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The purple b-proteobacterium *Rubrivivax* (*R.*) *gelatinosus* expresses high amounts of active cbb_3 cytochrome *c* oxidase when grown under micro-aerobic conditions (Khalfaoui-Hassani *et al., J. Biol. Chem.*, 2010). Cytochrome *c* oxidases are the terminal members of the electron transport chains in mitochondria (aa_3 oxidase) and many bacteria (cbb_3, caa_3 and aa_3 oxidases). They belong to the heme-copper oxidase superfamily. The four genes coding for the cbb_3 oxidase (*ccoNOQP*) were identified and cloned; they encode respectively for four subunits: the membrane-integral catalytic subunit CcoN containing heme *b* and heme b_3-Cu_B binuclear centre, the monoheme cytochrome CcoO, a short cofactor-less subunit CcoQ and the diheme cytochrome CcoP. The major features of this enzyme are the lack of Cu_A-containing subunit and the presence of three *c*-type hemes in CcoP and CcoO. Here we report the importance of each subunit for the stability of the cbb_3 oxidase in the membrane, for the oxidase activity and for micro-aerobic growth of *R. gelatinosus*. Individual mutants of each subunit were constructed. They were all analysed for the O_2 consumption under micro-aerobic growth, for the oxidase activity of solubilised membranes on BN-PAGE, for the presence of cytochrome *c* and subunits identified respectively by gel detection with TMBZ and western blots. Our data revealed the importance of both CcoN and CcoO subunits for activity and stability of the complex in the membrane. However in the absence of CcoP or CcoQ, active complexes were produced but displaying lower activities compared to the wild type. Altogether these data allowed us to conclude that CcoN and CcoO can assemble in the absence of CcoP or CcoQ and produce a partially active and functional core-complex.

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13P.3 Coordination of mitochondrial and nuclear genes expression in *Arabidopsis* mutant with impaired mitochondrial translation

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In *Arabidopsis thaliana* the *Rps10* gene encodes the S10 protein, which is part of the small subunit of mitochondrial ribosomes. Transgenic lines obtained by RNA-i mediated silencing of *Rps10* gene expression were utilized to investigate how impaired mitochondrial translation influences biogenesis of mitochondria. It is well known that subunits of oxidative phosphorylation complexes are encoded by both nuclear and mitochondrial genomes and synthesized in cytosol and mitochondria, respectively. Therefore, first we have focused on changes in expression of oxidative phosphorylation genes in *Rps10* mutants. All of mitochondrial encoded transcripts increased approximately four-fold, whereas the abundance of nuclear-encoded transcripts were constant or altered in less degree. Analysis at the protein level revealed that both mitochondrial- and nuclear-encoded subunits of oxidative phosphorylation complexes were at much lower level in *Rps10* mutants compared to wild type plants. Thus, silencing of *Rps10* gene altered expression of mitochondrial genes at the transcript and protein levels, while for nuclear genes, significant changes in expression were observed only at the protein level. These results suggest that mitochondria try to compensate the lower amount of mitochondrial proteins by the increase in abundance of mitochondrial encoded transcripts, but the level of nuclear-encoded transcripts is insensitive to impaired mitochondrial translation. Furthermore, differential abundance in transcripts of genes encoding mitochondrial and nuclear proteins from the same oxidative phosphorylation complex indicates that coordination of expression between mitochondrial and nuclear genes in the *Rps10* mutants occurs at the posttranscriptional level. Now, we are checking the hypothesis that biogenesis of oxidative phosphorylation complexes in the *Rps10* mutants is limited by the abundance of mitochondrial encoded subunits and the excess of nuclear-encoded proteins are degraded by mitochondrial ATP-dependent proteases.

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13P.4 Assembly of cytochrome cbb_3 oxidase

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Cbb_3-type cytochrome oxidases (cbb_3-Cox) represent a subfamily of the heme-copper oxidase superfamily. Due to their high oxygen affinity, they are considered to be important factors for the bacterial adaptation to different oxygen concentrations. In particular, many pathogenic bacteria like *Vibrio cholerae*, *Helicobacter pylori*, *Bordetella pertussis*, *Campylobacter jejuni* or *Neisseria meningitidis* seem to depend on the cbb_3-Cox for colonizing the human host. In contrast to well studied aa_3-type cytochrome oxidase, only little is known about the assembly of cbb_3-Cox. By combining Blue–Native–Page analyses with *in vitro* transcription/translation assays and chemical cross-linking, we have been able to determine a first model for cbb_3-Cox assembly in the model organism *Rhodobacter capsulatus*. Our data reveal that the functional assembly is initiated by the formation of two independent subcomplexes. The catalytic subunit CcoN first assembles with the mono-heme cytochrome CcoO subunit to form a 200 kDa complex. To this complex, a 40 kDa complex containing the di-heme cytochrome subunit CcoP and the small CcoQ subunit is recruited to form the active 230 kDa cbb_3-complex [1]. Further analyses of this process led to the identification of several assembly proteins which are essential for cbb_3-Cox assembly. One is CcoH, a small integral membrane protein, which is essential for cbb_3-Cox biogenesis and appears to be required for the recruitment of the CcoPQ complex into the CcoNO complex. In agreement with this, we show by cross-linking that CcoH is able to interact directly with CcoP and CcoN subunits. In addition, CcoH appears to form a stable complex with both the CcoNO and the CcoQP subassemblies as well as with fully assembled 230 kDa complex on BN-PAGE. It suggests that CcoH serves as an adapter protein that by dimerization assembles both sub-complexes into a functional unit and is a permanent component of an active form of cbb_3-type oxidase.

Reference

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13P.5 Developmental changes of mitochondrial DNA content and expression of genes involved in mtDNA transcription and maintenance in human fetal liver and muscle tissues

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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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