can be coupled to energy-requiring cellular processes. The nitric oxide reductase (NOR) members of the superfamily catalyze the reduction of nitric oxide (NO) to nitrous oxide (N_2O) in microbes capable of either canonical or nitrifier denitrification.

We used comparative genomics, coupled with structural and biochemical analyses, to characterize the diversity of the heme-copper oxidoreductase superfamily. A number of previously unidentified oxygen and nitric oxide reductase families were discovered. Many of these new families have a wide environmental distribution and may play important roles in nitrogen cycling and aerobic respiration in low O_2 environments. Evolutionary analysis of the superfamily suggests that aerobic respiration originated after the evolution of oxygenic photosynthesis. In most currently known phyla early branching groups are anaerobic, implying that at the time of the major diversification of microbial life aerobic respiration had not yet evolved. It is also shown that nitric oxide reductase activity evolved many times independently, from various oxygen reductase members within the superfamily, suggesting that canonical denitrification evolved after aerobic respiration. The different oxygen reductase and nitric oxide reductase families vary in their substrate (O_2 and NO) apparent binding affinities and energy conservation efficiencies (H^+/e^- translocation ratio). These differences, which are due to structural variations within the proteins, have great ecological importance.

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14P12

The catalase activity of the aa_3 cytochrome *c* oxidase from *Paracoccus denitrificans*

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The four subunit (SU) aa_3 cytochrome *c* oxidase (CcO) from *Paracoccus denitrificans* is one of the terminal enzymes of the respiratory chain. It uses electrons from cytochrome *c* to reduce O_2 to H_2O. Its binuclear active center, residing in SU I, contains heme a_3 and Cu_B, the latter being ligated by three histidine residues. Apart from its oxygen reductase activity, CcO also possesses peroxidase and catalase activities.

To compare variants and the wild type (WT) protein in a more stringent way, a recombinant (rec.) WT CcO was created, carrying the gene for SU I on a low copy number plasmid. This rec. WT showed, as expected, no difference in oxygen reductase activity compared to the American Type Culture Collection (ATCC) WT CcO but, astonishingly, its catalase activity was increased by a factor of 20.

The slight overproduction of SU I due to plasmid coding and the resulting deficiency in metal inserting chaperones impairs the correct insertion of heme a_3 and Cu_B. This could lead to subtle differences in side chain orientation and changes in the water network. However, these slight changes might cause an increased accessibility of the active center for H_2O_2, resulting in an increased catalase activity. The availability of chaperones was improved by cloning the genes for the two metal inserting chaperones CtaG and Surf1c on the same plasmid together with the SU I gene. This new rec. WT CcO in fact showed a reduced catalase activity. To fathom the structural difference resulting in increased catalase activity capillary DSC was used. No significant difference in thermal stability between the ATCC WT CcO and the rec. WT CcO was detected. However, upon aging, the thermal stability of the rec. WT CcO was reduced faster than that of the ATCC WT CcO pointing to a decreased structural stability of the rec. WT CcO.

Several known inhibitors were used to probe the contribution of the metal cofactors in the catalase reaction. In addition, variants in aromatic amino acid positions near the active center were constructed to assess a possible reaction mechanism of the catalase activity of CcO. Moreover, GC-coupled MS-measurements were used to analyze the oxygen produced in the catalase reaction. As a result of these experiments, a reaction cycle of the catalase activity of CcO is postulated and the structural difference between the ATCC and rec. WT CcO is outlined. The catalase activity appears to be a true catalase activity and not a "pseudocatalase" activity.

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14P13

Heterologous production and purification of the cytochrome *bd* ubiquinol oxidase of *Aquifex aeolicus* in *Escherichia coli*

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The cytochrome *bd* ubiquinol oxidase is one of the terminal oxidases of the respiratory chain of many prokaryotes. The enzyme is a heterodimeric membrane protein expressed under microaerophilic conditions and has a high affinity towards oxygen. It catalyzes the reduction of dioxygen to water while oxidizing ubiquinol to ubiquinone. The redox reaction is coupled with a vectorial translocation of four protons across the membrane, thus, contributing to a proton motive force crucial for ATP production. The cytochrome *bd* ubiquinol oxidase is expected to cope with oxidative stress and as it is only expressed in bacteria, including a number of human pathogens, it may be an attractive drug target.

The cytochrome *bd* ubiquinol oxidase from various microorganisms has been studied by secondary structure predictions and orientation of subunits. Its cofactors were characterized and the role of possible proton channels was examined [1]. However, a crystal structure of the enzyme complex at high resolution is not yet known. To fill this gap, the stable cytochrome *bd* ubiquinol oxidase from *Aquifex aeolicus*, a hyperthermophilic organism with an optimal growth temperature of 85 °C, is used as a model. The *A. aeolicus* cytochrome *bd* ubiquinol oxidase was heterologously produced in *Escherichia coli*, purified by chromatographic steps and the heme-cofactors were spectroscopically characterized. However, the yield of the purification is still low. To facilitate protein purification, a green fluorescent protein combined with a hexahistidine-tag is fused to the terminal oxidase. The protein is produced in an *E. coli* strain depleted of the cytochrome *bd* ubiquinol oxidase genes [2].

References

[1] V.B. Borisov, R.B. Gennis, J. Hemp, M.I. Verkhovsky, The cytochrome *bd* respiratory oxygen reductases, Biochim. Biophys. Acta 1807 (2011) 1398–1413.

[2] D.L. Court, J.A. Sawitzke, L.C. Thomason, Genetic engineering using homologous recombination, Annu. Rev. Genet. 36 (2002) 361–388.

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14P14

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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14P15

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 shaked 100 mL flask).

References

[1] J.A. Garcia-Horsman, B. Barquera, J. Rumbley, J. Ma, R.B. Gennis, The superfamily of heme-copper respiratory oxidases, J. Bacteriol. 176 (1994) 5587–5600.
[2] J.F. Heidelberg, I.T. Paulsen, K.E. Nelson, et al., Genome sequence of the dissimilatory metal ion-reducing bacterium *Shewanella oneidensis*, Nat. Biotechnol. 20 (2002) 1118–1123.

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14P16

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 (C*c*O) [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 C*c*Os [2]. One of these has two isoforms, Cox5A and 5B, which have 68% sequence identity and are selectively expressed under normoxic