different types of diseases. These channels are nonselective and permeable to Na+, Ca++ and other cations and they are modulated by changes in the extracellular and intracellular concentrations of these cations. Our studies also show that the translocation of these proteins to the cell membranes is stimulated by defined chemical chaperones, proteasome inhibitors and modulators of ion homeostasis. They play a role in membrane trafficking and their translocation between different subcellular compartments is modulated by hormones involved in the homeostasis of Na+ and other ions. Cells expressing mutated TRPP and related proteins show abnormal trafficking of subcellular membrane vesicles. Such abnormalities may explain the symptomatology and the pathogenesis of the disorders caused by the mutations of the genes encoding these proteins.

904-Pos Board B783

Binding of Syntaxin 1A to the C-terminus of hERG Channels Affects Channel Trafficking and Inactivation

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The SNARE protein, syntaxin 1A (STX1A), functionally regulates cardiac ion channels, including the human ether-à-go-go related gene (hERG) which encodes the pore-forming voltage-gated K^+ channel underlying I_{Kr} in the heart. The primary mode for STX1A-dependent inhibition of hERG channel function is trafficking impairment which can be rescued by reduced temperature or the high-affinity channel blocker, E4031. A secondary mode is achieved by the production of a hyperpolarizing shift in the voltage dependence of steady-state inactivation. Here we report the STX1A binding region on hERG channels. GST pulldown and coimmunoprecipitation demonstrates that the cytosolic SNARE motif-containing STX1A-H3 domain preferentially binds to hERG. This cytosolic domain is attached to the TM region by a short inflexible linker and is 60 residues in length. Use of N- (hERG-Δ2-16 and hERG-Δ2-354) and C-terminal truncation mutations (hERG-Δ1120, hERG-Δ1045, hERG-Δ1000, hERG-Δ960, hERG-Δ899, hERG-Δ860-899, hERG-Δ860, and hERG-Δ814) demonstrates that STX1A binds to all truncation mutations tested. The hERG C-terminus begins at approximately residue 670 immediately adjacent to the cytosolic portion of the S6 helices. Therefore, we deduce that the STX1A-H3 domain interacts with the C-terminus of hERG channels between residues 670 and 814. Functional analysis of C-terminal truncation mutations demonstrates that STX1A inhibits the trafficking of truncations up to hERG-Δ1000, but has no effect on hERG-Δ960 while enhancing trafficking and function of hERG-Δ899. We infer that STX1A-interaction disrupts normal protein folding thereby inhibiting channel trafficking, and may alter the movement of S6 helices affecting the hERG channel inactivation gate, shifting the voltage-sensitivity of channel inactivation. SNARE protein-mediated regulation of cardiac ion channels represents a novel biological mechanism that may have universally intrinsic implications for normal and diseased heart function.
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Rapidly Inducible Protein Modification Using Rapamycin-mediated Complementation Of Tobacco Etch Virus (TEV) Protease

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Traditional modalities of protein knock down rely on transcriptional or translation suppression and are thus hampered by slow endogenous protein turnover rates and compensatory mechanisms. Directly targeting proteins, rather than synthetic pathways, facilitates more rapid depletion of the targeted protein. Tobacco etch virus protease (TEVp), a potyvirus nuclear inclusion protein, cleaves at a canonical sequence, ENLYFQ/G, with sufficient specificity to be useful for targeting specific proteins. Starting with a TEVp mutant (S219V) deficient in autocatalysis (Kapust et al., *Protein Eng.* 14:993, 2001), we have humanized the codon bias and introduced mutations (van den Berg et al., *J. Biotechnology* 121:291, 2006) to increase solubility. Expression of the modified TEVp was well tolerated by Hela cells and resulted in efficient cleavage, as determined by Förster resonance energy transfer, of a co-expressed reporter consisting of fluorescent proteins (Cerulean and Venus) fused with a linker containing a TEVp cleavage sequence. Splitting the TEVp at residue 119 (Wehr et al., *Nat. Methods* 3:985, 2006) and fusing the fragments to FKBP12 and the c-terminus of FRB resulted in inducible enzyme activity following addition of rapamycin to living cells. Cleavage at 37°C proceeded exponentially with a time constant of approximately 100 minutes. In an effort to accelerate cleavage, the two halves of the complementation system were fused to form circular permutated unitary constructs. However, these constructs demonstrated constitutive activity without rapamycin. Fusing one half of TEVp to the reporter protein (cleavage in *cis*) also resulted in non-regulated activity. Current efforts are aimed at targeting one half of TEVp to the plasma membrane and recruiting the other half from the cytoplasm upon addition of rapamycin. Targeted protein cleavage was monitored by measuring $K_V3.4$ inactivation of a reporter construct consisting of EGFP-TEVP site-$K_V3.4$.

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Resolving the Structural-Functional Interaction Between HIV-1 Vpu and TASK Channels by FLIM/FRET

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An established membrane potential is fundamental to the survival of a cell. The majority of cellular and physiological processes foremost require orchestration of various ion channel activities and interactions in a spatially and temporally interdependent fashion. Previously we found that Vpu, a single transmembrane protein encoded by HIV-1, could interact with the assembly of endogenous K_{2P} channels that set resting potentials. This Vpu function reflects its viroporin structure, conferring the propensity to oligomerize into homomeric channels or promiscuously with homologous endogenous channel subunits. By interfering with normal assembly of background K^+ channels, Vpu suppresses the stabilizing K^+ conductance and dissipates the transmembrane voltage constraint on viral particle discharge. In this report, we measured membrane potential changes with respect to TASK-Vpu interaction in single cells by implementing a FRET pair with potential-tracking imaging. Our combined measurements from FLIM/FRET and confocal microscopy showed a structural-functional coupling between the two physical parameters (protein-protein interaction; membrane potential), and confirmed the previous experimental results obtained by independent means.

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The Positive Effect Of STREX On BK Channels

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The large conductance voltage and calcium sensitive potassium channel (BK) is encoded by a single gene KCNMA1. The inclusion of a stress regulated exon (STREX) at a splice site (C2) in the intracellular carboxyl (C) terminus of BK confers differing properties to the channel. STREX insertion generates a putative polybasic region. In a number of other channels, polybasic regions are suggested to interact with negatively charged phospholipids, such as phosphoinositides, to control ion channel gating and membrane targeting. We hypothesised that the polybasic region including STREX may serve as a membrane targeting domain of the STREX BK channel C-terminus. To test this, a GFP-tagged carboxyl terminal construct spanning the S6 transmembrane domain to the COOH end region of the intracellular carboxyl terminus was constructed (S6-COOH-STX) and transiently transfected into HEK293cells. The construct localised at the plasma membrane and this was abolished when the STREX insert was deleted. To test whether the polybasic region is important for plasma membrane targeting, two approaches were taken: Firstly, site directed mutagenesis to change selected positive residues into neutral (alanine) residues, also abolished membrane targeting of S6-COOH-STX to the plasma membrane. Secondly, to discern whether the polybasic region may interact with negatively charged phosphoinositides at the plasma membrane, the S6-COOH-STX construct was co-transfected with a 5′phosphatase, IPP. Cells co-expressing IPP displayed significantly reduced plasma membrane targeting of S6-COOH-STX, however a phosphatase null mutant of IPP did not effect plasma membrane expression. These data suggest that the polybasic region generated by inclusion of the STREX insert is an important determinant of STREX domain interaction with the plasma membrane. The functional role of the polybasic region and the interaction of phospholipids on BK channel calcium and voltage sensitivity were elucidated using patch clamp electrophysiology and high throughput dyes.

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Regulation Of Kcnq2/3 Channels By The Transcriptional Repressor REST In Nociception

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Understanding how excitability of sensory neurons is regulated is an important goal since this excitability underlies pain transmission and unfortunately almost everyone will suffer from inflammatory pain at some point in their life. Recent studies have identified expression of M-type K^+ channels (encoded by KCNQ genes) in damage-sensing (nociceptive) sensory neurons, where they are thought to control excitability. Accordingly, receptor-induced inhibition of M-current in these neurons has been shown to contribute to peripheral sensitisation and inflammatory pain. Here we describe a new mechanism for downregulation of KCNQ channel expression in nociceptors. We identified binding sites for the transcriptional repressor REST within both the KCNQ2 and