rhodopsin, 11-cis retinal, to all-trans retinal. This event results in formation of the active species Metarhodopsin II (Meta II), which binds and activates several copies of the G-protein transducin, leading to signaling to nearby nerve cells. Metarhodopsin II is also the substrate for rhodopsin kinase, which phosphorylates the receptor to allow binding of the signal-quenching protein arrestin. Metarhodopsin II is not stable and decays within minutes into late photoproducts and ultimately the apoprotein opsin. In a rod cell exposed to the bright light associated with daytime, nearly all rhodopsin photoreceptors are ''bleached'' and exist as a heterogeneous population of these various late-photoproducts. Intriguingly, arrestin has been observed to translocate to the photoreceptor-rich disc membranes of the rod outer segment upon exposure to light, and arrestin remains there for the duration of light exposure.

To better understand the molecular mechanisms involved in this phenomenon, we have studied the interactions of arrestin with phosphorylated opsin (opsin-P) and the effects of all-trans retinal on this interaction. We find that, although arrestin has poor affinity for opsin-P (Kd ~ 40 μM) compared to Meta II-P (Kd ~ 20 nM), the addition of all-trans retinal to opsin-P increases its affinity for arrestin by approximately two orders of magnitude. Given that all rhodopsin photoproducts eventually decay to opsin and free all-trans retinal, this result could explain the persistence of arrestin in the outer segment during constant light-exposure. Finally, we find that arrestin binds opsin-P differently than Meta II-P but undergoes a similar conformational change upon the addition of all-trans retinal. The structural and physiological implications of our results will be discussed.

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Revamped Outer Segment Structure and Photoresponse in Retinal Rods Over-expressing Rhodopsin

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Visual phototransduction begins with photon capture by rhodopsin. Dense packing of rhodopsins in the outer segment membranes improves photon capture but can hinder the photoresponse by impeding the lateral diffusion of transduction proteins on the membrane. We attempted to investigate this effect by over-expressing rhodopsin in rods of transgenic mice. Increased rhodopsin expression was confirmed by Western analyses and by single cell microspectrophotometry. However, electron microscopy revealed that the excessive rhodopsins did not increase the packing density. Rods simply expanded the size of their membranous disks to accommodate the extra rhodopsins without increasing membrane congestion. Rod sensitivity increased due to improved photon capture. The dispersal of transducin and phosphodiesterase within the more spacious outer segment and the greater interdiskal volume delayed the photoresponse onset and reduced the cascade amplification. Flash responses from mutant and wild-type rods were fit with a mathematical model that adjusted for the alteration in outer segment structure.

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Structural Changes of Cephalopod Rhodopsin and β-Arrestin Measured by FTIR Difference Spectroscopy and Isotope Editing

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Invertebrate rhodopsin is the primary photoreceptor found in the eyes of cephalopods. Importantly, unlike vertebrate rhodopsin, invertebrate rhodopsins such as sepia rhodopsin (s-Rh) can be activated by light and then rapidly cycled back to the original state with a second red-shifted photon, thereby facilitating a variety of novel biophysical studies. Additionally, invertebrate rhodopsins can bind to the ubiquitous β-Arrestin2 which is used in regulating signal transduction in many GCPRs. In this study, we used static and time-resolved FTIR difference spectroscopy to investigate the photocycle of s-Rh complexed to β-Arrestin2. In the spectrum of s-Rh alone, difference spectra obtained using two colors to cycle between the ground (rho) and acid meta state show an 11-cis to all-trans photocycle as previously described. Several bands between 1750-1700 cm-1 are assigned using a D2O induced shift to an as yet unknown carboxyl groups. Other large bands are seen especially in the amide I and II regions which indicate significant backbone structural changes. Upon addition of β-Arrestin2, the difference spectrum is altered, especially in the amide I and II regions, reflecting additional structural changes occurring in β-Arrestin2 upon photoactivation. A negative band at 1742 cm-1 was shifted higher in the complex indicating the β-Arrestin2 is perturbing at least one carboxyl group in s-Rh. In order to assign these changes, total 15N isotope labeling of β-Arrestin2 was utilized. Comparison of difference spectra from s-Rh complexes containing unlabelled and 15N labeled β-Arrestin2 reveals ~ 3 cm-1 downshift of a negative/positive feature at 1668/1655 cm-1 indicating that these bands reflect at least partially conformational changes of the β -arrestin involving α-helical structure. These results offer a promising new tool to investigate the molecular mechanism of β-Arrestin interactions with GCPRs to regulate downstream signaling.

2702-Pos Board B672

Estimating The Rate Constant Of Cyclic GMP Hydrolysis By Activated Phosphodiesterase In Photoreceptors

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The early steps of light response occur in the outer segment of rod and cone photoreceptor. They involve the hydrolysis of cGMP, a soluble cyclic nucleotide, that gates ionic channels located in the outer segment membrane. This process has been characterized experimentally by two different rate constants beta_d and beta_sub: beta_d accounts for the effect of all spontaneously active PDE in the outer segment, and beta_sub characterizes cGMP hydrolysis induced by a single light-activated PDE. We estimate the experimental values of beta_d and beta_sub from a theoretical model. Considering diffusion in the confined rod geometry, we derive analytical expressions for beta_d and beta_sub by calculating the flux of cGMP molecules to an activated PDE site. We obtain the dependency of these rate constants as a function of the outer segment geometry, the PDE activation and deactivation rates and the aqueous cGMP diffusion constant. Our estimations show good agreement with experimental measurements.

2703-Pos Board B673

Revealing The Linear Aggregates Of Light Harvesting Antenna Proteins In Photosynthetic Membranes

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How light energy is harvested in a natural photosynthetic membrane through energy transfer is closely related to the stoichiometry and arrangement of light harvesting antenna proteins in the membrane. Their specific architecture helps them to absorb photons in the primary process of photosynthesis that is subsequently followed by a rapid and efficient energy transfer among the light harvesting proteins (LH2 and LH1) and to the reaction center. In this work, using atomic force microscopy (AFM) imaging, single membrane fragment FRET spectroscopy, spectral fluctuation analysis, and time-resolved spectroscopic analysis, we show the identification of linear aggregates of light harvesting proteins, LH2, in the photosynthetic membranes under ambient conditions. Our results suggest that the light harvesting proteins, LH2, mostly exist in two states, the aggregated and non-aggregated states in the photosynthetic membranes. Our results shed a light on understanding the complex intramolecular energy transfer dynamics and mechanism of the light harvesting in the photosynthetic membranes.

2704-Pos Board B674

Prolonged Illumination Up-regulates Arrestin And Two GCAPs: A Novel Mechanism For Light Adaptation

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In vertebrate photoreceptors, light adaptation is mediated by multiple mechanisms but the genomic contribution to these mechanisms has never been studied before. Therefore, we have investigated changes of gene expression using microarrays and real-time PCR in isolated photoreceptors, in cultured isolated retinas and in acutely isolated retinas. In all these three preparations after 2 hours of exposure to a bright light, we observed an up-regulation of almost two-fold of three genes *Sag, Guca1a and Guca1b,* coding for proteins known to play a major role in phototransduction: arrestin and guanylate cyclase activators 1 and 2. Gene up-regulation depends on light intensity and half up-regulation occurs for a light intensity corresponding to 5×10^3 Rh*/sec/rod. Gene up-regulation leads to an increase in the related protein content. Indeed, after three hours of