similar. While biochemists have typically focused on how small molecules, pH, and other proteins modulate the activity of a protein of interest, it is clear that mechanical forces can play a large role. We provide some new insights into the mechanical properties of F-actin, and suggest how actin can act as a tension sensor in many cell biological systems. In contrast to the long held view that F-actin is almost inextensible, we show how subdomain 2 of actin cooperatively and allosterically modulates both the bending and stretching stiffness of F-actin. Further, we show that the ability of actin-binding proteins to change actin's structure depends upon the intrinsic plasticity and cooperativity of actin.

124-Symp
Rupture and Contraction of Crosslinked Actin Networks by Myosin Motor Activity
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Self-organized contractile arrays of actin filaments and myosin motors drive cell division, migration, and tissue morphogenesis. Biophysical studies have provided detailed mechanistic insights into the mechanisms of force production by individual motor molecule. However, it is not well understood how motors and actin filaments collectively self-organize into force-generating arrays. It is for instance poorly understood how network connectivity (or crosslinking) influences active contractility. We addressed this problem by reconstituting cell-free model systems from purified actin, myosin, and actin crosslinking proteins. By studying motor-driven activity over a broad range of network connectivities, we discovered that myosin motors contract actin networks into clusters that exhibit a scale-free distribution of sizes, characteristic of a critical state. Surprisingly, this critical behavior occurs over a broad range of network connectivities. To explain this robustness, we performed simulations of contractile networks taking into account network restructuring: motors reduce connectivity by promoting crosslink unbinding. We demonstrate that this coupling between activity and connectivity drives initially well-connected networks to a critically connected state. This model provides new avenues to understand contraction and rupture phenomena occurring during cell and tissue morphogenesis.

125-Symp
Shape Changes Induced by Actin Dynamics and Contraction
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In order to unveil generic mechanisms of cell movements, we design stripped-down experimental systems that reproduce cellular behaviours in simplified conditions. Actin-based motility is mimicked using beads or oil droplets placed in an appropriate in vitro system that contains the actin machinery. Cortices of cells and their contractility are mimicked using liposomes covered with actin filaments that are straight or growing in branches, in the presence of myosin. We find that the efficiency of contraction or motility depends on the concentrations of proteins, on the length of the filaments, and on the strength of their attachment to the liposome membrane. Moreover, the mechanics of bio-mimicking liposomes can be characterized using tube pulling experiment, and we will present an unexpected result, that membrane dynamics is not only affected by the presence of the cytoskeleton, but to a large extend by membrane composition and liposome preparation.

Platform: Other K Channels

126-Plat
Functional Modulation of Cardiac ATP-Sensitive Potassium Channels by Nitric Oxide via Intracellular Signaling
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ATP-sensitive potassium (K_{ATP}) channels are crucial for stress adaptation in the heart. Nitric oxide (NO) has been shown to stimulate cardiac K_{ATP} channels; however, the mechanistic details remain poorly understood. Here we sought to delineate the intracellular mechanism responsible for NO modulation of sarcolemmal K_{ATP} ($sarcK_{ATP}$) channels in ventricular cardiomyocytes. Cell-attached patch recordings were performed in combination with pharmacological, genetic and biochemical approaches. Bath application of the NO donor NOC-18 increased the single-channel activity of Kir6.2/SUR2A (*i.e.*, the cardiac-type K_{ATP}) channels in transfected HEK293 cells, which was abolished by selective suppression of cGMP-dependent protein kinase (PKG), extracellular signal-regulated protein kinase (ERK)1/2, Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII), and reactive oxygen species (ROS) (hydrogen peroxide H_2O_2 in particular), respectively. Importantly, NO donors potentiated function of $sarcK_{ATP}$ channels preactivated by the channel opener pinacidil in adult rabbit ventricular myocytes, through destabilizing the longest closed state and facilitating opening transitions, and the potentiation was nullified when PKG, calmodulin, CaMKII or ERK1/2 was inhibited. Exogenous H_2O_2 also stimulated ventricular $sarcK_{ATP}$ channels in intact cells in an ERK1/2- and CaMKII-dependent manner. Genetic ablation of CaMKIIδ, the predominant cardiac CaMKII isoform, diminished PKG stimulation of mouse ventricular $sarcK_{ATP}$ channels (compared with wild-type controls). Kinase activity and Western blot assays further supported that NO-PKG activation augmented CaMKII activity in ventricular myocytes, which was mediated by ERK1/2. Collectively, we demonstrate that NO stimulates ventricular $sarcK_{ATP}$ channels via a cGMP/PKG/ROS(H_2O_2)/ERK/calmodulin/CaMKII signaling cascade that alters channel gating. This novel signaling pathway may control cardiac excitability and mediate, in part, cytoprotection against ischemia-reperfusion injury, by opening myocardial K_{ATP} channels.

127-Plat
Awakening Loss-of-Function KATP Channel Mutants with an Engineered 'Forced Gating' Approach
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Regulation of inwardly-rectifying potassium channels by intracellular ligands couples membrane excitability to important signaling cascades and metabolic pathways. A significant barrier to understand coupling mechanisms between ligand binding and gating is that many functionally important channel motifs are highly sensitive to mutagenesis, resulting in a loss of function phenotype. We have developed a 'forced gating' approach that rescues function in electrically silent channel mutants, enabling characterization of channel motifs that are otherwise intractable to electrophysiological recording. This approach involves substitution of a glutamate in the hydrophobic Kir channel bundle crossing (F168E mutation in Kir6.2), generating channels that are pH sensitive and open upon alkalization, due to mutual repulsion of introduced negatively charged side chains in the channel gate. We have implemented this 'forced gating' approach in mutagenic scans of the Kir channel slide helix and G-loop, two motifs proposed to play a role in ligand dependent gating of Kir channels. Both motifs are also highly sensitive to mutagenesis, with alanine mutations causing nearly complete loss-of-function at 7/20 slide helix positions, and 8/13 G-loop positions. Without exception, expression of silent mutants on the Kir6.2[F168E] background permitted activation of functional channels in alkaline pH, and measurement of kinetics and potency of ATP inhibition. Our results highlight an essential 'aspartate anchor' (Kir6.2 residue D58) that bridges the slide helix and multiple interacting residues in the cytoplasmic domain. Disruption of the highly conserved 'aspartate anchor' uncouples the transmembrane and cytoplasmic domains, reducing ATP sensitivity of Kir6.2 far more than any other G-loop or slide helix mutants. These findings indicate a central role for the 'aspartate anchor' in coupling ligand binding to Kir gating, and also emphasize the potential general utility of this 'forced gating' method to study loss-of-function channel mutants.

128-Plat
Distant Cytosolic Residues in Kir Channels Control Channel Gating and Modulation by Cholesterol and $PI(4,5)P_2$
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In recent years, cholesterol has been emerging as a major regulator of ion channel function. Channels regulated by cholesterol include the Kir2 channels subfamily of constitutively active, strongly inwardly rectifying K^+ channels that set the resting membrane potential and modulate membrane excitability. Yet, the mechanism by which cholesterol affects channel function is unclear.
We have previously shown that Kir2 channels are suppressed by the elevation of membrane cholesterol and enhanced by cholesterol depletion. We thus hypothesized that cholesterol modulates the function of Kir2 channels by