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,