1030-Plat
Mathematical aspects of Variability and Variability Suppression of the Single Photon Response in Vertebrate Phototransduction
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The study of visual transduction has reached a level of maturity unmatched by other cellular signaling processes. It has long served as a model for G protein coupled receptor signaling in other cells. Given the deep understanding of the biochemical and biophysical basis of visual transduction, it is ripe for systems-level analysis. A spatio-temporal mathematical model is proposed that separates the activation/deactivation module of the visual transduction cascade from the transduction module. The effects of the layered geometry of the ROS and the incisures and their shape and number on the single photon response are analyzed. Generation and suppression of variability following isomerization are also analyzed. The transduction module of the cascade, and specifically the diffusion of the second messengers in the cytoplasm, is identified as a dominant variability suppressor. The deactivation module, and specifically the randomness of the sojourn times of activated rhodopsin in its phosphorylation states, are identified as a dominant generator of variability.

1031-Plat
Melanopsin Signalling: Low Pigment Density, Large Single-Photon Response, and High-Efficiency Transmission
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A subset of retinal ganglion cells has recently been discovered to be intrinsically photosensitive. These cells use melanopsin as the visual pigment and are the only photoreceptors known in mammals besides rods and cones. They project primarily to brain centers that serve non-image-forming visual functions such as the pupillary light reflex and circadian photoentrainment. How well these cells signal intrinsic light absorption to drive such behaviors remains unclear. We have used perforated-patch and loose-patch recordings to study mouse ipRGCs that are genetically labeled by a fluorescent protein, tdTomato. Here we report fundamental parameters that govern their intrinsic light responses, and the associated spike generation. They express melanopsin at a membrane density that is four orders of magnitude lower than that of rod and cone pigments (~3 μm^2), giving rise to a low photon-catch and thus a phototransducing role in relatively bright light. Nonetheless, the single-photon response is even larger than that of rods, suggesting high amplification in melanopsin phototransduction. It is also extraordinarily prolonged, with an integration time that is ~20-fold longer than that of rods, and has a unique shape among known photoreceptors. Remarkably, ipRGCs are capable of signalling single-photon absorption to the brain. Moreover, a flash resulting in a few hundred isomerized melanopsin molecules in a retina is sufficient for reaching the threshold of the pupillary light reflex.

Platform P: Muscle: Molecular Mechanics and Sructure

1032-Plat
Thermodynamics Of The Myosin Nucleotide-binding Pocket Measured By Epr Spectroscopy Using Spin Labeled Nucleotides
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Structural models based upon crystal structures show the myosin nucleotide-binding pocket closes around the ATP. EPR spectroscopy using nitroxide spin label covalently attached to ADP on the ribose moiety detects the degree of closure around this pocket. Our work with spin labeled nucleotide indicates that the pocket opens up in actomyosin, presumably to facilitate ADP release. Actomyosin spectra show evidence that the open and closed states are in equilibrium. Deconvolution of the spectra allows a quantitative analysis of this equilibrium yielding a ΔG for this structural transition. We have looked at this equilibrium in a range of different myosins, ranging from slow myosins such as myosin V and smooth muscle to faster myosins from skeletal muscle and Drosophila flight muscle. Assuming the open state is a necessary intermediate for ADP release, we have found a counterintuitive result that the closed state is favored in faster myosins and the open state is favored in slow myosin. The ΔG for the closed to open transition is proportional to the in vitro motility and the ADP affinity. The ability to observe the structural equilibrium gives an insight into the energetics of the actomyosin ADP state. We hypothesize that this correlation represents a destabilization of the open state in actomyosin ADP that results in a faster ADP release rate. Extending the analysis, the ΔG also correlates to muscle efficiency, which suggests that the energetics in this state play a central roll in the tradeoff between speed and efficiency in myosin.

1033-Plat
Transient Dynamics of the Force-Generating Domain in Myosin During the Recovery Stroke
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We have used transient time-resolved FRET (TR^2-FRET) to detect structural changes within the relay helix in real time during the myosin recovery stroke. The most intriguing problem in myosin's function is the mechanism of signal transduction between the nucleotide binding site and the force-generating domain of myosin during the recovery stroke. Atomic structures of myosin provide clues, but structural measurements during transient kinetics are needed to reveal the details of this mechanism. Kinetics of nucleotide binding is reliably determined from the fluorescence of mant nucleotides or single Trp myosin mutants (W129, W131). Single Trp mutant W501 has provided insight into structural kinetics in the force-generating domain, but the complex fluorescence signals observed during the recovery stroke do not yield clear structural interpretation. To overcome this problem, we have performed FRET measurements that monitor well-defined structural changes in the relay helix, which is proposed to be a key structural element in myosin's force-generating domain. We engineered two double-Cys myosin mutants in the Cys-lite *D. discoideum* myosin construct (gift from J. Spudich), with one Cys placed at the C-terminus of the relay helix (K498C) and the other at one of two stable positions in the lower 50K domain (D515C or A639C). We labeled these mutants with a donor-acceptor FRET pair (IAEDANS-DABSYL), then used time-resolved FRET (TR-FRET, detects donor lifetime at nanosecond timescale) to detect interprobe distances in equilibrium states trapped by nucleotide analogs, reflecting pre- and post-powerstroke structures of myosin. We then performed these nanosecond time-resolved measurements repetitively every 0.1 ms, in the transient phase after ATP addition using stopped flow. These transient time-resolved FRET (TR^2-FRET) signals showed directly that the relay helix changes from straight to bent conformation in the recovery stroke, from the post-powerstroke to the pre-powerstroke state.

1034-Plat
Modeling The Mechanics and Enzymatic Activities of Doubly, Singly and Unphosphorylated Smooth Muscle Myosin
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Smooth muscle myosin II has two heads, each capable of ATP hydrolysis and actin binding. However, both heads are required for maximal force and motion generation (Tyska et al., 1999), where one head, by weakly interacting with actin, may serve as a ''tether and guide,'' to optimize the second head's mechanical performance (Kad et al., 2003). In addition to these non-equivalent mechanical roles, an asymmetric head-head interaction inhibits the mechanical and ATPase activities of unphosphorylated (0P) smooth muscle myosin where one head's (blocked head) actin-binding domain associates with the converter region of the second head (free head). How does the equilibrium between active and inhibited states depend on myosin's phosphorylation state and specifically when only one of its two heads is phosphorylated? Once fully active, how quickly do the heads exchange their mechanical roles? We have addressed these questions in a multistate kinetic and mechanical model.
Based on single ATP turnover and steady-state ATPase data for fully active doubly phosphorylated (2P) smooth muscle myosin (Ellison et al. 2000, Rovner et al. 2006), we predict that, surprisingly, the two heads exchange their mechanical roles (i.e. weak vs. strong-binding head) slowly - at a rate of 0.1-0.3s^-1. By comparing ATPase and single molecule mechanical data (step size, attachment time, force-velocity) from fully active (2P), inhibited (0P), and smooth muscle myosin with only one head phosphorylated (1P), we predict that an equilibrium between active and inhibited conformations, which is phosphorylation-state dependent, can explain the enzymatic and mechanical data for all three phosphorylation states (2P, 1P and 0P). However, an alternative model can be proposed where the active and inhibited state equilibrium in the 1P state depends on the mechanical role of the phosphorylated head.