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mechanistic insight into the gating of this channel and validates the use of VCF in this channel. Co-expression of KCNE1 dramatically separates the voltage dependence of fluorescence and current signals, suggesting multiple independent voltage sensor movements are now required for channel opening. This prediction is further supported by measurement of a Cole-Moore effect in KCNQ1/KCNE1 but not KCNQ1 alone, and by computer-based Markov modeling of channel kinetics. Taken together, our results indicate a KCNE1-dependent change in the coupling between the voltage sensor and the channel gate. This represents the first measurements of KCNQ1 voltage sensor using VCF and elucidates a novel role for a potassium channel beta-subunit: to alter the relationship between voltage sensor movement and channel opening.

154-Plat
Different Molecular Phenotypes of LQT2-Linked hERG1a Mutations in the Same Amino Acid

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The human ether-a-go-go-related gene (hERG) encodes the pore-forming α-subunits that underlie the cardiac rapidly activating delayed rectifier K+ current (IKr). Patients with mutations of hERG1a channels are associated with type 2 long QT syndrome (LQT2), and more than 500 mutations, predominantly missense mutations, have been identified in hERG1a channels. In vitro, hERG1a mutations cause reduced hERG current (IhERG) by several mechanisms, including nonsense-mediated mRNA decay, channel protein trafficking-deficiency, abnormal channel gating and abnormal ion permeation. Two independent LQT2 clinical cases have reported hERG nucleotide mutations for tyrosine at amino acid 475 (ΔY475 and Y475C), yet the mechanisms for altered IhERG are not known. In this study, we heterologously expressed WT and these LQT2 mutations in HEK 293 cells and characterized their biochemical and biophysical properties, and additionally we studied two engineered mutations Y475F and Y475A that substitute structurally different amino acids. Western blot analysis of WT, ΔY475, Y475F and Y475A showed 135 and 155kDa protein bands, whereas Y475C showed only the 135kDa protein band, suggesting that Y475C is trafficking-deficient. Whole-cell patch clamp experiments showed that the V1/2 of the activation relation for ΔY475 shifted negatively to -29.8 ± 1.4 mV compared to WT, Y475F and Y475A (-12.4 ± 2.3, -9.3 ± 0.4 and -14.6 ± 0.4 mV, respectively). At -50 mV, Y475A and ΔY475 hERG deactivated faster compared to WT, whereas Y475F has little effect on channel deactivation kinetics. The data suggest that, 1) multiple mutations in a single amino acid exert strikingly different molecular phenotypes, 2) Y475C results in reduced IhERG due to a protein trafficking-deficient mechanism, 3) ΔY475 results in reduced IhERG due to an abnormal channel gating mechanism, and 4) the aromatic side chain of Y475 appears to modulate hERG1a channels deactivation properties.

155-Plat
Deletion of the Amino-Terminus Uncovers an Inactivated State in Eag1 Channels

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Ether-a-go-go (Eag) family channels, which include hErg1, are voltage-gated K+ channels that are important in cardiac and neural function and have a well-documented role in disease. Eag1 channels in particular are found throughout the central nervous system and are crucial regulators of cell cycle and tumor progression. Wild-type Eag1 channels exhibit voltage-gated activation and deactivation, but no apparent inactivation. Here we find that deletion of the entire intracellular amino-terminal domain uncovers an inactivated channel state at depolarizing potentials. We characterized this new inactivated state in inside-out patches excised from Xenopus oocytes expressing the mutant Eag1 channels. Intriguingly, we find that application of the membrane-impermeable cysteine modifying reagent MTSES to the intracellular side of the amino-terminal deletion mutant abolishes this inactivation. We have localized this cysteine-modifying effect to one or more of three cysteine residues in the intracellular carboxy-terminal domain. One of these residues resides in a domain that shares sequence similarity with the cyclic nucleotide binding domain (CNBD) of other channels and enzymes, and the other two reside in the C-linker which connects the CNBD-like region to the S6 transmembrane domain. As the CNBD-like region in does not bind to, and these channels are not regulated by cyclic nucleotides, the role of domain in channel gating is unknown. Our results suggest that the entire amino-terminus, or a fraction thereof, may act as a ligand that binds to the CNBD-like region to prevent inactivation in the wild-type channel.

PLATFORM M: Exocytosis & Endocytosis

156-Plat
How Dynamin and Amphiphysin Sense and Generate Membrane Curvature

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A long matter of debate in membrane traffic biology is how proteins can create or sense membrane curvature. Recent studies have associated either ability of proteins involved in membrane traffic with their structure and the specific physico-chemistry that dictates their binding to the lipid membrane. By using a micromanipulation set-up combining a micropipette used to aspirate Giant Unilamellar Vesicles (GUV) and optical tweezers to extract a tubule out of them, we can precisely control and measure the size of the tubule, and the force needed to hold it (tube force). We studied the binding of both dynamin and its partner amphiphysin, a BAR domain protein. We show that both proteins are able to lower the tube force at high concentration (several micromolar). At these concentrations, both dynamin and amphiphysin are able to tubulate membranes. At much lower concentrations (nanomolar) however, both proteins interacts with the membrane in a curvature coupled manner. Dynamin polymerizes around tubules less than 20nm only, and the amount of amphiphysin bound is linear with increasing curvature. By estimating the coupling coefficient (in the range of 1000) between the amount bound and the curvature, we found that it is the highest value measured for endocytic protein. No modification of the tubule force is observed, indicating that the protein works in these conditions as a membrane curvature sensor.

Importantly, we show that proteins that transiently interact with membranes in a curvature-coupled manner can work both as curvature generator or curvature sensor, depending on the conditions. We will compare our *in vitro* results to the *in vivo* situation and give some clues about how to discriminate the function of these proteins *in vivo*.

Figure 1: Experimental set-up for the study of dynamin and amphiphysin polymerization.

157-Plat
Real-Time Imaging of Clathrin Dynamics in Three Dimensions

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Clathrin mediated endocytosis is a crucial factor in maintaining cellular dynamics. So far, real-time observation of this phenomenon has been achieved using methods that are limited to two dimensional spatial analysis even though cells have three dimensional geometries. Here we have used a fast, confocal based, three dimensional automated tracking scheme to analyze clathrin coat dynamics at the ventral and dorsal surfaces of mammalian cells. This technique presents a direct observation of a clathrin mediated virus entry at the apical surface of polarized cells for the first time. Our analysis has shown that a sub-population of clathrin-coated structures ascend from plasma membrane for hundreds of nanometers before they uncoat. Elevation of these structures depends on actin machinery and is enhanced by increasing membrane tension. On the migrating cells, we have found that clathrin-mediated endocytosis is constrained to the dorsal side of the plasma membrane at the sites of protrusion. Clathrin-coats emanating at the leading edge move towards the center of the cell with velocities inversely proportional to the migration speed of the cell.

158-Plat
A new Role for the Dynamin Gtpase as a timer that Controls Fusion Pore Expansion

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Dynamin is a master regulator of membrane fission in endocytosis. However, a function for dynamin immediately upon fusion has also been suspected from a variety of experiments that measured release of granule contents. The