sensors. The action of DHA is strikingly smaller in S1o1 + β2 (without inactivation) and in S1o1 alone, and DHA even decreases currents through S1o1 + β1 (LRCC26). The stimulatory effect of DHA on S1o1 + β1 and S1o1 + β4 critically depends on two potentially interacting residues in each N terminus. The mutagenesis based on DHA-sensitive and -insensitive S1o1 channels reveals the importance of a residue in the S6 segment. The consequence of the DHA interaction with the channel involving this S6 residue is greatly amplified by the presence of β1 or β4. The β1 subunit-dependent effect of DHA undermines the blood-pressure lowering effect of the fatty acid acutely injected into anesthetized mice, and the hypotensive effect is absent in S1o1 knockout mice.

59-Subg

Powerful and Ancient Embrace of Four-Domain Voltage-Gated Channels with Calmodulin

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A most ubiquitous Ca²⁺-sensing molecule throughout biology—calmodulin (CaM)—serves as a virtual subunit of numerous ion channels, conferring vital Ca²⁺-dependent modulation of channel opening. Nowhere is this Ca²⁺ modulation more prevalent than in voltage-gated Ca²⁺ channels, where CaM dynamically switches among differing interactions with a proximal carboxyl tail region (CI domain), thus translating Ca²⁺ fluctuations into profound adjustments in channel opening. Using new approaches, we here uncover striking ramifications for Ca²⁺ to channels itself imparts a striking boost in baseline open probability, with at least 10 splice variants detected in brain. All splice variants interact strongly with the C-terminus of all four channel isoforms (HCN1-4) at two different interaction surfaces. Whereas all TRIPβb isoforms inhibit channel gating by antagonizing the normal action of cAMP to facilitate opening, the various isoforms have distinct effects on channel trafficking. We identified two splice isoforms with opposing actions on HCN1 surface expression and distinct subcellular localizations that are critical for HCN1 dendritic targeting. Our recent results have identified the structural and functional bases for many of the regulatory actions of TRIPβb.

Subgroup: Motility

62-Subg

Movement of Signaling Receptors Inside Primary Cilia

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The primary cilium is a signaling organelle with a distinct complement of membrane and soluble proteins. How specific proteins are concentrated within cilia while others remain excluded is a major unanswered question. Recent work has uncovered a diffusion barrier for membrane proteins at the base of the cilium that functionally separates the topologically continuous plasma and ciliary membranes. Using a newly develop in vitro system for trafficking to cilia, we now demonstrate the existence a size-dependent permeability barrier for soluble proteins that allows the passive entry of proteins smaller than 75 kDa and forces large proteins to utilize active transport for entry into cilia. Interestingly, the ciliary permeability barrier is mechanistically unique and does not share features with the nuclear pore complex or the axonal diffusion barrier. Beyond ciliary entry, our in vitro system has enabled a study of soluble diffusion inside cilia which reveals that diffusion alone is sufficient for a rapid exploration of the ciliary space in the absence of active transport.

Further dissect the respective contributions of diffusion and active transport to the exploration of the ciliary space, we established a system for single molecule imaging of signaling receptors inside cilia. Here, we find that diffusion is the major driver of membrane proteins movement along primary cilia and that signaling receptors spend less than 25% of time undergoing motor-driven transport. Perturbation of either diffusion or active transport shows that cargo movements can be uncoupled from movements of the intraflagellar transport (IFT) machinery, and that diffusion is sufficient for membrane proteins to explore the ciliary surface. Taken together, our results indicate that signaling within cilia need not be entirely reliant on active transport and poses the question of the role of active transport in transducing signals through the primary cilium.

63-Subg

Probing Forces on Newly Generated Spindle Microtubule Minus-Ends

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The mitotic spindle is a dynamic self-organizing machine that coordinates cell division and preserves genomic stability. The ability to focus microtubule minus-ends into poles is crucial to spindle structure and function. However, our understanding of pole-focusing forces has been limited by the challenges of labeling and imaging microtubule minus-ends in established spindles. Here, we used laser ablation to sever kinetochoore-fiber microtubules in mammalian cells and probe how the cell detects and organizes newly generated microtubule minus-ends. Within a few seconds of ablation, the cell recognizes...