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

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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

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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

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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

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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

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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

channels independently of Bax, the permeabilization is enhanced by the addition of less than 5nM oligomeric Bax. As much as 50nM oligomeric Bax alone did not result in any significant MOMP. The Bax enhancement occurs with an apparent affinity that increases with an increase in ceramide-induced MOMP, indicating an underlying mechanism by which Bax enhances ceramide-induced MOMP. Bax also causes apparent ceramide channel enlargement in yeast mitochondria, which lack Bcl-2 family proteins, as well as in planar phospholipid membranes, which is a defined, protein free, system. By contrast, monomeric Bax has no effect on ceramide channels in the aforementioned systems. The Bax inhibitor, Bci2 [Bruno Antonsson], prevents Bax mediated channel enlargement but does not affect permeabilization induced by ceramide alone. **Conclusions:** Both pro- and anti-apoptotic proteins regulate ceramide channels, consistent with ceramide channels being the pathway by which proteins are released by mitochondria early in apoptosis. (Supported by NSF grant: MCB-0641208)

2726-Pos Board B696

Bcl-2 Does Not Inhibit Bax Insertion During Intrinsic Apoptosis

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Mitochondrial outer membrane (MOM) permeabilization and cytochrome c release from mitochondria into the cytosol are considered to be the commitment steps of the intrinsic apoptotic pathway. Cytochrome c release is regulated by the Bcl-2 family proteins, which contains both pro-apoptotic (e.g. BAX) and anti-apoptotic (e.g. Bcl-2) members. It is now well established that after a death signal, cytosolic BAX is translocated to the mitochondrial outer membrane, inserted then in the double leaflet and activated through a conformational change. Activated BAX oligomerizes and might be associated to other mitochondrial proteins, leading to the formation of the Mitochondrial Apoptosis-induced Channel (MAC) into the MOM. This channel allows the release of cytochrome c into the cytosol. Bcl-2 inhibits MAC formation and therefore, cytochrome c release. However, the molecular mechanisms through which Bcl-2 affects earlier steps of BAX-mediated apoptosis are not fully understood.

In this study we investigated the effects of Bcl-2 over-expression on BAX-mediated apoptosis. We were able to confirm that Bcl-2 over-expression inhibits BAX translocation to the MOM and activation/oligomerization, as previously reported. Bax translocation is generally considered as the primary target of Bcl-2. Surprisingly, Bcl-2 over-expression did not alter the insertion status of BAX into the MOM. These data point out the further step, BAX activation/oligomerization, as the primary target of Bcl-2. Since Bcl-2 does not inhibit BAX insertion, we hypothesize that an event occurring after cytochrome c release triggers somehow a positive feedback on Bax expression and translocation. Thus, the inhibition of cytochrome c release by Bcl-2 could explain the further blocking on BAX translocation.

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2727-Pos Board B697

Estrogen-induced Protection of Heart Ischemia-reperfusion Injury by the Inhibition of the Mitochondrial Permeability Transition Pore (mPTP) in Isolated Heart Mouse

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Although several studies have shown that the administration of 17ß-estradiol (E2) has a cardioprotective effect during ischemia-reperfusion (I/R), the mechanisms of this action are largely unknown. In this study, we investigated the effects of E2 on the opening of mPTP and as well on the myocardial infarct size after global myocardial I/R injury. Hearts of male mice were isolated and retrograde-perfused through aorta with the Langerdoff system at 37 oC. After 20 min of perfusion, hearts were subjected to 20min global ischemia followed by 40min reperfusion. Mitochondria were isolated to measure Calcium Resistance Capacity (CRC) and mPTP installation; infarct size was evaluated by triphenyltetrazolium chloride staining (TTC). Experiments were performed in hearts perfused with Krebs Henseleit solution or with Krebs Henseleit + E2 (100 pg/ml, corresponding to E2 peak concentration at proestrus). The E2-treated group had increased CRC (0.73+0.11 μM vs. 1.2+ 0.06, $p < 0.01$) and a reduced infarct size (43 ± 3% vs. 68 ± 5%, $p < 0.01$) compared to the control group. The E2-induced infarct size reduction was abolished by the specific estrogen receptor antagonist ICI 182,780 (100 nM). These results indicate that a brief E2 exposure favors CRC by inhibiting the mPTP installation resulting in a reduction of the infarct size. We propose these actions as a mechanism for E2-induced protection during I/R in isolated hearts.

2728-Pos Board B698

In vivo Imaging of Superoxide Flashes in Skeletal Muscle

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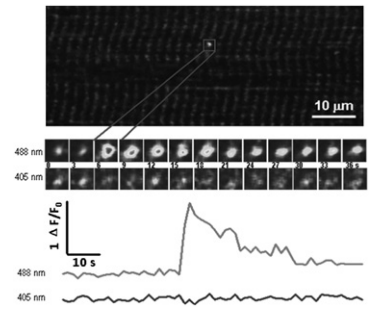
Emerging evidence suggests that reactive oxygen species (ROS) constitute a class of signaling molecules that regulate diverse cell functions including metabolism, muscle contractility and apoptosis. Recently we have developed and characterized a highly sensitive and reversible superoxide-selective probe, a circularly permuted yellow fluorescent protein (cpYFP), and demonstrated quantal and transient superoxide-producing events (superoxide flashes) within single mitochondria across multiple cell types (Wang et al, *Cell*, 132, 279).To further understand the physiological significance of flash events, we generated the pan-tissue mt-cpYFP transgenic mice expressing cpYFP in the mitochondria of multiple tissues. *In vivo* imaging of superoxide signals in gastrocnemius of transgenic mouse under anesthesia revealed mitochondrial superoxide flashes with similar properties (Fig). Further, superoxide flashes were also visualized in isolated skeletal muscle fibers transfected *in vivo* by electroporation with mt-cpYFP. Our findings support that mitochondrial superoxide flash activity is a physiologically relevant phenomenon that may participate in diverse aspects of cell function and signaling.



2729-Pos Board B699

Visualization Of Mitochondria-targeted Photodynamic Effects Of Hpph-in Coupled With Visible Laser 637 Nm In Osteosarcoma 143b Cells

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Photosensitizer HPPH cooperated with metal complex containing In (III) (HPPH-In) produces much efficient singlet oxygen production and photodynamic effects (PDE) as compared to HPPH alone. With the application of mitochondrial fluorescent probes and laser scanning imaging microscopy, mitochondrial level of PDE induced by HPPH-In coupled with visible laser 637 nm were investigated in detail. PDE of HPPH-In significantly enhances depletion of a mitochondria specific fluorescent probe MitoTracker Green at very earlier time points suggesting its primary targeting on the mitochondrial membrane. Mitochondria soon swelled and followed by plasma membrane blebing and later apoptotic condensation of nuclei and cell death. These mitochondria-associated apoptotic events induced by PDE of HPPH-In can be partially inhibited by a mitochondria antioxidant, MitoQ, and by the removal of extracellular Ca^{2+} suggesting a mROS- and Ca^{2+}-dependent mechanism is involved. When mitochondrial reactive oxygen species (ROS) formation and mitochondrial membrane potential depolarization ($\Delta\Psi$) were imaged simultaneously, PDE of HPPH-In significantly enhanced mROS formation and $\Delta\Psi$ depolarization with small delay. PDE of HPPH-In-induced increase in mROS soon propagated to adjacent non-irradiated mitochondrial population as well as that in adjacent cells and caused depolarization of$\Delta\Psi$ of these non-irradiated mitochondria. In addition to PDE of HPPH-In-enhanced mROS formation, we observed PDE of HPPH-In-induced sudden depolarization of $\Delta\Psi$effectively reduced mROS formation suggesting a possible protective preconditioning may exist. Finally, PDE of HPPH-In significantly altered mitochondrial dynamics by decreasing mitochondrial movement and enhancing fission of mitochondria. These observations suggest that multiple mitochondria-targeted devastating mechanisms provided by the PDE of HPPH-In coupled with 637 nm laser