oxygen species (ROS) production and to enzymatic activity assays. Submitochondrial particles (SMP) were treated with class A or B inhibitors. NADH addition initiated the electron transfer. In our system class A inhibitors (rotenone, piericidin A) increase ROS production from complex I, whereas class B inhibitors (stigmatellin, mucidin, CoQ2) have no effect on ROS production. We measured the presence of semiquinone (SQ) at 180 K and state of reduction of the iron sulfur cluster N2 at 12 K in SMP inhibited with class A and class B inhibitors. Our data confirm a strong SQ signal reduction in the presence of rotenone while the signal intensity is less reduced in samples treated with stigmatellin [2]. N2 spectra show different reduction state in presence of rotenone and stigmatellin. In presence of stigmatellin the center is mainly oxidized. We hypothesize a two-step reduction performed by N2, possibly following a rearrangement of the site [3]. Rotenone like inhibitors, not allowing the access of the quinone to the active site, would block the enzyme in a conformation that only permits electron delivery from N2 to oxygen. In this conformation hydrophilic quinones like CoQ1 can be reduced by N2 to a semiquinone species in a non physiological site. This semiquinone can rapidly react with molecular oxygen to form anion superoxide. Stigmatellin like inhibitors would block the enzyme in a conformation allowing only the first step of quinone reduction (Q→SQ) in the physiological reduction site, but blocking any further reduction; this conformation does not allow reaction of N2 with oxygen.

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doi:10.1016/j.bbabio.2010.04.052

1P.5 NADH binding to complex I: Implications for the mechanism

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Complex I plays a central role in cellular energy production, coupling electron transfer between NADH and quinone to proton translocation. We have determined several X-ray structures of the oxidized and reduced hydrophilic domain of complex I from *Thermus thermophilus* at up to 3.1 Å resolution. The structures reveal the mode of interaction of complex I with NADH, explaining known kinetic data and providing implications for the mechanism of ROS production at the flavin site of complex I. Bound metals were identified in the channel at the interface with the frataxin-like subunit Nqo15, indicating possible iron-binding sites. Conformational changes upon reduction of the complex involve adjustments in the nucleotide binding pocket, as well as small, but significant, shifts of several α-helices at the interface with the membrane domain. These shifts are likely to be driven by the reduction of nearby Fe–S clusters N2 (the electron donor to quinone) and N6a/b. Cluster N2 is coordinated by unique motif involving two consecutive (tandem) cysteines. An unprecedented "on/off switch" (disconnection) of coordinating bonds between the tandem cysteines and this cluster was observed upon reduction. Comparison of the structures suggests a novel mechanism of coupling between electron transfer and proton translocation, combining conformational changes and protonation/de-protonation of tandem cysteines.

doi:10.1016/j.bbabio.2010.04.053

1P.6 The role of the isolated [2Fe–2S] cluster adjacent to the flavin mononucleotide of mitochondrial complex I: Does it influence catalysis at the flavin site?

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Complex I is the proton pumping NADH:ubiquinone oxidoreductase of the mitochondrial inner membrane. Complex I from bovine mitochondria contains eight iron–sulphur clusters (two [2Fe–2S] clusters and six [4Fe–4S] clusters). Seven of them link the NADH oxidation site to the ubiquinone reduction site. The eighth cluster, named 2Fe[24] or N1a, is ligated by the 24 kDa subunit; it is isolated from the main chain of clusters but adjacent to the flavin mononucleotide and close enough to accept electrons from it. Whether the 2Fe[24] cluster has a role in the mechanism of complex I is not known. It is possible it minimises the lifetime of the semi-reduced flavin species, decreasing the rate of superoxide production and/or preventing direct hydrogen peroxide production by the fully reduced flavin. Complex I from *Escherichia coli* contains a homologous cluster with a reduction potential 0.1 V higher than that of the bovine cluster; complex I from *E. coli* also produces hydrogen peroxide rather than superoxide. The *E. coli* cluster is probably reduced during catalytic turnover, and so may be incapable of minimising the semi-reduced flavin. In this study, complex I from *Yarrowia lipolytica* was used to establish the role of the 2Fe[24] cluster. Mutations were generated in the closely homologous NUMH subunit, to increase the reduction potential of the [2Fe–2S] cluster to that observed in *E. coli*. The effects on catalysis and superoxide production by the complex are described.

doi:10.1016/j.bbabio.2010.04.054

1P.7 Mitochondrial acyl carrier proteins in *Yarrowia lipolytica*: Guilty by affiliation with complex I

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Mitochondrial acyl carrier proteins (ACPMs) were first discovered in the 1980s in *Neurospora crassa*. They are thought to be involved in mitochondrial fatty acid synthesis and in the production of octanoic acid via a phosphopantetheine group covalently attached to a conserved serine. Our group has previously demonstrated that *Yarrowia lipolytica* codes for two different mitochondrial acyl carrier proteins, ACPM1 and ACPM2, that both are *bona fide* subunits of complex I. Deletion of the ACPM1 gene is lethal, whereas ACPM2Δ strains are viable in a certain strain background. However, the ACPM2Δ cells showed an apparent lack of complex I, pointing towards a role in assembly/stability for the complex. In contrast, ACPM1 seems to have a function beyond complex I. The two ACPM protein sequences differ mostly in their putative mitochondrial targeting sequences. We thus created a protein consisting of the ACPM1 targeting sequence fused to the sequence of mature ACPM2. Two DNA constructs with different length of the putative ACPM1 targeting sequence were created and used for plasmid-based complementation of the ACPM1Δ strain. No viable spores were obtained, indicating that both chimeric proteins failed to take over the function of ACPM1. In the ACPM2Δ strain, both constructs led to the formation of assembled complex I, suggesting that the functional difference between the ACPM variants is mediated by the targeting sequence. Currently, various domain-swap constructs are underway

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

doi:10.1016/j.bbabio.2010.04.055

1P.8 Electron transfer in *Eschericha 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 $170 \pm 10\ s^{-1}$. 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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doi:10.1016/j.bbabio.2010.04.056

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.

doi:10.1016/j.bbabio.2010.04.057

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 form *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.

doi:10.1016/j.bbabio.2010.04.058

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