

to narrow down the sites conferring the functional specificity of ACPM1 and ACPM2.

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1P.8 Electron transfer in *Escherichia coli* respiratory complex I

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The respiratory complex I couples the transfer of electrons from NADH to ubiquinone with the translocation of protons across the membrane. One flavin mononucleotide and seven iron-sulfur (Fe/S)-clusters build an electron transfer path from the NADH binding site to the ubiquinone binding site. The role of the protein in electron transfer was recently questioned. It is generally accepted that electron transfer takes place via tunnelling, that means through-space electron transfer with the Fe/S-clusters as the only centers of excess electrons. Recently it was proposed that electron transfer is performed via a hopping mechanism involving conserved aromatic amino acids between the Fe/S-clusters as stepping stones. The calculated transfer rates of the individual intramolecular electron transfer steps are in agreement with the experimental determined total transfer rate of $170 \pm 10 \text{ s}^{-1}$. To examine this hypothesis several aromatic amino acids in complex I from *E. coli* were mutated by λ -Red recombineering. The mutations led to a dramatic decrease in the NADH:oxidase activity of the mutant membranes, when the aromatic amino acid was replaced by a non-aromatic amino acid. Conservative substitution with another aromatic amino acid had a mild effect on the activity. The mutations showed the same effect on the k_{cat} of the NADH:ubiquinone oxidoreductase activity of the isolated complex I variants.

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1P.9 On complex I of *Neurospora crassa* – Role of the chaperone B17.2L

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Complex I or the NADH:ubiquinone oxidoreductase deficiency is a common cause of mitochondrial oxidative phosphorylation diseases, including Leigh syndrome. Mutations in nuclear genes encoding structural subunits or assembly factors of complex I have been increasingly identified as the cause of the diseases. One such assembly factor is a paralogue (B17.2L) of the B17.2 structural subunit of the enzyme, but the mechanism by which it exerts its function is still unclear. To better understand the requirement of B17.2L for complex I assembly we analyzed the mitochondrial respiratory chain of *Neurospora crassa* strains using one and two-dimensional blue-native PAGE. The results obtained indicate that disruption of both *Neurospora* genes, the one encoding the structural subunit (*nuo-13.4*) and its paralogue (*nuo-13.4L*), has no effect on the assembly or activity of complex I. Moreover, an anti-13.4L antibody does not recognize the holoenzyme but specifically associates with subassemblies present in several

complex I mutant strains. The analyses performed revealed that the 13.4L protein associates with different subassemblies in different complex I mutants. Our results indicate that the 13.4L protein is a molecular chaperone involved in the assembly of complex I in *N. crassa*, probably stabilizing a subcomplex of assembly containing the membrane arm and the connecting part. Furthermore, dissociation of the 13.4L protein seems to occur only upon full complex I assembly, suggesting that the assembly factor functions as a sensor of integrity.

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1P.10 Crystallisation of the membrane domain of complex I from *Escherichia coli*

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Complex I is the first enzyme of the respiratory chain and plays a central role in cellular energy production, coupling electron transfer between NADH and quinone to proton translocation. The enzyme is L-shaped, consisting of peripheral and membrane arms. The structure of peripheral arm was previously determined, however the complete structure and the coupling mechanism of this large molecular machine are currently unknown. To determine the structure of the membrane domain of complex I from *E. coli*, we have developed an effective procedure for its separation from the hydrophilic domain. Isolated membrane domain was crystallised. Initially poor X-ray diffraction properties were improved after extensive optimisation of crystallisation conditions. After a broad screening of conditions for heavy atom derivatisation, MIRAS data were collected. The preliminary findings from crystallographic data will be discussed.

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1P.11 The Zn^{2+} inhibitive effects on the cytochrome *bc*₁ complex from *Rhodobacter capsulatus* as revealed by the FTIR difference spectroscopy

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The cytochrome *bc*₁ complex is the third enzyme in the respiratory chain and couples the redox reactions to the proton translocation across the membrane. It is widely accepted that the enzyme functions following the modified Q-cycle [1], first proposed by Mitchell [2]. Metal dications such as Zn^{2+} can bind to the proton translocating enzymes and blocks the proton transfer, preventing thus the generation of the proton gradient, ubiquitous for the ATP production [3, 4]. It is indeed crucial to understand the mechanism of inhibition of Zn^{2+} . The Zn^{2+} binding site was suggested to be located at the interface between the heme *b*_L and the Rieske protein. The binding site consists of two histidines, one aspartic acid, one asparagine, and one glutamic acid; all belong to the heme *b* subunit [5]. The effect of the Zn^{2+} binding to the *bc*₁ complex from *Rhodobacter capsulatus* is studied with the help of a redox induced FTIR difference spectroscopy combined with site directed mutagenesis. The experiment show that redox reaction rates of the protein becomes considerably slower in presence of Zn^{2+} . Furthermore, the FTIR difference spectroscopy shows that the mutation of the Glu295 to Val295 leads to a loss of the signature of the protonated acidic residue in