photobleaching (FRAP) to determine structures and dynamics of complexes formed by the photoreceptor G protein transducin, and its effector enzyme, cGMP phosphodiesterase, PDE6. Cryo-EM studies of complexes tagged with Fab fragments of monoclonal antibodies revealed that the inhibitory gamma subunit of PDE6, PDE6γ, stretches from the catalytic domain of PDE6 where its carboxyl-terminal region binds, to the GAFa domain, where its amino terminus binds, consistent with previous photo-crosslinking studies. FRET reveals that in unbound PDE6γ the amino and carboxyl termini are fairly close to one another. Upon addition of the catalytic subunits of PDE6, there is an initial very fast (near diffusion limit) binding to the catalytic domain, followed by a very slow (minutes) stretching out and binding of the amino terminal region to the distant GAFa domain. FRAP measurement of diffusion in living rod cells of transgenic *Xenopus laevis* revealed free diffusion of transducin along the long axis of cell, and the presence in disk membranes of cholesterol-dependent lipid microdomains, into which transducin complexes segregate upon activation.

1025-Plat
Mouse Cone Opsins Require An Arrestin For Normal Inactivation
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Arrestins are a family of proteins that arrest the activity of phosphorylated G-protein coupled receptors (GPCRs). While it is well established that rhodopsin, the GPCR of rod phototransduction, requires an arrestin (ARR1) for normal inactivation, the requirement for an arrestin for cone opsin inactivation has been disputed. We established that mouse cones express two distinct visual arrestins, Arr4 (alias "cone arrestin") and Arr1, and by recording electrical responses of the cones of WT mice, and mice with one or both the arrestins knocked out, established that an arrestin is required for normal inactivation of both mouse S-opsin and M-opsin. We also estimated the expression levels of Arr1 and Arr4 in cones, and established that both arrestins at their normal expression levels are competent to support inactivation. The complete absence of Arr1 and Arr4 from cones, however, slows down cone inactivation much less than does the absence of Arr1 from rods.

1026-Plat
Light-Dependent Translocation of Arrestin in Rod Photoreceptors is Signaled through a Phospholipase C Cascade and Requires ATP
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Light adaptation of rod photoreceptors induces translocation of arrestin from inner segments (IS) to outer segments (OS). Our study suggests that components of the G-protein linked phosphoinositide pathway play a role in signaling the initiating events of arrestin translocation. We show that arrestin translocation can be stimulated by activators of phospholipase C (PLC) and protein kinase C (PKC) in the absence of light. Conversely, arrestin translocation to the OS is significantly slowed by inhibitors of PLC and PKC.
In the second part of this study, we investigated the mechanism by which arrestin translocates in response to light. Other investigators have suggested that arrestin translocation between the OS and IS is a passive process, resulting from arrestin's binding affinity for light-activated, phosphorylated rhodopsin in the outer segments and affinity for microtubules in the inner segments. The central tenet of this model is that arrestin's translocation is an energy independent process. In our investigation of this process, we found that treatment of Xenopus retinas with potassium cyanide inhibits arrestin translocation to the OS in response to light, but that translocation can be restored by the removal of cyanide and addition of ATP. These results were confirmed in the mouse retina and clearly suggest that at least one step in arrestin translocation requires ATP. We also found that an arrestin with scrambled C-terminal 30 amino acids retained its binding for both activated rhodopsin and microtubules, but yet was unable to translocate in response to light. The results obtained from both investigating the signaling cascade and the mechanisms of arrestin translocation indicate that arrestin translocation between the IS and OS is more complex than previously proposed, and likely involves both diffusion and motor-assisted processes.

1027-Plat
NCKX Reaction Cycle: ATP, Voltage And Ion Regulation
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Ca^{2+} concentration in photoreceptor rod outer segment (OS) strongly affects the generator potential kinetics and the receptor light adaptation. The response to intense light stimuli delivered in the dark produce voltage changes exceeding 40 mV: since the Ca^{2+} extrusion in the OS is entirely controlled by the Na^+:Ca^{2+}, K^+ exchanger (NCKX), it is important to assess how the exchanger ion transport rate is affected by the voltage and, in general, by intracellular factors (like Mg-ATP, known to regulate the Na^+:Ca^{2+} exchanger). The NKCX regulation was therefore investigated in isolated OS, recorded in whole-cell configuration, using ionic conditions that activated maximally the exchanger in both forward and reverse mode. In all species examined (amphibia: *Rana esculenta* and *Ambystoma mexicanum*; reptilia: *Gecko gecko*), the forward (reverse) exchange current increased about linearly for negative (positive) voltages and exhibited outward (inward) rectification for positive (negative) voltages. Since hyperpolarization increases Ca^{2+} extrusion rate, the recovery of the dark level of Ca^{2+} (and, in turn, of the generator potential) after intense light stimuli results accelerated. Mg-ATP increased the size of forward and reverse exchange current by a factor of ~2.3 and ~2.6, respectively, without modifying their voltage dependence. This indicates that Mg-ATP regulates the number of active exchanger sites and/or the NCKX turnover number, although through an unknown mechanism. The ion transport mechanism was further investigated by using voltage and Ca^{2+} jumps (achieved via photolysis of caged-Ca^{2+} or fast solution changes) and by studying the NCKX selectivity in different ionic conditions. Ca^{2+} jumps, but not voltage jumps, produced current transients, possibly originating from electrogenic partial reactions. No monovalent cation substituted for Na^+ at the NCKX binding sites, but Rb^+ substituted for K^+, while Sr^{2+}, Ba^{2+}, Mg^{2+} substituted for Ca^{2+} with an apparent permeability ratio of 0.78, 0.20, $<$0.05:1, respectively.

1028-Plat
Regulation Of Photoreceptor Guanylyl Cyclase By Ca2+/Mg2+ Exchange In GCAPs
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Photon absorption by rods and cones activates transduction cascade that shuts down cGMP-gated channels and thus decreases free Ca^{2+} concentrations in outer segment. The Ca^{2+} feedback, which activates guanylyl cyclase (retGC) through guanylyl cyclase activating proteins (GCAPs), accelerates cGMP resynthesis in photoreceptors and thus expedites their recovery. GCAP1 and GCAP2, two ubiquitous among vertebrate species GCAPs that sequentially activate retGC during physiological response of rods to light, are Ca^{2+}/Mg^{2+}-binding proteins. They have one non-metal binding EF-hand, EF1, and three metal-binding EF-hands - EF2, EF3, and EF4. For each metal-binding EF-hand in GCAP1 we found point mutations that can block binding of Ca^{2+}, but not Mg^{2+}, and those that can block both Ca^{2+} and Mg^{2+} binding. We tested their effects on activation of retGC at physiological Mg^{2+} and either low Ca^{2+} (conditions representing light adaptation) or high Ca^{2+} (dark adaptation). Mg^{2+} binding in EF-2 and EF3 was essential for activation of retGC in the conditions of light adaptation. Mg^{2+} in EF2 was especially critical for the binding of GCAP1-GFP to retGC1 in co-transfected HEK293 cells, as revealed by confocal fluorescence microscopy. Mg^{2+} binding in EF4 contributed to neither retGC1 docking nor its activation. Instead, the replacement of Mg^{2+} by Ca^{2+} in this domain in the conditions of dark adaptation was the key event that switched the cyclase off. The Mg^{2+}/Ca^{2+}exchange in EF3 was required for the subsequent binding of Ca^{2+} in EF4. Contrary to EF3 and EF4, Mg^{2+}/Ca^{2+} exchange in EF2 was not essential for retGC inhibition. Binding of Mg^{2+} *versus* Ca^{2+} causes characteristic changes in the intrinsic Trp fluorescence of GCAP1 corresponding to its activator *versus* inhibitor states, including the non-metal binding EF1.

1029-Plat
Phototransduction Cascade Inactivation Kinetics Depend on Experimental Solutions
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Significant variation exists in published measurements of the kinetics and sensitivity of mouse rod photoreceptors; this variation includes ~2× differences in the amplitude of the single-photon response and the half-maximal flash strength, and ~1.5× differences in the time-to-peak of the dim flash response. The most obvious discrepancy between these studies is in the conditions used to store and perfuse the retina during the course of the experiment. Here we characterize changes in phototransduction produced by different recording conditions. Consistent with past work, the sensitivity and response kinetics depended strongly on recording conditions. These differences resulted, at least in part, from an apparent change in relative time constants of rhodopsin and transducin inactivation. These results underscore the importance of identifying experimental conditions that closely resemble the physiological environment of the retina to relate properties of phototransduction to downstream processing and visually-guided behavior.