and their role in sensory perception. We focus on temperature-sensitive ion channels, particularly TRPV1, TRPV4 and TRPA1. TRP channels are challenging structural biology targets because they are large multidomain eukaryotic membrane proteins and are not naturally abundant. We take complementary approaches to obtain structural and functional information on TRP channels, including crystallography, biochemistry and cell-based functional assays. The combined results advance our understanding of TRP channel function. TRPV channels play key roles in pain, thermo- and mechanosensation, and calcium homeostasis, and mutations in the TRPV4 channel have been implicated in both neuronal degeneration diseases and skeletal dysplasias. The N-terminus of TRPV channels contains six ankyrin repeats, short sequence motifs often involved in protein-ligand interactions. The isolated ARDs do not oligomerize, suggesting that they interact with regulatory factors instead. Our accumulated data provide information about how regulatory ligands interact with the ARD and other N- and C-terminal intracellular regions and alter the sensitivity of these TRPV channels.

43-Subg
Conformational Motions of K^+ Channel RCK Domains
Brad Rothberg.
Temple University School of Medicine, Philadelphia, PA, USA.
Ligand binding sites within ion channel domains can interact by allosteric mechanisms, to modulate binding and control channel function. By determining crystal structures of regulator of K conductance (RCK) domains from K^+ channels, we identify possible mechanisms of allosteric coupling among Ca^{2+} regulatory sites in the channel. Combining this structural analysis with complementary electrophysiological analysis of channel gating, we arrive at a working hypothesis for chemical interactions that are important for modulation of ligand binding and subsequent channel opening.

Subgroup: Motility

44-Subg
Multiplex Regulation of Cytoplasmic Dynein Motility
Richard J. McKenney, Walter Huynh, Minhajuddin Siarjuddin, Marvin Tanenbaum, Gira Bhabha, Ronald D. Vale.
UCSF, San Francisco, CA, USA.
Cytoplasmic dynein is the predominant minus-end directed microtubule motor in metazoan cells. Dynein transports diverse cargoes over long distances in neurons, and the motor is thought to be adapted for a myriad of cellular functions through the use of several accessory protein factors that impinge on its basic biophysical characteristics. One of these accessory factors is the multisubunit dynactin complex, which has been implicated in dynein-based cargo transport and the modulation of dynein processivity and directionality. While isolated dynein from Saccharomyces has been shown to be a strongly processive motor, dynein from other organisms displays weakly processive, bidirectional or diffusive motility. Here we show that, on its own, cytoplasmic dynein from humans and other metazoans is not a processive motor. Previous attempts to study dynein-dynactin co-complexes have found relatively modest effects on dynein processivity and directionality by dynactin. We show that multiple cargo-specific adapter proteins link dynein to dynactin to form a stable supercomplex (DDB complex) that is over 2MDa in size. Using multicolor single-molecule microscopy, we have found that, remarkably, the purified DDB supercomplex is unidirectional and ultra-processive, displaying runlengths that closely match those observed in vivo. Further we show that processive movement of the DDB complex requires specific modifications of the tubulin c-terminal tails, implicating post-translational modification of the microtubule track in the control of dynein motility. Our data suggest that the dynein motor is more plastic that previously thought, able to transition from a non-processive motor to an ultra-processive mode of motility upon association with external regulatory factors and specific subsets of microtubule tracks.

45-Subg
The Kinetics Underlying the Velocity of Smooth Muscle Myosin Filament Sliding on Actin Filaments in vitro
Brian D. Haldeman, Richard K. Brizendine, Diego Alcala, Kevin C. Facemyer, Josh E. Baker, **Christine R. Cremo**.
Biochemistry and Molecular Biology, University of Nevada School of Medicine, Reno, NV, USA.
Actin-myosin interactions are well-studied using soluble myosin fragments, but little is known about effects of myosin filament structure on mechanochemistry. We stabilized unphosphorylated smooth muscle myosin (SMM) and phosphorylated (pSMM) filaments against ATP-induced depolymerization using a cross-linker and attached fluorescent rhodamine (XL-Rh-SMM). XL-Rh-pSMM filaments moved processively on F-actin that was bound to a PEG brush surface. The ATP-dependence of filament velocities was similar to that for solution ATPases at high [actin], suggesting both processes are limited by the same kinetic step (weak to strong transition), therefore being attachment-limited. This differs from actin-sliding over myosin monomers, which is primarily detachment-limited. Fitting filament data to an attachment-limited model showed that ~½ of the heads are available to move the filament, consistent with the side-polar structure. We suggest that the subfragment 2 domain, which has a low stiffness, remains unhindered during filament motion in our assay. Actin-bound negatively-displaced heads will impart minimal drag force due to buckling of S2. Given the ADP release rate, the velocity, and length of S2, these heads will on average detach from actin before slack is taken up into a backwardly-displaced high-stiffness position. This mechanism explains the lack of detachment-limited kinetics at physiological [ATP]. These findings address how nonlinear elasticity in assemblies of motors leads to efficient collective force generation.

Subgroup: Exocytosis & Endocytosis

46-Subg
Mapping the Molecular Dynamics of Clathrin Mediated Endocytosis
Christien J. Merrifield.
Laboratoire d'Enzymologie et Biochimie Structurales, CNRS - UPR3082, Gif-sur-Yvette, France.
Clathrin mediated endocytosis is the principle mechanism by which cells internalize cell surface receptors. It can be thought of as a molecular machine which concentrates receptors into patches at the plasma membrane and which bends the membrane into a vesicle that pinches off. Quite how the many structural, enzymatic and cytoskeletal components of the endocytic machine are spatially and temporally organised is not fully understood. In this talk I will describe our efforts to address this problem by analysing single endocytic events using total internal fluorescence microscopy (TIR-FM).

47-Subg
Hair Cell Ribbon Synapse Function - Differently Optimized for Hearing and Balance
Soroush G Sadeghi[1], Sonja J. Pyott[2], Zhou Yu[3], **Elisabeth Glowatzki**[4].
[1]State University of New York at Buffalo, Buffalo, NY, USA, [2]University of Groningen, Groningen, Netherlands, [3]Neuroscience, Johns Hopkins University, Baltimore, MD, USA, [4]Johns Hopkins University, Baltimore, MD, USA.
In the cochlea, inner hair cells transmit the sound signal via type I auditory nerve fibers to the brain, and outer hair cells mainly operate as local amplifiers of the sound signal in the periphery. However, outer hair cells also contact a small group of afferent type II fibers, of unknown function. In the vestibular organs in the inner ear, hair cells transmit head rotation signals to the brain, via vestibular nerve fibers. All of these different hair cell types release glutamate onto their afferent contacts via ribbon synapses. Interestingly, synaptic transmission at these individual hair cell ribbon synapses operates differently in many respects, most likely optimized for their specific function.
For example, at the inner hair cell synapse, known for its high reliability and precision, EPSC are fast and amplitudes are quite variable in size and unusually large, with distributions from 20 pA to 1 nA (holding potential −94 mV). In comparison, at outer and vestibular type I hair cells, EPSCs show rather 'conventional' sizes of 20 - 50 pA. At inner hair cell ribbon synapses, EPSC amplitude distributions are quite diverse for individual nerve fibers, and we propose that specific distributions underlie 'high' and 'low spontaneous rate fibers carrying different aspects of the sound signal. Interestingly, EPSC distributions are calcium-independent, as hair cell depolarizations do not change the distributions. At the vestibular type I hair cell afferent synapse, hair cell depolarization shifts the amplitude distribution, suggesting a calcium-dependence. Postsynaptically, EPSCs are unusually slow, causing slow, summed depolarizations of the postsynaptic membrane and thereby a change in firing rate.
In summary, different mechanisms of ribbon synapse transmission and their function at different hair cells will be compared and discussed.
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