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its lack leads to an incomplete repression of mitochondrial respiration in presence of glucose [1]. Voltage Dependent Anion Channel 1 (VDAC1) is the protein that ensures the exchanges of metabolites and ions through the mitochondrial outer membrane (MOM). *Saccharomyces cerevisiae* cell lacking VDAC1 ( $\Delta$ por1) show a defective growth on not fermentable carbon source. In a recent work, we demonstrated that the expression of human SOD1 in  $\Delta$ por1 restores the yeast growth on glycerol, the mitochondrial functionality and the ROS level [2]. Moreover the presence of hSOD1 increases the gene expression of  $\beta$ -barrel MOM proteins. Among them, the second VDAC isoform (also known as porin2) was found 8 times over-expressed [2]. The pore-forming activity of yeast VDAC2 has never been characterized. Our results strongly suggest that it can substitute for VDAC1 function. Accordingly, we observed that the expression of SOD1 in a yeast strain devoid of both VDAC1 and VDAC2 is unable to reactivate the mitochondrial metabolism. In this work we present these observations and a preliminary characterization of purified *S. cerevisiae* VDAC2.

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#### 07.10

##### Mitochondria protect themselves from hypoxia-reoxygenation damage by recycling nitrite to NO and inhibiting iron mediated oxidative stress

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Nitrite is present in tissues in micro molar concentrations and recently has also been employed as a drug, because it ameliorates ischemia-reperfusion induced mitochondrial dysfunction and tissue damage. The mechanism of this action of nitrite is not completely understood. Here we provide evidence that this action of nitrite is due to the inhibition of iron-mediated oxidative stress caused by the release of ferrous ions upon hypoxia. We show that hypoxia and re-oxygenation substantially reduced mitochondrial respiration in state 3 with both complex I and II substrates due to increased permeability of outer mitochondrial membrane and the release of cytochrome c. The release of cytochrome c was accompanied by iron mediated lipid peroxidation. The experiments with nitrite pre-treatment showed that upon hypoxia mitochondria reduce nitrite to nitric oxide (NO) in amounts sufficient to inactivate redox active ferrous ions in close vicinity to mitochondria due to the formation of inactive dinitrosyl iron complexes (DNIC). The scavenging of iron ions in turn prevented lipid peroxidation in the outer mitochondrial membrane and the release of cytochrome c. This action of nitrite protected respiratory function of mitochondria during reoxygenation. However, at high concentrations nitrite, in addition to beneficial deactivation of iron ions, exerted also toxic effects inhibiting mitochondrial respiration. This explains why beneficial effects of nitrite are restricted to a narrow therapeutic window described previously in *in vivo* models. The formation of DNIC with nitrite-derived NO was also demonstrated in a

ischemia/reperfusion model on perfused liver. Our data suggest that the formation of DNIC is a key mechanism of nitrite-mediated protection of mitochondrial, cellular and organ function upon ischemia reperfusion.

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#### 07.11

##### Beneficial and deleterious roles of mitochondrial ROS upon inflammation

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The aim of this study was to clarify the impact of mitochondrial reactive oxygen species (mtROS) upon inflammation induced by bacterial toxin in rats and in rat hepatocyte and macrophage cultures incubated with inflammatory mediators (IM). Inflammation influenced mtROS generation in two manners, namely elevating mtROS generation within mitochondria and releasing mtROS into cytoplasm, tentatively via mPTP. The increase in mtROS levels inside and outside of mitochondria caused oxidative stress and activated specific signaling pathways. The oxidative stress was manifested as a drop in mitochondrial membrane potential and a damage to cellular membrane resulted in the release of intracellular enzymes (AST, LDH) in the blood. The increase in mtROS resulted in up-regulation of IM (iNOS and IL-6) accompanied by a drastic increase in intracellular and blood NO levels; in addition it resulted in an increase in NADPH-oxidase activity in macrophages. The treatment with mitoTEMPO, a mitochondria targeted antioxidant, decayed mtROS, normalized membrane potential, decreased expressions of iNOS and IL-6 in hepatocytes, decreased NADPH-oxidase activity in macrophages and reduced circulating NO and AST/ALT levels in the blood. In addition we demonstrated that the increase in mtROS was induced by late phase IM such as IL-4 released during compensatory anti-inflammatory response phase rather than by IM such as TNF-alpha released during systemic inflammatory response, an early acute phase. The nature of the IM is still unclear. All together our data suggest that mtROS predominantly affect inflammatory response not via oxidative stress reactions but via two signaling pathways, namely up-regulation of inflammatory genes and elevation NADPH-oxidase activity. Since both these pathways are components of the immune response, the application of mitochondria targeted antioxidants can be both deleterious weakening immune system and beneficial attenuating host tissue damage.

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#### 07.12

##### Structural insights into biological hydrazine synthesis

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Anaerobic ammonium oxidizing (anammox) bacteria are ubiquitously present in nature and contribute substantially to the global release of fixed nitrogen to the atmosphere. The biological novelty of the anammox process is the production of the reactive intermediate hydrazine (N<sub>2</sub>H<sub>4</sub>), which gets subsequently oxidized to dinitrogen gas (N<sub>2</sub>) yielding the necessary reducing equivalents for respiration. The protein complex catalyzing the formation of N<sub>2</sub>H<sub>4</sub> from nitric oxide (NO) and ammonium (NH<sub>4</sub><sup>+</sup>), that is Hydrazine Synthase (HZS), was purified to homogeneity as a dimer of heterotrimers (αβγ)<sub>2</sub>. Analyses of the crystal structure of HZS at 2.7 Å resolution together with spectroscopic studies led to the formulation of a working hypothesis. We suggest that hydrazine synthesis occurs via a two-step mechanism facilitated by two heme c active sites. First, NO undergoes a three-electron reduction to hydroxylamine (NH<sub>2</sub>OH) at the active site of the γ-subunit (heme γI). NH<sub>2</sub>OH is then transferred to the α-subunit through an intraprotein tunneling system that is presumably regulated by a short amino acid stretch of the β-subunit. The second active site heme of the HZS complex (heme αI) facilitates the condensation of ammonia (NH<sub>3</sub>) and NH<sub>2</sub>OH to the end product N<sub>2</sub>H<sub>4</sub>. Interestingly, the resolution of the structure of HZS revealed few oddities with regards to the architecture of the active site hemes. The γ-subunit is structurally similar to cytochrome c peroxidase and methylamine utilization protein G but displays a third covalent attachment between a cysteine residue and the porphyrin ring of the γI heme moiety. Heme αI also differs considerably from a canonical heme c site, as the histidine residue of the CXXCH heme binding motif does not coordinate the heme iron, but a zinc ion instead. The fifth ligand of heme αI appears to be a tyrosine, resembling the active site of catalases. These results provide the first insight into the molecular mechanism of biological hydrazine synthesis.

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### 07.13

#### A new answer to an old problem: The energization of brain mitochondria with pyruvate is regulated by cytosolic calcium via the mitochondrial gas pedal and does not require the mitochondrial Ca uptake via the Ca uniporter- New evidences from experiments with MCU<sup>-/-</sup> mice

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It is assumed that for activation of mitochondria, cytosolic Ca<sup>2+</sup> has to enter the matrix via the Ca uniporter (MCU) and to stimulate the matrix dehydrogenases. However, recent experiments from Pan et al., show that animals without MCU can live well although MCU<sup>-/-</sup> mitochondria cannot acutely take up Ca<sup>2+</sup>. Obviously the classic hypothesis is insufficient to explain these results. Recently, our group discovered that increasing Ca<sup>2+</sup><sub>cyt</sub> (R<sub>0.5</sub> = 300 nM) can activate via

aralar (glutamate/aspartate carrier) the activity of both the malate aspartate shuttle (MAS) and the formation of pyruvate without involvement of MCU. As result pyruvate and hydrid anions are increasingly pushed into mitochondria. We called this process mitochondrial gas pedal. To prove our hypothesis, we performed experiments with MCU ko and WT mice. By means of Morris water maze test we found that the MCU knock out did not affect hippocampal memory function and the swimming performance as well. Isolated brain MCU<sup>-/-</sup> mitochondria did not accumulate Ca<sup>2+</sup> and were stable against large Ca<sup>2+</sup> additions. Glutamate/malate but not pyruvate/malate driven respiration was at least doubled by increasing Ca<sup>2+</sup> in WT and ko mitochondria with and without ruthenium red. Respiration of glucose fed synaptosomes was decreased by BAPTA due to diminished Ca<sup>2+</sup>. Pyruvate addition partially recovered the respiration indicating the contribution of limited pyruvate to the inhibition of respiration. In contrast the inhibition of respiration by aminooxyacetate not affecting Ca<sup>2+</sup> could be completely reversed by pyruvate addition. We obtained similar results with MCU<sup>-/-</sup> and WT synaptosomes indicating, that the MCU is not involved in the Ca<sup>2+</sup>-regulation of pyruvate oxidation of brain mitochondria. In brain mitochondria the extent of the gas pedal mechanism is larger than the classic intramitochondrial Ca<sup>2+</sup>- activation of matrix DH. This is the reason that mitochondrial energization can also be regulated by cytosolic Ca<sup>2+</sup> in MCU ko animals.

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### 07.14

#### Respiratory complex II catalyzed ROS production

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Complex II plus complex III contribute about 20% to the overall ROS production by the mammalian respiratory chain components [1]. A bell-shaped dependence of ROS-producing complex II activity on succinate concentration have been described [2,3]. Here we present further analysis of complex II ROS generating activity. The succinate-reduced membrane-bound respiratory complex II in inside-out bovine heart submitochondrial particles freed of superoxide dismutase and treated with rotenone and myxothiazol reacts with oxygen (at 50 μM "optimal" succinate concentration) at the specific rate of about 5% of its NADH-supported fumarate reductase activity. The enzyme produces more than 80% of ROS as superoxide anion. The reaction is insensitive to Atpenin 5 (the inhibitor of iron-sulfur S3-ubiquinone junction site) and its rate decreases upon increase of succinate concentration. The contribution of complex II to the ROS production by myxothiazol-inhibited, NADH-reduced respiratory chain (complexes I + II) is about 15%. The dependence of ROS production on succinate/fumarate ratio shows that the midpoint redox potential of oxygen-reactive component is about 40 mV more positive than that of succinate/fumarate couple, a value close to that of S3 iron-sulfur center. A possibility of the dicarboxylate binding-induced conformational change of the enzyme structure propagated to S3 specific protein environment is proposed.

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