Oral Presentations

– 2. Channels and Ca^{2+} signaling –

O-15

Structural and functional studies of TRP channels

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Numerous physiological functions rely on distinguishing temperature by temperature-sensitive transient receptor potential (TRP) channels. While TRP channel function has been studied extensively, structural determination of their heat- and cold-activated states has remained a challenge. We determined cryo-EM structures of mouse TRPV3 in temperature-dependent closed, sensitized and open states. The heat-induced transformations of TRPV3 are accompanied by changes in the secondary structure of the N- and C-termini and represent a conformational wave that links these parts of the protein to a lipid occupying the vanilloid binding site. State-dependent differences in the behavior of bound lipids suggest their active role in TRP channel temperature-dependent gating. Our results provide an insight for understanding the molecular mechanism of temperature sensing.

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The open and closed pore conformations of the pacemaker channel HCN4

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HCN1-4 genes family generate the hyperpolarization-activated cation current I_f/I_h, which controls automaticity in cardiac and neuronal pacemaker cells. By single particle cryogenic electron microscopy (cryo-EM) we obtained structures of the HCN4 hyperpolarization-activated cyclic nucleotide-gated channel in open and closed pore conformations, with and without cAMP bound. Systematic comparison of open and closed states in HCN4 shows that a concerted movement of the S5 and S6 transmembrane helices opens a cytosolic gate. Furthermore, the open state structures, in combination with molecular dynamics analyses, provide atomic level insights into mechanisms of K^+ and Na^+ permeation, revealing unique ion-binding dependent adaptation in selectivity filter diameter. Thus, our ability to compare open and closed state structures for HCN4 channels provides fundamental insights into mechanisms of HCN channel gating, cyclic nucleotide-dependent modulation, and ion permeation.

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Structural insights into Ca^{2+} and cholesterol inhibition in the TRP channel PC2

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The TRP channel Polycystin-2 (PC2) is a Ca^{2+}-permeable, Ca^{2+}-activated channel located in the ER, the primary cilia, and the plasma membrane, respectively. PC2 is non-selectively permeable to both monovalent and divalent cations playing an important role in renal physiology. The inherited autosomal dominant polycystic kidney disease (ADPKD) is partly related to loss-of-function mutations in PC2. Although being not voltage-gated, PC2 displays sequence similarity in TM1-TM4 to the voltage-sensing domain of other TRP channels. The intracellular C-terminal domain includes a Ca^{2+}-binding EF hand, a coiled-coil domain, and an ER retention sequence. Consequently, the fraction of PC2 is retained in the ER, where it is suggested to play an important role as Ca^{2+}-leakage channel. Without the ER retention signal, PC2 is mis-trafficked within the kidney epithelial cells. We have solved the structure of a PC2 mutant missing the ER retention signal by cryo-EM to 3.1 Å. Our structure reveals an asymmetric opening of the lower gate, a conserved Ca^{2+}-binding site in the VSD, which together with new cholesterol binding sites point towards an intriguing inhibition mechanism. Our structural data are supported by a lipid MS analysis and Scanning transmission electron microscopy (STEM) of PC2 expressing ER membranes. Based on our data we suggest that cholesterol binding is an important feature to modulate PC2 Ca^{2+}- channel activity in the ER.

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Assessment of temperature sensitivity of the TRPM2 channel

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TRPM2 belongs to the TRP family, and is expressed in neurons of the central nervous system, bone marrow, phagocytes, β-cells, and cardiomyocytes. It forms Ca^{2+}-permeable nonselective cation channels that open under oxidative stress by simultaneous binding of intracellular Ca^{2+}, ADP-ribose (ADPR), and PiP_2 in the membrane. Malfunction of the channel has been linked to neurological disorders (AD, PD, ALS) as well as to pathological conditions that lead to apoptosis (cerebral stroke, myocardial infarction). Temperature-dependent activation of TRPM2 in warm-sensitive neurons underlies body heat control and the generation of fever. The channel may be a target to treat fever, chronic inflammations, diabetes or congenital hyperinsulinism. The biophysical background of temperature sensitivity of TRPM2 is yet unknown, and might reflect either temperature dependence of the intracellular concentrations of any of its three activating ligands, or intrinsic heat sensitivity of the TRPM2 itself. Therefore, we determined temperature dependence of TRPM2 gating parameters and apparent ligand binding affinities for Ca^{2+}, ADPR, and PIP_2 in inside-out patches from HEK-293 cells expressing human TRPM2, under temperature-controlled perfusion of the cytosolic surface. Our results unequivocally clarify and describe the mechanism of temperature sensitivity of the TRPM2 channel.

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