations from 0 to 110 mM, and intracellular buffers from EGTA to BAPTA, we proved that the decaying phase of I_{off} is completely calcium dependent. We tested and then rejected the hypothesis that calmodulin mediates the CDI of TRPP3 channels, by overexpressing the mutant form of calmodulin. Further mutagenesis analyses revealed that CDI would be abolished when the interactions between intracellular calcium ions and the EF-hand of carboxyl termini of TRPP3 channels were perturbed. Our work not only provides mechanistic insights into CDI of I_{off}, but also paves the way to the elucidation of calcium-dependent activation, thus the ultimate resolution of calcium-dependent regulations of TRPP3 channels.

1939-Plat
Identification of a Domain in Trpv4 C-Terminus Critical for Channel Function and Trafficking
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Like the majority of proteins, Transient Receptor Potential Vanilloid 4 (TRPV4) channels after synthesis in the endoplasmic reticulum (ER) undergo glycosylation and must be trafficked and targeted to the membrane surface where they will function. It has been shown that mutations in either N- or C-terminus of TRPV4 are associated with channelopathies, indicating the important role of cytoplasmic domains in the channel function. In this study we carried out biochemical, cell biological and electrophyisological experiments to identify domains/residues critical for TRPV4 function and trafficking. Confocal imaging of HEK293 cells revealed that a C-terminal deletion of TRPV4 caused the channel proteins trapped in the ER. Western blot analysis of serial C-terminal deletions identified a region/domain essential for complex glycosylation and maturity of channel proteins. Electrophysiological recordings combined with calcium influx assay confirmed that TRPV4 lacking the identified domain within the C-terminus resulted in a loss of channel function. Taken together, our results reveal a region/domain in the channel C-terminus critical for TRPV4 function and trafficking.

1940-Plat
Screening for TRPV1 Temperature-Sensing Domains with Peptide Insertion
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As the prototype of temperature-activated thermoTRP channels, the vanilloid transient receptor potential channel TRPV1 is involved in acute noxious thermosensation and thermal hyperalgesia. Its temperature sensing mechanism remains mysterious and controversial. Multiple heat-sensing domains that spread across the protein have been proposed, such as the intracellular N-terminal segment between the ankyrin repeats domain and S1, the outer pore region, and the intracellular C terminus. To test the contribution of various channel regions to heat activation, we introduced short peptide insertions to uncouple peripheral domains from the pore-forming core domain. Effects on heat- and agonist-induced channel activation were tested with both calcium imaging and electrophysiological recordings. While disruption of covalent periphery-core interaction weakened desensitization upon prolonged capsaicin application, none of the tested insertions appeared to significantly alter heat activation. Proton activation and Mg^{2+} activation, which are mediated by the outer pore, were also not significantly affected. These observations are consistent with the transmembrane core domain being intrinsically temperature-sensitive.