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Pet117 — Assembly factor of cytochrome *c* oxidase

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Cytochrome *c* oxidase is assembled of 13 subunits in mammals and 11 subunits in yeast *Saccharomyces cerevisiae*. The core subunits of complex IV encoded by mitochondrial genome are conserved among the *Eukaryotes* and contain active centers crucial for enzyme activity.

The assembly process of cytochrome *c* oxidase requires large number of assembly factors and includes formation of several intermediates. The crucial step in the assembly of the complex IV is formation of active centers in core subunits including copper and heme insertion into Cox1.

Here we show that Pet117, a conserved protein with a single transmembrane domain is a crucial assembly factor of cytochrome *c* oxidase.

We observed that deletion of yeast Pet117 leads to loss of complex IV assembly and formation of 'petit' colonies by cells grown on YPG medium. We also noticed decreased levels of several structural subunits of complex IV including Cox1 and Cox2 in Pet117 deficient strain. The wild type phenotype could be restored after complementation of the deletion strain with the plasmid containing Pet117 gene.

Import of radiolabeled Pet117 and carbonate extraction assay revealed that protein localizes in the inner mitochondrial membrane. Moreover, Blue Native gel analysis showed that Pet117 forms protein complexes of 120 kDa, 400 kDa, and above 600 kDa.

Using SILAC approach followed by pull-down experiments with tagged Pet117 we observed strong interaction with copper binding protein Cox11, known assembly factor responsible for Cu delivery to Cox1 subunit. Additionally, detailed analysis of elution fractions with the use of both SDS and Blue Native gels revealed interaction of Pet117 with structural components and assembly factors of complex IV including Cox1, and components of complex III. Similar results were obtained with the use of a strain containing tagged Cox11 protein. Moreover, Pet117-Cox11 interaction was observed in the absence of structural subunits of complex IV and some of assembly factors.

Our results suggest that Pet117 may participate in Cu transfer to Cox11 or cooperate with Cox11 during copper delivery process to Cox1 subunit. However, further analysis is necessary to clarify the exact role of Pet117 in assembly of complex IV.

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***Shewanella oneidensis* terminal oxidases**

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In aerobic respiration of prokaryotic and eukaryotic organisms, the reduction of molecular oxygen to water is catalyzed by terminal oxidases, cytochrome *c* or quinol oxidases, which are integral membrane multi-subunit enzymatic complexes pumping protons across membrane. Two types of terminal oxidases are known, the heme-copper oxidases and the cytochrome *bd*-type oxidases. The heme-copper oxidases are classified into three families: type A (mitochondrial like oxidases), type B (*ba*₃-type oxidases) and type C (*cbb*₃-type oxidases). The subunit composition of types A and B enzymes differs from one oxidase to another but these heme-copper oxidases always contain the catalytic subunit (subunit I) and a smaller subunit named subunit II [1].

Shewanella oneidensis, a gram-negative proteobacterium, inhabits a wide variety of niches in nature and has the characteristic ability to reduce, in addition to oxygen, a broad spectrum of electron acceptors such as metals, nitrate, thiosulfate, dimethyl sulfoxide, trimethylamine N-oxide, fumarate and azo dyes.

The *S. oneidensis* MR-1 genome sequence analysis revealed the presence of genes coding for enzymes potentially involved in oxygen reduction: two cytochrome *c* oxidases and a cytochrome *bd* quinol oxidase [2]. Based on sequence comparison, we proposed that the oxidase encoded by the genes SO4606-SO4609 is a cytochrome *c* oxidase (Cox) belonging to type A whereas cytochrome *c* oxidase encoded by SO2361-SO2364 is a C-type enzyme (*cbb*₃-type). The deduced amino acid sequence of Cox revealed that the subunit II contains two *c* type-heme binding motifs, an uncommon feature among type-A oxidases.

In the membrane of *S. oneidensis* MR1, we detected a cytochrome *c* oxidase activity. The preliminary study of the wild type strain as well as of three single oxidase deletants (lacking the gene encoding the catalytic subunit) shows that the *cbb*₃-type oxidase as well as the cytochrome *bd* quinol oxidase is present in the membrane of *S. oneidensis* MR-1, in our culture conditions (vigorously shaken 100 mL flask).

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Yeast mitochondrial cytochrome *c* oxidase: Effect of mutations in the hydrophilic channels within Cox1 and the adjacent supernumerary subunit Cox5A/B

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Yeast *Saccharomyces cerevisiae* has recently been developed for facile purification of mutant forms of a mitochondrial cytochrome *c* oxidase (CcO) [1]. It is composed of eleven subunits, three forming its catalytic core (Cox1, 2 and 3) and eight others, of unknown role, which are homologous to supernumerary subunits of mammalian CcOs [2]. One of these has two isoforms, Cox5A and 5B, which have 68% sequence identity and are selectively expressed under normoxic