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

Lorie R. Blakeley, Chunhe Chen, **Yiannis Koutalos**.
Medical University of South Carolina, Charleston, SC, USA.

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.

2717-Pos Board B687

Control Of Sensitivity Following Pigment Bleaching By NADPH In Salamander Rods

K. Joshua Miyagishima[1], M. Carter Cornwall[2], Alapakkam P. Sampath[1].
[1]USC Keck School of Medicine, Los Angeles, CA, USA, [2]Boston University School of Medicine, Boston, MA, USA.

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

Tomoki Isayama[1], Tetsuji Okada[2], James D. Looney[1], Rosalie K. Crouch[3], Anita L. Zimmerman[4], Clint L. Makino[1].
[1]Massachusetts Eye & Ear Infirmary, Boston, MA, USA, [2]Gakushuin University, Tokyo, Japan, [3]Medical University of South Carolina, Charleston, SC, USA, [4]Brown University, Providence, RI, USA.

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.

2719-Pos Board B689

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

Ryan O. Parker, Rosalie K. Crouch.
Medical University of South Carolina, Charleston, SC, USA.

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

Pablo V. Peixoto[1], Shin-Young Ryu[1], Bruno Antonsson[2], Kathleen W. Kinnally[1].
[1]New York University, New York, NY, USA, [2]Merck-Serono Pharmaceuticals, Geneva, Switzerland.

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

intermembrane space of mitochondria. MAC is a potential therapeutic target, as modulation of its opening could induce or prevent cell death. Compounds previously found to block Bax-induced release of fluorescein from liposomes were tested for their ability to directly inhibit the channel activity of MAC. Patch clamp techniques were applied to proteoliposomes containing mitochondrial outer membranes of apoptotic FL5.12 cells to monitor MAC activity. Several antagonists irreversibly blocked MAC with the IC50's ranging from 25 to 900 nM. These Inhibitors of MAC, or iMACs, were also effective in preventing cytochrome c release and progression of apoptosis induced by IL3 deprivation or staurosporine treatment. A matrix-targeted GFP facilitated visualization of the collapse of the mitochondrial network during staurosporine-induced apoptosis; this collapse was also prevented by iMACs. The action of these inhibitors demonstrates the tight links between MAC activity, cytochrome c release and apoptosis. Future studies will evaluate the impact of MAC on mitochondrial dynamics.

2721-Pos Board B691

Voltage-gated Potassium Channel In Brain Mitochondria

Krzysztof Dolowy[1], Piotr Bednarczyk[1,2], Joanna Kowalczyk[3], Adam Szewczyk[2], Barbara Zablocka[3].
[1]Warsaw University of Life Sciences - SGGW, Warszawa, Poland, [2]Nencki Institute of Experimental Biology, Warsaw, Poland, [3]Mossakowski Medical Research Institute, Warszawa, Poland.

Transient cerebral ischemia is known to induce endogenous adaptive mechanisms such as the activation of mitochondrial ATP regulated potassium channels or Ca^{2+} regulated large conductance potassium channels that can prevent or delay neuronal injury. In this study a single channel activity was measured after patch-clamp of the mitoplasts isolated from gerbil hippocampus. In 70% of the all patches, a potassium selective current was recorded with mean conductance 109 $\pm$ 6 pS in symmetrical 150 mM KCl solution. The patch-clamp single channel studies showed properties of the voltage-gated potassium channel (Kv channel). We found that ATP/Mg^{2+} complex and Ca^{2+} ions had no effects on observed activity of ion channel. Observed channel was blocked by negative voltage and margatoxin (MgTx) a specific Kv1.3 channel inhibitor. The inhibition by MgTx was irreversible. We conclude that gerbil hippocampus mitochondria contain voltage-gated potassium channel (mitoKv) with properties similar to the surface membrane Kv1.3 channel which can play a role in control function of mitochondria as well as in ischemia-reperfusion phenomenon.

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2722-Pos Board B692

Large-conductance Calcium-activated Potassium Channel In Neuronal Mitochondria

Adam Szewczyk[1], Marta Piwonska[1], Piotr Bednarczyk[1,2], Krzysztof Dolowy[2].
[1]Nencki Institute of Experimental Biology, Warsaw, Poland, [2]Warsaw University of Life Sciences - SGGW, Warsaw, Poland.

Large-conductance calcium-activated potassium (BK) channels are expressed in the plasma membrane of various cell types. Interestingly, recent studies provide evidence for existence of this channel also in mitochondria. The goal of the present study was to find a candidate for the regulatory component of the large conductance calcium activated potassium channel in neurons. A combined approach of western blot analysis, high-resolution immunofluorescence and immunoelectron microscopy with the use of antibodies directed against four distinct beta subunits demonstrated the presence of the BK channel beta4 subunit in the inner membrane of neuronal mitochondria in rat brain and cultured neurons. Additionally, channel activity was measured with the use of patch-clamp technique.

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2723-Pos Board B693

Biophysical Mechanism of Converting Apoptosis Regulator Bcl-2 from a Protector to a Killer in Cancer Cells By A Short Nur77-derived Peptide

Xuefei Tian[1], Siva Kumar Kolluri[2], Xiuwen Zhu[3], Bingzhen Lin[4], Ya Chen[4], Dayong Zhai[4], Feng He[1], Zhi Zhang[1], John C. Reed[4], Arnold C. Satterthwait[4], Xiao-kun Zhang[3], **Jialing Lin**[1].
[1]Univ of Oklahoma Health Sciences Center, Oklahoma City, OK, USA, [2]Cancer Biology Laboratory, Department of Environmental and Molecular Toxicology, Oregon State University, Corvallis, OR, USA, [3]4Burnham Institute for Medical Research, Cancer Center, La Jolla, CA, USA, [4]Burnham Institute for Medical Research, Cancer Center, La Jolla, CA, USA.

Bcl-2 can be converted into a pro-apoptotic molecule by nuclear receptor Nur77. The development of Bcl-2 converters as anti-cancer therapeutics has been explored by us. We reported recently the identification of a Nur77-derived Bcl-2 converting peptide (NuBCP) and its enantiomer, which induce apoptosis of cancer cells in vitro and in animals. The apoptotic effect of NuBCP enantiomers and their activation of Bax are not inhibited but rather potentiated by Bcl-2. Using fluorescence polarization assays, we determined that NuBCP enantiomers bind both quantitatively and stoichiometrically to the Bcl-2 loop, which shares the characteristics of structurally adaptable regions with many cancer-associated signaling proteins. NuBCP-9 enantiomers act as molecular switches to dislodge the Bcl-2 BH4 motif exposing its BH3 motif. Mechanistically we demonstrated, using fluorescence quenching based liposome assays, that NuBCP-9-induced Bcl-2 conformational change not only neutralizes Bcl-2's inhibition of Bax-mediated membrane permeabilization but also exposes the Bcl-2's BH3 motif that in turn neutralizes Bcl-X_L's inhibition of Bax like BH3 motif-derived peptides and compounds. Our results provide mechanistic insight into Bcl-2 conversion and identify a new direction for developing Bcl-2-based cancer therapeutics.

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2724-Pos Board B694

Respiratory Complex I Dysfunction Due to Mitochondrial DNA Mutations Shifts the Voltage Threshold for Opening of the Permeability Transition Pore toward Resting Levels

Anna Maria Porcelli[1], Alessia Angelin[2], Anna Ghelli[1], Elisa Mariani[1], Andrea Martinuzzi[3], Valerio Carelli[1], Valeria Petronilli[4], Michela Rugolo[1], **Paolo Bernardi**[2].
[1]University of Bologna, Bologna, Italy, [2]University of Padova, Padova, Italy, [3]IRCSS Medea, Conegliano, Italy, [4]CNR Institute of Neuroscience, Padova, Italy.

We have studied mitochondrial bioenergetics in HL180 cells (a cybrid line harboring the T14484C/ND6 and G14279A/ND6 mtDNA mutations of Leber hereditary optic neuropathy, leading to an about 50% decrease of ATP synthesis) and XTC.UC1 cells (derived from a thyroid oncocytoma bearing a disruptive frameshift mutation in the MT-ND1 gene, which impairs complex I assembly). Addition of rotenone to HL180 cells and of antimycin A to XTC.UC1 cells caused fast mitochondrial membrane depolarization that was prevented by treatment with cyclosporin A, intracellular Ca2+ chelators, and antioxidant. Both cell lines also displayed an anomalous response to oligomycin, with rapid onset of depolarization that was prevented by cyclosporin A and by overexpression of Bcl-2. These findings indicate that depolarization by respiratory chain inhibitors and oligomycin was due to opening of the mitochondrial permeability transition pore (PTP). A shift of the threshold voltage for PTP opening close to the resting potential may therefore be the underlying cause facilitating cell death in diseases affecting complex I activity. This study provides a unifying reading frame for previous observations on mitochondrial dysfunction, bioenergetic defects and Ca2+ deregulation in mitochondrial diseases. Therapeutic strategies aimed at normalizing the PTP voltage threshold may be instrumental in ameliorating the course of complex I-dependent mitochondrial diseases.

2725-Pos Board B695

Bax Enhances the Permeabilization of the Mitochondrial Outer Membrane Induced by Ceramide Channels: Implications on the Regulation of the Initiation of Apoptosis

Meenu N. Perera, Vidyaramanan Ganesan, **Marco Colombini**.
University of Maryland, College Park, MD, USA.

Background: Bax is a pivotal pro-apoptotic Bcl-2 family protein that localizes to the mitochondrial outer membrane (MOM) during apoptosis and causes MOM permeabilization to proteins (MOMP). Earlier studies have demonstrated that upon an apoptotic stimulus, ceramide levels often greatly increase in cell membranes, including in the MOM. Elevation of ceramide in the MOM is sufficient to cause MOMP without requiring Bcl-2 family proteins. Moreover ceramide induced MOMP is reversed/prevented by the anti-apoptotic protein, Bcl-xL. **Methods:** Using rat liver or yeast mitochondria, the MOMP was measured with a dynamic cytochrome c accessibility assay. Ceramide's channel-forming ability was also assessed using a defined system: planar phospholipid membranes. Only C_{16}-ceramide was used. **Results:** We found that Bax induces MOMP by apparently enlarging ceramide channels. While ceramide forms