ABCA4 is a member of the superfamily of ATP binding cassette (ABC) proteins that is localized in outer segment disc membranes of rod and cone photoreceptor cells. Mutations in the *ABCA4* gene are responsible for Stargardt macular dystrophy, cone-rod dystrophy and retinitis pigmentosa. Biochemical studies together with analysis of *abca4* knockout mice implicate ABCA4 in the transport of N-retinylidene-phosphatidylethanolamine across disk membranes. This transport process facilitates the complete removal of retinal derivatives from photoreceptors following the photobleaching of rhodopsin and cone opsin as part of the visual cycle. Loss in the activity of ABCA4 leads to the production of diretinal derivatives in disc membranes which accumulate in adjacent retinal pigment epithelial (RPE) cells as lipofuscin deposits following phagocytosis of outer segments. Progressive buildup of these toxic diretinal compounds causes the degeneration of RPE and photoreceptors and a loss in vision. Recently, we have investigated the effect of C-terminal deletion, including several disease related mutations, on the structural and functional properties of ABCA4. Our studies indicate that ABCA4 contains a conserved motif near the C-terminus that is crucial for proper protein folding and functional activity of ABCA4. Individuals missing this motif due to C-terminal truncation of ABCA4 exhibit a severe form of retinal degeneration known as cone-rod dystrophy.

2716-Pos Board B686

Formation Of All-*Trans* Retinol In Mouse Rod Photoreceptors

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Light detection destroys the visual pigment of vertebrate rod photoreceptors, rhodopsin, as its retinyl moiety is photoisomerized from 11-*cis* to all-*trans*. Rhodopsin is regenerated through a series of reactions that begin in the rod outer segment with the release of the all-*trans* retinal and its reduction to all-*trans* retinol. All-*trans* retinol is then transported to the neighboring retinal pigment epithelial cells where it is used to remake 11-*cis* retinal. The reduction of all-*trans* retinal to all-*trans* retinol is catalyzed by retinol dehydrogenase and requires metabolic input in the form of NADPH. We have used the fluorescence of all-*trans* retinol to monitor its concentration in isolated mouse rod photoreceptors. After the bleaching of rhodopsin, all-*trans* retinol formation proceeds with a rate of ~0.06 min^{-1}, which is faster than the rate of rhodopsin regeneration in whole animals; this would allow recycled chromophore to contribute to the 11-*cis* retinal used for regeneration. Inner segment metabolic pathways appear to make a significant contribution to the pool of NADPH needed for the reduction of all-*trans* retinal, as formation of all-*trans* retinol is suppressed in rod outer segments separated from the cell body. Finally, generation of all-*trans* retinol is suppressed in the absence of glucose, indicating a critical dependence of all-*trans* retinol formation on the level of metabolic activity.

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Control Of Sensitivity Following Pigment Bleaching By NADPH In Salamander Rods

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The recovery of sensitivity following photopigment bleaching requires the quenching of phototransduction, and the reduction of all-trans retinal is key. Retinol fluorescence increases after bleaching as a base to tip gradient in the rod outer segment and broadly matches the recovery of sensitivity. This gradient must result from a key component in retinal reduction, and we sought to determine how NADPH limits this process. Rod outer segment currents were recorded with suction electrodes, and responses were evoked by brief full-field flashes or by a narrow slit to stimulate selectively the base or tip of the outer segment. Simultaneous whole-cell recordings were made prior to bleaching to dialyze the cell with NADPH and track the recovery of sensitivity. After a 50% bleach rods remained in saturation for ~ 12 minutes. The base recovered sensitivity with tau ~ 160 s, but the tip recovered with tau ~ 450 s resulting in a tip-base tau ratio of ~ 3. Dialysis of 5 mM NADPH accelerated the recovery time by ~ 2 min, and eliminated the tip-base difference. Dialysis with 1.66 mM NADPH didn't influence recovery and failed to eliminate the tip-base difference. After a 90% bleach rods remained saturated for ~ 20 minutes with tip-base ratio ~ 10 (base tau ~ 250 s and tip tau ~ 2700 s). Thus 5 mM NADPH eliminates the gradient along the outer segment, while 1.66 mM fails to influence the recovery of sensitivity; suggesting intrinsic NADPH exceeds 1.66 mM. In addition, tau at the base following 50% or 90% bleach are remarkably equivalent, suggesting that NADPH availability is sufficient to reduce all-trans retinal. The slower tip tau following 90% bleach suggests NADPH originates predominantly near the base, which is adjacent to mitochondria.

2718-Pos Board B688

An Additional Retinoid Binding Site in Rhodopsin

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Recently we discovered that some dark-adapted salamander rods and cones generated an electrical response to the truncated retinal analogue, β-ionone. This finding was tempered by observations that exposure to β-ionone led to pigment bleaching, and that very high concentrations of β-ionone inhibited rod channels in patch experiments. Therefore we examined whether β-ionone could activate the visual pigments by attacking the chromophore-binding pocket or through an interaction at an alternate binding site. Microspectrophotometry showed an accumulation of β-ionone in green-sensitive rod outer segments that increased linearly with bath concentration indicating that β-ionone most likely partitioned into disk membranes. β-Ionone also went into blue-sensitive rod outer segments, however, uptake was higher than in green-sensitive rods at all bath concentrations tested, suggesting that β-ionone bound to at least one site on rhodopsin in addition to partitioning. X-ray diffraction of green-sensitive rod rhodopsin crystallized in the presence of millimolar concentrations of β-ionone revealed a binding site located near the extracellular/intradiskal side of rhodopsin. β-Ionone may have low efficacy and low binding affinity because rhodopsin with ligand bound retained the inactive state conformation. β-Ionone is not a native retinoid, so we also examined the effects of retinol. Dark-adapted green-sensitive rods exposed to retinol lost sensitivity to flashes due to direct rhodopsin activation. In addition, rods exhibited a relative increase in sensitivity to shorter wavelengths, consistent with the ability of retinol to act as an antenna chromophore. Similar effects were seen for a blue-sensitive rod. These results support the presence in opsins of a retinoid-binding site(s) in addition to the chromophore-binding pocket, and suggest that this alternate site(s) mediates a number of distinct ligand-specific effects.

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Normal Function of the Cone Visual System Requires the Interphotoreceptor Retinoid Binding Protein (IRBP)

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An adequate supply of 11-*cis* retinal is essential to the normal function and survival of photoreceptors. Rods and cones are activated when light isomerizes 11-*cis* retinal to all-*trans* retinal, and continuous function requires the recycling of all-*trans* photoproducts back into 11-*cis* retinal. Cones mediate color vision and are the daytime photoreceptors most important for human vision, and their ability to function in constant light may be linked to a novel cone-specific visual cycle. The interphotoreceptor retinoid-binding protein (IRBP) is a proposed retinoid transporter in the visual cycle, but retinoid metabolism in the rods of *Irbp*$^{-/-}$ mice is surprisingly normal. Our goal was to analyze the cone population in *Irbp*$^{-/-}$ mice and explore IRBP's contribution to normal cone function. *Irbp*$^{-/-}$ mice have cone densities equivalent to *C57Bl/6 (WT)* and express normal levels of cone opsins. However, cone function measured by electroretinogram (ERG) is reduced in *Irbp*$^{-/-}$ mice. Because a visual cycle disruptions could result in an 11-*cis* retinoid deficiency, cone ERGs were measured in *Irbp*$^{-/-}$ mice before and after injections of 9-cis retinal. Treatment with 9-*cis* retinal rescued the cone response in *Irbp*$^{-/-}$ mice, but had no effect on *Irbp*$^{-/-}$ rods or *WT* responses. These data show that the absence of IRBP results in an 11-*cis* retinal deficiency for cones but not rods and indicate that IRBP is essential to normal cone function.

Mitochondria in Cell Life & Death

2720-Pos Board B690

Apoptosis in FL5.12 cells is suppressed by inhibitors of the Mitochondrial Apoptosis-induced Channel MAC

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The Mitochondrial Apoptosis-induced Channel (MAC) forms early in apoptosis and orchestrates cell death by releasing cytochrome c from the