1L.7 A new hypothesis on thesimultaneous direct and indirect coupling mechanism in NADH–quinone oxidoreductase (Complex I)

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Earlier, T. Ohnishi and her collaborators detected two distinct protein-associated semiquinone species (SQNf and SQNs). Only SQNf signal intensity is strongly dependent on the proton motive force, thus we proposed a novel mechanistic hypothesis of a "proton pump gated by SQNf and redox-driven conformational change of its binding protein (Ohnishi & Salerno, FEBS Lett., 2005, 579: 4555–4561). This model is now modified to include both QNf and QNs molecules. To investigate the indirect proton-pump mechanism, we focused on the largest transmembrane NuoL subunit which has a high sequence similarity to the multi-subunit (Na^+/H^+) antiporter, like NuoM and N. (NuoN, however, does not contain active acidic residues, and localizes most likely the SQNs-binding site.) We constructed 13 NuoL mutants of highly conserved acidic residues. Similar amount of fully assembled protein were obtained in most of these mutants. Their dNADH–oxidase activities were mostly at the control level or modestly reduced. The proton pumping efficiency, however, was decreased in these NuoL mutants by 30–50% without affecting IC_{50} values for asimicin (a potent inhibitor for E. coli complex I). This suggests that the (H^+/e^-) stoichiometry has changed from ($4H^+/2e^-$) to either ($3H^+/2e^-$) or ($2H^+/2e^-$). Furthermore, 5-(N-ethyl-N-isopropyl)-amiloride (EIPA), a specific inhibitor for (Na^+/H^+) antiporters, caused a $38 \pm 5\%$ decrease in the initial (H^+) pump activity in the wild-type membranes. However, no change was observed in D178N, D303A and D400A mutants (in which the (H^+) pumping efficiency had already been decreased by the NuoL mutation). The electron transfer activities were unaffected by EIPA in all of the mutants. Our data indicate that the NuoL subunit is involved in the indirect proton pump, although the NuoM subunit may also be involved. Thus, we propose that electron–proton coupling in complex I consists of "QNf-gated (H^+) pump" and "QNs-induced conformationally-driven (H^+) pump", each of which transports ($2H^+$) coupled with 2 electron transfer. Both pumps must be operated by the redox energy. In the indirect pump, the conformational linkage to the (Na^+/H^+) antiporter homologs is provided by quinone binding site occupancy. After the uptake of scalar (H^+) from the N-side, the binding of quinone to the SQNs site, and the release of quinol from there, conformationally triggers ion pump reorientation, enabling vectorial (H^+) movement. We propose that QNf carries ($2H^+$) (direct pump) and QNs induces the transport of ($2H^+$) (indirect pump).

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1L.8 cAMP dependent protein kinase regulates expression and post-translational processing of respiratory chain complex I in mammalian cells

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Work is presented on the role of cAMP-dependent protein phosphorylation in post-translational processing and biosynthesis of complex I subunits in mammalian cell cultures [1, 2]. PKA-mediated phosphorylation of the NDUFS4 subunit of complex I promotes in cell cultures *in vivo* import/maturation in mitochondria of the precursor of this protein [3]. The import promotion appears to be associated with the observed cAMP-dependent stimulation of the catalytic activity of complex I. These effects of PKA are counteracted by activation of protein phosphatase(s). PKA and the transcription factor CREB play a critical role in the biosynthesis of complex I subunits. CREB phosphorylation by PKA and/or caMKs activates, at nuclear and mitochondrial level a transcriptional regulatory cascade which promotes the concerted expression of nuclear and mitochondrial encoded subunits of complex I and other respiratory chain proteins [4].

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1L.9 The architecture of bacterial respiratory complex I

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NADH–ubiquinone oxidoreductase (complex I) is the first and the largest enzyme in the respiratory chain of mitochondria and most bacteria. Complex I is implicated in many human neurodegenerative diseases, as well as in aging. We study bacterial complex I as a "minimal" model of human enzyme. It is an L-shaped assembly, with the hydrophobic arm embedded in the membrane and the hydrophilic arm protruding into the bacterial cytoplasm. Previously, we have determined the crystal structure of the hydrophilic domain of complex I from *Thermus thermophilus* [1,2]. The mechanism of coupling between the electron transfer and proton translocation in complex I is currently not established, mainly due to the fact that the high-resolution structures of the hydrophobic domain and of the intact complex remain unknown. We have now crystallised the membrane domain of complex I from *E. coli* and determined, by X-ray crystallography, its low-resolution α-helical structure. We have also crystallised the entire complex I from *T. thermophilus* and determined its structure by molecular replacement with the known structure of the hydrophilic domain and the α-helical structure of the *E. coli* membrane domain. The overall architecture of complex I, thus revealed, provides strong clues about the coupling mechanism.

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