Escherichia coli complex consists of 13 subunits called NuoA to NuoN. One FMN and 7 to 8 Fe/S-clusters participate in the electron transfer reaction. The three dimensional structure of the complex from *Thermus thermophilus* was determined at 4.5 Å resolution [1]. However, the structure does not show electron density from the substrate quinone, thus the quinone-binding site is structurally not defined. A large cavity is located at the interface between the peripheral arm and the membrane arm which is supposed to represent the quinone-binding site. We used site-directed spin labeling (SDSL) in combination with EPR/DEER spectroscopy to localize the quinone-binding site in *E. coli* complex I reconstituted in lipids. For this purpose several cysteine residues were introduced at the surface of the complex. The positions were labeled with (1-oxyl-2,2,5,5-tetramethyl-Δ3-pyrroline-3-methyl)-methanethiosulfonate (MTSL) containing a nitroxide radical. A decyl-ubiquinone derivative with the same label covalently linked to the end of the alkyl chain was synthesized [2]. Positions $R112^{B}$, $T337^{CD}$ and $A570^{CD}$ were chosen for spin labeling as the enzymatic activity of the labeled variants was not affected. The distance between the enzyme- and the substrate-bound label was measured by cw-EPR and DEER experiments. Triangulation of the distances point to a distinct position within the complex, where the alkyl chain of decyl-ubiquinone is located.

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The ion-translocation activities of proteins from the Mrp-antiporter family, evolutionarily related to complex I, analyzed in a *Bacillus subtilis* model system

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NADH:quinone oxidoreductase (Complex I) is a large membrane bound enzyme complex that has evolved from smaller functional building blocks. Intermediate size enzyme complexes exist in nature, that comprise some, but not all of the protein subunits in full size complex I. The complex I subunits NuoL, NuoM and NuoN are homologous to each other and to two proteins from one particular class of Na^+/H^+ antiporters, denoted MrpA and MrpD. In complex I, these subunits are prime candidates for harboring important parts of the proton pumping machinery. Using a model system consisting of *Bacillus subtilis* MrpA and MrpD deletion strains and a low copy expression plasmid, it was recently demonstrated that NuoN can rescue the strain deleted for MrpD but not that deleted for MrpA whereas the opposite tendency was seen for NuoL [1]. This demonstrated that although structural homologues, the MrpA-type and MrpD-type proteins have unique functional specializations. The simplest explanation for the experimental results is that MrpA and MrpD are single ion transporters that together form an antiporter. Since NuoL was previously shown to conduct Na^+ [2] we tentatively assigned the homologous MrpA as the Na^+-channel and MrpD as the H^+ channel. In this work the corresponding homologous protein subunit from the smaller enzymes evolutionary related to complex I was tested in the same model system. Interestingly, the NuoL, NuoM and NuoN subunits from 11-subunit complex I from *Bacillus cereus* behaved essentially as those of full size complex I, corroborating that this enzyme is indeed a bona fide complex I. The corresponding hydrogenase proteins tested exhibited less specified function. The homologous hydrogenase-3 protein HycC could substitute equally well for either MrpA or MrpD, suggesting that this transporter protein have no ion specificity, whereas the three homologous hydrogenase-4 proteins showed some differentiation. To identify the amino acid residues responsible for the specificity, a detailed sequence comparison of polypeptides with different demonstrated ion specificity was made. Based on these results, a set of positions in MrpA and NuoL was chosen and subjected to site-directed mutagenesis. The mutants were subsequently analysed in the *B. subtilis* model system.

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The role of subunit NuoL for proton translocation by the respiratory complex I

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The NADH:ubiquinone oxidoreductase, respiratory complex I, couples the electron transfer from NADH to ubiquinone with the translocation of protons across the membrane. The structure of the complex revealed the presence of a 110 Å long horizontal helix aligning almost the entire membrane arm of the complex. It was proposed that this helix is used as a 'piston' to transmit the energy that is released by the redox reaction at the ubiquinone binding site to the membrane arm, where proton translocation takes place [1]. Recently, we reported that the deletion of the horizontal helix results in a reduced H^+/e^- stoichiometry indicating its direct involvement in proton translocation [2]. In addition, we showed in *Escherichia coli* the effect of mutations of conserved acidic amino acids, which are part of the horizontal helix, on proton translocation [3]. The variants $D563X^{L}$ (X = N, E, Q and A) exhibited a reduced H^+/e^- stoichiometry, while the D542N and D546N variants showed the same stoichiometry as the parental complex. Cysteine scanning implies a movement of the horizontal helix during the redox reaction. The implications for the mechanism of proton translocation are discussed.

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