The mitochondrial biogenesis and adequate energy production are important for fetal growth and early postnatal adaptation. The aim of the study was to characterize mitochondrial DNA (mtDNA) content and expression patterns of *POLG*, *TFAM*, *NRF1*, *NRF2* and *PGC1* family of regulated coactivators (*PGC1A*, *PGC1B* and *PPRC1*) involved in the mtDNA transcription, regulation and maintenance in human fetal tissues during second trimester of gestation. Further the mRNA expression profiles of selected cytochrome *c* oxidase (COX) subunits were analysed. Moreover enzyme activities of COX and citrate synthase (CS) and protein levels of COX subunits were analysed.DNA, RNA and proteins were isolated from 26 pairs of fetal liver and muscle samples obtained at autopsy after termination of pregnancy for genetic indications unrelated to OXPHOS deficiency between the 13th and 28th weeks of gestation. This work offers a broad view on the mtDNA content changes in two different tissues during the second trimester of gestation and in the corresponding tissues after birth. The important differences in expression of *POLG*, *TFAM*, *NRF2* genes and family *PGC1* coactivators were found between the fetal tissues. The significant tissue-specific changes in expression of selected COX subunits on mRNA level (*COX4* and *MTCO2*) were observed. Further the considerable differences in enzyme activities of COX and CS are demonstrated between fetal and postnatal phases. In conclusion our study indicates that the fetal developing tissues might differ in the control of mitochondrial biogenesis depending on their energy demand and the age of gestation. Moreover the gene expression is changed mainly on transcriptional level through fetal period.

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13P.6 Oxidative protein folding in the intermembrane space of mitochondria

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For a long time, the endoplasmic reticulum (ER) was considered to be the only compartment of the eukaryotic cell in which proteins are folded by dedicated enzymes in an oxidation-driven process. However, it became recently evident that eukaryotic cells harbor another oxidizing compartment, the small lumen between the outer and inner membranes of mitochondria—the intermembrane space (IMS). In mitochondria, protein oxidation can be used to drive protein translocation from the cytosol across the outer membrane. Moreover, redox reactions have been implied in many IMS-connected processes like apoptosis, aging and the regulation of the respiratory chain. Major players of the oxidative pathway in the IMS are the oxidoreductase Mia40 that oxidizes substrates and the sulfhydryl oxidase Erv1 that re-oxidizes Mia40. Erv1 derives its oxidative power from the respiratory chain via its interaction with cytochrome *c*. To detailedly analyse the mechanism of this oxidative pathway and the interplay of its components we reconstituted the complete process *in vitro* using purified cytochrome *c*, Erv1, Mia40 and the substrate Cox19. Hereby, we demonstrate that Erv1 dimerizes non-covalently, and that the subunits of this homodimer cooperate in intersubunit electron exchange. Moreover, we show that Mia40 promotes complete oxidation of the substrate Cox19. The efficient formation of disulfide bonds is hampered by the formation of long-lived, partially oxidized intermediates. The generation of these side products is efficiently counteracted by reduced glutathione. Thus, our findings suggest a role for a glutathione-dependent proof reading during oxidative protein folding by the mitochondrial disulfide relay.

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13P.7 The role of SenC in assembly of the cytochrome cbb_3 oxidase in *Rhodobacter capsulatus*

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As a member of the heme copper oxidase superfamily, the cbb_3-type cytochrome oxidase (cbb_3-Cox) is composed of four subunits. Based on their high oxygen affinity they are considered to facilitate bacterial growth at low oxygen concentrations. Many pathogenic bacteria like *V. cholerae*, *H. pylori*, *C. jejuni* and *N. meningitidis* seem to be dependent on the cbb_3-Cox for colonizing the human host. SenC in *Rhodobacter capsulatus* (Sco1 in yeast and human) is an assembly protein which contains a copper binding motif and has been implicated so far in the assembly of the Cu_A centre of aa_3-type cytochrome oxidases. Sco1 also contains a thiol-disulfide oxidoreductase activity, which is discussed to keep the Cu-ligating residues in their correct redox-state. Although, the cbb_3-Cox, which is the only cytochrome-*c*-oxidase in *R. capsulatus*, does not contain a Cu_A centre, the *senC* knock-out strain shows a strongly reduced oxygen uptake activity. We could show that this is due to the absence of all four subunits CcoNOQP of the cbb_3-Cox in the *senC* knock-out. Because the loss of *senC* does not cause a major change in the transcriptional level of the cbb_3-Cox subunit CcoN, as shown by RT-PCR, the absence of the cbb_3-Cox is due to an instability or a defect in the assembly process. Furthermore the cbb_3-Cox can be restored by growing cells in the presence of increased Cu concentrations. This effect was copper specific and was not observed in the presence of iron or magnesium ions. To further elucidate the role of SenC in cbb_3-Cox maturation, we analysed direct interactions of SenC with other proteins. On Blue Native PAGE SenC is running in complexes of 230 kDa, 70 kDa and 40 kDa. Interestingly, the cbb_3-Cox also runs as a 230 kDa complex, which could indicate an interaction between SenC and the cbb_3-Cox. To further show a contact to an individual subunit of the cbb_3-Cox we performed copurification- and formaldehyde-crosslinking-experiments. While we could not detect interactions between SenC and the cbb_3-Cox main subunit CcoN, which contains a heme *b* and a heme b_3-CuB-center, we could copurify SenC with the subunit CcoP which is a *c*-type cytochrome and could also crosslink these two proteins with formaldehyde. These data indicate that SenC is a general assembly factor for cytochrome-oxidases and not only for cytochrome-oxidases which contain a Cu_A centre.

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13P.8 Mitochondrial biogenesis in human osteosarcoma cells with chronic mitochondrial stress

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Defects in mitochondrial genome lead to mitochondrial dysfunctions and chronic mitochondrial stress. In such conditions, altered mitochondria-to-nucleus signaling triggers specific adaptive changes in the expression of a number of nuclear genes to compensate for the defects. Since mitochondria are the main sites of ROS generation in the cells, it is suspected that these signaling molecules are implicated in mediating the retrograde communication from mitochondria to the nucleus. We have showed previously that ROS level was altered in human osteosarcoma NARP (Neuropathy, Ataxia and Retinitis Pigmentosa) cybrids with high heteroplasmy and in Rho0 cells depleted of mtDNA. In this presentation, we aimed to elucidate to what extent such severe mitochondrial impairment affected mitochondrial biogenesis whereas antioxidant (selenium) employment allowed us to establish whether regulation of this process was associated with ROS-dependent retrograde signaling. We found that the level/activity of nuclear respiratory factors 1 and 2 (NRF1, 2) was altered in NARP and Rho0 cells and followed by changes in the level of respiratory chain subunits. Moreover, the levels of NRF and the respiratory chain subunits were affected by selenium supplementation what showed that ROS-mediated retrograde signaling is involved in a regulation of mitochondrial biogenesis, not only in cells with mitochondrial defects but also in WT control.

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