Biochimica et Biophysica Acta 1817 (2012) 1785–1795

Contents lists available at SciVerse ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbabio

Review

# The coupling mechanism of respiratory complex I — A structural and evolutionary perspective ☆

Rouslan G. Efremov [1], Leonid A. Sazanov *

*Medical Research Council Mitochondrial Biology Unit, Wellcome Trust/MRC Building, Hills Road, Cambridge CB2 0XY, UK*

ARTICLE INFO

*Article history:*
Received 15 December 2011
Received in revised form 9 February 2012
Accepted 14 February 2012
Available online 22 February 2012

*Keywords:*
NADH:ubiquinone oxidoreductase
Hydrogenase
Modular evolution
Coupling mechanism
Redox reaction
Proton translocation

ABSTRACT

Complex I is a key enzyme of the respiratory chain in many organisms. This multi-protein complex with an intricate evolutionary history originated from the unification of prebuilt modules of hydrogenases and transporters. Using recently determined crystallographic structures of complex I we reanalyzed evolutionarily related complexes that couple oxidoreduction to trans-membrane ion translocation. Our analysis points to the previously unnoticed structural homology of the electron input module of formate dehydrogenlyases and subunit NuoG of complex I. We also show that all related to complex I hydrogenases likely operate via a conformation driven mechanism with structural changes generated in the conserved coupling site located at the interface of subunits NuoB/D/H. The coupling apparently originated once in evolutionary history, together with subunit NuoH joining hydrogenase and transport modules. Analysis of quinone oxidoreduction properties and the structure of complex I allows us to suggest a fully reversible coupling mechanism. Our model predicts that: 1) proton access to the ketone groups of the bound quinone is rigorously controlled by the protein, 2) the negative electric charge of the anionic ubiquinol head group is a major driving force for conformational changes. This article is part of a Special Issue entitled: 17th European Bioenergetics Conference (EBEC 2012).


## 1. Introduction

Complex I (NADH:ubiquinone oxidoreductase) plays a central role in the respiratory chain in mitochondria and many bacteria, providing about 40% of the proton flux during proton-motive force (pmf) generation for the synthesis of ATP [1–6]. It catalyzes the exergonic transfer of two electrons from NADH to ubiquinone ($\Delta G'_{NADH>UQ} \sim -80$ kJ/mol, depending on the redox state of NADH and quinone pools), coupled to the translocation of four protons (current consensus value [7–9]) against the electrochemical potential of ~180 mV across the inner mitochondrial membrane (conserving ~69 kJ/mol) [10]. Complex I is a reversible machine, able to reduce $NAD^+$ by ubiquinol, utilizing the trans-membrane (TM) potential [11].

Bacterial complex I presents a minimal model of the mitochondrial enzyme [12]. It is an L-shaped assembly normally consisting of 14 "core" subunits (7 hydrophilic and 7 hydrophobic, ~550 kDa combined mass) conserved from bacteria to humans [1,2,5]. The complex contains 8 to 10 iron–sulfur (Fe–S) clusters and a flavin mononucleotide, located in the peripheral hydrophilic arm (Fig. 1A). The mechanisms of coupling between redox reaction and proton translocation have been discussed widely: both "direct" (redox-driven) and "indirect" (conformation-driven) [2,3,5,13] coupling mechanisms were suggested.

Recent years were marked with great progress in understanding the structure of bacterial complex I, which answered many mechanism related questions. The atomic structure of the peripheral arm established the electron transfer pathway between NADH and ubiquinone binding sites [14]. The structure of six membrane domain subunits and a lower resolution structure of intact complex I established plausible proton translocation pathways and the relative positions of peripheral and membrane arms [15,16]. NuoH is the only subunit for which an atomic model is still not known; so far the arrangement of trans-membrane helices for this subunit, connecting two arms, was determined [15]. The structure of complex I clearly indicates that it operates by a unique conformation driven mechanism [15,17], not observed in any oxidoreductases of known three dimensional structure. While the general principles of oxidoreduction catalysis for NADH and quinones [11,18,19], electron transport by metal prosthetic groups [20] and directed ion transport across membrane [16,21] are understood at various levels of detail, the energy transformation from transfer of electrons into mechanical movement is still enigmatic in complex I.

*Abbreviations:* FHL, formate hydrogenlyases; Mrp, multiple resistance and pH adaptation; $F_{420}H_2$, 8-hydroxy-5-deazaflavin; TM, trans-membrane; pmf, proton motive force; MPh, methanophenazine; Fpo, $F_{420}H_2$: MPh dehydrogenase; Ech, *Escherichia coli* hydrogenase-3-type hydrogenase; Mbh, membrane bound hydrogenase; FdhF, formate dehydrogenase; Hyd, *E. coli* hydrogenase; Q, ubiquinone or menaquinone; Q-site, quinone-binding site; $Fd_{red}$ and $Fd_{ox}$, reduced and oxidized ferredoxin

☆ This article is part of a Special Issue entitled: 17th European Bioenergetics Conference (EBEC 2012).

* Corresponding author. Tel.: +44 1223252910; fax: +44 1223252915.
*E-mail address:* sazanov@mrc-mbu.cam.ac.uk (L.A. Sazanov).
[1] Present address: Max-Planck-Institute for Molecular Physiology, Otto-Hahn Str. 11, Dortmund 44227, Germany.

0005-2728/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbabio.2012.02.015

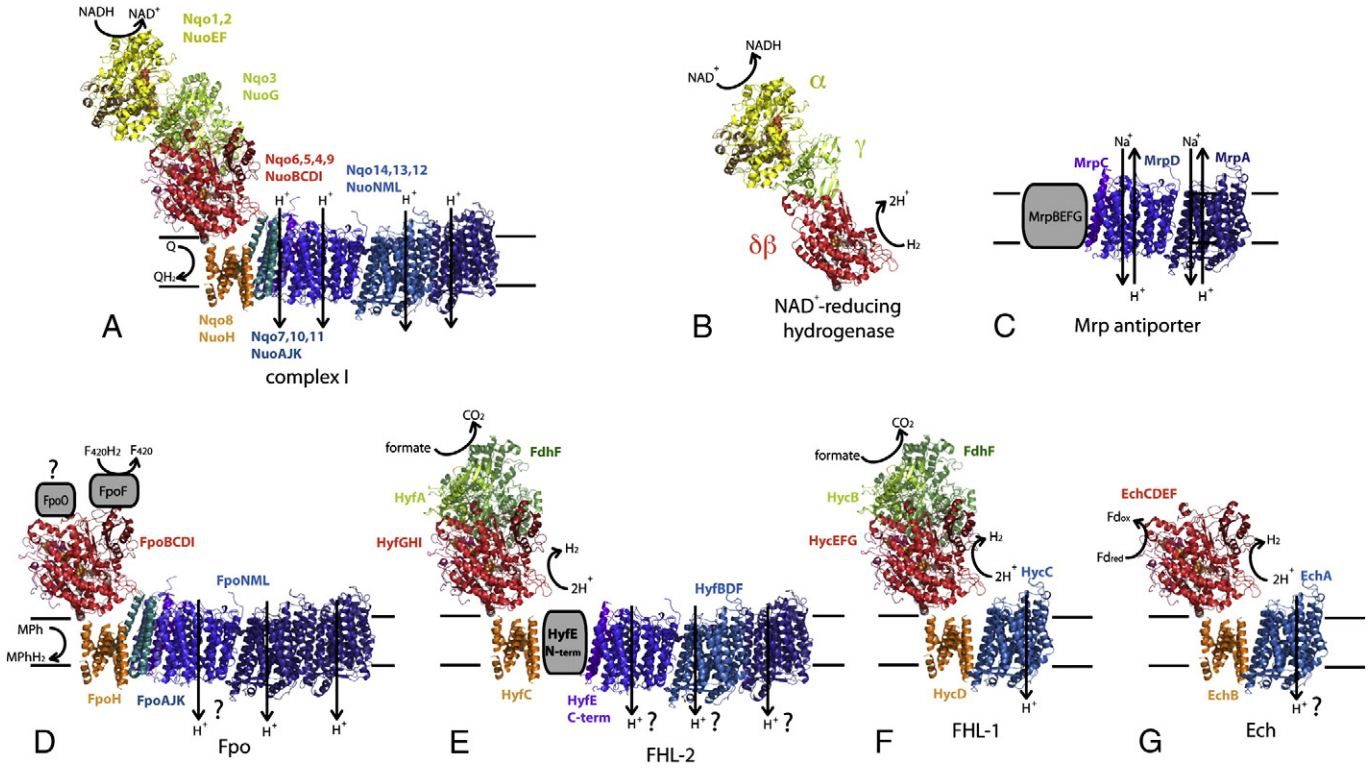


**Fig. 1.** Evolutionary relatives of complex I (see also Table 1). (A) Structure of the entire *T. thermophilus* complex I, with color-coded evolutionary modules (the same coding is used throughout): yellow and green, N-module; red, Q-module; orange, NuoH-like subunits; blue, P-module. (B, C) Two separate origins of complex I: closely related protein complexes that have either only oxidoreductase activity, bidirectional $NAD^+$-reducing NiFe-hydrogenase (B), or transport activity, Mrp antiporters (C). (D–G) Evolutionary and functionally related to complex I complexes: (D) 11 subunit homologue, Fpo complex from *M. mazei* (NuoBCDIHAJKLMN); (E) FHL-2 of *E. coli*, (F) FHL-1 of *E. coli*, (G) the simplest known complex I-related proton-pumping oxidoreductase, Ech. Names of subunits constituting the complexes are indicated. Subunits unrelated to complex I are shown as gray rectangles. Question marks indicate characteristics that have not been unambiguously established experimentally, such as proton-pumping of Ech and FHL-2, $H^+/e^-$ stoichiometry of Fpo, as well as association of subunit FpoO with the Fpo complex.

Complex I is a member of a family of membrane-residing oxidoreductases found in all kingdoms of life. This family couples oxidation of a range of soluble electron donors and reduction of membrane bound quinone analogues to translocation of ions against TM potential [22]. Moreover, complex I also relates to a class of membrane-bound NiFe-hydrogenases coupling substrate oxidation and reduction of hydrogen to active proton transport [23]. Complex I has an intricate evolutionary history with independently pre-evolved building modules joined to form the machinery [13].

Here we reanalyze the modular structure evolution of complex I (previously discussed in Refs. [14,24–26]) with an emphasis on novel structural information [14,15,17]. In this paper complex I evolution is understood in a broad sense as a process of formation of the currently existing complex I from individual subunits or functional subcomplexes (modules). The evolutionarily related complexes are those sharing homologous functional modules. Our analysis suggests that complex I, and all related oxidoreductases, operate via a common coupling mechanism, the principles of which are derived.

## 2. Modular structure of complex I

Homology of complex I subunits with those in other protein complexes reveals that complex I originated from the unification of pre-evolved subcomplexes [24]. These have distinct functions, can be combined in different ways and are referred to here as evolutionary modules. The modules are color coded in Fig. 1A [3]: the NADH oxidizing or N-module (subunits NuoEFG), serving for electron input from NADH into the chain of iron–sulfur clusters (Fig. 2A), the Q-module, which comprises subunits NuoBCDI, conducting electrons to quinone binding site; and proton translocating P-module (subunits NuoLMNKAJ). Subunit NuoH, joining the peripheral and membrane arms, does not belong to these modules. It is very specific and unique to complex-I like oxidoreductases and hydrogenases. NuoH is functionally unrelated to the other known proteins. Apart from the junction point, the two arms of complex I are functionally and evolutionarily independent of each other: the peripheral arm catalyzes oxidation/reduction reactions and the membrane arm proton transport. The modules trace back to two unrelated protein families: hydrogenases that gave origin to modules Q and N, constituting the peripheral arm, and Mrp (multiple resistance and pH adaptation) cation/$H^+$ antiporters, which are homologous to the P-module [27] (Fig. 1B,C).

Hydrogenases catalyze reversible oxidation of molecular hydrogen ($H_2$) and are among the most ancient energy converting enzymes [28]. A modular arrangement is typical for hydrogenases, which are often built like combinations of Lego blocks [28]. The N-module is related to group-3 bidirectional soluble NiFe-hydrogenases, which use soluble cofactors, like $F_{420}$, NADH or NADPH to reversibly oxidize hydrogen. Subunits NuoEFG are homologous to NAD-linked formate dehydrogenase (Table 1, yellow and green modules in Fig. 1). The Q-module is related to the class of NiFe-hydrogenases [14,26], which include water-soluble as well as membrane bound complexes. NiFe-hydrogenases are the most numerous and most studied of these enzyme classes; they share a core consisting of large and small subunits, homologous in structure and sequence with subunits NuoD and NuoB of complex I, respectively [14] (red in Fig. 1).

Recent sequence analysis of complex I-like enzymes revealed that N-modules have likely been added to complex I in 2 steps: subunit NuoG first, with E and F recruited later [22]. The structure of the entire complex is consistent with the modular evolution hypothesis. The modules form distinct domains within complex I, and can be