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Review

The bacterial energy-transducing NADH-quinone oxidoreductases

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Abbreviations: Q, quinone; NDH-1 or complex I, energy-transducing NADH-quinone oxidoreductase; NDH-2, NADH-quinone oxidoreductase lacking an energy coupling site; URF, unidentified reading frame; DCCD, *N,N'*-dicyclohexylcarbodiimide; FP, IP and HP, respectively, the flavoprotein, the iron-sulfur protein, and the hydrophobic protein fractions of bovine complex I; complex III, quinol-cytochrome *c* oxidoreductase; ORF, open reading frame; LHON, Leber's hereditary optic neuropathy; EPR, electron paramagnetic resonance; FeS cluster, iron-sulfur cluster; mitochondrial DNA, mtDNA.

I. Historical overview of bacterial NADH-quinone oxidoreductases

In 1967, Bragg and Hou [1] reported the separation of an *Escherichia coli* membrane homogenate into a number of fractions of which several contained NADH dehydrogenase activity (see Table I). This classical paper opened the door to a flood of research on bacterial NADH-quinone (Q) oxidoreductases. Thus, shortly thereafter, a Japanese group found that the NADH-Q oxidoreductase segment of *Paracoccus deni-*

TABLE I

A chronological table of research in NADH-quinone oxidoreductases

Year	Bacterial NADH-Q oxidoreductases	Mitochondrial complex I
1961		isolation of complex I from bovine heart mitochondria [23]
1967	isolation of several different NADH dehydrogenase fractions from *E. coli* membranes [1]	
1968	discovery of the energy coupling site 1 in *Paracoccus denitrificans* [2,3]	
1975		reconstitution of bovine complex I with phospholipid [24]
1980		identification of the NADH-binding subunit of bovine complex I [181]
1981	gene cloning of *E. coli* NDH-2 [4]	
1983		determination of amino acid sequence of bovine FP 24 kDa subunit [110]
1985		assignment of 6 URFs of mitochondrial DNA for structural genes of complex I [25]
1986	isolation of NDH-1 [11,14]	
1987	recognition of two types of NADH dehydrogenase in *E. coli* [7] reconstitution of *Vibrio* NDH-1 with phospholipid [13]	identification of rotenone-binding subunit of bovine complex I [126]
1988	isolation of two types of NADH dehydrogenase from *Thermus thermophilus* HB-8 membranes [8]	
1989		sequencing of cDNA encoding polypeptides of complex I [29]
1990	identification of the NADH-binding subunit of the *Paracoccus denitrificans* NDH-1 [15]	
1991	cloning and DNA sequencing of the *Paracoccus* NDH-1 gene cluster [16]	electron microscopic analysis of *Neurospora crassa* complex I [53]

trificans membranes bears an energy-coupling site, is rotenone sensitive, and contains multiple EPR-visible iron-sulfur clusters [2,3]. Subsequently, a number of reports were published regarding the isolation and characterization of NADH-Q oxidoreductases from various bacterial sources. Young's group [4] carried out the gene cloning of the single polypeptide NADH-Q oxidoreductase from *E. coli*. In addition, Bragg and Hou [1] and Owen et al. [5,6] presented data suggesting that there may be at least two types of NADH dehydrogenase in *E. coli* and *Micrococcus lysodekticus* membranes. In 1987, Matsushita et al. [7] suggested

TABLE II

Bacterial NADH-quinone oxidoreductases

	NDH-1	NDH-2
Energy coupling site	present	absent
Cofactors	noncovalently bound FMN FeS clusters	noncovalently bound FAD
Inhibitors	rotenone piericidin A capsaicin DCCD	flavone (?)
Polypeptide composition	multiple subunits	single polypeptide
Origins	*Paracoccus denitrificans* [11] *Thermus thermophilus* HB-8 [8] *Escherichia coli* [12] *Rhodobacter capsulatus* [182] *Synechocystis* PCC6803 [67] *Thermus aquaticus* [10] *Vibrio alginolyticus* [14]	*Bacillus subtilis* [65] *Thermus thermophilus* HB-8 [8] *Escherichia coli* [72] *Rhodobacter capsulatus* [183] *Synechocystis* PCC6803 [184] *Thermus aquaticus* [10] *Halobacterium cutirubrum* [185] *alkalophilic Bacillus* YN-1 [186] *Sulfolobus acidocaldarius* [66] *Bacillus caldotenax* [187] *Bacillus stearothermophilus* [188] *Photobacterium phosphoreum* [189,190] *Vibrio alginolyticus* [141]

that in *E. coli* one type of NADH dehydrogenase bears an energy coupling site while the other does not. Subsequent to these reports Yagi et al. [8] isolated and characterized two types of NADH-Q oxidoreductase from *Thermus thermophilus* HB-8 membranes. These two enzymes are distinct from each other in terms of energy transduction, cofactors and polypeptide composition. A complete compilation of the published data has led to the concept that in many, if not all, bacterial species there are at least two types of NADH-Q oxidoreductases [9,10]. One of these is the enzyme which bears the energy coupling site (designated NADH dehydrogenase 1 (NDH-1)) and the other is the enzyme which lacks the coupling site (designated NADH dehydrogenase 2 (NDH-2)). To date, NDH-1 type enzymes have been isolated from *P. denitrificans* [11], *T. thermophilus* HB8 [8], *E. coli* [12], and *V. alginolyticus* [1] [13,14] whereas the NDH-2 type enzymes have been isolated from a large number of bacteria as shown in Table II.

Since the discovery of the two different types of NADH-Q oxidoreductase, several investigators have begun to characterize these individual enzyme complexes. In 1990, the location of the NADH-binding subunit of the *P. denitrificans* NDH-1 was determined by a direct photoaffinity labeling procedure using [^{32}P]NAD(H) [15]. In addition, the gene cloning of the NADH-binding subunit from the same species was completed [16]. Furthermore, it was discovered that the structural genes encoding the *Paracoccus* NDH-1 subunits constitute a single gene cluster [16–19]. This gene cluster is composed of 14 structural genes and 6 URFs [20]. More recently, Weiss' and Dupuis' groups have succeeded in cloning the *E. coli* and *R. capsulatus* NDH-1 gene clusters, respectively [21,22].

A thorough understanding of the studies of the mitochondrial complex I that both preceded and accompanied the work on the bacterial NDH-1 is necessary to a good understanding of the work that has been done on these bacterial NDH-1 enzyme complexes. A chronological table of the progress made in the study of the mitochondrial complex I from various species is shown in Table I. Work done on the bacterial NADH-Q oxidoreductases is also included. Mitochondrial complex I was first isolated from bovine heart by Hatefi et al. [23] in 1961. In 1975, reconstitution of the bovine complex I with phospholipids was carried out by Ragan and Hinkle [24]. More recently, Chomyn et al. [25,26] have reported data which suggest that 7 URFs (1, 2, 3, 4, 4L, 5 and 6) present in the mitochondrial DNA encode polypeptides of complex I. Those URFs have been sequenced by Anderson et al. [27] and have been designated the ND1, ND2, ND3, ND4, ND4L, ND5 and ND6 genes, respectively. 1989 marked the beginning of a series of reports by both English (Walker's) and German (Weiss' and Werner's) groups in which attempts were made to determine the primary structures of both bovine and *Neurospora crassa* complex I [28–47] (see also review Ref. 48). Japanese (Matsubara's) and U.S. (Attardi and Chomyn's) groups have also contributed to the determination of the primary structures of several polypeptides present in the bovine complex I [49–52]. According to Walker's group [47], bovine complex I appears to be composed of at least 42 dissimilar polypeptides. The *N. crassa* complex I is considered to be composed of more than 30 unlike polypeptides [48]. Recently, Leonard and his coworkers on the basis of analysis of electron microscope images of two-dimensional crystals of *N. crassa* complex I [53] have suggested that this enzyme complex has an unusual L-shaped structure with two domains which appear like 'arms' arranged at right angles.

To date several excellent reviews summarizing the work done on the mitochondrial complex I have been published [48,54–56]. However, there are only two reviews concerning the bacterial NADH-Q oxidoreductases. One by Unemoto and Hayashi [57], concentrates on the sodium-transporting NADH-Q oxidoreductase of *Vibrio alginolyticus*. The other, which deals with the bacterial NADH-Q oxidoreductases, was published by Yagi in 1991 [10]. Since these two reviews were published, rapid progress has been made in structural studies on the *Paracoccus* NDH-1. Therefore, I will attempt, in this review, to describe primarily the present state of knowledge of the NDH-1 of both *Paracoccus* and other bacteria.

II. Two types of NADH-Q oxidoreductase: characteristics and comparison

As described in the previous section, the NADH-Q oxidoreductases of the bacterial respiratory chains can be divided into two groups depending on whether they do (NDH-1) or do not (NDH-2) bear an energy-coupling site. Table II compares the characteristics of NDH-1 and NDH-2. NDH-1 generally contains non-covalently bound FMN and iron-sulfur clusters as prosthetic groups [10–12]. On the other hand, NDH-2 bears non-covalently bound FAD and has no FeS clusters [8–10]. NDH-1 is generally inhibited by one or more of the potent mitochondrial complex I inhibitors (rotenone, piericidin A, capsaicin and DCCD) while

[1] The Na-transport NADH-Q oxidoreductase of *Vibrio alginolyticus* contains FAD and FMN and lacks FeS clusters. These characteristics appear to correspond to those of neither NDH-1 nor NDH-2. Therefore, Rich and his coworkers have proposed that this sodium-pumping enzyme may belong to a third class of NDHs which is distinct from NDH-1 or NDH-2 (P.R. Rich, personal communication). Although this may be the case, we have temporarily classified the *Vibrio* sodium-transport enzyme into the NDH-1 category on the basis of its energy-transducing function.

TABLE III

Characteristics of the FeS clusters of the NDH-1 segments of P. denitrificans, T. thermophilus HB-8, E. coli and bovine heart mitochondria

Organisms	Cluster	Field positions $g_{x,y,z}$	E_m (mV)	Cluster structure	pH dependence of E_m
P. denitrificans [a]	N1a	1.92, 1.94, 2.03	−150	Binuclear	yes(?)
	N1b	1.93, 1,94, 2.02	−260	Binuclear	no
	N2	1.92, 1.92, 2.05	−130	Tetranuclear	yes
	N3	1.86, 1.94, 2.01	−240	Tetranuclear	no
	N4	1.88, 1.94, 2.09	−270	Tetranuclear	no
T. thermophilus HB-8 [b]	N1a	1.93, 1.94, 2.02	−274	Binuclear	?
	N2	1.89, 1.95, 2.04	−304	Tetranuclear	?
	N3	1.80, 1.83, 2.06	−289	Tetranuclear	?
E. coli [c]	N1	1.92, 1.94, 2.03	−220	Binuclear	?
	N2	1.90, 1.91, 2.05	−240	Tetranuclear	?
Bovine heart [d]	N1a	1.91, 1.95, 2.03	−370	Binuclear	yes
	N1b	1.92, 1.94, 2.02	−245	Binuclear	no
	N2	1.92, 1.92, 2.05	−20	Tetranuclear	yes
	N3	1.86, 1.93, 2.04	−245	Tetranuclear	no
	N4	1.87, 1.93, 2.10	−245	Tetranuclear	no

[a] From Meinhardt et al. [82].
[b] From Meinhardt et al. [85].
[c] From Meinhardt et al. [191].
[d] From Ingledew and Ohnishi [192].
The names of the EPR-visible FeS clusters of various NDH-1 are based on each paper cited. It should be noted that N2 cluster of *Thermus* or *E. coli* NDH-1 do not correspond with N2 clusters of bovine and *Paracoccus* NDH-1 in terms of redox potentials.

NDH-2 is not affected by these inhibitors [58–61]. Although the NDH-2 type rotenone-insensitive NADH-Q oxidoreductases of *Arum maculalum* and *Saccharomyces cerevisiae* mitochondria [62,63] have been reported to be inhibited by flavone, the same inhibitor does not inhibit the *Thermus* or *E. coli* NDH-2 [10]. Thus, no general inhibitor specific to NDH-2 has yet been found. Because such inhibitors can be very useful in determining the mechanism of action of the enzyme complexes, the discovery of a general NDH-2 specific inhibitor would contribute significantly to the study of NDH-2 type NADH-Q oxidoreductases. With respect to polypeptide composition, NDH-1 type enzymes are multiple-polypeptide enzyme complexes whereas NDH-2 type enzymes are composed of a single polypeptide [9,10,64]. However, the *Sulfolobus* NDH-2 has been reported to constitute a homodimer structure [8,10,11]. *P. denitrificans* membranes appear to bear only NDH-1 [11]. On the other hand, *Bacillus subtilis* and *Sulfolobus acidocaldarius* membranes have been reported to contain only NDH-2 [65,66]. As described above, *T. thermophilus, E. coli, Thermus aquati-*

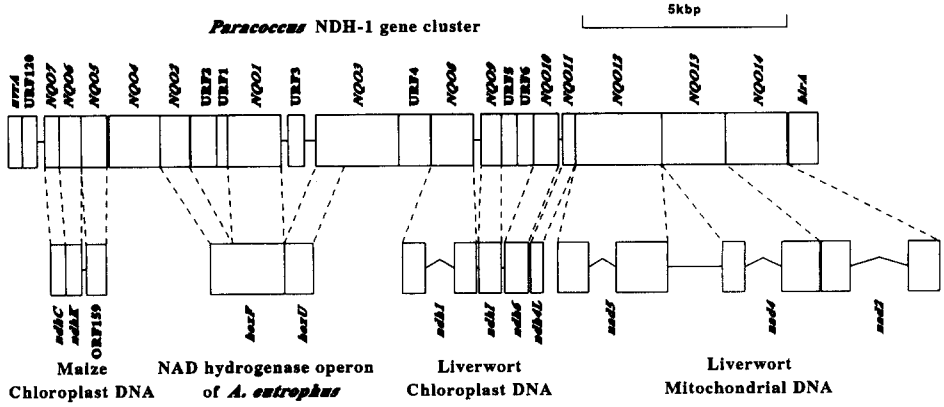

Fig. 1. Gene map of the Paracoccus NDH-1 gene cluster. The polypeptides encoding the *NQO1, NQO2, NQO3, NQO4, NQO5, NQO6, NQO7, NQO8, NQO9, NQO10, NQO11, NQO12, NQO13* and *NQO14* genes are homologous, respectively, to bovine 51 kDa (FP), 24 kDa (FP), 75 kDa (IP), 49 kDa (IP), 30 kDa (IP), 20 kDa (IP), ND3 (HP), ND1 (HP), 23 kDa (HP), ND6 (HP), ND4L (HP), ND5 (HP), ND4 (HP) and ND2 (HP). For comparison, the gene arrangements of *Synechocystis* PCC6803, liverwort chloroplast DNA and liverwort mitochondria DNA are shown. — and ∧ indicate, respectively, the non-coding region and the intron.

cus, Rhodobacter, Synechocystis and *Vibrio* all appear to have both types of NADH-Q oxidoreductases [8,10,12,67,68]. DNA sequences of the structural genes encoding subunits of NDH-1 have been determined in *P. denitrificans* [16–19] and *Synechocystis* [69,70] (K. Steinmüller, unpublished data registered in EMBL database with the accession number x62517). NDH-2 structural genes, on the other hand, have been cloned in *E. coli* [4] and alkalophilic *Bacillus* YN-1 [71]. In the case of the *E. coli* NDH-2, overexpression of the NDH-2 gene has been carried out [72]. Guest and his coworkers [73,74] have suggested that oxygen regulates the expression of the NDH-2 gene of *E. coli* such that under conditions at higher oxygen concentration the expression of NDH-2 increases, whereas under lower oxygen conditions the expression of NDH-2 decreases. Such regulation has not been detected in the NDH-1 expression of *E. coli*.

At the present time it appears that mammalian mitochondria contain only complex I. However, plant and fungal mitochondria bear complex I and one or two NDH-2 type enzyme(s) [10]. The NDH-2 type enzymes of plant and fungal mitochondria can be divided into at least two groups; one directs to the cytoplasmic side and the other faces to the matrix side [75–78]. It is of interest to note that in the case of plant mitochondria the activities of the NDH-2 type enzymes are stimulated by the presence of Ca ions [76,78]. *S. cerevisiae* mitochondria lacking complex I bear two NDH-2 type enzymes similar to plant mitochondria [63,79,80]. Recently, Møller's and Wiskich's groups have isolated complex I from beetroot mitochondria (Ref. 81, and I.M. Møller, personal communication).

TABLE IV

Comparison of the subunit composition of the Paracoccus NDH-1, bovine complex I and N. crassa complex I

	Paracoccus NDH-1	Bovine complex I	*N. crassa* complex I
FP	NQO1	51 kDa	51 kDa
	NQO2	24 kDa	? [b]
	– [a]	9 kDa	?
IP	NQO3	75 kDa	78 kDa
	NQO4	49 kDa	49 kDa
	NQO5	30 kDa	30.4 kDa
	NQO6	20 kDa [c]	?
	–	18 kDa	?
	–	15 kDa	?
	–	13 kDa (A)	?
	–	13 kDa (B)	?
HP	NQO8	ND1	ND1
	NQO14	ND2	ND2
	NQO7	ND3	ND3
	NQO13	ND4	?
	NQO11	ND4L	ND4L
	NQO12	ND5	ND5
	NQO10	ND6	ND6
	NQO9	23 kDa	?
	–	42 kDa	?
	–	39 kDa	?
	–	?	29.9 kDa
	–	?	21.3 kDa (A)
	–	?	21.3 kDa (B)
	–	19 kDa	20.8 kDa
	–	?	18.3 kDa
	–	10 kDa (acyl carrier)	9.6 kDa (acyl carrier)

[a] –, Not present in the *Paracoccus* NDH-1 gene cluster.
[b] ?, Not yet reported.
[c] This polypeptide has been isolated from the bovine IP by Masui et al. [50]. Whether the 20 kDa polypeptide is actually a component of IP remains to be seen [50].

III. Structure of NDH-1

III-A. Cofactors

As described above, one of the characteristics of NDH-1 that distinguishes it from NDH-2 is that it bears FMN and FeS clusters as prosthetic groups. The discovery that all the known NDH-1 and complex I contain FMN suggests that FMN may be an indispensable component of the energy coupling site 1 [10]. On the other hand, the contribution of the FeS clusters to energy coupling is less clear. Table III compares the properties of the FeS clusters of NDH-1 of *P. denitrificans, T. thermophilus* HB-8, *E. coli*, and bovine heart mitochondria. Although the *Paracoccus* NDH-1 is similar to mitochondrial complex I in terms of FeS clusters [82,83], the *E. coli* or *Thermus* NDH-1 FeS clusters are much simpler [84,85]. Thus, for example, the high potential FeS cluster N2 present in *Paracoccus* NDH-1 and the mitochondrial complex I has not been detected in *E. coli* or *Thermus* NDH-1. Based on the demonstration that E_m values of cluster N2 are dependent on energized state or the pH of the membrane, this high potential FeS cluster N2 has been considered to play a role in H^+ translocation. Additional support for this theory is supplied by the discovery that *Paracoccus* membranes grown under limited sulfate conditions or in the presence of rotenone lack not only coupling site 1 but also the cluster N2 signals [86]. However, because such high potential FeS clusters have not been observed in certain bacteria as described above, it remains to be seen whether the high potential FeS cluster is an essential component for energy coupling site 1.

III-B. Subunit composition

Recently, the gene cluster encoding the *Paracoccus* NDH-1 has been isolated and sequenced [16–20]. The gene cluster is approx. 18 kbp long and is composed of 14 structural genes and 6 URFs (see Fig. 1) [20].

Although the complete assignment of all the gene products has not been carried out, the number of subunits of the *Paracoccus* NDH-1 estimated from the gene structure appears to match the number of polypeptides of the enzyme complex isolated by hydroxyapatite column chromatography after dodecylmaltoside extraction or immunoprecipitated with anti *Paracoccus* NADH dehydrogenase complex antiserum (T. Yagi, unpublished results) [2]. It is, therefore, conceivable that all the structural genes encoding subunits of the *Paracoccus* NDH-1 are present in this gene cluster. If that is the case, the *Paracoccus* NDH-1 would be composed of at least 14 subunits. As shown in Fig. 1 and Table IV, the structural genes encoding the 14 subunits are designated *NQO1–NQO14*. When compared to bovine complex I which is composed of at least 42 dissimilar polypeptides, the *Paracoccus* NDH-1 has a relatively simple structure. However, it is similar to the mitochondrial complex I in terms of EPR-visible FeS clusters [87,88]. It is interesting to note that the other energy-transducing enzyme complexes (complex III and cytochrome oxidase) of bacterial respiratory chains are composed of only three subunits [89–94] (see also reviews Refs. 95–98). This raises the question as to why nature created such an intricate structure for the energy coupling site 1. Ragan, in his 1987 review [56], provides a possible answer by suggesting that some polypeptides of complex I do not have a role in the catalytic function of complex I but serve instead as receptors for other dehydrogenases. This hypothesis follows from finding that several TCA cycle dehydrogenases in mitochondria appear to channel NADH to complex I [99,100]. Therefore, it will be interesting to determine whether similar channeling of NADH to NDH-1 are also present in bacteria.

Bovine complex I can be resolved into a water-soluble fraction and a water-insoluble fraction (hydrophobic protein fraction, HP) by treatment with $NaClO_4$ [54,55,101,102]. The water-soluble fraction can be further resolved into two fractions by ammonium sulfate fractionation [54,55,101,102]. The two water-soluble fractions have been designated the flavoprotein fraction (FP) and the iron-sulfur protein fraction (IP). As shown in Table IV, bovine FP is composed of three dissimilar polypeptides. Bovine IP contains eight dissimilar polypeptides. The HP appears to be composed of at least 31 unlike polypeptides. In Table IV, the polypeptide composition of the *Paracoccus* NDH-1 is compared with that of bovine and *N. crassa* complex I. In the case of FP, homologues of the bovine 51 kDa and 24 kDa polypeptides are present in the *Paracoccus* NDH-1 but a counterpart of the bovine 9 kDa polypeptide is absent in the *Paracoccus* enzyme complex. With respect to IP polypeptides, polypeptides similar to the high molecular mass polypeptides (75 kDa, 49 kDa, 30 kDa and 20 kDa polypeptides), but not to the lower molecular mass polypeptides (18 kDa, 15 kDa, 13 kDa(A) or 13 kDa(B)), are present in the *Paracoccus* enzyme. In terms of HP polypeptides, homologues of the bovine 23 kDa and all seven mitochondrial DNA-encoded polypeptides are present in the *Paracoccus* enzyme complex. Other polypeptides of bovine HP do not appear to be present in the *Paracoccus* enzyme complex. The lack of lower molecular mass mitochondrial counterparts in bacterial respiratory chain complexes is not unique to the NADH-Q oxidoreductase [96,98,103]. For example, although bacterial complex III contains homologues to the mitochondrial complex III cytochrome *b*, cytochrome c_1, and FeS protein, it lacks homologues to the 14 kDa, 12 kDa, 11 kDa, and 8 kDa polypeptides [98,104]. The question arises as to what role these lower molecular mass polypeptides of the mitochondrial complex I play in the enzyme function. As reported in [105], these polypeptides may play a role in the assembly of complex I in mitochondria rather than being involved in the catalytic function of the enzyme complex.

III-C. Characteristics of the subunits

The 50 kDa polypeptide of the *Paracoccus* NDH-1 has been identified as the NADH-binding subunit by direct photoaffinity labeling method with [^{32}P]NAD(H) [15]. This result has been confirmed by immuno-crossreactivity between the *Paracoccus* 50 kDa subunit and the bovine complex I NADH-binding (51 kDa) subunit [15,106]. That the *NQO1* gene encodes the 50 kDa subunit of the *Paracoccus* NDH-1 has been biochemically demonstrated [16]. The hydropathy plot of the *NQO1* product, in agreement with the results of topological studies of its bovine counterpart using monospecific antibodies [102], does not show a hydrophobic stretch typical of membrane-spanning regions. The deduced amino acid sequence of the *Paracoccus* NADH-binding subunit has 64% identity to its bovine and *N. crassa* counterparts [28,43,52]. Such high sequence identity explains the similarity of amino acid composition and immunocross-reactivity between the *Paracoccus* NADH-binding subunit and its bovine counterpart [15]. The analysis of consensus sequences of the NADH-binding site by Scrutton et al. [107] suggests that residues 54–63 of the primary structure of the *Paracoccus* NADH-binding subunit constitute the most likely candidate for the NADH-binding site. In addition, the same structural motif has been reported in its bovine [28,52] and *N. crassa* counterparts [43]. The deduced primary structure of the 50 kDa

[2] These results suggest that the preparation of the *Paracoccus* NADH dehydrogenase complex using NaBr may lack several subunits [11].

subunit also contains the structural motif (CxxCxx-$C(x)_nC$) typical of tetranuclear FeS clusters from ferredoxins of bacteria and chloroplasts [108]. In view of the suggested interaction between FMN and cluster N3 [109], and the likelihood that FMN is the primary oxidant of NADH, both FMN and cluster N3 can be provisionally assigned to the *Paracoccus* 50 kDa subunit [16,28,52]. Patel et al. [52] and Weiss et al. [48] have proposed the conserved glycine rich region between residues 167 and 186 as the FMN-binding site. However, the location of the FMN-binding site is less clear because of insufficient data for the consensus sequence motif [28].

The *NQO2* gene encodes the 25 kDa subunit of the *Paracoccus* NDH-1 [17]. The *NQO2* subunit is homologous to the 24 kDa polypeptide of bovine complex I [29,110]. Analysis of the primary structure and topological studies using monospecific antibodies suggest that the *Paracoccus* 25 kDa subunit and its mammalian counterpart are both peripheral proteins [17,102]. Bovine FP contains two EPR-visible FeS clusters, the binuclear center N1b and the tetranuclear center N3. Both the bovine 51 kDa subunit fraction and the 24 kDa + 9 kDa subunit fraction contain non-heme iron and acid-labile sulfide after resolution of FP [111]. Analysis of the non-heme iron content in these 2 fractions appears to suggest that the binuclear cluster is in the 24 kDa + 9 kDa subunit fraction [111]. Since the 9 kDa subunit does not contain any cysteine residues [34,50] and since a subunit corresponding to the bovine 9 kDa subunit is not present in the *Paracoccus* NDH-1 [17], this binuclear cluster (N1b) would most likely be associated with the bovine 24 kDa subunit (see Table V). The cluster N1b has also been reported to be present in the *Paracoccus* NDH-1 [82]. Therefore, it is conceivable that the 25 kDa subunit bears the cluster N1b [17,55]. Before the primary structure of the *Paracoccus* 25 kDa subunit was determined, the primary structures of the 24 kDa subunits of complex I were available from human, bovine, and rat mitochondria [29,51,110,112]. These three subunits show more than 93% identity to each other, and all five cysteines and three histidines are conserved in each, the one exception being one additional cysteine in the rat 24 kDa subunit. Of these residues four cysteines and only one histidine are conserved in the *Paracoccus* 25 kDa subunit. This has made it possible to speculate on the following binding sites for cluster N1b: H_{92}xxxCxxxxC_{101} and C_{137}xxxC_{141} [17].

The *Paracoccus NQO3* gene product (66 kDa) has about 49% and 48% sequence identity, respectively, to its bovine and *N. crassa* counterparts [19]. The monospecific antiserum to the bovine complex I 75 kDa subunit crossreacted with the *NQO3* (66 kDa) encoded subunit of the *Paracoccus* NDH-1 [19]. Hydropathy plots of this *Paracoccus* 66 kDa (*NQO3*) subunit suggest that this subunit may lack hydrophobic membrane-spanning segments. Similar results have been reported for the bovine and *N. crassa* counterparts of this subunit [30,43]. However, surface labeling and immunochemical studies using bovine complex I suggested that the bovine 75 kDa subunit is exposed on both sides of the inner mitochondrial membrane [56,101]. One possible explanation for this apparent discrepancy is that the *Paracoccus* 66 kDa subunit and its bovine homologue do actually span the membrane. However, the membrane spanning segments of the subunit may be hydrophilic in nature with these hydrophilic residues being shielded from the lipid bilayer by an annulus of other complex I or NDH-1 subunits [19,30]. The *NQO3* encoded subunit appears to contain a putative tetranuclear iron-sulfur cluster (probably cluster N4) and possibly a binuclear iron-sulfur cluster (possibly cluster N1a) (see Table V) [19]. These putative iron-sulfur cluster-binding sites are located approx. 250 amino acid residues from the N-terminus of the 66 kDa subunit. The function of the remaining two thirds of the polypeptide is not clear. However, the bovine 75 kDa homologue of this subunit has been reported to polymerize into filamentous structures subsequent to extraction and purification. In fact, this bovine subunit has been purified using a method originally developed for the isolation of a cytoskeletal component [113]. As described by others [114,115] the α-helical domain of all intermediate filamentous proteins studied to date contains a repeating heptad sequence of the form $(a\text{-}b\text{-}c\text{-}d\text{-}e\text{-}f\text{-}g)_n$ where residues a and d are usually hydrophobic residues. The residues 443–521 of the *Paracoccus NQO3* subunit and the residues 283–349 and 450–508 of the bovine 75 kDa subunit contain repeating heptad organizations which are similar to these intermediate filament proteins. Therefore, it may be speculated that the *NQO3* subunit plays a dual role in the NADH-Q oxidoreductase, one as an electron carrier and the other as a structural polypeptide [19]. It should be noted, however, that to date no protein having dual roles of this nature have been reported.

The *Paracoccus* NDH-1 48 kDa (*NQO4* encoded) and 21 kDa (*NQO5* encoded) subunits crossreacted with monospecific antibodies to the bovine complex I 49 kDa and 30 kDa polypeptides, respectively [116]. Both *Paracoccus* subunits appear, on the basis of hydropathy plots, to be peripheral membrane proteins similar to their bovine counterparts [101,116]. Neither of these *Paracoccus* subunits contain typical structural motifs associated with the iron-sulfur clusters present in the ferredoxins of either bacteria or chloroplasts [108]. However, EPR studies using the bovine enzyme showed that the isolated fraction composed of the bovine IP 49 kDa, 30 kDa, and 13 kDa polypeptides bears one tetranuclear and one binuclear iron-sulfur

cluster [55]. This fraction can be further resolved into two subfractions: one consisting of the 49 kDa polypeptide and the other of the 30 kDa plus 13 kDa polypeptides. Each of these subfractions has been reported to contain non-heme iron and acid-labile sulfides, suggesting that both the 49 kDa and the 30 kDa polypeptides should bear iron-sulfur clusters. On the basis of amino acid sequence similarity and immuno-crossreactivity, the *NQO4* and *NQO5* encoded subunits might then also be expected to bear iron-sulfur clusters. The absence of typical iron-sulfur cluster binding structural motifs may then be an indication that two or more of subunits contribute ligands to the iron-sulfur cluster jointly [117,118].

The *NQO6* product has significant sequence homology to the putative polypeptides encoded by the gene of *Paramecium* mitochondrial DNA (mtDNA) and by the *ndhK* gene of chloroplast DNA [69]. The *ndhK* gene (previously designated *psbG*) was first discovered in chloroplast DNA [119,120] and was believed to encode a polypeptide of Photosystem II [119,120]. Subsequently, an open reading frame homologous to *ndhK* was detected in the *Paramecium* mtDNA [121,122]. In addition, Nixon et al. [123] have suggested that the *ndhK* gene is not a photosystem II gene. It has been shown immunochemically that these genes are being expressed in cyanobacterium, *Synechocystis* [67]. Furthermore, we have discovered that the *NQO6* gene is located in the *Paracoccus* NDH-1 gene cluster [116]. In addition, Masui et al. [50] have isolated and partially sequenced a polypeptide ($M_r = 20\,000$) from the bovine IP fraction which is also homologous to the *ndhK* encoded polypeptide. The bovine 20 kDa polypeptide has striking similarity to the *Paracoccus NQO6* gene product [47,50,116]. In view of these results, the *NQO6* product and its homologues are considered to be subunits of NDH-1 and the mitochondrial complex I. The chloroplast *ndhK* subunits have a unique stretch of approx. 40 amino acids at the C-terminal region which are not found in the mitochondrial subunits [47,116]. Two structural genes similar to the *NQO6* gene have been cloned and sequenced in *Synechocystis* [69,124]. Both *Synechocystis* gene products are more closely related to the chloroplast subunits than to mitochondrial subunits [116].

The *NQO9* encoded subunit bears two putative binding sites for tetranuclear iron-sulfur clusters [20] (T. Yagi, X. Xu and A. Matsuno-Yagi, unpublished results). A homologue of the *NQO9* subunit has been found in HP of bovine complex I [31]. According to Refs. 54 and 55, bovine HP appears to contain the EPR-visible tetranuclear FeS cluster N2 and possibly as many as two binuclear clusters. Taken together, these data suggest that the *NQO9* subunit is most likely the bearer of the high potential cluster N2 (see Table V). Sequences similar to the *NQO9* subunit have been found in the *ndhI* genes (previously designated *frxB*) of chloroplast [31], *Rhodobacer* [22] and *Synechocystis* DNA (K. Steinmüller; the sequence is registered in the EMBL database with the accession number X62517).

The subunits of the *Paracoccus* NDH-1 encoded by the *NQO7, NQO8, NQO10, NQO11, NQO12, NQO13* and *NQO14* genes are, respectively, homologous to the ND-3, ND-1, ND-6, ND-4L, ND-5, ND-4 and ND-2 gene products of the bovine mtDNA. The ND-1, ND-3 and ND-4L products have been immunochemically detected in HP of bovine complex I [64,125,126]. In addition, the ND-2 product has been chemically identified in HP of bovine complex I (T. Yagi and Y. Hatefi, unpublished results). These data together with the fact that homologs of the seven ND gene products are all present in the *Paracoccus* NDH-1 gene cluster support the suggestion made by Chomyn et al. that all seven ND genes of the human mtDNA encode polypeptides of complex I [25,26]. Hydropathy plots of these seven gene products suggest that these polypeptides are all highly hydrophobic. Furthermore, when *N. crassa* was grown in the presence of chloramphenicol, complex I was transformed into a rotenone-insensitive enzyme complex (so-called small complex I) which lacked ND gene products, the tetranuclear cluster N2 and a large membrane-associated section of complex I [53,127]. Taken together, these data suggest that the *NQO7, NQO8, NQO10, NQO11, NQO12, NQO13* and *NQO14* subunits probably constitute the membrane-associated sector of the *Paracoccus* NDH-1. The bovine *NQO8* homologue (ND1 gene product) has been identified as the rotenone- and DCCD-binding polypeptide [58,64,126]. The *Paracoccus NQO14* subunit and its bovine homologue (ND2 gene product) are extracted from both NDH-1 and complex I with a chloroform-methanol solution, suggesting, in agreement with the above hypothesis, that the *NQO14* subunit is highly hydrophobic [128] (T. Yagi, unpublished results). None of the these seven subunits has structural motifs associated with the FeS clusters found in the ferredoxins of either bacteria or chloroplasts [108]. In addition to the subunits of NDH-1 discussed so far, the mitochondrial complex I contains approx. 28 additional polypeptides. One of these polypeptides is believed to be the acyl-carrier protein although the *E. coli* homologue of this subunit is located in the cytoplasm rather than in the membrane [36,42]. Otherwise, the structural and/or functional roles of these 20 polypeptides remain unknown.

Synechocystis contains not only cytoplasmic membranes but also thylakoid membranes. The *Synechocystis* genes homologous to the *Paracoccus* NDH-1 *NQO4, NQO5, NQO6, NQO7, NQO8, NQO9, NQO10* and *NQO11* genes have been isolated and sequenced [69,124] (K. Steinmüller; unpublished sequences have

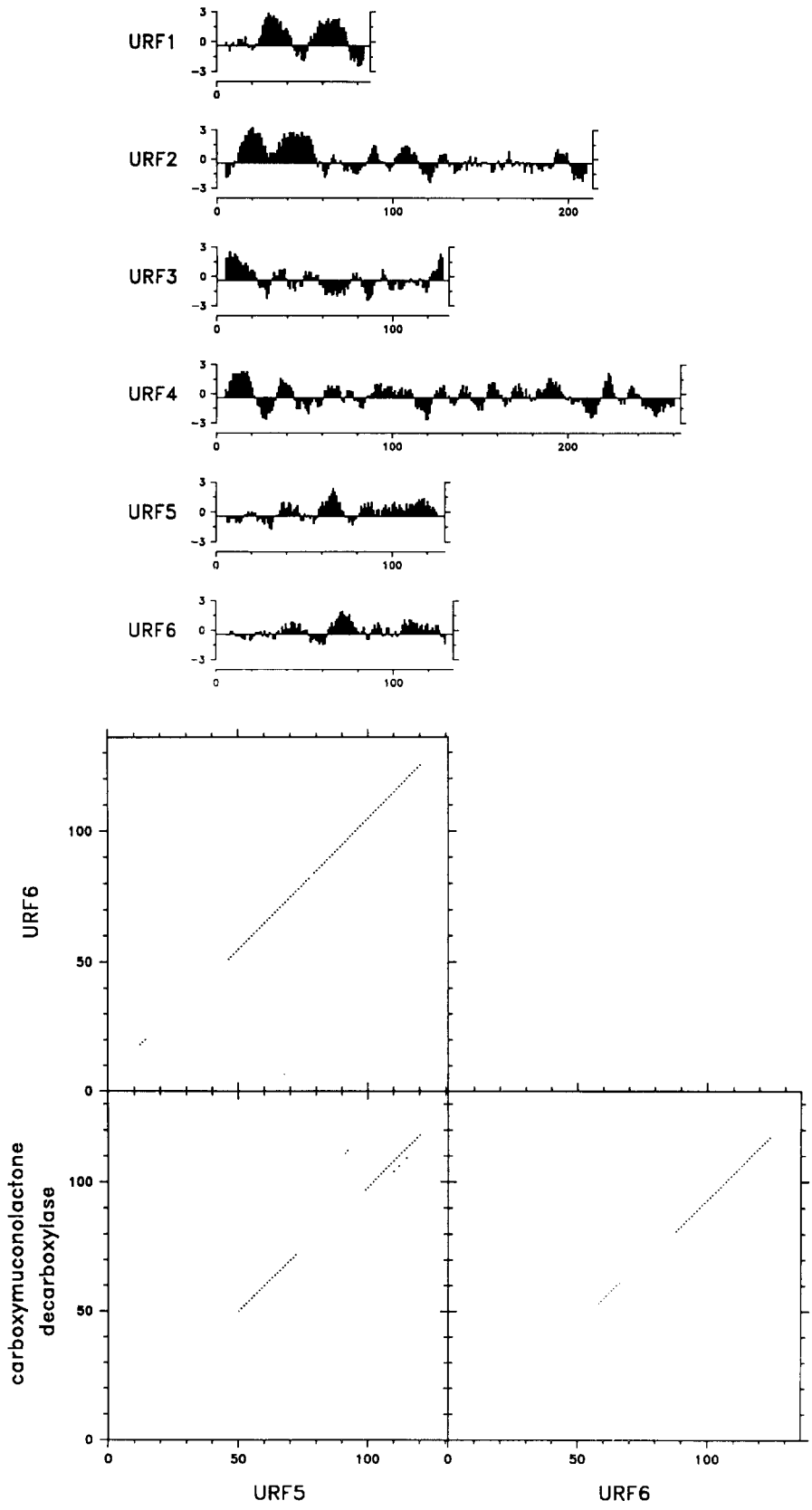

Fig. 2. Hydropathy plots of the 6 URFs-encoding polypeptides (A) and comparison of the deduced primary sequences of the polypeptides encoding URF5 and URF6 and γ-carboxymuconolactone decarboxylase of *Acinetobacter calcoaceticus* (B). On the basis of the data of Kyte and Doolittle [209], hydropathy indices were calculated with a window of nine residues by using the KDPLOT program written by A. Matsuno-Yagi (The Scripps Research Institute, La Jolla).

been deposited with EMBL database under accession number X62517). In these structural genes of *Synechocystis*, the *ndhC*, *ndhK* and ORF157 genes (homologous to the *Paracoccus NQO7, NQO6* and *NQO5* genes, respectively) constitute an operon [69]. A comparison of amino acid sequences of the polypeptides encoded by the *Synechocystis* genes with the respiratory NDH-1 (including complex I) and chloroplast NDH-1 type enzyme homologues [3] suggests that the primary structure of the polypeptides encoded by the cloned *Synechocystis* genes resemble those of the chloroplast more than those of the respiratory NDH-1 homologues [116]. These data suggest that one of the following is most likely the case: (1) only the chloroplast type NDH-1 is present in *Synechocystis*. This chloroplast-type enzyme complex is responsible for the respiration of this organism. (2) Two types of NDH-1 are present in *Synechocystis*, a chloroplast-type and a second type which is similar to the respiratory NDH-1. The genes encoding the polypeptides of this latter enzyme complex have not been cloned yet. This issue remains to be resolved.

As touched on briefly above, homologues to the 11 *Paracoccus* subunits encoded by the *NQO4–NQO14* genes have been found in the predicted polypeptides encoded by genes of chloroplast DNA from a number of species. Furthermore, as shown in Fig. 1, the arrangements of the *NQO7,6,5* and *NQO8,9,10,11* genes of the *Paracoccus* NDH-1 correspond with the arrangement of homologous chloroplast genes [69,120], supporting the possible presence of NDH-1 type enzymes in chloroplasts and suggesting that the *Paracoccus* NDH-1 might have an evolutionary relationship with the predicted enzyme complex of chloroplasts. In terms of amino acid sequences of the *NQO4, NQO5* and *NQO6* homologues, the NDH-1 of *Synechocystis*, a cyanobacterium containing both cytoplasmic and thylakoid membranes, appears to be more closely related to the NDH-1 type subunits of chloroplast than of complex I, supporting the hypothesis that a NDH-1 type enzyme is present in thylakoid membranes of chloroplasts. However, direct physicochemical or biochemical evidence is required before it can be stated that chloroplasts contain an NDH-1 type of NADH-Q oxidoreductase.

The complete mitochondrial DNA sequence of liverwort has been determined [129]. A gene arrangement similar to that of the *NQO12, NQO13* and *NQO14* genes of the *Paracoccus* NDH-1 gene cluster is observed in liverwort mitochondrial DNA. In addition, Takahashi et al. [130] have recently demonstrated that in the filamentous cyanobacterium *Plectonema boryanum* the *ndh1*, $frxB_3$, *ndh6* and *ndh4L* genes (homologous to the *Paracoccus NQO8, NQO9, NQO10* and *NQO11* genes, respectively) are cotranscribed. This gene arrangement is also consistent with that of the *Paracoccus* NDH-1 gene cluster.

III-D. Possible essential polypeptides of NDH-1

The gene cluster encoding the *Paracoccus* NDH-1 contains 6 unidentified reading frames (URFs). Fig. 2 illustrates the hydropathy plots of the putative polypeptides encoded by these six URFs. Except for URF5 and URF6 these URFs have no significant homology to any of the sequences in the GenBank/EMBL databases. Nor was any significant relationship found between these URFs and the known amino acid sequences of any of the other proteins of mitochondrial complex I. In terms of URF5 and URF6, however, the predicted URF5 encoded polypeptide has high identity from the 50th residue to the C-terminus to that encoded by URF6 (see Fig. 2B). In addition, this region is also similar to the primary structure of the γ-carboxymuconolactone decarboxylase of *Acinetobacter calcoaceticus* [131–133]. This enzyme is involved in the metabolism of β-ketoadipate and catalyzes the decarboxylation of γ-carboxymuconolactone to produce β-ketoadipate enol-lactone [133]. Both quinone and lactone have been reported to induce expression of FMN-dependent DT-diaphorase in *E. coli* [134,135]. It could be speculated, therefore, that the URF5 and URF6 products might be related to the regulation of the NDH-1 biosynthesis.

Recently, Tzagoloff and coworkers [136,137] reported that the *COX11* and *COX10* products of *S. cerevisiae* have considerable sequence identity with the *Paracoccus* ORF3 (now designated *ctaG*) and ORF1 (now named *ctaB*) products, respectively, located in the cytochrome oxidase operon, and concluded that the *COX11* and *COX10* products are essential for terminal step of biosynthesis of the cytochrome oxidase. However, neither the *COX11* product nor the *COX10* product is a subunit of this enzyme complex. Therefore, the *Paracoccus ctaB* and *ctaG* genes probably encode essential polypeptides for biosynthesis of cytochrome oxidase but not subunits of this enzyme complex. Since a gene located in a gene cluster carrying a particular enzyme complex is believed to code for an essential polypeptide (but not always a subunit) of that enzyme complex, it will be of interest to investigate whether these six URFs are expressed, and if so,

[3] According to the references [31,116,120], chloroplasts appear to bear NDH-1 type enzymes. When the respiratory NDH-1 is compared with the predicted NDH-1 like enzymes of chloroplasts, there are a number of unique stretches of amino acid sequence that are found in the respiratory NDH-1 but not in the chloroplast NDH-1 and vice versa (Ref. 114; T. Yagi, X. Xu and A. Matsuno-Yagi, unpublished results).

what the functions of their products are discovered to be.

IV. Mechanism of action of NDH-1

Albracht and his coworkers [138,139] have proposed that bovine complex I has a dimeric structure in which one protomer contains FMN and FeS clusters 1–4 in equal amounts and the other protomer is identical to the first one except for the absence of cluster N1. NADH is able to react with both protomers. However, NADPH reacts only with the former protomer. This proposal is based on the fact that NADPH acts as the substrate for bovine complex I only at approx. pH 6. However, in this laboratory it has been discovered that NADPH is not oxidized by the *Paracoccus* NDH-1 even at a pH of 6 (T. Yagi, unpublished data). In addition, NADPH has been reported not to act as the substrate for the *Vibrio* Na-transporting NADH-Q oxidoreductase [140]. Therefore, it may be speculated that the bacterial NDH-1 does not have a dimeric structure with a parallel electron transfer mechanism as is proposed for bovine enzyme. On the other hand, since NADPH does act as the substrate for plant mitochondrial complex I, although the K_m and V_{max} values for NADPH differ from those for NADH [75], the same mechanism may apply to both plant and mammalian enzyme complexes. Additional work is required to resolve the monomer/dimer issue.

A semiquinone signal which may belong to NDH-1 has been detected in *T. thermophilus* HB-8 membranes [85]. These data suggest that single electron transfer may be occurring to and from Q in the *Thermus* NDH-1 [85]. In contrast, NDH-2, which lacks single electron carriers, is only able to conduct two electron transfers [141]. Certain quinone analogues such as piericidin A and capsaicin inhibit NDH-1 activity but not NDH-2 activity [10,58,59,142]. One possible explanation is that these inhibitors may be competing for Q radical binding to NDH-1 rather than for Q which binds to both enzymes. Recent studies on the spin relaxation of the rotenone-sensitive ubisemiquinone signal of bovine submitochondrial particles indicated that the signal may interact with one of rapidly relaxing tetranuclear FeS clusters, most probably cluster N2 [143]. These data suggest that the Q radical binding site in the bovine enzyme is located in the vicinity of cluster N2. However, as described above, *Thermus* and *E. coli* NDH-1 appear to lack this high potential FeS cluster. Therefore, whether the same mechanism operates in both the bacterial and mitochondrial NDH-1 remains to be seen. However, it is very clear that NDH-1 and NDH-2 type enzymes operate by different mechanisms.

Several groups [56,144] including Vinogradov's group [145] have proposed that bovine complex I contains two H^+ translocation sites. One site appears to include the FMN cofactor. One of the characteristics of the bacterial NDH-1 that distinguishes it from the NDH-2 is that the NDH-1 complex bears FMN and translocates protons while the NDH-2 does not contain an FMN and does not translocate protons [10]. In addition, the Na-translocating NADH-Q oxidoreductase of *Vibrio* bears an FMN and does not appear to contain FeS clusters (P.R. Rich, personal communication). Since both of these FMN containing NADH-Q oxidoreductases translocate proton, it is conceivable that the FMN is involved in H^+ translocation. The other site of H^+ translocation in bovine complex I is proposed to be located between a high potential FeS cluster and the Q pool [48,56,145]. As described above, *E. coli* and *Thermus* NDH-1 appear to lack a high potential FeS cluster. Furthermore, the Na-transporting NADH-Q oxidoreductase of *Vibrio* probably lacks FeS clusters. It may be speculated, therefore, that these NADH-Q oxidoreductases may contain only a single proton translocating site. The determination of accurate H^+/e values of various bacterial NDH-1 segments will provide useful information for elucidating the mechanism of energy coupling site 1 and how this mechanism differs in various species and types of NADH-Q oxidoreductase.

V. Superfamily of NDH-1

When the amino acid sequences of the *Paracoccus NQO* gene products were used to search the GenBank/EMBL database [4], the only sequences with significant similarities to these gene products, other than complex I and the NDH-1 subunits from other species, were the NAD-linked hydrogenase of *Alcaligenes eutrophus* H16 and the formate hydrogenlyase of *E. coli*.

The NAD-linked hydrogenase, located in the cytoplasm of the bacterium *A. eutrophus* H16, catalyzes the reaction

$$H_2 + NAD^+ \rightleftarrows NADH + H^+$$

This enzyme is composed of four dissimilar subunits of molecular mass 63 kDa, 56 kDa, 30 kDa and 26 kDa (designated, respectively, α, β, γ and δ). It has been suggested by Tran-Betcke et al. [146] that the NAD-linked hydrogenase bears one FMN, one Ni ion, and four tetranuclear, one trinuclear, and one binuclear FeS clusters. According to recent work from the author's laboratory [16,17,19], the α and γ subunits have significant similarity to the *Paracoccus NQO2 + NQO1* and *NQO3* gene products, respectively. In addition, the

[4] The FASTA program of the University of Wisconsin Genetics Computer Group was used [177].

TABLE V

Possible location of Paracoccus NDH-1 prosthetic groups

Subunit	Prosthetic groups
NQO1	FMN
	tetranuclear cluster N3
NQO2	binuclear cluster N1b
NQO3	tetranuclear cluster N4
	binuclear cluster N1a
NQO9	tetranuclear cluster N2

gene arrangement of the NAD-linked hydrogenase α and γ subunit is consistent with that of the *Paracoccus NQO2 + NQO1*, and *NQO3*, respectively (see Fig. 1) [19]. These similarities add support to the proposed evolutionary relationship between the NAD(H) catalytic fractions of the *Paracoccus* NDH-1 and the NAD-linked hydrogenase of *A. eutrophus* [16,28,52].

Formate hydrogenlyase of *E. coli* is one of three enzymes which are involved in hydrogen metabolism [147]. Formate hydrogenlyase is part of the non-energy conserving formate hydrogenlyase pathway and functions unidirectly, releasing H_2 via proton reduction coupled to formate oxidation. Although the formate hydrogenlyase complex has not yet been isolated [148], gene cloning of this enzyme has been performed and its structural genes have been sequenced [149]. The operon encoding formate hydrogenlyase is composed of eight open reading frames. These have been temporarily designated (from the upstream) ORF1–8. The ORF3 product is homologous to the *Paracoccus NQO13* product and has some similarity to the *Paracoccus NQO12* product. The ORF4 gene product has some similarity to the *Paracoccus NQO8* product. The ORF5 and ORF7 products appear to be similar to the *Paracoccus NQO4*, and *NQO6* gene products, respectively. Each of the ORF2 and ORF6 products contain two sequence motifs associated with the tetranuclear FeS clusters found in ferredoxins of bacteria and chloroplasts [108], suggesting that these gene products bind two FeS clusters each.

While NDH-1 catalyzes H^+ translocation across membranes, both NAD-linked hydrogenase and formate hydrogenlyase lack H^+ pumping functions. This may suggest that the *Paracoccus* NDH-1 subunits homologous to polypeptides of the NAD-linked hydrogenase and formate hydrogenlyase might not be directly involved in the H^+ channel in the membrane. If that is the case, the *Paracoccus* subunits lacking homology to the NAD-linked hydrogenase and formate hydrogenlyase must be responsible for the H^+ channel in the NDH-1 complex. In fact, Weiss and coworkers hypothesize that in the mitochondrial system the ND-1 gene product (homologous to the *Paracoccus NQO8* gene product) is involved in H^+ translocation. This hypothesis is based on similarities in both primary structure and piericidin A sensitivity between the ND-1 product and the bacterial glucose dehydrogenase [150]. As described above, the ND-1 product bears DCCD-binding site [5] and rotenone-binding site [64,126]. Although the ND-1 gene product has sequence similarity to the ORF4 gene product of formate hydrogenlyase complex, the similarity is limited to a short hydrophobic stretch [149]. Taken together, it may be conceivable that ND-1 (*Paracoccus NQO8*) product is involved in the proton channel.

VI. NDH-1 as a promising model for the study of mitochondrial diseases

Human mitochondrial DNA is composed of 16.5 kbp and encodes 22 transfer RNAs, two ribosomal RNAs, and 13 polypeptides. These polypeptides are cytochrome *b* of complex III, subunits I, II and III of cytochrome oxidase, subunits A6 and A8 of ATPsynthase, and the ND1, 2, 3, 4, 4L, 5 and 6 gene products of complex I [27,151]. Mitochondrial DNA has a much higher mutation rate than does nuclear DNA [152–154] because not only is the environment rich in free radicals [155], but mitochondria also lack effective DNA repair mechanisms [156] and contain only the γ DNA polymerase which is a relatively inefficient enzyme [157]. However, the significance of single mtDNA mutations is reduced by the protective effect of a very high mtDNA copy number in most mammalian cells (up to several hundred) and of several mtDNA copies per mitochondrion [158,159]. Although this heterogeneity is clearly physiologically beneficial, it complicates biochemical studies of mitochondrial diseases.

The first human mitochondrial disease was discovered over 30 years ago by Luft et al. [160] in a particular hypermetabolic patient. Loose mitochondrial coupling was found to be responsible for the hypermetabolic state of this patient. Since that time the advent of muscle histochemistry and electron microscopy has led to the identification of markers for a number of mitochondrial diseases. Thus, for example histochemistry is used to identify ragged red fibers and electron microscopy is able to locate both abnormal crystal morphology and paracrystalline inclusions. At the present time chronic progressive external ophthalmoplegia (CPEO) and its variant, the multisystem Kearns-Sayre syndrome (CPEO, heart block, retinitis pigmentosa and variable degeneration of the central nervous system) [161], the myoclonic epilepsy ragged red fiber syndrome (MERRF, a common form of progressive

[5] Hassinen's group [178–180] and this laboratory [58] suggest that DCCD might affect the proton channel of the energy coupling site 1.

myoclonic epilepsy) [162] and mitochondrial encephalopathy lactic acidosis stroke-like episode syndrome (MELAS) [163] are all believed to be related to mitochondrial dysfunction. Morgan-Hughes' group [164] has reported that patients with CPEO or the Kearns-Sayre syndrome have mitochondria with large mtDNA deletions coexisting with a population of normal mtDNA. As described above, this heteroplasmy makes it difficult to conduct biochemical investigations and to find etiological causes of mitochondrial diseases. However, bacterial systems generally have no heteroplasmy in terms of their DNA. Therefore, the *Paracoccus* NDH-1 which contains homologues of all seven human ND genes, appears to be a useful model system for conducting biochemical studies of mitochondrial diseases resulting from deletions of ND genes.

In addition to the diagnostic methods listed above, since mitochondrial DNA is exclusively inherited through the maternal line, recognition of a maternal inheritance pattern in some disorders is another indication that a disorder may result from a mitochondrial DNA mutation [157,165]. An example is Leber's hereditary optic neuropathy (LHON) which is a maternally inherited disease associated with the late onset of bilateral loss of central vision and cardiac dysrhythmias [166]. Wallace's group [167] found that in most Leber's disease patients a single nucleotide mutation at position 11778 of the mtDNA is present. This mutation converts the 340th amino acid of the ND4 gene product from an arginine to a histidine. As shown in Table VI, this arginine is conserved in the ND4 homologue of *Paracoccus* NDH-1. In some Leber's disease patients mtDNA mutations have also been detected in the ND1 (positions 3460, 4136, 4160 and 4216), ND2 (positions 4767, 4917 and 5244), and ND5 (position 13708) subunits. These 8 mutations result in single amino acid substitutions within the ND1 (residues 52, 277, 285 and 304), ND2 (residues 100, 150 and 259), and ND5 (residue 458) products (Table VI). Of the eight wild type residues, four are conserved in the ND homologues of the *Paracoccus* NDH-1. In addition, the fact that the *Paracoccus NQO14* product bears a valine rather than a methionine at the position corresponding to the residue 100 of ND2 product (this is identical to the amino acid placed in that position by the mutated gene), suggests that the position 4767 mutation might be irrelevant to the etiological cause of LHON. Although preliminary studies of electron transport activities of mitochondria from LHON patients suggest that complex I activities and/or activities of the enzymes related to complex I might be decreased in LHON patient mitochondria [168,169], more detailed biochemical studies are required to clarify the effect of the ND gene mutations on the structure and function of complex I. However, the limited amount of material available from patient tissues coupled with the mtDNA heteroplasmy observed in these tissues presents a serious impediment to biochemical research in neuromuscular mitochondrial diseases. The use of bacterial model systems would alleviate this difficulty since these materials are abundant. The *Paracoccus* NDH-1 seems to be a promising model system for the investigation of mitochondrial diseases resulting not only from the deletion of mtDNA but also from point mutations within the mtDNA.

In addition to the human mitochondrial diseases described above, Parkinson's and Huntington's diseases also appear to be related to structural and functional defects in complex I [170,171]. Therefore, studies on NDH-1 together with complex I will provide useful information for clinical medicine.

VII. Conclusions and perspective

The mammalian complex I is composed of at least 42 dissimilar polypeptides. Study of the gene cluster carrying the *Paracoccus* NDH-1 suggests that the *Paracoccus* NDH-1 is composed of between 14 and 20 subunits. The NDH-1 isolated from *Thermus thermophilus* HB-8 membranes is composed of approx. 10

TABLE VI

Comparison of the mutated amino acid residues of ND gene products in Leber's disease with those of their Paracoccus homologues

Gene	Position		Amino acid Wild → LHON		Reference	*Paracoccus*	
	DNA	amino acid				Gene	amino acid
ND1	3460	52	A	T	[193]	NQO8	A
	4136	277	Y	C	[194]		V
	4160	285	L	P	[194]		L
	4216	304	Y	H	[195]		V
ND2	4767	100	M	V	[195]	NQO14	V
	4917	150	N	D	[195]		W
	5244	259	G	S	[196,197]		G
ND4	11778	340	R	H	[167,197] [a]	NQO13	R
ND5	13708	458	A	T	[195,196]	NQO12	A

[a] See also Refs. 195,198–208.

dissimilar polypeptides. In the case of *Synechocystis* NDH-1, although the biochemical data are not yet available, it is postulated on the basis of gene sequence homology to the mitochondrial complex I polypeptides to have a subunit composition similar to the *Paracoccus* NDH-1. Together these data indicate, as has been anticipated, that the bacterial NDH-1 has a simpler structure than the mitochondrial complex I.

The relative simplicity of the bacterial NDH-1 coupled with the ease of working with bacterial systems allows many options not available when studying the mitochondrial system. For example, recently, replacement of subunits of complexes III and cytochrome oxidase has been successfully conducted at the genetic level in the *Paracoccus* system [95,96,103,172–174]. Similar gene replacement methods applied to the NDH-1 systems should enhance the investigation of not only the 14 subunits but also 6 URFs products. In addition, recently, Haltia et al. [175] have succeeded in producing a site-directed mutant of subunit III of the *Paracoccus* cytochrome oxidase. This technology should also be useful for the study of both the structure and the mechanism of action of NDH-1 and complex I. Finally, it is known that the *Paracoccus* membranes grown under sulfate-limited conditions or in the presence of rotenone lack coupling site 1, rotenone sensitivity, and the high potential cluster N2 [86,176]. The above technologies will be useful in determining which process of NDH-1 biosynthesis is regulated by these growth conditions.

Although the number of investigators concentrating on NDH-1 and complex I is steadily increasing, the number is too small to even begin addressing all the interesting issues unique to these enzyme complexes. It is the author's goal in this review to stimulate others to participate in the investigation of this enzyme complex which is not only complicated but important and 'beautiful' [6] as well.

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[6] Weiss referred to complex I (or NDH-1) as Sleeping Beauty.

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