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Procaryotic complex I (NDH-1), an overview

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1. Preamble

First of all, we will discuss the terminology of the NADH-quinone oxidoreductase in this article. Although the NADH dehydrogenases in the bacterial respiratory chains are referred to as NADH-ubiquinone (UQ) oxidoreductase by some researchers, many bacteria have been known to bear quinones (Q) other than UQ (e.g., menaquinone (MQ), phylloquinone, chlorobiumquinone, demethylmenaquinone, plastoquinone, α-tocopherolquinone, rhodoquinone (RQ), epoxyubiquinone and caldariellaquinone) [1]. Mitochondria do not seem to be exemptions for this issue, although mitochondria in most organisms are believed to carry UQ as Q. For example, in mitochondria of adult *Ascaris suum*, complex I and succinate-quinone oxidoreductase (but not complex III or cytochrome oxidase) are present in its respiratory chain and the only Q detected is RQ [2–4]. In view of these facts, the name NADH-*quinone* (Q) oxidoreductase for the enzyme especially in bacteria seems more appropriate than NADH-*ubiquinone* (UQ) oxidoreductase. Therefore, in this article, we designate the NADH dehydrogenase in the respiratory chain as NADH-Q oxidoreductase. In addition, the bacterial proton-translocating NADH-Q oxidoreductase is designated NADH dehydrogenase I (NDH-1) because Kaback and his colleagues [5–8] initially designated this enzyme complex as NADH dehydrogenase I (NDH-1) to distinguish it from NADH dehydrogenase II (NDH-2), which lacks an energy coupling site. Therefore, we use this terminology after the pioneering work of Kaback and his colleagues on the bacterial NADH-Q oxidoreductases. In this article, NDH-1 and complex I refer to bacterial and mitochondrial proton-translocating enzyme complexes, respectively.

In the previous chapter, the articles have reviewed present knowledge of structure and function of mitochondrial complex I. This chapter is concerned with its bacterial counterparts, NDH-1. Following this article, Friedrich [9] and Dupuis et al. [10] describe recent progress in studies of the NDH-1 in *Escherichia coli* and *Rhodobacter capsulatus*, respectively. Therefore, we attempted to provide general and wide information about the NDH-1 and also cover present knowledge of other bacterial NDH-1.

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Table 1
Bacterial NADH-quinone oxidoreductases

	NDH-1	Na^+-NDH	NDH-2
Ion-pump	H^+	Na^+	Absent
Cofactors	Noncovalently bound FMN	Noncovalently bound FAD (FMN?)	Noncovalently bound FAD
	8–9 iron–sulfur clusters		
	2 [2Fe–2S]	1 [2Fe–2S]	
	6–7 [4Fe–4S]		
Subunit composition	13–14[a]	4–6[b]	1[c]
Inhibitors	Rotenone	Ag^+	Flavone (?)
	Piericidin A	HQNO	Pentagalloy glucose (?)
	Capsaicin		
	Annonaceous acetogenins (e.g., Rolliniastatin-1)		

[a] *E. coli* NDH-1 is composed of 13 different subunits instead of 14 different subunits (see text).
[b] Na^+-NDH isolated from *V. alginolyticus* is composed of four different subunits and is capable of Na^+ translocation, whereas the NQR operon encoding Na^+-NDH contains six structural genes.
[c] The NDH-2 of certain bacteria have been reported to form a homodimer.

The NADH-Q oxidoreductases of the bacterial respiratory chains can be divided into three groups [11–13]. They are the H^+-translocating NADH-Q oxidoreductase (designated NDH-1 or bacterial complex I), the Na^+-translocating NADH-Q oxidoreductase (designated Na^+-NDH or Na^+-NQR) [14–16], and the NADH-Q oxidoreductase lacking an energy coupling site (designated NDH-2). Table 1 summarizes the characteristics of these three NADH-Q oxidoreductases. The NDH-1 contains one non-covalently bound FMN and at least 5 EPR-detectable iron–sulfur clusters (2[2Fe–2S] and 3[4Fe–4S]) as prosthetic groups [17–20]. Na^+-NDH bears one non-covalently bound FAD and possibly FMN and one EPR-detectable [2Fe–2S] cluster [15,21]. NDH-2 bears non-covalently bound FAD and no iron–sulfur clusters [11,12]. The NDH-1 and Na^+-NDH are multiple subunit enzyme complexes. They are composed of at least 13–14 and four to six different subunits, respectively. On the other hand, NDH-2 is composed of a single subunit. NDH-1 is inhibited by the potent mitochondrial complex I inhibitors (rotenone, piericidin A, capsaicin, rolliniastatin-1, etc.) while Na^+-NDH and NDH-2 are not affected by these inhibitors. Na^+-NDH is inhibited by Ag^+ and 2-*n*-heptyl-4-hydroxyquinoline N-oxide (HQNO) [15]. In terms of cofactors, subunit sequence, and inhibitors, NDH-1 is believed to be a counterpart of mitochondrial complex I.

2. Genes encoding bacterial NDH-1

At the present time, complete DNA sequences of the NDH-1 operon (gene cluster) are available only from three bacteria, namely, *Paracoccus denitrificans*, *E. coli*, and *Thermus thermophilus* HB-8 [22–28]. In addition to these organisms, cloning and DNA sequencing of the NDH-1 operons from *R. capsulatus*, and *Salmonella typhimurium* are now in progress in other laboratories [29–33]. Furthermore, the various bacterial genome projects will provide DNA sequences of other bacterial complex operons (gene clusters) in near future. So far, it is generally accepted that operons encoding bacterial NDH-1 contain 14 structural genes (see Fig. 1). Those are designated *NQO1-14* (*P. denitrificans* and *T. thermophilus* HB-8) [13,28] and *nuoA-N* (*E. coli* and *R. capsulatus*) [30–34]. It seems interesting that the gene arrangements in NDH-1 operons reported so far are consistent among bacteria (see Fig. 1) [35]. The NDH-1 operons of *P. denitrificans* and *R. capsulatus* bear several URFs in addition to the subunit genes. URFs deletion of *R. capsulatus* NDH-1 operon

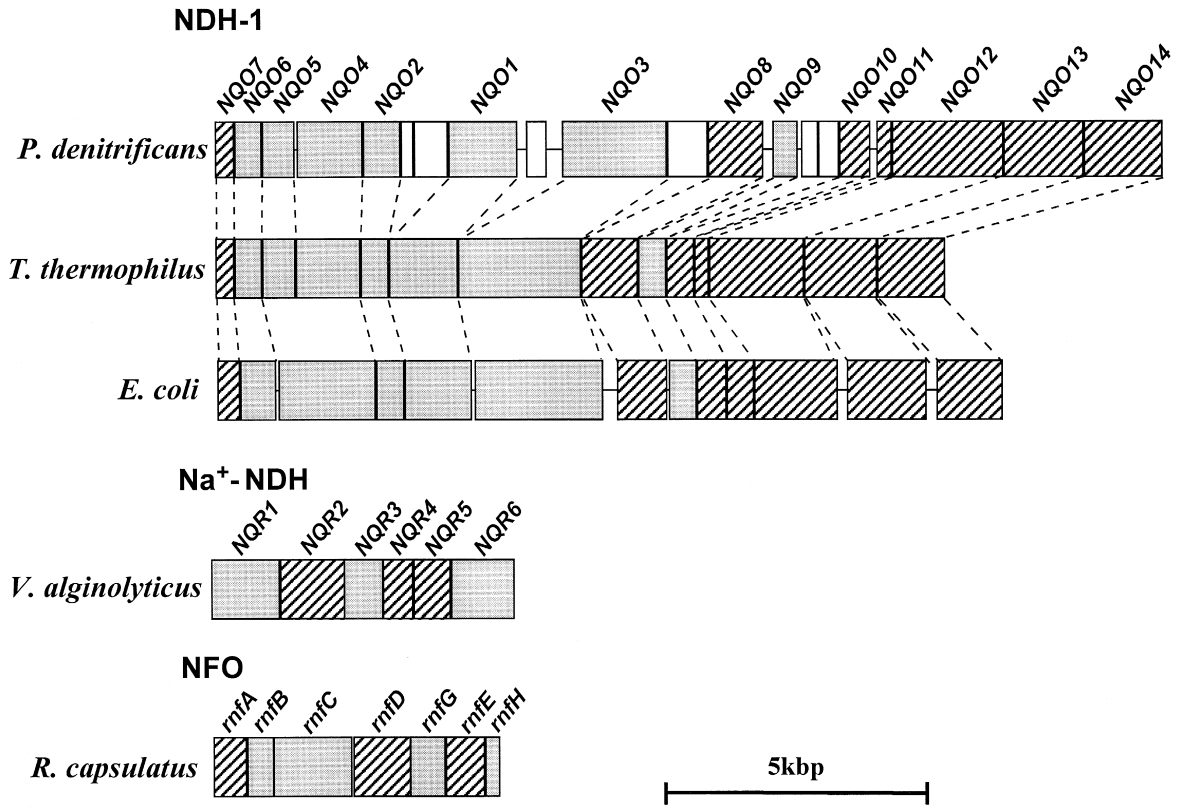

Fig. 1. Comparison of gene arrangements of operons encoding NDH-1, Na^+-NDH, and *N*ADH-*f*erredoxin (?) *o*xidoreductase (tentatively designated NFO). Gray, hatched, and open bars indicate genes encoding hydrophilic subunits, hydrophobic subunits, and URF, respectively. GenBank Accession Numbers are: *P. denitrificans NQO* operon (M64432, M74171, M84572, M93015 and L02354), *T. thermophilus* HB-8 *NQO* operon (U52917), *E. coli nuo* operon (X68301 and U00096/b2288–b2276), *V. alginolyticus NQR* operon (AB008030) and *R. capsulatus rnf* operon (Y11913).

scarcely affected the activity of its NADH dehydrogenase (A. Dupuis, personal communication). The function of URF-encoded products remains to be clarified.

The *ndh* gene that encodes the *E. coli* NDH-2 is anaerobically repressed by FNR (regulator of fumarate and nitrate reduction) which is a transcriptional regulator that controls gene expression in response to oxygen limitation in *E. coli* [36]. However, in the absence of FNR, *ndh* expression is enhanced by anaerobic growth in media containing amino acids. Meng et al. [36] speculated that this activation may involve two potential regulatory proteins, Arr (amino acid response regulator) and Nbp (*ndh*-binding protein) [37,38]. In contrast, the expression of the *nuo* genes encoding the *E. coli* NDH-1 is stimulated by the presence of fumarate during anaerobic respiration [39]. Expression of *E. coli nuo* genes is regulated by O_2 and nitrate via ArcA (anaerobic respiration control A), NarL (nitrate reductase L), FNR, and IHF (integration host factor) at sites within the -277 region and by other factors including C4 dicarboxylates at a site between -277 and -899 [40]. At the present time, reports concerning gene regulation of *NQR* operons encoding Na^+-NDH enzyme complexes are not available.

3. Subunit composition

It is generally accepted that the NDH-1 is composed of 14 different subunits. A subunit comparison among bovine mitochondrial complex I and bacterial NDH-1 has been illustrated in Table 2. It should be noted that, according to a recent report on the DNA sequence of the whole *E. coli* K-12, the *E. coli*

Table 2
Subunit comparison

Fraction	Bovine complex I	*P. denitrificans* and *T. thermophilus* NDH-1	*E. coli* and *R. capsulatus* NDH-1
FP	51 k	NQO1	NUOF
	24 k	NQO2	NUOE
	9 k		
IP	75 k	NQO3	NUOG
	49 k	NQO4	NUOD
	30 k	NQO5	NUOC
	20 k (PSST)	NQO6	NUOB
	18 k		
	15 k		
	13 k (A)		
	13 k (B)		
HP	ND1	NQO8	NUOH
	ND2	NQO14	NUON
	ND3	NQO7	NUOA
	ND4	NQO13	NUOM
	ND4L	NQO11	NUOK
	ND5	NQO12	NUOL
	ND6	NQO10	NUOJ
	23 k (TYKY)	NQO9	NUOI
	remaining 24 subunits including acyl carrier protein		

NDH-1 is likely to consist of 13 different subunits because the *nuoC* (*NQO5*) and *nuoD* (*NQO4*) genes are probably fused and encode a single fused subunit (tentatively designated NuoCD) [41]. This means that the minimum subunit composition of the NDH-1 may be 13 subunits [41] instead of 14 subunits reported for *E. coli* NDH-1 previously by the Düsseldorf group [27,42]. Similar gene fusion of *NQO4* and *NQO5* was observed in *Buchnera aphidicola* (endosymbiont of aphids) [43]. Nevertheless, in terms of amino acid sequences, the bacterial NDH-1 from different sources are akin to each other. It is known that MQ ($E_m = -75$ mV) and RQ ($E_m = -63$ mV) have lower midpoint potentials than UQ ($E_m = +110$ mV) [3,4,28,44]. Certain bacteria contain only MQ as described above (especially gram positive bacteria). *T. thermophilus* HB-8 has been reported to bear only MQ (major component MQ-8 and minor component MQ-7) as Q [1] and the rotenone-sensitive proton-translocating NADH-Q oxidoreductase [18,19]. The operon encoding its NDH-1 has been cloned and sequenced [28]. It turned out that the *T. thermophilus* HB-8 NDH-1 is composed of 14 different subunits similar to the NDH-1 of bacteria having UQ only (e.g., *P. denitrificans, R. capsulatus, R. sphaeroides*) [1]. The *T. thermophilus* HB-8 *NQO2* gene was overexpressed in *E. coli*. The expressed *T. thermophilus* HB-8 NQO2 subunit carries one [2Fe–2S] cluster whose physicochemical properties are similar to those of the *P. denitrificans* NQO2 subunit [28]. This suggests that the different species of Q in situ does not affects the structure and cofactors of NDH-1 regardless of the differences in the midpoint redox potential of Q. This is not surprising because NDH-1 and complex I can reduce various Q and the Q reduction is sensitive to rotenone and coupled to the proton translocation [11]. On the contrary, the succinate-Q oxidoreductase (which also acts as fumarate reductase) of *A. suum* adult muscle mitochondria has been reported to bear a strong specificity for RQ-10 but not for UQ-10 [4,45].

Determination of subunit stoichiometry and subunit topology of the NDH-1/complex I is a prerequisite to study structure and mechanism of action of this enzyme complex. Bovine heart complex I has been resolved into three fractions with the use of

chaotropes; a flavoprotein fraction (FP), an iron–sulfur protein fraction (IP), and a hydrophobic protein fraction (HP) [46–48]. FP + IP fractions contain at least 10 different subunits (FP, 51, 24, and 9 kDa; IP, 75, 49, 30, 18, 15, 13, and 11 kDa). Recently, Belogrudov and Hatefi [49] have immunochemically determined a stoichiometry of subunits constituting the water-soluble fraction (designated FP + IP subcomplexes) of isolated bovine complex I. According to their results, per mole of bovine heart complex I, there are 2 mol of the IP 15 kDa subunit and 1 mol each of FP and other IP subunits. Subunit stoichiometries of the NQO1-6 of the *P. denitrificans* NDH-1 have been determined by radioimmunoassay [50]. The results show that there is one copy each of the NQO1-6 subunits in the *P. denitrificans* NDH-1. The only information available to date on the subunit stoichiometry of the membrane-associated fraction of complex I/NDH-1 is from human mitochondria as reported by Chomyn et al. [51]. On the basis of [^{35}S]methionine incorporation into subunits isolated by immunoprecipitation, they reported that the subunit stoichiometry of ND1: ND2: ND3: ND4: ND4L: ND5: ND6 in human mitochondrial complex I is 2:8:2:2:1:2:2. However, the use of this immunoprecipitation approach to study the subunit stoichiometry is associated with a number of problems which might possibly lead to erroneous results. Among these problems is the fact that chase radiolabeling of subunits is affected by the turnover rate of each subunit in situ. Thus, if turnover rates of the subunits differ the incorporated radiolabel will not accurately reflect the relative ratio of each subunit. Secondly, the use of detergents for immunoprecipitation tends to cause errors in the stoichiometry of the hydrophobic subunits of the membrane-bound enzyme complex because they dissociate the complex resulting in the loss of subunits from the complex. The use of subunit specific antibodies together with membrane preparations is believed to be a superior method for determining the subunit stoichiometry of the membrane-bound enzyme complex. Therefore, it would be important to reinvestigate the subunit stoichiometry of hydrophobic subunits using the methods determined for soluble subunits.

Treatment under alkaline conditions or with chaotropes are believed to extract the peripheral part of the membrane-bound enzyme complexes [52,53]. The *P. denitrificans* NQO1-6 and 9 subunits were extracted from the membranes by these treatments [35,50]. The *P. denitrificans* NQO1–5 subunits were overexpressed mainly in the soluble fraction in *E. coli* [54–56]. On the contrary, the *P. denitrificans* NQO6 and 9 subunits overexpressed in *E. coli* are located in the membrane fraction [35,50]. The NQO1–5 subunits are present in the NADH dehydrogenase complex isolated from the *P. denitrificans* membranes by treatment with NaBr. Recently, the 3D structure analysis of bovine and chicken complex III indicated that its iron–sulfur protein subunit which is extracted by strong chaotrope is mainly located in the peripheral segments but bears one helix which traverses the membrane [57] (E. Berry, personal communication). Taken together, it is conceivable that NQO1–5 subunits are present in the peripheral part and NQO6 and 9 subunits are mainly located in the peripheral part but are partly present in the membrane segment as seen in the case of the iron–sulfur protein subunit of complex III. The NQO7, 8, and 10–14 subunits are believed to be intrinsic membrane subunits on the basis of extraction methods of mitochondrial homologues of these subunits and hydrophobicity plots of their deduced amino acid primary structure [46–48,58–62]. If these conclusions are correct, it would be of interest to investigate which hydrophobic and hydrophilic subunits interact with NQO6 and 9 subunits.

4. Substrate and cofactor binding site(s)

To date, the NADH-binding subunits (NQO1) in the NDH-1 have been identified in *P. denitrificans* and *T. thermophilus* HB-8 by direct photoaffinity labeling with [^{32}P]NAD(H) [63,64]. In complex I, Deng et al. and Chen and Guillory [65,66] identified the 51 kDa subunit of FP as the NADH-binding subunit by using tritiated A3′-*o*-{3-[*N*-(4-azido-2-nitrophenyl)amino]-propionyl}NAD$^+$. Unfortunately, direct evidence regarding the NADH-binding site(s) has not been available at the present time although putative NADH-binding sites are speculated from the amino acid sequences of the NQO1 and its mitochondrial homologues [22].

So far the quinone-binding subunit in the NDH-1 has not been identified. However, rotenone has been

reported to bind to the ND-1 subunit (NQO8 homologue) [67]. The binding site of rotenone is considered to be in the proximity of quinone-binding site(s) [68]. This is because an analogue of rotenone showed similar inhibitory effects to capsaicin which is believed to be a competitive inhibitor for quinone in either NDH-1 or complex I [69,70]. It has been speculated that the IP 14 kDa subunit of bovine complex I [71] and 9.5 kDa subunit of *Neurospora crassa* complex I [72,73] may be quinone-binding subunits. However, homologues of these subunits do not exist in the NDH-1. Regarding the binding site of quinone of the NDH-1, there is no information as far as our present knowledge is concerned.

The NDH-1 and complex I have all been reported to bear noncovalently bound FMN [17,18,46,47]. Although there is no direct evidence that the FMN moiety is associated with the NQO1 and 50 kDa subunits, FMN can be provisionally assigned to the subunits, because the FMN is the primary oxidant of NADH on the basis of the reconstitution experiments of the NQO1 and NQO2 subcomplex with FMN [56]. However, it is possible that FMN may bind to the NQO1 and NQO2 subunits. Therefore, the assignment of FMN to subunit(s) must be more thoroughly investigated in the future.

NDH-1 and complex I are thought to be the most elaborate iron–sulfur proteins. It is generally accepted that NDH-1 and complex I contain at least five iron–sulfur clusters; N1a, N1b, N2, N3, and N4 although the electron transfer mechanism of NDH-1/complex I has not been clarified. Clusters N1a and N1b are binuclear and N2, N3, and N4 are tetranuclear. In addition, *E. coli*, *S. typhimurium*, and *T. thermophilus* (but not *P. denitrificans* or *R. capsulatus*) are believed to bear another binuclear iron–sulfur cluster which is tentatively designated cluster N1c. Furthermore, another tetranuclear cluster (designated cluster N5) has been reported to be present in bovine heart complex I [46,47,74,75]. However, at the present time, there is no report concerning the presence of N5 signals in the NDH-1. Table 3 summarizes the present knowledge about the iron–sulfur clusters in the NDH-1.

Cluster N1a shows rhombic symmetry in the NDH-1 and complex I and is believed to bind to the NQO2 subunit [54]. The physicochemical properties of the N1a is similar to the binuclear ferredoxin of *Clostridium pasteurianum* which is known to be an orphan of the binuclear clusters [76]. Site specific mutagenesis experiments showed that the four conserved cysteine residues (C96, C101, C137, and C141, numbering of the *P. denitrificans* NQO2) ligate the [2Fe–2S] cluster of the NQO2 subunits [77].

Table 3
Tentative assignment of iron–sulfur clusters of the NDH-1 to subunit

Clusters	Species	Symmetry	Subunit(s)
N1a	[2Fe–2S]	rhombic	NQO2
N1b	[2Fe–2S]	axial[a]	NQO3
N1c(?)	[2Fe–2S]	rhombic (?)	NQO3(?)
N2	[4Fe–4S]	axial	NQO6 or 9
N3	[4Fe–4S]	rhombic	NQO1
N4	[4Fe–4S]	rhombic	NQO3
spin $S = 3/2$ (?)	[4Fe–4S]		NQO3

[a]N1b of mammalian complex I is reported to exhibit rhombic symmetry.

Cluster N1b exhibits axial and rhombic symmetry, respectively, in the NDH-1 and mammalian complex I [55,74]. The properties of the expressed NQO3 subunit of the *P. denitrificans* NDH-1 suggested that the NQO3 subunit ligates the [2Fe–2S] cluster N1b [13]. The residues involved in ligation to the N1b are assumed to be cysteines in the cysteine clusters of the N-terminal region of the NQO3 subunit [13,24,28].

EPR signals of cluster N1c (rhombic symmetry) have been detected only in the *E. coli* NDH-1 at the present time. It should be noted that the NQO3 subunits of *T. thermophilus* HB-8, *E. coli*, and *S. typhimurium* contain a cysteine cluster in the middle of the deduced amino acid sequence of the subunits (C256, C259, C263, and C291, *Thermus* numbering) [28]. It is interesting to investigate whether *T. thermophilus* HB-8 and *S. typhimurium* NDH-1 bear cluster N1c.

Cluster N2 shows EPR signals with axial symmetry and the highest values of the midpoint potential in iron–sulfur clusters in the NDH-1/complex I. In addition, the E_m values of cluster N2 in the *P. denitrificans* and *R. sphaeroides* NDH-1 and bovine heart complex I are dependent on pH. Therefore, it is believed that cluster N2 plays an important roles in proton translocation and Q reduction. Concerning subunits ligating cluster N2, there are two candidate subunits, one is the NQO6 and the other is the

NQO9. The NQO6 subunit bears 4 conserved cysteines (C53, C54, C118, and C148, *Paracoccus* numbering) [50]. The NQO9 subunit contains typical $2 \times$ [4Fe–4S] ferredoxin type sequence motif. Direct evidence for this assignment is not available.

Clusters N3 and N4 exhibit EPR signals with rhombic symmetry. N3 and N4 are thought to be ligated by the NQO1 and NQO3 subunits, respectively [55,56]. Both subunits have typical tetranuclear binding cysteine motif [13].

The NQO3 subunit of the *P. denitrificans* NDH-1 expressed in *E. coli* was subjected to EPR and low-temperature magnetic CD measurements. It was found that the expressed NQO3 subunit ligates one [4Fe–4S] cluster with ground state spin $S = 3/2$. Preliminary results also suggested that a [4Fe–4S] cluster with spin $S = 3/2$ is present in the isolated bovine heart complex I. It is of interest to study the role of this iron–sulfur cluster in electron transfer in NDH-1 and complex I.

5. Energy-coupling mechanism

The proposed hypotheses of energy transducing mechanism of the NDH-1 and complex I can be divided into two types. One is that electron transfer carrier(s) is directly involved in the proton translocation. The other is that catalytic sector and proton translocating sector are separate entities just as the proton-translocating nicotinamide nucleotide transhydrogenase (TH) and ATP synthase [49,78,79]. The direct coupling mechanism of NDH-1 and complex I was intensively discussed in the recent review by Brandt [44]. In addition, Dutton provides his own direct coupling hypothesis in this issue [80]. Although many of the proposed hypotheses belong to the category of the direct coupling mechanism, a possibility of mechanism other than the direct-coupling mechanism may not be excluded because structural and functional information of the NDH-1/complex I seems to be too little to evaluate the hypotheses. Therefore, the latter hypothesis proposed by Belogrudov and Hatefi [49] will be discussed in this article. The NDH-1/complex I can be divided into a water-soluble fraction and a water-insoluble fraction. As described in Section 3, subunit composition, the water-soluble fraction of the *P. denitrificans* NDH-1 is composed of NQO1-6 and 9 subunits and its water-insoluble fraction contains NQO7, 8, and 10–14 subunits. Finel et al. [81] reported that so-called complex I λ isolated from bovine heart complex I houses FMN and all the EPR-visible iron–sulfur clusters, but does not contain any of the seven ND products. If there is no further cofactor(s) in NDH-1/complex I, these may suggest that all putative cofactor binding subunits are present in the water-soluble fraction. Furthermore, *N,N′*-dicyclohexylcarbodiimide (DCCD), which is a specific inhibitor of the proton translocation of ATP synthase, is also believed to inhibit the proton translocation of complex I and NDH-1 [58,82,83]. This inhibition is correlated with modification of the hydrophobic ND1 subunit by DCCD [58]. Therefore, it seems possible that the analogy to TH and ATP synthase is extended such that the water-soluble segment of NDH-1 would catalyze the scalar reaction of the enzyme and the membrane-bound segment would provide a proton channel (or water-wire) through the membrane [49]. As described above, experimental tests are required to verify various hypotheses in the future.

6. Family and superfamily

When the amino acid sequences of NDH-1 were used to search the GenBank/EMBL database, the only sequences showing significant similarities to the *P. denitrificans* NDH-1 gene products, other than NDH-1/complex I subunits from other species, were the NAD-linked hydrogenase [84] and the formate hydrogenlyase [85]. The previous review extensively discussed these two enzyme complexes [12,61,86], which will not be repeated here. Nowadays, it is generally accepted that Na^+-NDH is a member of NDH-1/complex I family in terms of enzyme functions in spite of no significant sequence similarities between Na^+-NDH and NDH-1/complex I [12,21]. Furthermore, Kumagai et al. [87] reported that *R*hodobacter capsulatus *n*itrogen *f*ixation enzyme complex encoded by the *rnf* operon may be an additional new member of NDH-1/complex I family (see Fig. 1). The *rnf* operon is composed of 7 structural genes (designated *rnf ABCDGEH*). Analyses of deduced amino acid sequences of subunits suggest that RnfA, D and E are hydrophobic sub-

units, whereas RnfB, C, G, and H are water-soluble subunits (see Fig. 1). The RnfB and RnfC contain a sequence motif of plant type 2[4Fe–4S] ferredoxin. In addition, RnfC has a sequence similar to the putative NADH-binding site of the NADH-binding subunit of the NDH-1/complex I. The Rnf enzyme complex appears to have a chimeric construction between the NDH-1 and Na^+-NDH. The subunits RnfA, RnfC, and RnfG are similar to the subunits NQR4 and 5, NQR2, and NQR3 of Na^+-NDH, respectively. The gene cluster encoding homologues of the Rnf subunits have been found in *Haemophilus influenzae* (hi1683–hi1688 and hi0395) [88] and *E. coli* (b1627–b1632 and b2618) [41]. It has been speculated that the Rnf enzyme complex catalyzes electron transfer from NADH to ferredoxin coupled to proton or Na^+ ion translocation across the chromatophore membranes. It is of interest to investigate the physiological roles and structure of the Rnf type enzyme complex in various bacteria.

General information regarding Complex I/NDH-1 is available at http://www.scripps.edu/mem/biochem/CI/.

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