Bioenergetics

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Inside View of a Giant Proton Pump

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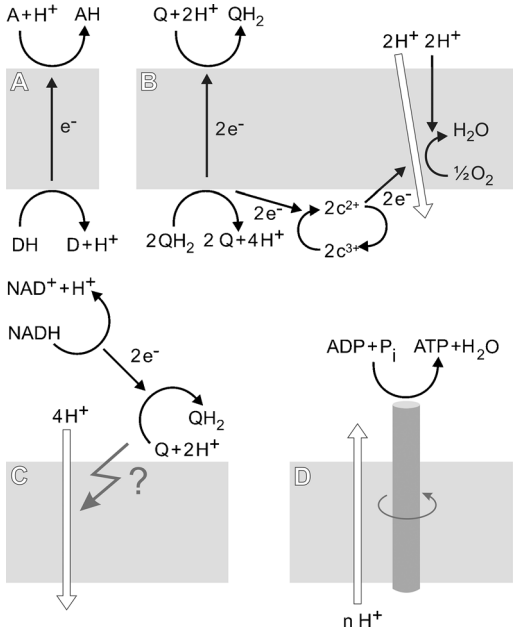
Aerobic cells meet their energy demands predominantly by synthesizing ATP through oxidative phosphorylation. In this process hydrogen extracted from foodstuffs is transferred to a chain of redox enzymes to eventually reduce molecular oxygen to water. In eukaryotes this takes place in the mitochondria, the cellular "power plants", which in a resting human being, turnover the equivalent of about 3000 litres of H_2 per day.

Defining the fundamental principles of such biological fuel cells more than 50 years ago, Peter Mitchell published the now well-established concept of chemiosmotic coupling between respiratory electron transfer and ATP synthesis through the generation of a proton gradient across a bioenergetic membrane.[1] While Mitchell's original suggestion that the so-called proton motive force is generated by means of redox loops combining the transmembrane transport of electrons with the sided uptake of protons by the substrates is still valid (Scheme 1 A), biochemical and structural analysis of the respiratory complexes involved has revealed a remarkable variety of molecular mechanisms for proton pumping (Scheme 1 B), like the more sophisticated substrate-linked protonmotive Q-cycle in the cytochrome bc_1 complex (complex III) and localized pump–gate mechanisms like in cytochrome c oxidase (complex IV).

Remarkably, for respiratory complex I (proton pumping NADH: ubiquinone oxidoreductase), biochemical evidence[2] and the emerging structures of the bacterial[3] and mitochondrial[4] enzymes revealed that the redox reactions take place in the hydrophilic domain entirely separated from the proton pumps in the membrane (Scheme 1 C) implying yet another, indirect coupling mechanism. Such spatial separation of chemistry and transmembrane proton translocation was found previously in ATP synthase (complex V), where ATP synthesis is driven by the proton motive force through a unique rotary mechanism (Scheme 1 D). Already the initial structural data suggested a different kind of long-range energy transfer for complex I, but the actual mechanism



Scheme 1. Principles of proton pumping in oxidative phosphorylation. A) basic redox loop; B) protonmotive Q-cycle of comple III (left) and gated proton pump in complex IV (right); C) indirect pump in complex I; D) rotary mechanism of ATP synthesis in complex V. Q = ubiquinone, c = cytochrome *c*.

how redox chemistry and proton pumping are coupled remained elusive.[3,4]

Complex I couples the electron transfer from NADH to the hydrophobic hydrogen carrier ubiquinone with the vectorial translocation of four protons across the bacterial plasma membrane or the inner membrane of mitochondria.[5] In some cases, the enzyme uses menaquinone as an alternative substrate. It is by far the largest complex of the respiratory chain. Even in bacteria, where it typically consists of the functional core of 14 central subunits with very few additional proteins, the L-shaped membrane-integral enzyme has a molecular mass of more than 500 kDa. The mitochondrial complex that comprises a large number of additional, so-called accessory subunits reaches a mass of almost 1 MDa. Complex I can be subdivided into functional modules[5] that have defined structural correlates (Figure 1).[4] From the NADH-oxidizing N-module with the two- to one-electron converter FMN, electrons are transferred to the Q-module where the ubiquinone reduction site resides at the end of

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a chain of seven iron–sulfur clusters. The Q- and N-modules form the peripheral arm of complex I that sits almost perpendicular at one end of the 180 Å long membrane arm. This P-module harbors the proton pumps and is subdivided into a proximal and a distal domain each of which is known to pump half of the protons.[6]

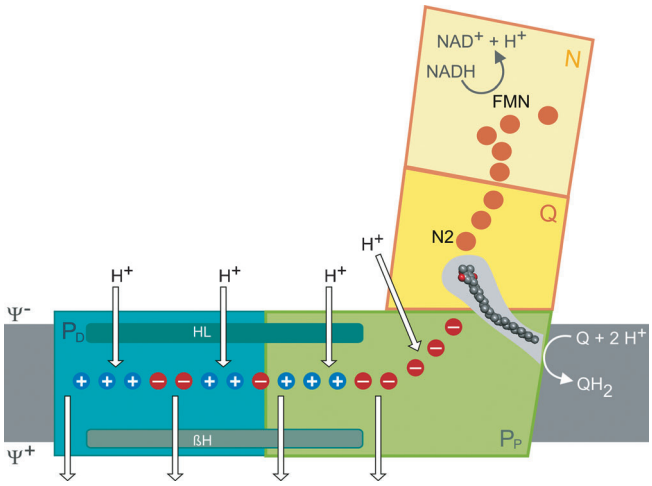

Figure 1. Schematic overview of the functional elements of complex I. In the N-module, electrons from NADH enter a chain of seven iron–sulfur clusters (orange dots). They are transferred from the terminal cluster N2 of the chain onto a ubiquinone molecule (space-filling model) bound in a pocket of the Q-module reaching into the membrane domain. A long chain of charged residues passing through the middle of the membrane integral P-module connects the Q-binding pocket to four putative pump sites consisting of separate proton input and output channels. The long lateral helix (HL) and a series of β-hairpin-helix elements (βH) bridge the proximal (Pp) and distal (PD) domain of the P-module.

The crystal structure of the entire bacterial complex I from *Thermus thermophilus*[7] at 3.3 Å resolution now provides a complete picture of all the components of this remarkably large and complicated enzyme. It offers detailed insights into the connection between the redox prosthetic groups of the peripheral arm and the proton pumps of the membrane arm. The roughly 300 Å long, continuous array of functional elements encompasses all parts of the giant multiprotein complex and reaches from the NADH oxidation site at the top of the peripheral arm all the way to the most distal pump site at the end of the membrane arm (Figure 1). In addition to the putative proton uptake and release channels described previously within the three subunits homologous to Mrp-type Na^+/H^+ antiporters,[3] a fourth set of putative channels is now clearly described that runs through the membrane right next to the docking region of the peripheral arm and possibly even involving it in part. Although the number of four pump sites suggested from the structure fits the experimental pumping stoichiometry of complex I of $4H^+/e^-$, the functionality of all the putative channels must still be tested experimentally.

Confirming earlier reports,[2,4,8] the headgroups of the substrate ubiquinone and the quinone-analogous inhibitor piericidin A are shown to bind to a conserved tyrosine next to the terminal iron–sulfur cluster N2 residing about 25–30 Å above the membrane surface in a large amphipathic pocket of the Q-module.[7] This pocket extends as a narrow channel into the P-module, thereby providing access to the membrane domain. However, such a constrained path does not seem compatible with efficient substrate exchange. This may be overcome during turnover by the transient opening of the ubiquinone pocket to some extent. On the other hand, the formation of a well-shielded reaction chamber is well in line with the proposal that tight control of the protonation of anionic ubiquinone intermediates is critical for the energy-converting mechanism of complex I.[9] In good agreement with this idea and corroborating an earlier proposal that energy transmission within complex I may occur through electrostatic coupling,[10] the ubiquinone binding pocket now appears as the starting point of a unique uninterrupted chain of positively and negatively charged residues that for the most part runs through the middle of the P-module and connects the four putative proton-input and -output channels observed in the structure of the membrane domain.[7] While it is tempting to speculate that this unique feature of complex I represents the key energy-transducing element driving the proton pumps, to prove this, several pertinent questions need to be answered both theoretically and experimentally. How are the charges shielded securely from access by water and ions to ensure tight coupling and prevent the uncontrolled dissipation of energy? Does the mechanism proceed through conformational changes at all, or does it operate exclusively by electrostatically controlled changes in the apparent pKs of the residues involved? How is the gating of proton access and release ensured? Do all pumping elements operate in sync? At any rate, the strikingly long lateral horizontal helix bridging the distal and the proximal part of the P-module seems to merely stabilize the structure of the membrane domain together with a set of β-hairpin-helix elements on the opposite side of the membrane rather than playing an active part in the pumping mechanism as suggested earlier.[3,4]

The structure of the entire bacterial complex I offers a stunning inside view into a unique and fascinating molecular machine.[7] We are only just starting to understand how complex I might work as a redox-driven proton pump. Its sheer size and complexity, a binding site for its hydrophobic substrate in the hydrophilic domain and a peculiar long chain of charge residues running through the middle of its membrane domain pose major challenges for future structural and functional studies.

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