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Organization and evolution of structural elements within complex I

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1. Introduction

The mitochondrial H^+-translocating NADH: ubiquinone oxidoreductase (complex I) is a large membrane enzyme that contains many protein subunits and redox centres. The precise number of Fe–S clusters and the possible presence of other redox centres in addition to FMN and Fe–S clusters are not yet known with certainty. There are at least 42 different protein subunits in bovine complex I, the primary structure of which was determined (see Ref. [1] and references therein). Most of the subunits of complex I from the fungus *Neurospora crassa* were also cloned and sequenced, and several of them have already been inactivated. This provided valuable information about the location of one Fe–S cluster [2] and insight into the assembly of the mitochondrial enzyme [3,4].

Seven subunits of complex I, ND1–ND6 and ND4L, are encoded and synthesized in the mitochondrion. This semi-autonomous organelle has a unique genetic system, and at present it is technically very difficult to manipulate its genes. Due to this none of the ND's have been deleted or mutagenized. Nonetheless, several interesting mutants have been identified in some of them by sequencing mitochondrial DNA from human patients suffering from maternally inherited mitochondrial diseases [5].

Much progress was made during the last few years in cloning and sequencing the genes encoding subunits of NDH-1, the bacterial complex I [6–9]. These genes are clusters into one operon in each bacterium, and these operons contain a minimal set of 14 genes (or 13 in cases where *nuoC* and *nuoD* are fused, see below). Seven of the NDH-1 genes are homologous to the seven ND's of complex I, and the rest to the nuclear-encoded subunit 'PSST', 'TYKY', 30, 49, 24, 51 and 75 kDa. In some bacteria, there are additional genes or open reading frames within the NDH-1 operon, but they are not homologous to any subunit of mitochondrial complex I [6,8]. Therefore, it appears that the 14 subunits of NDH-1 contain between them all the structural components required for full enzymatic activity.

In this minireview, we will analyse published results from recent studies on mitochondrial and bacterial complex I that shed light on the organization of the different proteins and redox centres into functional and structural domains within the enzyme. Homology to other enzymes will be presented, and a proposal for the evolution of complex I from pre-existing elements will be discussed in some details.

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2. The NADH binding and oxidation domain

The domain of complex I (and NDH-1) that binds and oxidises NADH contains FMN and several Fe–S clusters. The subunit composition of this part of the enzyme has been discussed extensively following the observation that the 24 and 51-kDa subunits are homologous to the N-and C-terminal regions, respectively, of HoxF of the NAD^+-reducing hydrogenase from the bacterium *Alcaligenes eutrophus*, and that the N-terminal region of the 75-kDa subunit is homologous to HoxU of this hydrogenase [10]. This homology suggested that the electron input domain of complex I is made up of the 24 and 51-kDa subunits as well as the N-terminal segment of the 75-kDa subunit. The former two subunits were known to be part of this domain due to their presence in the flavoprotein fraction (FP) of bovine complex I [11], but the 75-kDa subunit has not previously been detected in this fraction. Furthermore, a bacterial-type FP fragment that contained FMN but no Fe–S clusters was recently purified from the bacterium *Rhodobacter capsulatus* [12]. The latter finding suggests that FMN is the only cofactor needed for catalysis of NADH oxidation by ferricyanide, and that the 75-kDa subunit (i.e., its bacterial homologue, NUOG or NQO3) is not an integral part of the NADH oxidation domain of NDH-1. On the other hand, the results obtained from fractionation of NDH-1 of *Escherichia coli* [13] and the homology to the NAD^+-reducing hydrogenase from *A. eutrophus* suggest that NUOG does belong to this domain.

The primary structure of some subunits of the $NADP^+$-reducing hydrogenase from the bacterium *Desulfovibrio fructosovorans* provided further support for the notion that the N-terminal region of NUOG (NQO3) belongs to the NADH oxidation site. The operon encoding this hydrogenase contains genes that are homologous to the 24 and 51-kDa subunits, but no homologue of the 75-kDa subunit was reported [14]. Nevertheless, the N-terminal region of the HydD subunit of the $NADP^+$-reducing hydrogenase is homologous to the N-terminal region of the 75-kDa subunit, while the rest of the protein is not (not shown). Thus, it is suggested that the N-terminal region of the 75-kDa subunit, its 'HoxU' segment, might not be essential for the binding and oxidation of NADH, but it probably plays an important role in electron transfer from this domain to the other parts of complex I.

3. A link between the NADH oxidation domain and the high potential Fe–S cluster(s)

The 75-kDa subunit of complex I (NQO3 or NUOG in bacteria) appears to be build from two segments that were fused together during evolution. The N-terminal segment, about 220 amino acids, is homologous to *HoxU* of *A. eutrophus*. It is probably located very close to the NADH oxidation domain, and it binds at least one tetranuclear and one binuclear Fe–S cluster [10]. The second segment of the 75-kDa subunit is longer and less conserved. In mitochondrial complex I and some bacterial enzymes, e.g., NDH-1 of *Paracoccus denitrificans*, the second segment lacks conserved cysteines. However, in some other NDH-

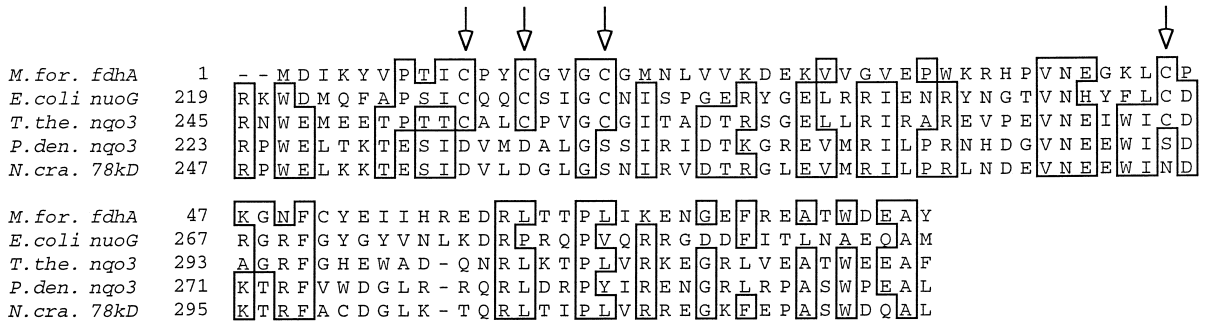

Fig. 1. The 'FDHA' segment of the 75-kDa subunit, and the possible ligands of cluster N-1c. Alignments of the N-terminal region of *fdhA* from *M. formicicum* (acc. no. J02581) with selected bacterial and fungal homologues of the 75-kDa subunit. Accession numbers for these genes are: *E. coli* (P33602), *T. thermophilus* (U52917), *P. denitrificans* (M84572) and *N. crassa* (X57602). The four arrows indicate cysteine residues that are conserved only among some of the NDH-1's, and which might ligate cluster N-1c.

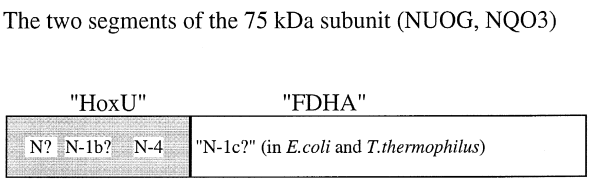

Fig. 2. A schematic representation of the two segments of the 75-kDa subunit, and the Fe–S clusters that were suggested to be bound in each segment. N? stands for a possible third Fe–S cluster at the N-terminal region of this protein.

1's, it contains a cysteine-rich motif, the possible role of which is discussed below. The second segment of the 75-kDa subunit begins with a stretch of about 80 amino acids that exhibits high homology to the N-terminal part of the α subunit of formate dehydrogenase from *Methanobacterium formicicum* (FDHA, Fig. 1). The homology to FDHA extends towards the C-terminus of the 75-kDa subunit (unpublished results), and part of it was already reported [15]. Due to this homology, the lower part of the 75-kDa subunit may be called the 'FDHA' segment (Fig. 2).

The N-terminal region of FDHA of *M. formicicum* has four cysteine residues in a $CX_2CX_3CX_{27}C$ arrangement that might bind an Fe–S cluster (Fig. 1). A similar motif is present in the NUOG subunit of NDH-1 of *E. coli*, but not in the enzyme from *P. denitrificans* or mitochondria (represented by *N. crassa*, Fig. 1). NDH-1 of *E. coli* contains an additional binuclear Fe–S cluster called N-1c [16,13], and these four cysteines might bind it. On the other hand, NQO3 of *T. thermophilus* also contains such a cysteine-rich motif (Fig. 1), but EPR experiments on its NDH-1 have not detected a cluster similar to N-1c in its line shape [17]. Nonetheless, at present it cannot be excluded that a binuclear cluster is ligated by these cysteines in the thermophilic enzyme as well as in *E. coli*.

There is an interesting correlation between the presence of the cysteine-rich motif at the beginning of the 'FDHA' segment, the 'N-1c' motif, and menaquinone reductase activity. The NDH-1's of *E. coli* and *T. thermophilus* that contain such a motif (Fig. 1) are both menaquinone reductases under physiological growth conditions [18,9]. It may thus be speculated that N-1c plays a role in the electron transfer to menaquinone, an electron acceptor the midpoint potential of which is lower than ubiquinone. The involvement of N-1c in such an activity may, however, be indirect. Future studies may show if the correlation between that 'N-1c' motif and menaquinone reduction is a coincidence or a meaningful observation.

4. Subcomplexes and the location of cluster N-2 with respect to the membrane

Several different subcomplexes have been isolated from complex I by relatively mild means. The 'peripheral arm' of complex I from *N. crassa* was isolated from fungi that were grown in the presence of chloramphenicol [19], and later it was characterized in cells from which a gene encoding a subunit of the 'membrane arm' was disrupted [3]. The 'peripheral arm' is composed of at least 10 subunits, and analyses by EPR showed that it contained the Fe–S clusters N-1, N-3 and N-4, but lacked N-2 [20,3].

Another interesting finding was the accumulation of the 'membrane arm' of complex I in fungi that were grown under manganese limitation. Mitochondrial membranes from such cells contained cluster N-2, and lacked all the other EPR-detectable Fe–S clusters of complex I [21]. Taken together, the results from *N. crassa* are consistent with the suggestion that cluster N-2 is bound within the membrane section of complex I.

In contrast to the 'peripheral arm' from *N. crassa*, all the enzymatically active subcomplexes of bovine complex I contained cluster N-2, as well as the other EPR-detectable clusters [22,23]. However, the presence of cluster N-2 in these subcomplexes does not necessarily mean that it is located outside the membrane region of complex I. The reason for this is that the subcomplexes were not water-soluble, and the line-shape of cluster N-2 in all of them was modified. The latter suggests that some structural changes occurred at the vicinity of this cluster during the isolation [23]. These results led us to suggest that cluster N-2 is bound close to the interphase between the membrane and the outer membrane sectors of complex I [23]. But whether or not it is located within the membrane dielectric could not be answered from these experiments.

Two laboratories have recently shown that the g_z transition of cluster N-2 in coupled submitochondrial

particles splits under high membrane potential [24,25]. The splitting was assigned to either magnetic interaction between cluster N-2 and a semiquinone radical [24], or to interaction between two adjacent N-2 clusters [25]. In any case, these results demonstrated that cluster N-2 is sensitive to the electrochemical gradient across the membrane, and suggested that it is functionally located within the membrane.

NDH-1 of *E. coli* was purified and dissociated into three fragments [13]. The connecting fragment included four (or three, see below) proteins, two of which, TYKY and PSST, are the current candidates for being the cluster N-2-binding subunit. In addition to them it contained the 30 and 49-kDa subunits which are fused to a single protein in *E. coli* (see genebank, acc. no. AE000317). The connecting fragment was not water soluble even though none of its protein subunits is predicted to be an integral membrane protein [13]. This is significant since the main basis for the assumption that N-2 is located outside the membrane was the lack of predicted trans-membrane α-helices in the TYKY and PSST subunits. In this respect, it may be noted that some homology between a segment of the 49-kDa subunit and a 'membrane attached' α-helix of the bacterial reaction centres has been reported [26]. In addition, homologues of all the subunits of the connecting fragment are encoded by the *Hyc* operon of *E. coli*, together with homologues of ND1 and ND5 or ND4 (see below). This might suggest that the true domain in this region of the enzyme may include two hydrophobic proteins, in addition to the 'PSST', 'TYKY' 30 and 49-kDa subunits.

5. The integral membrane proteins

The primary structure of each of the mitochondrially-encoded subunits of complex I, the ND's, predicts that they are integral membrane proteins containing multiple trans-membrane α-helices. Mitochondrial complex I also contains other proteins that are predicted to traverse the membrane at least once [27], but no homologues to any of them were found in bacterial NDH-1's. The only predicted integral membrane proteins in NDH-1 are the homologues of the ND's, and they were named NQO 7, 8 and 10–14 [6], or NUO A, H and J–N [13]. These subunits contribute about half of the enzyme's mass, but currently there is not much information about the structural elements that must be present within them.

Information about the location of some important sites within the ND's has been derived from studies on inherited mitochondrial diseases. A good example in this respect is the Leber's hereditary optical myopathy (LHON) which may be inflicted by point mutations in mitochondrially-encoded genes [5]. Recent experiments using isolated mitochondria from cultured lymphocytes suggested that the ND1/3460 mutation affects a ubiquinone binding site, while the ND4/11778 does not [28]. It may be noted that both these mutations are predicted to reside outside the membrane bilayer. The latter, and the high conservation of primary structure in the 'non-membranous' segments of the ND's, may suggest that the enzyme's region that is located at the surface of the membrane, possibly on the 'inside' or 'negative' side, is involved in the function of complex I.

6. Homology to subunits of the formate hydrogenlyase(s) of *E. coli*

The gene composition of the *Hyc* operon of *E. coli*, encoding most of the subunits of formate hydrogenlyase (FHL) indicates close evolutionary relation to complex I [29,30]. It contains homologues of the ND1, ND4 or 5, 49-kDa and 'PSST' subunits [29,30], as well as the 'TYKY' and 30-kDa subunits of complex I [31,32].

An operon that encodes a second FHL has recently been characterized in *E. coli*, and named *Hyf* [33]. The enzyme encoded by the *Hyf* operon will be called FHL-2, and the FHL encoded by the *Hyc* operon [29,34] will be called FHL-1. The *Hyf* operon contains all the homologues of subunits of complex I that are found in the *Hyc* operon (Table 1), and in addition to them three hydrophobic proteins that are not present in FHL-1. HYFD and HYFF are related to both ND4 and ND5 [33]. The third protein, HYFE, is 216 amino acid long and its C-terminal half may be related to ND4L (NQO11 or NUOK in bacteria) (Fig. 3).

The homology between the central subunits of complex I, excluding the NADH oxidation domain, and the subunits of FHL-2 (and to a large extent

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E.coli nuoK      1   - M I P L Q H G L I L A A I L F V L G L T G L V I R R N L L F M L I G L E I M I N
T.the. nqo11     1   - - - - - M S Y L L T S A L L F A L G V Y G V L T R R T A I L V F L S I E L M L N
E.coli hyfE    120   M A T G L K P A L A V A L G H F L L G L L C I V S Q R N I L R Q I F G Y C L M E N

E.coli nuoK     41   A S A L A F V V A G S Y W G Q T D G Q V M Y I L A I S L A A A E A S I G L A L L L
T.the. nqo11    37   A A N L S L V G F A R A Y G L - D G Q V A A L M V I A V A A A E V A V G L G L I V
E.coli hyfE    161   G S H L - - V L A L L A W R A P E L V E - - - I G I A T D A I F A V I V M V L L A

E.coli nuoK     82   Q - L H R R R Q N L N I D S V S E M R G
T.the. nqo11    77   A - I F R H R E S T A V D D L S E L R G
E.coli hyfE    197   R K I W R T H G T L D V N N L T A L K G
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Fig. 3. Alignment of the homologues of ND4L in NDH-1 of *E. coli* (*nuoK*, acc. no. P33606) and *T. thermophilus* (*nqo11*, acc. no. U52917), and the C-terminal half of HYFE of FHL-2 from *E. coli* (acc. no. P77524).

FHL-1, too) may suggest a similarity in the function of these enzymes. A considerable amount of information is available on factors affecting the expression of FHL activity, but the knowledge about its structure and function is limited [34]. A schematic picture of FHL-1 is presented in Fig. 4, and the proposed electron transfer pathway within it is drawn with thick arrows (after Ref. [34]). The homology of HYCG and HYCE to the small and large subunit of Ni hydrogenases, respectively, and the presence of Ni in HYCE [35] suggest that these two subunits harbour the terminal segment of the electron transfer pathway within FHL-1. The thin arrows in Fig. 4 are labelled with question marks since they indicate open questions that may concern complex I as well as FHL. Among them are whether FHL can donate or accept electrons from the quinone pool in the membrane, and whether FHL-1 or FHL-2 can translocate protons

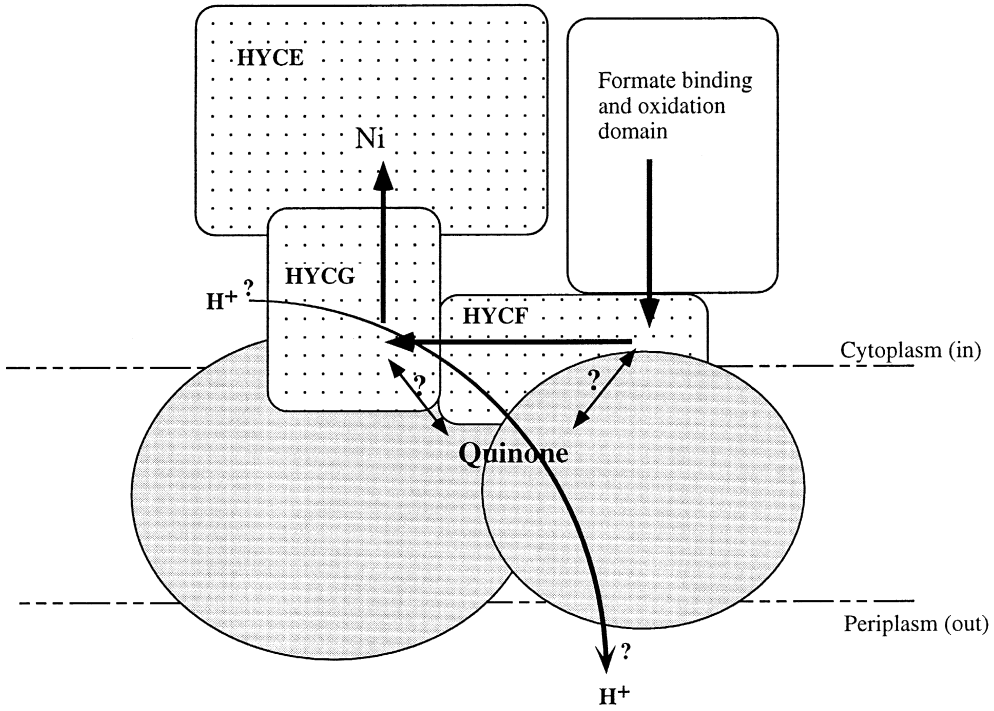

Fig. 4. Schematic view of the electron transfer pathway in FHL-1, and of open questions regarding its interaction with quinones and proton translocation activity. See text for further discussion.

accross the membrane. In case the answer to both these question is negative, one would like to ask why the FHL's contain integral membrane proteins. Could the membrane binding be due to an unknown redox centre that plays an important role in the internal electron transfer? And if so, is it a bound quinone? The answers to some of these questions may not be easy to obtain, but the discovery of FHL-2 might stimulate further research on FHL in different directions, and that will eventually reveal useful information about its structure and function.

7. How did complex I evolve?

The proposal for modular evolution of complex I from several pre-existing domains in other enzymes has been reviewed very recently [36]. It will not be repeated here but rather it will be extended by the addition of new and detailed suggestions for some steps within the general scheme of modular evolution. The following model starts from the proposed common ancestor of complex I and FHL [36], and it is assumed that such a multi-subunit protein was present in anaerobic bacteria that were employing formate dehydrogenases and different hydrogenases. The appearance of high redox potential oxidants, and the increased concentration of molecular oxygen in the atmosphere presented new opportunities as well as new problems to these organisms. More energy could be derived from electron transfer to the new acceptor(s), and it might have affected the quinone/quinol ratio in the membrane so that more quinone was available. On the other hand, many of the quinone reductases such as hydrogenases and perhaps also FHL (see above) were sensitive oxygen, or the concentration of their substrate was affected by it. Hence, the appearance of a new H^+-translocating quinone reductase might have provided an important advantage to such bacteria in their adaptation to aerobic environment, and possibly to considerable fluctuation in the oxygen tension in their surrounding.

The proposed evolution of complex I is presented as a series of consecutive steps, and some alternative steps are also included. Ancestors of present-day genes and proteins are given in square brackets (e.g., [*hyc B*] or [FDHA]). The genes encoding the subunits of NDH-1, their corresponding complex I subunits and homologues from other systemes that are relevant for this discussion are listed in Table 1.

(1) Two operons encoding FHL isozymes are currently known in *E. coli*. The *Hyc* operon that encodes most of the subunits of FHL-1, except the formate dehydrogenase subunit [34], and the *Hyf* operon which encodes most of the subunits of FHL-2 [33]. It is not known which of them is closer to the ancestor, and whether FHL-1 has developed from the latter by deletion of several genes, or if FHL-2 has evolved from it by duplication of certain genes. For the sake of this discussion it is assumed that FHL-1 is the ancestor, and that FHL-2 has evolved after two duplication events in [*hycB*], and incorporation of one more gene, *hyfE*, from a yet-unknown origin. The new integral membrane proteins, [HYFD], [HYFE] and [HYFF] could have provided the possibility to bind quinone, if it was not already present in the [HYFB] and [HYFC] subunits of FHL-1. Alternatively, the new subunits might have provided better insulation of a proton pump or proton channel that might be present in FHL.

(2) A fusion event between [*hoxU*] of *A. eutrophus* and [*fdhA*] of *M. formiciccum* created the primordial gene of the 75-kDa subunit, [*nuoG*]. An example of such a gene in a presently living organism is the α-subunit of the formate dehydrogenase of *Moorella thermoacetica* (accession no. U73807).

(3) The 'FDH' subunit of FHL which carries the formate binding and oxidation site, together with [HYFA], were replaced by [NUOG] (or [FDHA] of *M. thermoacetica*). This would have resulted in a new FHL that might function as a membrane bound formate:quinone oxidoreductase (in case FHL was not already capable of such an activity). Alternatively, only the [*fdhA*] of *M. formiciccum* was replacing the formate binding subunit(s) of FHL, and the fusion with [*hoxU*] (or the N-terminal region of [*HndD*] of *D. fructosovorans*) took place subsequently, perhaps together with [*hoxF*] (step 4).

4. The [*hoxF*] of *A. eutrophus* is added to [*nuoG*] of the new enzyme, thereby changing it to an NAD(P)H:quinone oxidoreductase. The new enzyme could have functioned as an $NAD(P)^+$-reducing hydrogenase that accepts electrons not only from its own hydrogenase section ([*hycE*] or [*hyfG*]) but also

Table 1
Selected homologues of subunits of NDH-1 in evolutionary-related systems

NDH1	Complex 1	Hyc operon (FHL-1)	Hyf operon (FHL-2)	$NAD(P)^+$-reducing hydrogenases and formate dehydrogenases
nuoA or *nqo7*	ND3	–	–	
nuoB or *nqo6*	PSST	*hycG*	*hyf1*	
nuoC or *nqo5*	30 kDa	upper part of *hycE*	upper part of *hyfG*	
nuoCD (in *E. coli*)	30 + 49 kDa	*hycE*	*hyfG*	
nuoD or *nqo4*	49 kDa	lower part of *hycE*	lower part of *hyfG*	
nuoE or *nqo2*	24 kDa	–	–	a. upper part of *HoxF* (A. *eutrophus*)
		–	–	b. *HndA* (*D. fructosovorans*)
nuoF or *nqo1*	51 kDa	–	–	a. lower part of *HoxF* (*A. eutrophus*)
			–	b. *HndC* (*D. fructosovorans*)
upper part of *nuoG* or *nqo3*	upper part of 75 kDa	–	–	a. *HoxU* (*A. eutrophus*)
		–	–	b. upper part of *HndD* (*D. fructosovorans*)
lower part of *nuoG* or *nqo3*	lower part of 75 kDa	–	–	*fdhA* (*M. formicicum*)
nuoG or *nqo3*	75 kDa	–	–	*fdhA* (*Moorella thermoacetica*)
nuoH or *nqo8*	ND1	*hycD*	*hyfB*	
nuoI or *nqo9*	TYKY	*hycF*	*hyfH*	
nuoJ or *nqo10*	ND6	–	–	
nuoK or *nqo11*	ND4L	–	lower part of *hyfE*	
nuoL or *nqo12*	ND5	*hycC* *	*hyfB*, *hyfD*	
nuoM or *nqo13*	ND4	*hycC* *	*hyfF*	
nuoN or *nqo14*	ND2	–	–	

* *hycC* exhibit similarity to both ND4 and ND5 [30].

from the quinone pool that under anaerobic conditions might have been kept highly reduced by quinone-reducing hydrogenases such as Hyd-1 and Hyd-2 of *E. coli* [34]. Such a membrane-bound $NAD(P)^+$-reducing hydrogenase has an advantage over the soluble enzyme(s) since the protons resulting from the reactions of Hyd-1 or hyd-2 are released on the periplasm side of the membrane and not in the cytoplasm [34]. In combination with the proton uptake from the inside for the reduction of $NAD(P)^+$ this is equivalent to proton translocation across the membrane. Such an advantage might have been instrumental in the evolutionary success of the new enzyme.

(5) After the appearance of a functional NAD(P)H:quinone reductase and an increase in the oxidation level of the quinone pool, the hydrogenase activity of FHL and the formate dehydrogenase activity of [FDHA] became redundant. Hence, it was eliminated by mutations in amino acids that ligate the molybdenum cofactor in the [FDHA] segment of [NUOG], and the nickel centre in [HYFG] (or [HYCE]) of FHL [37,35]. The appearance of oxygen in the atmosphere might have coincided with this step since these two metal centers are unstable under aerobic conditions.

(6) Rearrangements of genes and proteins: (a) Splitting of [*hox F*] into [*nuo E*] and [*nuo F*] (24 and 51-kDa subunits). (b) Further rearrangement of the genes encoding integral membrane protein such as [*hyf B-F*] yielding ND2, 3 and 6.

These events would have led to the appearance of a true NDH-1, similar to that found today in *E. coli*. It is assumed that shortly thereafter a splitting of [*hyfG*] into [*nuoC*] and [*nuoD*] took place (30 and 49-kDa subunits or NQO3 and NQO4), resulting in an enzyme that is close to the present-day NDH-1 of *T. thermophilus*.

(7) Elimination of the putative Fe–S cluster (N-1c) at the N-terminal region of the [FDHA] segment of

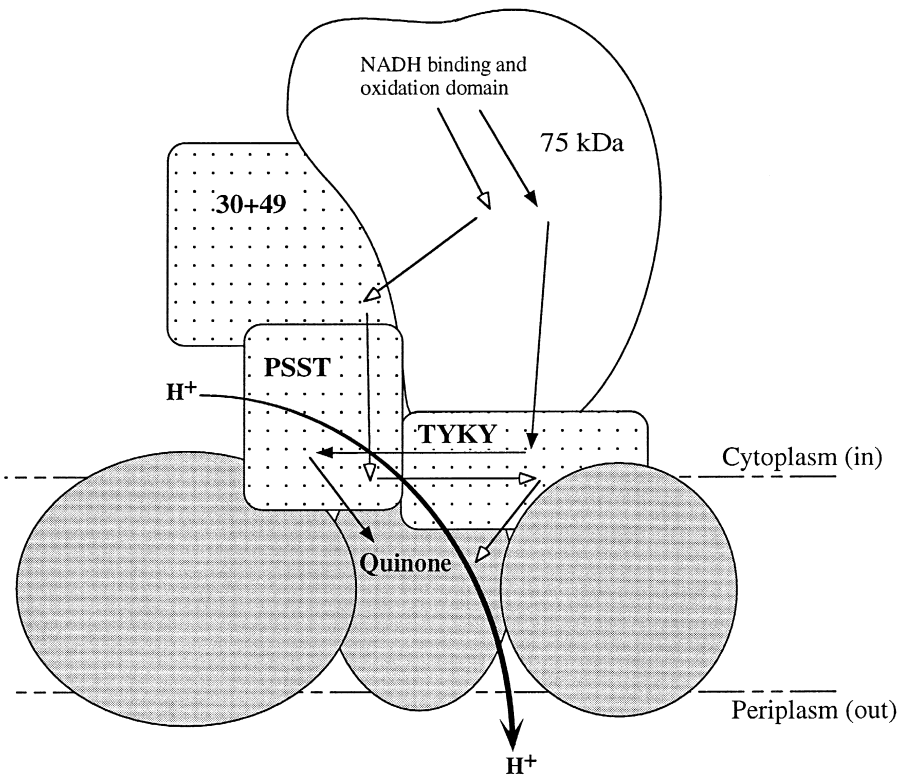

Fig. 5. Schematic view of NDH-1 with an emphasis on its evolutionary relation to FHL (Fig. 4). The dotted subunit are homologous to the dotted subunits in similar position in FHL-1 (Fig. 4), and they form the 'connecting fragment' in NDH-1 of *E. coli* [13]. See text for dicussion of suggestions concerning electron transfer pathways in this segment of the enzyme.

[NUOG] in the bacterial line leading to the present day *P. denitrificans* (Fig. 1).

(8) Endosymbiosis of an ancestor of *P. denitrificans* by an eucaryotic cell and the appearance of mitochondria.

(9) Transfer of the genes encoding the mainly hydrophilic subunits of complex I from the mitochondrial to the nuclear genome, and addition of many other nuclear-encoded subunits that are not found in NDH-1. The result of this is the appearance of mitochondrial complex I.

This proposal makes some predictions that may be examined in the future, e.g., by finding 'missing links' and 'intermediate steps' during genomic sequencing of different bacteria. One outcome of the model is that the hydrophobic fragment of complex I which contains the ND's has not evolved independently of the nuclear-encoded subunits (cf. Ref. [38]). Rather, the subunits of the 'connecting fragment', i.e., PSST, TYKY, 30 and 49-kDa subunits, have evolved together with the ND's until they were transferred from the mitochondrial chromosome to the nucleus.

One implication of the above proposal is that the 49-kDa subunit might be involved in the electron transfer pathway within complex I. Its homologues in FHL, HYCE and HYFG, bind nickel and harbour the terminal part of the electron transfer pathway within these enzymes (Fig. 4) [34]. Complex I (and NDH-1) lacks the nickel centre and the hydrogenase activity, but some parts of the primordial electron transfer pathway might still be in use, and in close contact with the putative redox centre in the PSST subunit (Fig. 5). Another suggestion is that the segment of the electron transfer pathway which contains parts of the 49 and PSST subunits is used in complex I, but in the opposite direction than in FHL. According to this the electrons may arrive at the 49-kDa subunit from the NADH oxidation domain (through the 75-kDa subunit) and then be transferred to the PSST subunit from which they will continue to the TYKY subunit and eventually to quinone (Fig. 5, white-head arrows). Another possibility is that only the electron transfer between the Fe–S clusters in TYKY and PSST is similar in FHL and NDH-1. The latter suggestion is presented in Fig. 5 by the black-head arrows, and according to it the absence of a redox centre in the 49-kDa subunits eliminated electron transfer to this protein. These suggestions are not the only possibilities, but further elaboration is beyond the scope of this article.

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