reduction of NO and O_2. Previous studies showed that NOR is taking both electrons and protons from the periplasm during both NO and O_2 reduction and is thus electrically silent. Our data suggest that for the cbb_3s proton pumping and transfer of protons against an electrochemical gradient varies with substrate. The cbb_3s, despite their presumed ability to pump protons during O_2 reduction, are taking electrons as well as protons from the same side of the membrane, the periplasm, when reducing NO. We take this surprising finding as an indication of the importance of the formation of distinct chemical intermediates in order to be able to perform endergonic vectorial proton transfer.

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S11.20 Oxidase assembly factor surf1 in *Paracoccus denitrificans*: Two copies specifically act on two different terminal oxidases

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Biogenesis of mitochondrial cytochrome *c* oxidase (COX) is a complex mechanism and involves a large number of assembly proteins, one of them being Surf1. The particular function of Surf1 is not yet understood, but a role in heme *a* insertion in COX subunit I and the stabilization of the nascent complex has been proposed. In humans, a fatal neurodegenerative disorder, the Leigh syndrome, is associated with the loss of Surf1 function. Still, Surf1 is not essential for COX assembly since mutant cells have residuals of fully assembled and active oxidase. Alignments revealed Surf1 homologues not only in eukaryotes, but also in prokaryotes. In the bacterium *Paracoccus denitrificans*, two homologous genes for Surf1 proteins have been identified: *surf1q* is the last gene of the *qox* operon coding for a ba_3-type ubiquinol oxidase (QOX), and *surf1c* is found at the end of the *cta* operon encoding subunits of the aa_3-type COX. We introduced chromosomal single and double deletions for both *surf1* genes, leading to significantly reduced oxidase activities in membrane. Our experiments show that both Surf1c and Surf1q are functional and act independently for the aa_3-type COX and the ba_3-type QOX, respectively. This is the first direct experimental evidence for the involvement of a Surf1 protein in the assembly of a quinol oxidase.

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S11.21 The BA_3 cytochrome *c* oxidase from *Thermus thermophilus*

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Terminal oxidases catalyse the energy-transducing reduction of molecular oxygen to water, coupling the redox energy to proton translocation through the cytoplasmic (or mitochondrial) membrane. Three main families of oxygen reductases can be identified on the basis of common features of their core subunits. Most of the residues which are critical for proton pumping in other cytochrome *c* oxidases ("signature") are not present in the sox B-like ba_3 oxidase. Even though the structure of this three-subunits enzyme was solved in 2000, still very little is known about in what way it deviates from canonical oxidases. To shed light on this, an internal his-tag was placed into subunit I to facilitate the purification of this enzyme and its variant forms. The spectral properties and enzymatic activities of the recombinant ba_3 oxidase are comparable to the wild-type enzyme. Mutants in critical residues involved in possible proton channels or in radical formation, were produced and compared to the native protein complex.To provide a genetically "clean" background for homologous expression of mutant enzyme(s), a chromosomal deletion of the ba_3-encoding *cba* operon was introduced in *T. thermophilus* via double-homologous recombination.

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S11.22 Biogenesis of cytochrome *c* oxidase subunit I: A cell-free expression approach

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Aim of the project is the development of a cell-free expression system suitable for biogenesis studies on cytochrome *c* oxidase (COX). Crucial steps during COX-biogenesis are the cofactor incorporation reactions into subunits I and II which generate the redox active centres. A transient mode of interaction has been proposed for the heme and copper insertion factors. *In vitro* translation, as a promising technique for the high-level production of membrane proteins, is used to address the biogenesis of COX subunit I. Unlike the *in vivo* situation the cell-free system offers the great advantage that immature biogenesis intermediates are not degraded and therefore accumulate. Moreover, samples can easily be manipulated by the addition of purified cofactors, chaperones and intact membrane vesicles deleted in specific biogenesis factors. In an *E. coli* extract expression of the full-length subunit I was achieved either as a precipitate or in a soluble form by the addition of suitable detergents. On a preparative scale subunit I was produced at a concentration of 500 µg per ml reaction mixture and purified by Immobilized Metal Affinity Chromatography. This approach should allow future expression and biogenesis studies in the presence of various maturation factors.

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S11.23 Synthesis of functional *Paracoccus Denitrificans* cytochrome *c* oxidase by *Escherichia coli* cell-free coupled transcription/translation system

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Time-resolved infrared spectroscopy could provide direct information about changes in protonation state and polarity of amino acid functional groups driving the proton pumping process catalyzed by cytochrome *c* oxidase. For assignment of the infrared signals, it is prerequisite to site-specifically label the amino acid residue with stable isotopes by the cell-free synthesis of the enzyme. Subunits I, II and III of the *Paraccocus denitrificans* enzyme were synthesized in the