mouse hearts were subjected to ischemia followed by reperfusion. The infarct size was ~4 fold larger in LP animals compared to NP both in the in-vivo rat model and in the ex-vivo mouse model. The hemodynamic parameters were similar between NP and LP before ischemia. However, after ischemia, the functional recovery was extremely poor in LP mice comparing to NP mice. RPP was reduced from 12818 ± 1485mmHg*beats/min in NP to 1614 ± 438mmHg*beats/min in LP mice. Interestingly, the poor functional recovery and the larger infarct size in LP was partially restored one day post-partum(PP1) and almost fully restored one week post-partum(PP7) to their corresponding levels in NP hearts(e.g. RPP=4716 ± 584mmHg*beats/min in PP1 and 9604 ± 1216mmHg*beats/min in PP7). To explore the role of mithocondiral function in the higher vulnerability of LP hearts to I/R injury, mithocondria was isolated from NP and LP hearts. State 3 oxygen consumption and respiratory control index(RCI) in malate and glutamate energized mitochondria were significantly decreased in LP((93 ± 5 vs. 162 ± 19nAO/min/mg in NP, $P<0.05$; RCI=2.6 ± 0.24 in LP vs. 4.2 ± 0.22 in NP , $P<0.05$). The threshold for opening of the mitochondrial permeability transition pore (mPTP) was much lower in LP hearts (CRC=167 ± 10 vs. 233 ± 18nM/mg protein in NP, $P<0.01$). In conclusion, the higher vulnerability of LP hearts to I/R is associated with increased sensitivity of the mPTP opening to calcium overload and reduced mitochondrial respiration.

822-Pos Board B608

Ischemia Reperfusion Induced Arrhythmia are Prevented by Mitochondrial IK,ATP Opening by Mesenchymal Stem Cell (MSC) Paracrine Factors

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MSC transplantation after I/R injury reduces infarct size and improves cardiac function. There is evidence that this cardioprotective effect is mediated by paracrine signalling, but the mechanism remains yet to be determined. Isolated mouse ventricular myocytes (VMs) were used in an in vitro model of I/R to determine the effect of MSC conditioned tyrode (ConT) on the recovery of VMs from an ischemic challenge (15 min). Measurements of the mitochondrial membrane potential (Ψmito) with TMRM (100 nM), the intracellular Ca concentration (fluo-4/AM) and cell shortening were used as functional readouts. During ischemia VMs exhibit a depolarization of Ψmito and an increase in diastolic calcium concomitant to a decrease in cell contractility. Reperfusion with either Ctrl or ConT resulted in an increase in Ca transient amplitudes. Early After Depolarizations (EADs) frequently observed in Ctrl cells were reduced during ConT reperfusion (EADs: ConT:1% vs. Ctrl: 24%; at 1 min). ConT prolonged VM survival (ConT: 58% vs Ctrl: 33%; at 20 min) and Ca transients returned to pre-ischemic values. After I/R, Ψmito rapidly recovered in Ctrl as well as ConT; however, in Ctrl an exaggerated hyperpolarization of Ψmito (Ctrl: 6 of 9; ConT: 0 of 5 cells) was observed. This hyperpolarization could be prevented by supplementing Ctrl solution after I/R with the ROS scavenger mitoTEMPO (5 μM) or the IK,ATP opener (diazoxide, 200 μM). Enhanced hyperpolarization was induced by supplementing ConT with a blocker of IK,ATP 5-HD (500 μM) or PI3K/Akt inhibitors (LY: 10 μM; Akt iV: 20 μM). In conclusion we could demonstrate that MSC ConT protects VMs from I/R injury by attenuation of arrhythmic Ca release events and by delaying the recovery of Ψmito through PI3K/Akt mediated opening of IK,ATP.

823-Pos Board B609

Reduced Mitochondrial Dynamics in Skeletal Muscle of an Amyotrophyic Lateral Sclerosis Mouse Model

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Mitochondria are highly dynamic organelles that constantly undergo fusion and fission in order to maintain their normal functionality. Impairment of mitochondrial dynamics is implicated in various neurodegenerative disorders. Amyotrophic lateral sclerosis (ALS) is a fatal disease involving degeneration of motor neurons and atrophy of skeletal muscle. Our recent studies on ALS mouse model G93A have found that its skeletal muscle shows defective mitochondria associated with elevated intracellular Ca^{2+} transients during muscle contraction (*Yi JBC 2011*). It is known that an elevated intracellular [Ca^{2+}] alters mitochondrial mobility in various cell types (*Liu and Hajnóczky Int J Biochem Cell Biol 2009*). Expecting alterations, we evaluated mitochondrial mobility by expressing a photoactivatable green fluorescence protein (PA-GFP) (obtained from Addgene) in G93A skeletal muscle. The PA-GFP was first photo-activated in a small area (~ 10 μm × 10 μm) of a muscle fiber. The time-dependent migration of PA-GFP out of the original area was evaluated in both longitudinal and transversal directions. Migration of PA-GFP over one sarcomere distance (~2.2 μm) was defined as one migration step (*ms*). While mitochondria in normal muscle fibers showed 11.0 ± 1.6 *ms* in 10 min and 18.7 ± 2.3 *ms* in 20 min (n=13), mitochondria in G93A muscle only showed 1.8 ± 0.4 *ms* and 2.0 ± 0.4 *ms* in the respective intervals (n=12). This constitutes a near 10-fold reduction of mitochondrial mobility in ALS muscle. Studies to identify the causes of the reduced mitochondrial dynamics are ongoing. Supported by MDA and NIAMS/NIH.

824-Pos Board B610

Mitochondrial Respiration-Coupled Superoxide Production Underlies Glutamate Excitotoxicity in Motor Neurons

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Glutamate excitotoxicity is responsible for neuron death during both acute neuron injury and chronic neurodegeneration. However, its specific mechanism is not clear. Cytosolic calcium influx and excessive reactive oxygen species generation have been suggested to play important roles. We hypothesized that glutamate-induced neuron death is mediated by cytosolic calcium influx, subsequent mitochondrial permeability transition pore (PTP) openings, and triggered mitochondrial superoxide production. Cultured NSC-34 cells, the motor neuron-like cell line, were differentiated to NSC-34D cells, which express glutamate receptors. Confocal imaging of the NSC-34D cells expressing a newly developed superoxide indicator, mt-cpYFP, revealed a bursting superoxide production event in individual mitochondria, named superoxide flash. The frequency of superoxide flash was positively correlated to mitochondrial respiration. Interestingly, glutamate (1 mM) incubation stimulated superoxide flash activity up to 24 hr, but inhibited it after 48 hr. The glutamate-induced superoxide flash activity was accompanied by a transient cytosolic calcium influx, which was blocked by glutamate receptor inhibitors, MK-801 (10 μM) and NBQX (1 μM), and mitochondrial calcium uniporter inhibition. We previously showed that superoxide flash is a triggered event by PTP openings, which is modulated by calcium. Simultaneous imaging of mitochondrial membrane potential using TMRM and superoxide flash showed that dissipation of membrane potential accompanied each flash in NSC-34D cells. Further, glutamate-induced flash activity was potentiated by atractyloside, a PTP opener, and abolished by cyclosporine A, a PTP blocker. Finally, cell death occurred at 24 hr after glutamate incubation. SOD1 and SOD2 overexpression blocked the glutamate-induced superoxide flash activity and cell death. In summary, we identified a signaling pathway mediating the glutamate excitotoxicity in motor neurons. This pathway includes cytosolic calcium influx-associated mitochondrial calcium uptake, calcium induced PTP opening, and bursting superoxide production coupled to mitochondrial respiration.

Photosynthesis & Photoreceptors

825-Pos Board B611

Correlated AFM-Spectroscopy Imaging of Linear Light Harvesting Protein Aggregates in Bacterial Native Photosynthetic Membrane

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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. The specific photosynthetic architecture facilitates a rapid and efficient energy transfer among the light harvesting proteins (LH2 and LH1) and to the reaction center. Here we report the identification of linear aggregates of light harvesting proteins, LH2, in the photosynthetic membranes under ambient conditions by using atomic force microscopy (AFM) imaging and spectroscopic analysis. Our results suggest that the light harvesting proteins, LH2, can exist as linear aggregates of 2 to 8 proteins in the photosynthetic membranes, and the protein distributions are highly heterogeneous. LH2 antenna proteins are responsible for absorbing most of the light energy for photosynthesis, and efficient intra- and intermolecular energy transfers of LH2 complexes are important for the overall efficiency of the light harvesting mechanism. We combined AFM imaging and spectroscopic analysis with *J* aggregate theoretical calculations to characterize the linear aggregation of LH2. AFM images reveal the linear aggregation of LH2, where the LH2 complexes are tilted to the plane of the photosynthetic membrane. The spectroscopic results support the attribution of LH2

complexes in the membrane to linear aggregates. The calculated values for the absorption, emission and lifetime using a model developed from *J* aggregate theory are consistent with the experimentally determined spectroscopic values, further proving a *J* type aggregation of the LH2 complexes in the photosynthetic membrane.

826-Pos Board B612

Maximal Coherence at Room Temperature in the Bacterial Photosynthetic Reaction Center

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The earliest steps in bacterial photosynthesis require that an antenna system efficiently capture incident photons and shuttle the excitation energy to the ''special pair'' bacteriochlorophylls within the membrane-bound reaction center where charge separation occurs. Previous work has shown coherent energy transfer - a wavelike transfer process - among peripheral chromophores, bacteriopheophytins and accessory bacteriochlorophylls, at cryogenic temperatures. Whether or not this coherent transfer extends to the special pair, however, has remained elusive at any temperature. Here we report direct evidence that the special pair is coherently coupled to the accessory bacteriochlophylls and that this coherence dephases only upon transfer to the special pair - the maximal amount of coherence physically possible. We employ Gradient Assisted Photon Echo Spectroscopy to simultaneously excite the bacteriopheophytins, accessory bacteriochlorophylls and the special pair in the reaction center from *Rhodobacter sphaeroides*. These results suggest the bacteria exploits coherent energy transfer at room temperature.

827-Pos Board B613

Efficient Intrinsic Photoprotection in Strongly Coupled (Bacterio) Chlorophyll Complexes

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Chlorophyll (Chl) molecules in photosynthetic proteins are known to produce highly toxic singlet oxygen under illumination as a result of energy transfer from their triplet excited states to oxygen. To prevent the formation of singlet oxygen, carotenoids (Car) are typically positioned close to Chl to ensure rapid triplet-triplet energy transfer from Chl to Car, which can then safely dissipate the energy of the excited states. Our recent studies revealed a new, unconventional, but very efficient photoprotection mechanism in strongly coupled natural and artificial light-harvesting complexes that does not rely on the presence of carotenoids. Experimental studies on carotenoid-free chlorosomes and artificial bacteriochlorophyll (BChl) aggregates show that these structures are at least three orders of magnitude more stable to photodamage than monomeric forms of BChl. It was proposed that this photoprotection in strongly coupled arrays of pigments is due to triplet exciton formation, which lowers the energy of the triplet exciton substantially below that of singlet oxygen.

In this report we present the results of our ongoing comprehensive study of the properties of triplet excited states of monomeric BChls, Chl aggregates and chlorosomes by means of EPR, time-resolved optical pump-probe spectroscopy and steady-state IR phosphorescence spectroscopy.

828-Pos Board B614

Computational Modeling of Excitation Energy Transfer in Xanthorhodopsin, a Model Light-Harvesting System

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Xanthorhodopsin (xR), a light-driven proton pump, is unique among the rhodopsin family because it contains not one, but two strongly interacting chromophores, the retinal and a carotenoid. The carotenoid is bound to the protein and acts as a light-harvesting antenna, transferring energy to the retinal with ~40% quantum efficiency (Balashov et al., *Science***309**, 2005). Unlike photosynthetic complexes in which excitation energy transfer (EET) occurs from a large collection of antennas to a reaction center, the EET in xR occurs directly between the retinal and a single bound carotenoid. Thus, xR serves as a simple (and computationally accessible, albeit challenging) model system for understanding EET in light-harvesting systems. Here, we present the results of long (aggregate length $>$ 350 ns) classical molecular dynamics (MD) simulations, starting from the x-ray crystal structure of xR (Luecke et al., *PNAS* **105**, 2008), coupled with single-point quantum mechanical (DFT) calculations performed on hundreds of simulation snapshots, to address two points. First, because the resolution of the crystal structure is not sufficiently high to resolve protons, the protonation state of the counterion complex (specifically, the His62-Asp96 pair) is unknown, so we ran four MD simulations in parallel, each with a different combination of protonation states for the His-Asp pair. The MD simulations, as well as DFT calculations of the retinal excited state energies, identify a single state that is most consistent with experimental data. Second, we probe the effects of small structural changes, which occur within the context of a thermally disordered lipid (POPC) bilayer, on the retinal excitation energies, and we conclude that the environment could play an important role in the EET between the bound carotenoid and the retinal.

829-Pos Board B615

Structural Dynamics in Chloroplast Signal Recognition Particle (cpSRP) Proteins Studied with Single Molecule Fluorescence

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The interdomain structural dynamics of cpSRP43 protein interacting with cpSRP54 protein is studied by single molecule FRET, FCS, and ensemble fluorescence spectroscopy in order to understand the protein-protein interactions that help to transport LHCP into the thylakoid membrane for photosynthesis. Several double Cys and single Cys mutants of cpSRP43 and cpSRP54 proteins are cloned, expressed, purified and then specifically labeled with fluorescent dyes. The single molecule FRET and FCS equilibrium binding results suggest that the fluorescence labeling does not affect the cpSRP43-cpSRP54 interaction. Furthermore, we find that cpSRP43 proteins are very heterogeneous in structure and that cpSRP43 proteins undergo complex interdomain structural dynamics when interacting with cpSRP54 protein.

830-Pos Board B616

Kinetic Model for Assessing the Effect of pH-Dependent Nonphotochemical Quenching of Chlorophyll Excitations on the Energetic Output of Chloroplasts

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The controlled dissipation of chlorophyll excitations protects photosynthetic organisms from inhibition of photosystem II. This dissipation, commonly known as nonphotochemical quenching (NPQ), enhances plants' fitness in natural conditions where sunlight intensity fluctuates. To identify the properties of feedback loop(s) controlling NPQ that enable it to effectively balance light harvesting and photoprotection, we have developed a mathematical model of photosystem II that incorporates molecular mechanisms for nonphotochemical quenching containing the PsbS protein and the xanthophyll cycle. The model accurately reproduces measurements of chlorophyll fluorescence over several minutes in intact leaves of the plant Arabidopsis thaliana. The model enables calculation of the effect of NPQ on both photoinhibition and energy consumption by the carbon reactions. This calculation provides a framework for quantifying the role of feedback-regulated photoprotection in enhancing the ability of plants to thrive in variable light conditions. Because the model incorporates mechanistic details, it has the potential to inform on modifications to improve the feedback loop controlling rapid nonphotochemical quenching to optimize the role of PSII regulation in maximizing biomass production.

831-Pos Board B617

Measurement of the Microscopic Dynamics of Photoprotection in Living Cells of Green Algae

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Photosynthetic organisms reduce inhibition of photosystem II (PSII) in variable light conditions by using a suite of photoprotective mechanisms called