attenuated the lifespan of Anti1-deficient mice. Our results prove mtDNA dictates the penetrance of age-related cardiomyopathy and mammalian lifespan. Therefore, therapeutics that most effectively preserve mitochondrial DNA and bioenergetics will provide the most promise for healthy aging.

**3066-Pos Board B498**

EPR Data Support the Existence of a Symmetric BH3-in-Groove Homodimer in Oligomeric BAK

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The BAX or BAK oligomeric pore formation in the mitochondrial outer membrane is a critical step in apoptosis, yet their structures are not clearly understood. Czabotar et al. (Cell 2013, 152, 519) reported a crystal structure of a water-soluble tetramer of the BAX fragment (helices a2-a5) fused to the green-fluorescent protein (GFP), in which the a2-a3 extended helices and the a5 helix, respectively, were juxtaposed to each other, in an anti-parallel orientation, forming a symmetric “B3-in-groove homodimer (BGH).” We have constructed a GFP-BAK fusion protein using the a2-a5 helices of mouse BAK, designated as GFP-BAK2-a5, which also forms a soluble tetramer. To determine whether the BGH exists in the BAK oligomers in the membrane, or not, we spiked the GOH- or BH3-bearing BH3-in-groove soluble form of mouse BAK (helices a1-a8) at residues 84, 122, 128 and 135 and the corresponding residues in GFP-BAK2-a5. We then compared the continuous wave (CW) EPR spectra of the spin-labeled residues from the tetrameric GFP-BAK2-a5 with those from the oligomeric BAK in membrane. Spin labeled residue 122R1, located in the loop connecting helices a4 and a5 in the homology model of the GFP-BAK2-a5 tetramer, displayed a mobile lineshape. The corresponding residue in the oligomeric BAK also had a remarkably similar lineshape, indicating that the two residues are in similar structural environments. Residues 84R1, 128R1 and 135R1, located at the anti-parallel helical interfaces in the BGH also had remarkably similar immobile lineshapes both in the GFP-BAK2-a5 tetramer and in the oligomeric BAK in membrane, further strengthening the above conclusion. The intra-dimer distances between 84R1 spin label pairs in the GFP-BAK2-a5 and the oligomeric BAK, determined by the double electron electron resonance (DEER) method, also support this interpretation.

**3069-Pos Board B499**

Modulation of Membrane Interactions of Anti-Apoptotic Regulator Bcl-xL by Lipids

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The Bcl-2 family of proteins (e.g., pro-apoptotic Bax and anti-apoptotic Bcl-xL) regulates the mitochondrial outer membrane permeabilization during the early stages of apoptosis. The prevalent Embedded Together Model of Bcl-2 action suggests that the membrane environment is critical for their proper functional interactions, consistent with the increasing evidence of lipids being involved in the regulation of apoptotic response. In this study, we apply a collection of fluorescence-based methods to investigate the effect of various lipids on the pH-triggered membrane interactions of Bcl-xL. The initial membrane association was studied using a FRET assay with donor-labeled Bcl-xL and acceptor-labeled vesicles, while the insertion/refolding of Bcl-xL into the membrane was monitored using negatively charged anionic lipids and the pH-triggered membrane interactions of Bcl-xL. The initial membrane association and subsequent insertion/refolding step is more complex and appears to be influenced by the size of the lipid headgroup. The kinetics of both the membrane association and membrane insertion/refolding is affected by the presence of non-bilayer forming lipids commonly found in mitochondria. While the presence of phosphatidylethanolamine accelerated the process, addition of lysophosphatidylcholine had the opposite effect, suggesting that mechanical properties of the bilayer also play a role. Taken together our results indicate that lipids can modulate the membrane interactions of Bcl-xL in multiple ways, providing an additional regulatory mechanism that ensures proper control of a complex cascade of apoptotic reactions leading to cell death or survival. NIHGM-069783, Fullbright-CONICYT, BRTP.
ergic stimulation confirmed that only two tyrosine sites were phosphorylated in response to adrenergic signaling induces activation of proline-rich tyrosine kinase 2 (Pyk2) and
mitochondrial outer membrane in the presence of UDCA and/or TUDCA.

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3071-Pos Board B501

MAC Inhibitors Neutralize the Pro-Apoptotic Effects of Tbid
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Since our initial characterization of the iMACs, different di-bromocarbazole derivatives with anti-apoptotic function have been developed and tested in several mouse models of brain injury and neurodegeneration [13-21]. Owing to the increased therapeutic potential of anti-apoptotic di-bromocarbazole derivatives, we sought to expand our knowledge of the mechanism of action of these small molecule inhibitors. We investigated the kinetics of MAC inhibition in mitochondria from wild type, Bak, and Bak knockout cell lines using patch clamp electrophysiology, fluorescence microscopy, ELISA, and quantitative western blot analyses. Our results show that iMACs work through at least two mechanisms: 1) by blocking relocation of the cytoplasmic Bax protein to mitochondria and 2) by disassembling Bax oligomers in the outer membrane. A comparison of the inhibitory effects over channel conductance and cytochrome c release suggests that the iMACs interacted with both Bak and Bax with similar kinetics. Interestingly, wild type mitochondria were more susceptible to inhibition than the Bak or Bax knockouts. A quantitative western blot analysis showed that wild type mitochondria had lower steady state levels of Bak, which suggests an uneven stoichiometry of the MAC components.

3072-Pos Board B502

Tyrosine Phosphorylation of Mitochondrial Ca2+ Unipporter Regulates Mitochondrial Ca2+ Uptake
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Mitochondrial Ca2+ has a critical role for balancing cell survival and death. Ca2+ influx into mitochondrial matrix is mediated primarily by the mitochondrial calcium uniporter (MCU). However, the signaling pathways that regulate MCU channel functions via post-translational modifications of MCU are completely unknown. Here we show that adrenergic signaling induces MCU tyrosine phosphorylation and accelerates mitochondrial Ca2+ uptake in cardiac cells. Adrenergic signaling induces activation of proline-rich tyrosine kinase 2 (Pyk2) and translocation into the mitochondrial matrix; enhancing the interaction between Pyk2 and MCU, which subsequently accelerates mitochondrial Ca2+ uptake via Pyk2-dependent MCU tyrosine phosphorylation. MCU contains 15 tyrosine residues (5 in the N-terminus, 0 in the pore-forming region, 4 in transmembrane domains and 6 in the C-terminus), which are conserved across all eukaryotic species. Among them, only 3 of these tyrosine residues (Y157 at N-terminus, Y288, and Y1061 at C-terminus) in mouse MCU were found to be tyrosine phosphorylation candidate sites for protein tyrosine kinases using phosphorylation prediction programs. We mutated these tyrosine residues to phenylalanine and generated non-phosphorylation mimetic MCU mutants (MCU-YFs). We confirmed that only two tyrosine sites were phosphorylated in response to adrenergic stimulation in situ using cell lines stably expressing MCU-YFs. In addition, overexpression of these MCU-YFs failed to increase mitochondrial Ca2+ uptake in response to cytosolic Ca2+ elevation by thapsigargin, whereas wild-type MCU transfections dramatically accelerated mitochondrial Ca2+ uptake compared to non-transfected cells. In summary, MCU contains Pyk2-specific phosphorylation site(s) and Pyk2-dependent tyrosine phosphorylation of MCU can modulate its channel functions and regulate mitochondrial Ca2+ uptake.

3073-Pos Board B503

Cardioprotective Roles of Neuronal Ca2+ Sensor-1 during Stress
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Disregulation of Ca2+ homeostasis in cardiomyocytes often results in heart failure. Identifying molecular targets that regulate cardiomyocyte survival is of therapeutic importance. Neuronal Ca2+-sensor-1 (NCS-1) is an EF-hand Ca2+-binding protein, which is important for excitable cell functions. We previously found that NCS-1-deficient (Ncs1−/−) mice had excess neonatal mortality (Circ. Res. 2011). The aim of the present study is to examine whether NCS-1 plays beneficial roles in cardiac survival during stress and the possible mechanisms under-lying these effects. Neonatal mouse ventricular myocytes or whole hearts from wild-type (WT) and Ncs1−/− mice were subjected to stressors, and the resistance to stress was evaluated. Ncs1−/− mice hearts were more susceptible to stress induced by oxidative impairment and ischemia-reperfusion injury. Stress-induced activation of phosphatidylinositol 3-kinase (PI3K)/Akt signaling, a major survival pathway, was substantially reduced in the Ncs1−/− group, and overexpression of NCS-1 or the constitutive active form of Akt increased the survival rate of Ncs1−/− myocytes. Cellular ATP levels, as well as mitochondrial respiration rate (both basal and maximal O2 consumption) were significantly depressed in Ncs1−/− myocytes; especially with oxidative stress. Furthermore, intracellular Ca2+ handling was more easily dysregulated in stressed Ncs1−/− myocytes than WT myocytes. Since NCS-1 levels were increased by stress, the data suggest that NCS-1 is a survival-promoting factor, which is upregulated by stress stimuli. Interestingly, however, supra-physiological NCS-1 expression was toxic to cells. Taken together, our data suggest that moderate NCS-1 expression during stress promotes cardiomyocyte survival by maintaining proper Ca2+ handling, which is required for activation of Akt survival pathways and mitochondrial function.

3074-Pos Board B504

Initiation of Electron Transport Activity and a Decrease of Oxidative Stress Occur Simultaneously during Embryonic Heart Development
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Mitochondria in early embryonic hearts are not thought to produce ATP, yet they do produce reactive oxygen species (ROS) that regulate myocyte differentiation. To understand changes in ATP and ROS genes over time during heart development, we measured mitochondrial oxygen consumption, the activity of the complexes (Cx) 1 and 2 of the electron transport chain (ETC), ETC supercomplex assembly, and ROS in embryonic mouse hearts. At embryonic day (E) 9.5, mitochondrial ETC activity and oxidative phosphorylation (OXPHOS) are not coupled, even though the ETC complexes are present. We show that Cx-1 is able to accept electrons from the Krebs cycle, but enzyme assays that specifically measure electron transport activity show that cytochrome c and ubiquinone show no activity at this early embryonic stage. At E11.5, mitochondria appear functionally more mature; ETC activity and OXPHOS are coupled and respond to ETC inhibitors. In addition, the assembly of highly efficient respiratory supercomplexes containing Cx-1, -3, and -4, ubiquinone, and cytochrome c begins at E11.5, the exact time when Cx-1 becomes functional activated. At E13.5, ETC activity and OXPHOS of embryonic heart mitochondria are indistinguishable from adult mitochondria. In contrast, generation of reactive oxygen species (ROS), as measured with Amplex Red, is high at E9.5 and drops significantly by E11.5, coinciding with activation of one of the ETC. In summary, our data suggest that between E9.5 and E11.5 dramatic changes occur in the mitochondria of the embryonic heart, which result in a decrease of ROS generation and an increase in OXPHOS due to the activation of Cx-1 and the formation of supercomplexes.

3075-Pos Board B505

The Stoichiometry between MICU1 and MCU Determines the Different Mitochondrial Ca2+ Uptake Phenotypes in Heart and Liver
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Mitochondrial Ca2+ uptake is central to oxidative metabolism and cell death signaling. The first clues to its molecular mechanism have emerged from the recent identification of the mitochondrial Ca2+ uniporter’s pore forming protein (MICU) as well as its regulators. Among the regulators, MICU1 shows striking