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co-expression and co-evolution with MCU. MICU1 has been demonstrated to be a Ca^{2+}-sensing protein, which both sets a threshold for low Ca^{2+} concentration while it assures cooperative activation during high Ca^{2+} rises. Mitochondrial Ca^{2+} uptake shows tissue specific differences and interestingly, mRNA level for MICUs and MCUs also displays tissue specificity. We set out to investigate if the stoichiometry between MICU1 and MCU could account for the previously described differences between heart and liver in mitochondrial Ca^{2+} uptake. Immunoblotting showed higher expression for all MICU1, MICU2 and MCU in mouse liver versus heart mitochondria, and a 4.5 fold higher MICU1 to MCU ratio in liver. In fluorometric measurements of mitochondrial Ca^{2+} uptake, heart mitochondria displayed a decreased threshold and lesser cooperativity compared to liver mitochondria. Additionally, NAD(P)H elevation was detectable after exposure to moderate $[Ca^{2+}]$ elevations only in heart mitochondria. Overexpression of MICU1 in the heart using AAV9-MICU1 tail-vein injection significantly increased the MICU1 protein level without any changes of MICU2 or MCU. This increased the MICU1 to MCU ratio in the heart and led to increased thresholding and cooperativity, reproducing the liver-like mitochondrial Ca^{2+} uptake phenotype. Vice versa MICU1 downregulation in the liver has been shown to lower the threshold and cooperativity of mitochondrial Ca^{2+} uptake in hepatocytes. Thus, heart and liver mitochondria show different levels of Ca^{2+} threshold and cooperative activation of Ca^{2+} uptake, which seem to result from differential quantitative relationship between MICU1 and MCU.

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ER Calcium Release is Tuned by Mitochondrial Redox Nanodomains

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Spatially and temporally controlled increases of H_2O_2 emerge as an intracellular signal. We hypothesized that ROS and Ca^{2+} interact locally, in the restricted volume of the ER-mitochondrial interface. These physically tethered structures host enrichments of ion transport proteins such as the IP_3 receptor, which support elevated nanodomains of Ca^{2+} during signalling events and are sensitive to H_2O_2. We used the genetically encoded H_2O_2 sensor HyPer incorporated into an inducible linker system to probe the redox environment at the ER-mitochondrial interface in HepG2 cells. We found a moderately elevated H_2O_2 nanodomain which developes into a H_2O_2 transient following IP_3 receptor-mediated ER Ca^{2+} release and mitochondrial Ca^{2+} uptake. Pharmacological inhibition showed that the transient was dependent upon ER Ca^{2+}, mitochondrial membrane potential and functional electron transport chain. HyPer measurements of the mitochondrial intermembrane space revealed significantly elevated H_2O_2 within this volume. Using electron microscopy we found that HepG2 mitochondria possess a cohort of dilated cristae, which disappeared following IP_3-linked Ca^{2+} release. Paxilline that inhibits mitochondrial BKCa channels blocked the cristae reshaping and also abolished the H_2O_2 transient at the interface. Furthermore, paxilline caused suppression of the IP_3-linked calcium signal, whereas interface targeted killer red, a photoactivated H_2O_2 source, induced sensitization to the IP_3-linked agonist. We conclude that the intermembrane/cristae volume of mitochondria represents an oxidized pool fed by the electron transport chain. Ca^{2+}-uptake stimulates expansion of the mitochondrial matrix via K^+ and concomitant water uptake, squeezing the oxidized volume of the cristae to the interface. Here, a transient H_2O_2 nanodomain sensitizes IP_3 receptors to further stimulation. We demonstrate a physiological setting where Ca^{2+} release may autoregulate using mitochondrial H_2O_2 released from the cristae.

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Reactive Oxygen Species (ROS) Suppress Mitochondrial Motility

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Mitochondrial distribution and transport play pivotal roles for many cellular functions, including cell differentiation, cell division to ensure proper inheritance, apoptosis, ATP supply at the local sites of demand, Ca^{2+} buffering for intracellular Ca^{2+} homeostasis.

We previously showed that mitochondrial motility (mito-motility) is regulated by the cytosolic Ca^{2+} concentration ($[Ca^{2+}]c$), providing the basis for a homeostatic circuit in which the organelles decrease their movements along microtubules to locally buffer high $[Ca^{2+}]c$ and contribute to ATP supply. Mitochondria are also a major site for production and scavenging of ROS that serve as both a messenger and regulator of calcium signaling and are particularly relevant for the control of mitochondrial function. Here we tested the hypothesis that ROS target mito-motility to control mitochondrial function. H9c2 myoblast cells were transfected with a mitochondrial matrix targeted YFP and then loaded with fura2, to monitor the mito-motility simultaneously with $[Ca^{2+}]c$. H2O2 (100 μM) caused a decrease in mito-motility (64 ± 8 %) and an elevation in $[Ca^{2+}]c$ (from 55 ± 8 to 91 ± 8 nM) at the same time. When the cells were incubated in a Ca^{2+}-free medium and were pretreated with thapsigargin to prevent Ca^{2+} entry and intracellular Ca^{2+} mobilization, respectively, H2O2 continued to inhibit the mito-motility dose-dependently without any changes in $[Ca^{2+}]c$. These results indicate that H2O2 can cause suppression of mito-motility through a Ca^{2+} independent mechanism we are currently analyzing.

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Miro1 is Dispensable for Calcium-Mediated Inhibition of Mitochondrial Movement

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Miro1 and 2 are (*Rhot1 and 2*) are two highly similar GTPases that are bound to the surface of mitochondria and possess EF-hand calcium binding motifs. Several groups have reported that Miro is involved in mitochondrial motility and inheritance, and particularly its calcium regulation, but the roles of the two isoforms have not been established. Genetic deletion of Miro1 in mouse is lethal at birth (Nguyen et al., 2014) and fibroblasts (MEFs) derived from Miro1 KO embryos show abnormal mitochondrial distribution, but the calcium-dependent inhibition of motility is unaffected and the respiratory and calcium buffering capacities are normal. Neuron-specific knockout of Miro1 leads to progressive deficits of upper motor neuron function, however mitochondria in processes of cortical neurons from Miro1 KO and wild-type embryos showed comparable calcium-sensitive motility inhibition. While no significant increase in Miro2 protein was observed in Miro1 KO MEFs, these data raise two possibilities: Miro1 and 2 are interchangeable with regard to calcium regulation of mitochondrial motility or Miro2 is the key player in this regard. To finally resolve this question, we are in the process of generating Miro2 KO and Miro1/2 KO cell lines.

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Mitochondrial Fusion Dynamics in the Heart

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Heart physiology depends on oxidative metabolism that likely requires dynamic and permanent reorganization of mitochondria by fusion and fission. To directly evaluate mitochondrial fusion dynamics in cardiomyocytes (CM), mitochondrial matrix-targeted photoactivatable GFP and DsRed were introduced either in vitro or in vivo by adenovirus and were followed by confocal microscopy. Four conditions were analyzed: 24 to 48 h cultured neonatal and in vitro transduced adult CM, and CM from in vivo infected rat hearts. In the latter case, CM were isolated 7-10 days after infection and were imaged promptly or 24-48 h post harvesting. Neonatal CM mitochondria form a highly connected network, whereas both in vitro and in vivo transformed cultured CM displayed only some connectivity. Impressively, in vivo transduced adult CM that were imaged promptly after harvesting, unveiled a significantly higher connectivity among mitochondria than the 24-48h cultured adult CM. Furthermore, fusion events (f.e./75 square micrometers/min) were almost absent in cultured in vitro transduced CM, meanwhile in vivo transduced and cultured CM showed 0.4 ± 0.2 f.e./min, whereas isolated, freshly-imaged CM displayed 1.4 ± 0.1 f.e./min. Imaging in perfused whole heart ex vivo, showed considerable mitochondrial continuity and fusion activity in ventricular CM. To study more directly the role of CM's contractile activity in mitochondrial fusion, CM were incubated with Verapamil (10μM), that blocked spontaneous contraction and partially suppressed the fusion activity of mitochondria. Also, mitochondrial fusion activity appeared to be higher after spontaneous contraction or short term field stimulation in isolated freshly-imaged CM. Thus, mitochondria are dynamic in both neonatal and adult CM, but under culture conditions, adult CM lose mitochondrial fusion activity. This might be at least in part, because cardiomyocyte contractile activity is altered in culture and contractions likely provide some factors to support mitochondrial fusion activity.

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Mechanistic Characterization of the Thioredoxin System in the Removal of Hydrogen Peroxide

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The thioredoxin system plays a critical role in the defense against oxidative stress by removing harmful hydrogen peroxide (H2O2). Specifically,

thioredoxin (Trx) donates electrons to peroxiredoxin (Prx) to remove H2O2 and then thioredoxin reductase (TrxR) maintains the reduced Trx concentration with NADPH as the cofactor. Despite a great deal of kinetic information gathered on the removal of H2O2 by the Trx system from various sources/species, a mechanistic understanding of the associated enzymes is still not available. We address this issue by developing a thermodynamically-consistent mathematical model of the Trx system which entails mechanistic details and provides quantitative insights into the kinetics of the TrxR and Prx enzymes. Consistent with experimental studies, the model analyses of the available data show that both enzymes operate by a ping-pong mechanism. The proposed mechanism for TrxR, which incorporates substrate inhibition by NADPH and intermediate protonation states, well describes the available data and accurately predicts the bell-shaped behavior of the effect of pH on the TrxR activity. Most importantly, the model also predicts the inhibitory effects of the reaction products (NADP+ and Trx(SH)2) on the TrxR activity for which suitable experimental data are not available. The model analyses of the available data on the kinetics of Prx from mammalian sources reveal that Prx operates at very low H2O2 concentrations compared to their human parasite counterparts. Furthermore, the model is able to predict the dynamic overoxidation of Prx at high H2O2 concentrations, consistent with the available data. The integrated Prx-TrxR model simulations show that the coupling of TrxR- and Prx-dependent reduction of H2O2 allowed ultrasensitive changes in the Trx concentration in response to changes in the TrxR concentration at high Prx concentrations.

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Higher Mitochondrial Membrane Potential Induces ROS Production in the Familiar Form of Frontotemporal Dementia with *MAPT* Mutations

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Frontotemporal dementia and parkinsonism linked to chromosome 17 (FTDP-17) is a neurodegenerative disorder caused by mutations in the *MAPT* gene encoding tau protein. Mitochondrial alterations have been associated with neuronal death in several diseases. The objective of our study was to analyse the mitochondrial function in human iPS cells from a patient of FTDP-17 carrying the 10+16 *MAPT* mutation. In addition, we have selected three different time points of the differentiation from pluripotent stem cells to cortical neurons to study how mitochondrial alterations develop.

We have used fluorescence imaging techniques to examine the mitochondrial function: TMRM to measure the mitochondrial membrane potential ($\Delta\psi_m$) and dyhidroethidium (DHE) to measure the rate of reactive oxygen species (ROS) production.

$\Delta\psi_m$ was higher in iPS-derived neurons from the patient bearing the *MAPT* mutation ($158.3 \pm 30.2\%$ of control). Higher $\Delta\psi_m$ was also found in non-differentiated pluripotent stem cells ($133.4 \pm 10.1\%$) and in the neural rosettes, which represent an earlier stage of the differentiation ($151.5 \pm 12.4\%$). In contrast, mitochondrial mass was lower in mutant iPS-derived neurons ($85.1 \pm 3.9\%$), although it was similar in non-differentiated cells.

We have also found that the rate of ROS production, measured using DHE, was also higher in iPS-derived neurons from the patient ($127 \pm 13.9\%$ of control). The increased rate of ROS production in these cells may be the consequence of the enhanced membrane potential. Consistently, the rate of ROS production in non-differentiated cells and in neural rosettes was also significantly higher ($123 \pm 12.9\%$ and $130 \pm 6.9\%$, respectively).

Our study indicates that this *MAPT* mutation leads to a higher mitochondrial membrane potential, which induces a higher ROS production that may be a trigger for neurodegeneration.

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The Overexpression of Superoxide Dismutase 1 Restores Growth Defect in a Porin1-Less Yeast Strain and Improves Mitochondrial Metabolism

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Metabolic exchanges between cytosol and mitochondria are made possible by the presence of the pore-forming protein VDAC1 on the outer mitochondrial membrane [1-3]. VDAC1 is directly involved in ATP/ADP, glucose and ions transportation, calcium homeostasis and apoptosis regulation. Moreover, it shows high level of sequence conservation in all eukaryotes: the homologous por1 in yeast S. cerevisiae shows 70% of identity and similar functional properties [1]. Recent studies have highlighted the existence of a link between VDAC1 and SOD1 enzyme, the most important cytosolic defense against superoxide anion. In yeast, SOD1 is required to protect VDAC1 from oxidation but also from carbonylation induced by ROS [3]; in addition, yeast strains devoid of endogenous SOD1 show down-regulated VDAC1 and TOM40 levels, and VDAC shows a significantly less pronounced voltage dependence and conductance [4]. To unravel SOD1 metabolic role in relation to VDAC1-mediated metabolism, we expressed human SOD1 in yeast devoid of endogenous VDAC (Δpor1). While Δpor1 strain cannot grow in the presence of a not-fermentable carbon source, possibly due to altered mitochondria, our results indicates that the overexpression of hSOD1 in Δpor1 strain relieves the growth defect, suggesting that SOD1 participates in the mitochondrial metabolic intersection with the cytosol.

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The Role of Complex I in Mitochondrial Reactive Oxygen Species Formation in Cochlear Sensory and Supporting Cells during Ototoxic Aminoglycoside Exposure

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Aminoglycosides (AGs) are the most widely used class of antibiotics in the world despite causing permanent hearing loss by damaging inner ear sensory cells. Although the mechanisms of cochlear sensory cell damage are not fully known, reactive oxygen species (ROS) are clearly involved. During normal mitochondrial metabolism low levels of ROS, primarily superoxide, are produced at complexes I and III in the electron transport chain. These levels can increase when mitochondrial dysfunction occurs. Complex I-specific ROS formation was evaluated in acutely-cultured murine cochlear explants exposed to gentamicin (GM, 300 μg/ml), a representative ototoxic AG antibiotic. Mitochondrial membrane potential and pro-apoptotic signaling were measured using Tetramethylrhodamine and apoptosis-inducing factor (AIF) labeling, respectively. Fluorescence intensity-based measurements of nicotinamide adenine dinucleotide (NADH) were used to detect changes mitochondrial metabolism. Relative amounts of superoxide and hydrogen peroxide produced during acute GM exposure were measured using MitoSox Red and Dihydrorhodamine 123, respectively. GM increased NADH fluorescence intensity in low- and high-frequency sensory cells. The complex I inhibitor rotenone (250 nM) significantly increased superoxide, not hydrogen peroxide, in low- and high-frequency sensory cells ($p < 0.01$). GM significantly increased superoxide and hydrogen peroxide formation in low- and high-frequency sensory cells ($p < 0.05$). Rotenone increased GM-induced superoxide formation but decreased GM-induced hydrogen peroxide formation. This effect was greatest in high-frequency cells indicating fundamental differences in ROS formation in high- and low-frequency sensory cells exposed to ototoxic antibiotics. This project provides a base for understanding the underlying mechanisms of mitochondrial ROS production in cochlear cells during exposures to ototoxic antibiotics.

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Mitochondrial Iron and Sphingosine Synergize Initiation of Hepatocyte Death by Augmenting Oxidative Stress

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Hepatocytes exposed to ischemia/reperfusion (I/R) succumb to cell death after reperfusion. Sphingosine and ceramide profiles revealed substantial accumulation of sphingosine after 4h of ischemia to rat hepatocytes, whereas other sphingoid bases did not change. A lysosomotropic inhibitor of acid ceramidase suppressed I/R-induced death, indicating a lysosomal origin of sphingosine. Addition of exogenous sphingosine to hepatocytes increased cell death, which was insensitive to the ceramide synthase inhibitor, fumonisin B1. This finding indicates that accumulation of sphingosine, not ceramide formed from sphingosine, promoted cell death. Exogenous sphingosine also inhibited complex IV (cytochrome oxidase), the terminal component of the respiratory chain, in