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The purple β -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 O₂ 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.

doi:10.1016/j.bbabbio.2010.04.325

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.

doi:10.1016/j.bbabbio.2010.04.326

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 *Rhodospirillum rubrum*. 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

[1] Kulajta C. *et al* (2006) *J. Mol. Biol.* **355**: 989–1004.

doi:10.1016/j.bbabbio.2010.04.327

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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