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Structure and function of the C-terminal end of MrpA — The evolutionary progenitor of the long, membrane parallel helix domain in Complex I

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MrpA is a subunit of the Mrp-antiporter, and the subunit that is evolutionary most closely related to the Complex I subunit NuoL. In addition to the 14 TM-helix protein part conserved in both MrpA and MrpD- type proteins in this family, both MrpA and NuoL contain a C-terminal extension. The recent structures of the Complex I membrane-spanning domain revealed this part of NuoL as one TM helix, followed by a long, membrane parallel helix and a final TM helix ending in the bacterial periplasm [1]. The long helix was immediately believed to have an important functional role for the coupling of the electron-transfer reactions to proton pumping. However, attractive, such a "piston" function has later been disowned [2], but an important structural role of the NuoL C-terminus seems indisputable.

In this work we have investigated the putative evolutionary progenitor of the long, membrane parallel (lmp) helix; the C-terminal domain of MrpA. Sequence alignments revealed that in MrpA, the putative lmp-helix is shorter, owing to the fact that the MrpA lmp-helix only needs to cover one partner protein (MrpD) whereas the NuoL lmp-helix is enclosing both NuoM and NuoN. Transmembrane topology studies showed that the remaining part of MrpA, that is absent from NuoL, instead seem to correspond to NuoJ in Complex I, although the primary sequence conservation is low in this part of the protein. We have previously demonstrated that the subunit NuoK, which is surrounded by NuoJ in the solved structure [1], is homologous to MrpC. The function of the MrpA C-terminus was subsequently assessed in a *B. subtilis* strain deleted for MrpA. At neutral pH, when antiporter-function can be exhibited without protein complex formation, the truncated MrpA could still operate. But at pH 8.4, the C-terminal domain of MrpA was absolutely essential for function, demonstrating an important structural role for this domain also in the Mrp antiporter complex.

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The structure of the NADH:ubiquinone oxidoreductase from *Vibrio cholerae*

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The sodium pumping NADH:ubiquinone oxidoreductase (Na^+-NQR) from the pathogenic Gram-negative bacterium *Vibrio cholerae* is an integral membrane protein complex [1,2]. The Na^+-NQR consists of six different subunits NqrA-F [3] and contains a series of cofactors to transport electrons from NADH to ubiquinone. The energy that arises during this process is used to pump sodium ions from the cytoplasm to the periplasm to generate a sodium motif force (SMF). This SMF is essential for substrate uptake, motility, pathogenicity or efflux of antibiotics.

NQR was isolated and crystallized by Marco Casutt and Günter Fritz [4]. The structure was solved at a resolution of 3.7 Å, which provides the first detailed information about this respiratory enzyme. In some regions, especially in the flexible NqrA subunit, the quality of the electron density was too low to assign a distinct structure.

In order to obtain further detailed structural information, we aim to determine the structure single subunits at a high resolution. Recently, a C-terminal truncated version of NqrA (residues 1-377), was crystallized and the structure was determined at 1.95 Å.

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Overproduction of *Aquifex aeolicus* complex I in *E. coli nuo*-deletion strains

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The NADH:ubiquinone oxidoreductase, respiratory complex I, is the main entrance point of electrons into the respiratory chains. The complex couples the electron transfer from NADH to ubiquinone with proton translocation across the membrane. The enzyme comprises a noncovalently bound flavin mononucleotide and several iron-sulfur clusters as cofactors. In *Aquifex aeolicus,* a hyperthermophilic bacterium with an optimal growth temperature of 95 °C, 13 out of totally 24 *nuo*-genes coding for NuoA-N subunits of complex I, are organized in three different loci [1, 2]. Expression plasmids were constructed containing all 13 *A. aeolicus nuo*-genes and sequences for polyhistidine affinity tags were fused at several positions. Heterologous production of *A. aeolicus* complex I is attempted in an *Escherichia coli* strain missing the chromosomal *nuo*-operon (C43Δ*nuo*) [3, 4]. The entire *A. aeolicus* complex I was successfully produced and assembled into the bacterial membrane. However, only subcomplexes of the overproduced protein were isolated due to the instability of the overproduced protein.

The NuoEF subcomplex was crystallized and the structure was resolved at 1.7 Å and 1.9 Å when soaked with substrates. It is attempted to crystallize the other preparations containing subcomplexes in order to understand the structure and function of complex I.

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The role of Ndufaf2 in the assembly of mitochondrial complex I

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Mitochondrial complex I (NADH: ubiquinone oxidoreductase) is the first and largest enzyme complex in the mitochondrial respiratory chain. It is the major entry point to oxidative phosphorylation, catalysing the transfer of electrons from NADH to coenzyme Q, which is coupled to pumping protons out of the mitochondrial matrix. It plays a central role in mitochondrial metabolism, as nearly 50% of oxidative phosphorylation disorders result from a complex I deficiency. In humans, complex I is composed of 45 subunits, with a total molecular mass of 980 kDa. It has been shown that the assembly of complex I follows a semi-sequential order and requires a number of assembly factors, with defects of these assembly factors responsible for complex I deficiency. Ndufaf2 is an assembly factor involved in the late stages of complex I assembly. However, its actual function is still unclear. We have identified three patients with complex I deficiency harbouring mutations in *NDUFAF2*. The loss of Ndufaf2 in these patients results in reduced levels of mature complex I, with the subsequent accumulation of membrane arm subunits in a stalled ~600 kDa complex. The steady-state levels of the nDNA-encoded subunits, NDUFA9 and NDUFS2, are also affected. However, the translation of complex I mtDNA-encoded subunits is normal. Additionally, the assembly factor Ndufaf4 is up-regulated, with its accumulation in an ~150 kDa complex with the complex I subunit NDUFS3. These studies will help us to solve the puzzle of complex I assembly and to understand the function of Ndufaf2 in the assembly process.

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Semiquinone intermediates formed during catalysis by complex I

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Understanding quinone reduction by mitochondrial complex I remains a challenge due to limited structural and mechanistic data. Ubisemiquinones are considered intermediates of mitochondrial complex I catalysis on the basis of electron paramagnetic resonance studies on isolated protein, proteoliposomes and native membranes, however the number of species and their location remain poorly defined. Up to three species have been associated with complex I: SQ_{Nf}, SQ_{Ns} and SQ_{Nx}. The extremely slow relaxing specimen, SQ_{Nx}, is no longer considered relevant. The fast relaxing specimen, SQ_{Nf}, has never been observed in isolated complex I or tightly coupled proteoliposomes, so its association with complex I remains questionable. Finally, SQ_{Ns}, the slow relaxing radical, while well documented to be originating from complex I, has not been located in the structure. Some studies employ also hydrophilic quinone analogues that can react with the flavin site of complex I and form intermediates not specific to energy-transducing quinone reduction. Here, we revisit the semiquinone formation in complex I under physiologically relevant conditions, to further elucidate the mechanism of quinone reduction.

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Site-directed mutagenesis of residues involved in proton translocation by *Escherichia coli* complex I

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NADH:ubiquinone oxidoreductase (complex I) is the first and largest enzyme of mitochondrial respiratory chain. Mutations in the hydrophobic part of the enzyme have been linked to many neurodegenerative diseases. Complex I catalyses the transfer of two electrons from NADH to a quinone molecule coupled to the translocation of protons, resulting in the establishment of a proton motive force. The enzyme is present in a wide variety of species and is well conserved in the core subunits. The enzyme from *Escherichia coli* is an excellent model for the eukaryotic complex I and a recent crystal structure of the membrane domain of *E. coli* complex I heralds a significant breakthrough. However, the detailed mechanism of the action of complex I is still largely unknown. Mechanism of proton translocation through channels in the membrane is widely disputed, as well as the nature and extent of the conformational changes in the protein upon proton pumping. Several residues in antiporter-like subunits are predicted from the membrane domain structure to be important for proton translocation. This study focuses on the site-directed mutagenesis of those residues in order to elucidate the mechanism of proton translocation in *E. coli* complex I.

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A combined electrochemical, Resonance Raman and Far Infrared study on the respiratory complex I

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