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.

2700-Pos Board B670

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.

2701-Pos Board B671

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

light exposure, the protein concentration of arrestin and GCAPs increases by about 30-50%. The up-regulation of these proteins in bright light conditions is expected to reactivate the photocurrent and thus to mediate a late phase of light adaptation. Functional *in vivo* electroretinographic tests show in fact that a partial recovery of the dark current occurs 1-2 hours after prolonged illumination with a steady light that initially causes a substantial suppression of the photoresponse. These observations demonstrate that a prolonged illumination results in the up-regulation of genes coding for proteins involved in the phototransduction signaling cascade, possibly underlying a novel component of light adaptation occurring 1-2 hours after the onset of a steady bright light.

2705-Pos Board B675

His75 in Proteorhodopsin, a Novel Component in Light-Driven Proton Translocation by Primary Pumps

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Proteorhodopsins (PRs), photoactive retinylidene membrane proteins ubiquitous in marine eubacteria, exhibit light-driven proton transport activity similar to that of the well-studied bacteriorhodopsin from halophilic archaea. However, unlike bacteriorhodopsin, PRs have a single highly conserved histidine located near the protein's photoactive site. Time-resolved FTIR difference spectroscopy combined with visible absorption spectroscopy, isotope labeling, and electrical measurements of light-induced charge movements reveal participation of His75 in the proton translocation mechanism of PR. Substitution of His75 with Ala or Glu perturbed the structure of the photoactive site and resulted in significantly shifted visible absorption spectra. In contrast, His75 substitution with a positively charged Arg did not shift the visible absorption spectrum of PR. The mutation to Arg also blocks the light-induced proton transfer from the Schiff base to its counterion Asp97 during the photocycle and the acid-induced protonation of Asp97 in the protein's dark state. Isotope labeling of histidine revealed that His75 undergoes deprotonation during the photocycle in the proton-pumping (high pH) form of PR, a reaction further supported by results from H75E. Finally, all His75 mutations greatly affect charge movements within the PR and shift its pH dependence to acidic values. A model of the proteorhodopsin proton transport process is proposed whereby (i) in the dark state His75 is positively charged (protonated) over a wide pH range and interacts directly with the Schiff base counterion Asp97; and (ii) photoisomerization-induced transfer of the Schiff base proton to the Asp97 counterion disrupts its interaction with His75 and triggers a histidine deprotonation.

2706-Pos Board B676

Slow quinone diffusion limits the photosynthetic rate in Phaeospirillum molischianum

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We have investigated the organization of the photosynthetic apparatus in Phaeospirillum molischianum using AFM, fractionation, functional kinetic measurements and modeling. The various proteins of the apparatus do not co-localize and specific membrane domains appear to be involved in light-collection and quinone reduction, while other regions are specialized in quinol oxidation or ATP synthesis. The overall turnover time of cyclic electron transfer is about 25 msec in vivo, and can be slowed to over 100 msec under oxidizing conditions. We show that the photosynthetic rate in this organism appears to be limited by a very slow quinone diffusion between the reaction center and cytochrome bc1 complex, a process that takes about 250 msec. This particularly slow diffusion appears to be compensated in part by the size of the quinone pool. In this context the details of the organization of the photosynthetic apparatus would seem critical to conserving a competitive bioenergetic system. It is possible that quinone excluding antennae domains are important for maintaining photosynthetic competence by channeling quinones between domains.
Our measurements highlight that the functional organization of the photosynthetic apparatus varies greatly between organisms, and that we observe in Phaeospirillum molischianum is very different from that observed in Rhodobacter sphaeroides.

2707-Pos Board B677

Characteristics of the Dark-Stable Multiline EPR Signal of Ca^{2+}-Depleted Photosystem II

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Photosystem II (PSII), which produces molecular oxygen using energy from light absorption, requires Ca^{2+} and Cl^- ions as inorganic cofactors. PSII shows two electron paramagnetic resonance signals that have been associated with the depletion or disruption of Ca^{2+} at the catalytic Mn_4Ca cluster: a dark-stable multiline signal from an S_2 state that decays very slowly, and a broad metalloradical signal from an S_2Y_Z state that is unable to proceed to higher oxidation states. The conditions for their formation were explored to help clarify how they are correlated. The dark-stable multiline signal was found to form in PSII prepared at pH 5.5 using itaconic acid buffer, a relative of citrate. The signal was very similar to the previously reported signal that is observed after EDTA treatment of PSII lacking the PsbP and PsbQ subunits. Both of these treatments, which employ Ca^{2+} chelators, also resulted in formation of the S_2Y_Z signal when PSII was illuminated in the presence of an electron acceptor. Treatment of intact PSII with fluoride, which is a competitor of Cl^- activation, resulted in formation of the S_2Y_Z signal, but not the dark-stable multiline signal. Fluoride may also interfere with Ca^{2+} function as a result of the high stability of the CaF_2 complex. These findings are examined in relation to the requirements of PSII for Ca^{2+} and Cl^-. (Supported by UNCG Office of Research).

2708-Pos Board B678

Photosystem II Supercomplexes Of Higher Plants: Isolation And Determination Of The Structural And Functional Organization

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Photosystem II is a supercomplex composed of 27-28 different subunits and it represents the most important machinery of the plants photosynthetic apparatus, having the ability to split water into oxygen, protons and electrons. In the last few years the structures of most of the photosynthetic complexes have been resolved, allowing to organize in a "visual framework" the large body of information obtained by genetics, biochemical and spectroscopic methods about the function and organization of the complexes. Only the structure of PSII-LHCII from higher plants is still lacking due to the impossibility to obtain a homogeneous and stable preparation of the supercomplex, which has also prevented functional and spectroscopic studies.
In this work homogeneous and stable Photosystem II supercomplexes with different antenna size were isolated. A full gallery of complexes, from the core to the largest C2S2M2, was characterized by electron microscopy and biochemical and spectroscopic methods, allowing to relate for the first time the supramolecular organization to the protein and pigment content and the energy transfer processes. A new complex containing a monomeric core, a trimeric LHCII (S) and a monomeric CP26 was isolated, showing that the antenna proteins can bind to the monomeric core in contrast to the current belief. The comparison of the supercomplexes obtained from WT plants and knock out mutants of several Lhcb proteins allowed determining the hierarchy of the assembly and to suggest a role for the individual subunits. The data also provides information about the organization of the oxygen evolving complex. For the first time it was possible to study the energy transfer process in the supercomplexes with the use of picosecond fluorescence spectroscopy.
The functional implication of these results on photoinhibition, state transition and energy transfer are discussed.

2709-Pos Board B679

Type I reaction center from the green sulfur bacterium *Chlorobium tepidum*: is Chl *a* a primary electron acceptor?

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The green sulfur bacterium *Chlorobium tepidum* has one of the simplest type I reaction center (RC) complexes. While its structure is still unknown, biochemical and protein sequence analyses suggest that it is similar to photosystem I (PS I), with two BChl *a* forming a special pair P840, four Chl *a* serving as pairs of accessory and primary electron acceptor (A_0) pigments and 14 BChl *a* constituting as an immediate RC antenna. This is a dramatic simplification compared to PS I RC, where 90 Chl *a* antenna pigments serve as antenna and 6 additional Chl *a* molecules function as electron transfer cofactors. The resulting spectral congestion has prevented direct visualization of ultrafast electron transfer processes within PS I RC and even the sequence of primary electron transfer processes in PS I is still under debate. The suggested presence of two types of pigments in RC from *Chlorobium tepidum* removes spectral congestion and opens a way to directly visualize electron transfer steps in type I RC using ultrafast spectroscopy, since the Chl *a* and BChl *a* pigments absorb at ~670 nm and ~800 nm, respectively. To confirm the proposed functional role of Chl *a* as