

### S3.14 In *Saccharomyces cerevisiae* cells VDAC mediates the cytosol redox state and subsequently the expression levels of protein import channels of the mitochondrial outer membrane

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The aim of this study was to explain the mechanism of the upregulation of protein import channels of the mitochondrial outer membrane (Tom40 and Tob55/Sam50) in *S. cerevisiae* mitochondria depleted of either VDAC isoform. Our data show that these isoforms are crucial for the cytosol redox state that changes between growth phases (exponential and stationary) in a VDAC isoform dependent way. The observed changes of the redox state can be imitated for a given VDAC isoform mutant by the addition of an oxidant (menadione) or an antioxidant (ascorbate) to the culture medium. Moreover the cytosol oxidation during stationary and the modified exponential growth phases requires only the presence of VDAC1, although VDAC2 seems to be important for the oxidation degree. Finally, the cytosol redox status is decisive for the expression levels of Tom40 and Tob55/Sam50 as well as MnSOD and the expression levels of these proteins increase when the cytosol redox state shifts towards oxidation. Thus, redox regulation of protein expression, postulated for proteins encoded by mitochondrially located genes seems to occur also in the case of mitochondrial proteins encoded by nuclear genes. Furthermore, the regulatory process is mediated by VDAC and at least partially does not depend on its channel activity.

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### S3.15 Novel proteins involved in mitochondrial $K^+/H^+$ exchange

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As postulated by P. Mitchell, the existence of a mitochondrial  $K^+/H^+$  exchanger (KHE) plays a vital role, since it allows the extrusion of excess  $K^+$  from the matrix and counterbalances the electrophoretic  $K^+$  uptake. For the first time, we have molecularly characterized yeast Mdm38 and human LETM1 as components of the KHE. Since these proteins are part of a high molecular weight complex, our aim is to identify other members of this complex. In absence of Mdm38/LETM1, mitochondrial  $K^+$  overload causes osmotic swelling, loss of membrane potential, fragmentation of mitochondria and eventually mitophagy. Importantly, all these phenotypes ensuing loss of Mdm38/LETM1 could be efficiently reversed by nigericin, an ionophore mediating  $K^+/H^+$  exchange. To qualify the proteins Mdm38/LETM1 as essential for  $K^+/H^+$  exchange activities and measure the  $K^+$  fluxes, we have developed a novel method involving  $H^+$  and  $K^+$  sensitive fluorescent dyes entrapped in submitochondrial particles (SMPs). Our data showed that the  $K^+/H^+$  exchange activity was nearly abolished in mutant SMPs. A genome wide screen for multi-copy suppressors of *mdm38*  $\Delta$  cells led to the isolation of 2 novel genes which were able to restore the  $K^+/H^+$  exchange activity in *mdm38*  $\Delta$  SMPs. Deletion of all 3 genes resulted in a total loss of residual  $K^+/H^+$  exchange across the mitochondrial inner membrane and in dramatic alteration of mitochondria and vacuole morphology and lowering of cell viability.

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### S3.16 Molecular and functional characterization of new pathogenic mutations in mitochondrial ornithine and aspartate/glutamate transporters

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Mutations in the SLC25A13 gene, coding for a liver-specific isoform of the mitochondrial aspartate/glutamate carrier (AGC2), and in the SLC25A15 gene, coding for ornithine carrier isoform 1 (ORC1), cause type 2 citrullinemia (CTLN2) and hyperornithinemia-hyperammonemia-homocitrullinuria (3H syndrome), respectively. The aim of this work was to identify and characterize novel mutations of these two genes in patients presenting symptoms suggestive of AGC2 or ORC1 deficiency. In the AGC2 transcript of a Pakistani man living in Europe suspected of being affected by CTLN2 (a highly prevalent disease in Southeast Asia), a homozygous mutation, c.1763G>A, was found which produces an R588Q change in the protein. In the ORC1 transcript of patients suspected of 3H syndrome and having different ethnic origin, six new homozygous mutations (c.110T>G, c.212T>A, c.337G>T, c.815C>T, c.818T>A and c.847C>T) were found that produce M37R, L71Q, G113C, T272I, M273K and L283F substitutions, respectively, in the protein. Each mutation was functionally characterized in liposomes reconstituted with AGC2 or ORC1 carrying the above-mentioned amino acid replacement. They all reduced transport activity by approximately 90% in comparison to the activity of the wild-type proteins suggesting that they are disease-causing mutations.

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### S3.17 A profile of free fatty acid specificity in *Acanthamoeba castellanii* mitochondrial uncoupling

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Uncoupling proteins, forming a subfamily within the mitochondrial anion carrier protein family, fulfill a physiological function through a  $\Delta\mu H^+$  dissipation by a free fatty acid (FFA)-activated, purine nucleotide-inhibited  $H^+$  cycling process driven by membrane potential ( $\Delta\Psi$ ) and pH (both constituting  $\Delta\mu H^+$ ). Oxygen consumption and membrane potential of isolated *A. castellanii* mitochondria were measured with Clark type oxygen and TPP<sup>+</sup> sensitive electrodes. We have observed the uncoupling specificity of thirteen different FFAs in stimulation of resting respiration, decreasing of membrane potential, and in decreasing of oxidative phosphorylation efficiency. The results show that in *A. castellanii* mitochondria the most active as uncouplers and protonophores are unsaturated (C18–20) FFA, with linoleic acid (C 18:2) as most effective. Among saturated FFAs which all are weaker uncouplers when compared to unsaturated FFAs, the potency to diminish  $\Delta\Psi$  and to stimulate respiration decreased with a decreasing carbon chain length (except for stearic acid C 18:0, which was slightly active). This indicates that the energy-dissipating ability of uncoupling protein in *A. castellanii* mitochondria depends on the uncoupling and protonophoretic efficiency of FFAs, therefore on their character (carbon chain length, saturation degree).

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