Phylogenomic analysis of the type I NADH:quinone-oxidoreductase

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NADH:quinone-oxidoreductase of type I (EC 1.6.5.2, Complex I, hereafter NDH-I) is the largest enzyme in the respiratory chain of mitochondria. It couples the transfer of two electrons from NADH to the membrane ubiquinone pool with the translocation of (supposedly) four protons across the membrane [1-3]. The genes of NDH-1 are found in many bacterial and archaeal genomes [4], although the products of these genes were functionally characterized only in a few cases.

Although the number of subunits varies in NDH-I complexes, reaching 44 different subunits in mitochondria [2], the catalytic core of this enzyme, which is conserved between eukaryotes and prokaryotes, comprises only 11 subunits [4]. The core subunits belong to the two functional modules, namely, the quinone module (Q-module) and the proton translocation module (P-module). The Q-module consists of four proteins that protrude into the bacterial cytoplasm or mitochondrial matrix; these subunits in prokaryotes are usually designated NuoA to NuoD (from NADH:ubiquinone oxidoreductase). The P-module is built of seven membrane-spanning subunits, from NuoH to NuoN. The enzymes of mitochondria and many bacteria also contain a separate NADH dehydrogenase module (N-module, subunits NuoE to NuoG).

Such a modular structure indicates that the NDH-I could evolve through combining of smaller functional building blocks [4-9]. One evolutionary scenario relies on the similarity between the membrane subunits of NDH-I and the Mrp-type Na^+/H^+ antiporters, on one hand, and on homology of some soluble subunits of NDH-1 and [Ni-Fe] hydrogenases, on the other hand; this scenario suggest that a soluble hydrogenase and a large, Mrp-type Na^+/H^+ antiporter may have merged to form the ancestor of the NDH-I [4, 7]. Alternatively, it has been suggested that Mrp-antiporters may have emerged from a later split of a large, NDH-I-related enzyme and not *vice versa*. In the corresponding scenarios, NDH-I has been traced to a membrane-bound [Ni-Fe] hydrogenase with only two membrane subunits [6, 9].

In this study, we have addressed the evolution of the NDH-I by phylogenomic analysis, using the cluster of orthologous groups (COGs) approach [10, 11]. For the major core subunit of the NDH-I the corresponding COGs were identified. From each of the COGs, sequences were retrieved to build the multiple alignments with the Muscle software [12]. A phylogenetic tree was constructed for every alignment by using the neighbor-joining algorithm implemented in MEGA 6.0 package [13].

The phylogenetic trees for the subunits NuoH and NuoN of the P-module showed distinct archaeal and bacterial clades, which suggests that the ancestors of these membrane proteins could be present already in the Last Universal Cellular Ancestor (LUCA). The subunits NuoL and NuoM, although homologous to the NuoN subunit, formed several archaeal clades, which might point to several gene duplication and/or horizontal gene transfer (HGT) events. The phylogenetic trees for the core NuoB and NuoD subunits of the Q-module showed strong archaeal clades, but with admixture of several bacterial sequences (all sequences from the *Thermotoga* phylum, as well as few sequences of *Aquificae* and *Proteobacteria*), and, in addition, few small archaeal clades. This pattern, although not quite unambiguous, is compatible with the presence of the ancestors of these two subunits in the LUCA and a few later HGT events. The phylogenetic trees for the subunit NuoC of the Q-module, as well as for the subunits NuoE, NuoF, and NuoG of the N-module showed the archaeal sequences spread among the

bacterial ones; the presence of the ancestors of these subunits in the LUCA is unlikely and their evolution requires additional case-by-case analysis.

The data obtained are compatible with evolutionary models assuming a simple ancestor of NDH-I with only two membrane subunits [6, 9]. Our analysis indicates that these two subunits could be the ancestors of subunits NuoH and NuoN (and not of the NuoL subunit as suggested earlier [9]) and that they could be present already in the LUCA. Recently the NuoH subunit was shown to show structural and sequence similarity to homologous subunits NuoL/M/N [9,14] [14, 15]. The data from [9, 14], taken at face value, indicate that NuoH and NuoN subunits could be traced to a primordial gene duplication event. Then, according to our analysis, this duplication event may have happened even before the LUCA. In modern NDH-I, the Q-module interacts with the NuoH subunit, but not with the NuoN subunit [2, 3, 14]. In the mitochondrial enzyme, the NuoH subunit was suggested to retain the ability to translocate Na^+ ions [3]; this ability however, showed up only in a deactivated enzyme [16]. It is tempting to speculate that the pre-LUCA ancestor of NDH-I could contain only one membrane subunit, ancestral both to NuoH and NuoN, and operate as a redox-driven sodium pump, functionally similar to the sodium-motive ferredoxin:NAD^+ oxidoreductase [17]. As argued elsewhere [18], such enzymes could help to keep the $[K^+]/[Na^+]$ ratio over unity within primordial cells [19]. The duplication of this single membrane subunit may have yielded a more adaptable two-subunit membrane module capable of translocating two cations. . The further duplication events could yield a membrane module with four homologous ion-translocating subunits, as in the modern Complex I.

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