Current Topics

The Proton-Translocating NADH−Quinone Oxidoreductase in the Respiratory Chain: The Secret Unlocked†

Takao Yagi* and Akemi Matsuno-Yagi

Department of Molecular and Experimental Medicine, MEM-256, The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, California 92037

Received November 12, 2002; Revised Manuscript Received January 6, 2003

The respiratory chain of mammalian mitochondria is composed of four enzyme complexes as depicted in Figure 1. These complexes, which are all located in the mammalian inner-mitochondrial membrane, are referred to as the proton-translocating NADH−quinone oxidoreductase (complex I),[1] the succinate−quinone oxidoreductase (complex II), the bc_1 complex (complex III), and cyt *c* oxidase (complex IV). Complex I is composed of at least 46 different subunits (*1*) with a total molecular mass of approximately 1 MDa. This enzyme complex, which spans the inner-mitochondrial membrane, bears one noncovalently bound FMN and eight iron−sulfur clusters (*2*). It translocates protons from the matrix side of the membrane to the cytoplasmic side, generating a proton gradient across the inner-mitochondrial membrane. Because of its ability to pump protons in concert with oxidation of a substrate, complex I constitutes the energy coupling site 1 of the oxidative phosphorylation system. In addition to being present in mammalian mitochondria, complex I is also present in plant and fungal mitochondria (*3*).

In contrast to mammalian, plant, and fungal mitochondria, the respiratory chain of certain bacteria harbors a proton-translocating NADH−Q oxidoreductase which, although quite similar to complex I in terms of electron carriers, inhibitor specificity, and amino acid sequence of subunits, is much simpler in structure. This bacterial equivalent of complex I has been referred to as NDH-1 or bacterial complex I (*4*−*7*). The bacterial NDH-1 is composed of only 14 dissimilar subunits, all of which have homologues in mammalian complex I (Table 1) (*4*, *5*). Because the subunit composition of NDH-1 is relatively simple compared to that of its mitochondrial counterpart, the NDH-1 is a useful model system for studying the structure and function of complex I. In this article, the current status of knowledge regarding complex I/NDH-1 is presented along with issues and questions still unanswered with respect to this enzyme complex. To avoid confusion of terminology, subunit names are given for the *Paracoccus* NDH-1. However, the homologous subunit names for the bovine complex I are presented in parallel.

Structures. Of the four respiratory chain enzyme complexes, X-ray structures have been published for the succinate dehydrogenase,[2] bc_1 complex, and cyt *c* oxidase. In contrast, the only available structural information on complex I/NDH-1 is based on EM analyses. Figure 2 illustrates the three-dimensional models of complex I/NDH-1 as they have developed over time. In 1987, on the basis of EM analysis of two-dimensional crystals, Leonard et al. (*8*) proposed that complex I isolated from *Neurospora crassa* mitochondria has a structure similar to that of a stone age ax. Later, the same group reported that the *N. crassa* complex I has an L-shaped structure (*9*). The L-shaped model contains two major segments, the peripheral domain and the membrane domain. Whereas most of the membrane domain is embedded in the lipid bilayer of the inner-mitochondrial membrane (or the bacterial cytoplasmic membrane), the peripheral segment protrudes into the matrix side of the inner-mitochondrial membrane (or the cytoplasmic phase in bacteria). The same group also reported a modified version of this *N. crassa* enzyme which has a longer peripheral segment (*10*). In addition, they claimed that the *Escherichia coli* NDH-1 has a structure similar to that of *N. crassa* but smaller in size (*10*). Similarly, Grigorieff (*11*) advocated the L-shaped structure for bovine heart complex I except that the peripheral segment of the bovine complex has a ball-like structure. One additional difference noted for the bovine complex is that the junction between the peripheral domain and the membrane domain is much thinner than in other models. The same L-shaped model was proposed for the yeast (*Yarrowia lipolytica*) complex I as determined by single molecule EM analyses (*12*). However, Friedrich's group recently claimed

† This work was supported by U.S. Public Health Science Grant R01GM33712. Synthetic oligonucleotides and DNA sequencings were, in part, supported by the Sam & Rose Stein Endowment Fund. This is Publication 15372-MEM from The Scripps Research Institute, La Jolla, CA.

* To whom correspondence should be addressed. Tel: (858) 784-8094. Fax: (858) 784-2054. E-mail: yagi@scripps.edu.

[1] Abbreviations: complex I, mitochondrial proton-translocating NADH−quinone oxidoreductase; cyt *c*, cytochrome *c*; DB, 6-*n*-decylubiquinone; DCCD, *N*,*N*′-dicyclohexylcarbodiimide; E_m, midpoint redox potential; EM, electron microscope; EPR, electron paramagnetic resonance; MQ, menaquinone; ND, mitochondrial DNA-encoded subunits of complex I; NDH-1, bacterial proton-translocating NADH−quinone oxidoreductase; PQ, plastoquinone; Q, quinone; RQ, rhodoquinone; SMP, submitochondrial particles; SUQ, semiubiquinone; UQ, ubiquinone; UQ2, 6-geranylubiquinone.

[2] To date the 3D structure of succinate dehydrogenase is not available. However, high-resolution 3D structures of bacterial fumarate reductases, which are believed to be counterparts of succinate dehydrogenase, have been solved.

10.1021/bi027158b CCC: $25.00
Published on Web 01/28/2003

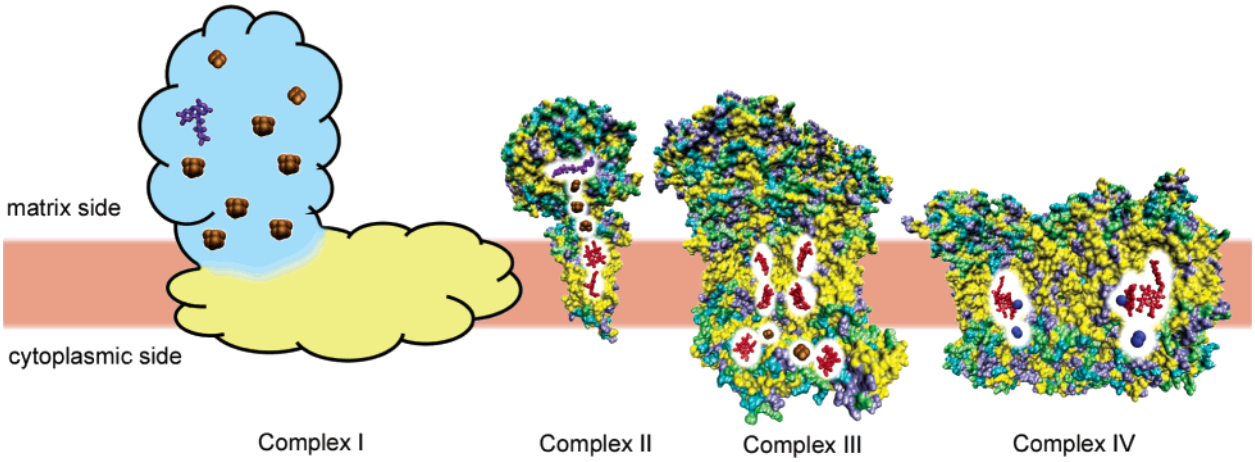

FIGURE 1: Schematic representation of the respiratory enzyme complexes in mammalian mitochondria. The 3D structures of complexes II, III, and IV were obtained from the PDB database. The coordinates used are as follows: complex II, 1QLB, as represented by fumarate reductase of *Wolinella succinogenes*; complex III (bc_1 complex), 1BCC, 1BE3, and 1QCR; complex IV (cyt *c* oxidase), 2OCC. Complexes III and IV are drawn as dimers. The molecular surface was constructed using the Surf option of the VMD program (*91*). Yellow, purple, cyan, and lime balls indicate nonpolar, polar, acidic, and basic amino acid residues, respectively. Cofactors, heme, copper, flavin, and iron−sulfur clusters are drawn in red, blue, purple, and brown, respectively.

Table 1: Subunits and Cofactors of Complex I/NDH-1

bovine complex I	*Paracoccus* and *Thermus* NDH-1	*E. coli* and *Rhodobacter* NDH-1	cofactors	Fe-S center	E_m (mV)
51K	Nqo1	NuoF	FMN		−340
			[4Fe-4S]	N3	−250
24K	Nqo2	NuoE	[2Fe-2S]	N1a	−370
75K	Nqo3	NuoG	[2Fe-2S]	N1b	−250
			[4Fe-4S]	N4	−250
			[4Fe-4S]	N5	−250
			[4Fe-4S]	N1c[b]	?
49K	Nqo4	NuoD[a]			N/A
30K	Nqo5	NuoC[a]			N/A
PSST	Nqo6	NuoB	[4Fe-4S]	N2	−50 to −150
TYKY	Nqo9	NuoI	2[4Fe-4S]	N6	?
ND1	Nqo8	NuoH			N/A
ND2	Nqo14	NuoN			N/A
ND3	Nqo7	NuoA			N/A
ND4	Nqo13	NuoM			N/A
ND4L	Nqo11	NuoK			N/A
ND5	Nqo12	NuoL			N/A
ND6	Nqo10	NuoJ			N/A
>32 other subunits	none	none	phospho-pantetheine, NADPH		N/A

[a] In some species of bacteria, NuoC and NuoD are fused. [b] This [4Fe-4S] cluster is only present in some species of bacteria (e.g., *T. thermophilus*, *E. coli*).

that the active form of *E. coli* NDH-1 assumes a horseshoe-like structure and that the L-shaped structure is actually an inactive form (*13*). More recently, Sazanov's group reported data contradicting that conclusion and showing that active *E. coli* NDH-1 remains L-shaped (*14*). One possibility, although remote, is that these two apparently distinct structures represent two dynamically different conformational states of the enzyme during catalysis. Indeed, there are data pointing to a significant conformational change in the presence of NADH. To date, no horseshoe-like structure has been observed for mitochondrial complex I.

Although the overall L-shaped (two-domain) structure of complex I/NDH-1, as derived from EM data, agrees with earlier observations derived from biochemical work, such studies, which have included subunit fractionation, extraction of subunits, and cross-linking experiments, have provided additional information to that obtained by EM. A summary of the biochemical data is provided in Table 2. Briefly, these data indicate that for bacterial systems the peripheral and membrane domains are each made up of seven subunits (*15*−*17*). In addition, the subunit stoichiometry of the peripheral segment (Nqo1−6 and 9) of the *Paracoccus* NDH-1 was immunochemically determined to be 1 mol of each subunit/mol of enzyme complex (*15*, *18*). The only information available for the stoichiometry of the membrane domain, which was obtained by human mitochondrial complex I [^{35}S]-methionine incorporation studies, indicated that the stoichiometry is 2:8:2:2:1:2:2 for subunits ND1−ND4, ND4L, ND5, and ND6 (see Table 2 for bacterial equivalents) (*19*). In terms of determining nearest neighbor relationships, cross-linking studies have revealed that among the peripheral subunits, Nqo6/PSST and Nqo9/TYKY are likely to be located near the membrane and serve as connectors between the two domains (*18*, *20*). In fact, this interpretation is supported both by Di Bernardo and Yagi's data (*21*), demonstrating that the Nqo6/PSST subunit directly interacts with the membrane domain subunit Nqo7/ND3, and by the discovery that, in a mutant of *E. coli* lacking Nqo7/ND3, the peripheral subunits are no longer associated with the membrane (Di Bernardo et al., unpublished result). Other data supporting the location and role of the peripheral domain subunits include cross-linking experiments completed by Hatefi's and Ragan's groups showing subunit−subunit interactions between the 51K, 24K, and 75K subunits and between the 30K and 49K subunits (Table 2) (*22*, *23*). The fact that Nqo4/49K and Nqo5/30K genes are fused in certain bacteria supports the cross-linking results obtained for those subunits using bovine complex I (*5*). Furthermore, the proximity and relationship between the 51K, 24K, and 75K subunits are supported by the following facts:

The Nqo1/51K, Nqo2/24K, and Nqo3/75K subunits are assembled in homologous expression in *E. coli* (*24*).

The Nqo1/51K and Nqo2/24K subunits are associated in heterologous expression experiments and together exhibit NADH dehydrogenase activity (*25*).

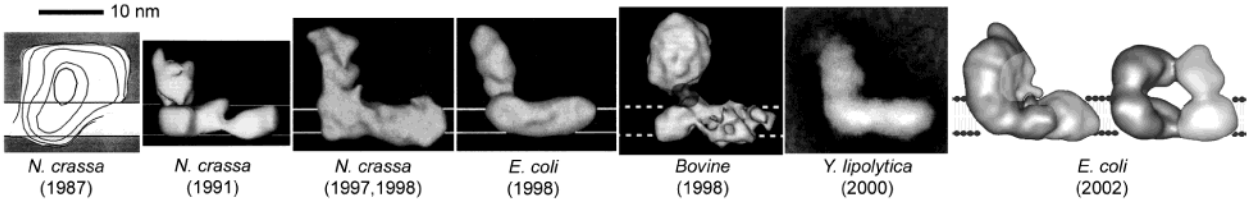

FIGURE 2: Comparison of 3D models or images of complex I/NDH-1 as revealed by EM analyses. From left to right: *N. crassa* in 1987 (*8*); *N. crassa* in 1991 (*9*); *N. crassa* in 1997 (*92*); *E. coli* in 1998 (*10*); bovine in 1998 (*11*); yeast (*Y. lipolytica*) in 2000 (*12*); *E. coli* inactive form and active form in 2002 (*13*).

Table 2: Evidence in Support of 3D Structure of Complex I/NDH-1

structural features	EM data	biochemical data	ref
shape[a]	L-shaped[b]	N/A	*9*−*14*
domains[a]	peripheral domain	subunits Nqo1−6 and 9 (51K, 24K, 75K, 49K, 30K, PSST, and TYKY)	*15*−*17*
		stoichiometry is 1 mol of subunit/mol of complex	*15*, *18*
	membrane domain	subunits Nqo7, 8, 10−14 (ND1−6 and 4L)	*15*−*17*
		Iβ fraction: ND4 (Nqo13), ND5 (Nqo12), and 11 other subunits	*26*, *27*
		Iγ fraction: ND1 (Nqo8), ND2 (Nqo14), ND3 (Nqo7), ND4 (Nqo11), and 1 other subunit	*26*, *27*
		stoichiometry[c] is 2:8:2:2:1:2:2 for ND1:ND2:ND3:ND4:ND4L:ND5:ND6 (Nqo8:Nqo14:Nqo7:Nqo13:Nqo11:Nqo12:Nqo10)	*19*
subunit locations			
peripheral domain	N/A	Nqo6 and Nqo9 (PSST and TYKY) located near membrane; may connect peripheral and membrane domains	*18*, *20*
membrane domain	N/A	Nqo7 (ND3) interacts with Nqo6 (PSST)	*21*
subunit relationships			
peripheral[d] domain	N/A	51K and 24K (Nqo1 and Nqo2)	*22*, *23*
		75K and 51K (Nqo3 and Nqo1)	*22*, *23*
		30K and 49K[e] (Nqo5 and Nqo4)	*5*

[a] Based on *N. crassa* complex I. Also appears to be true for *E. coli* NDH-1, bovine heart complex I, and yeast complex I. [b] Also reported (*13*) that the active form may have a horseshoe-like structure with the L-shaped form being inactive. [c] Based on incorporation of [^{35}S]methionine into human mitochondrial complex I by Attardi's group (*19*). [d] Based on cross-linking experiments with bovine complex I. [e] Fused in some species of bacteria (*5*).

In an Nqo3/75K deletion mutant the Nqo2/24K subunit is not associated with the membranes or is absent in subcomplex I (Nakamaru-Ogiso et al., unpublished results).

Unfortunately, although a great deal of information is known regarding the peripheral domain of this enzyme complex, information on the membrane domain is particularly limited. Sazanov's group, however, has succeeded in resolving the membrane domain of bovine complex I into two subcomplexes, designated Iβ and Iγ (*26*, *27*). The Iβ fragment contains subunits ND4/Nqo13 and ND5/Nqo12 along with 11 accessory subunits. The Iγ fragment is composed of ND1/Nqo8, ND2/Nqo14, ND3/Nqo7, and ND4L/Nqo11 along with 1 accessory subunit. Attardi's group reported that null mutants lacking the ND4/Nqo13 and the ND5/Nqo12 subunits have NADH−$K_3Fe(CN)_6$ reductase activity but lack NADH−UQ reductase activity (*28*, *29*), suggesting that these subunits may be specifically involved in the reduction of ubiquinone (Q). In addition, a null mutant lacking the ND6/Nqo10 subunit lost both activities (*30*). These results may suggest that the ND6/Nqo10 subunit might be located near the peripheral segment. Although many questions remain unanswered with regard to the structure of the complex I/NDH-1 enzyme complex, when the available data for both peripheral and membrane segments are taken together, a model for the architecture of complex I/NDH-1 can be proposed as illustrated in Figure 3.

Substrates and Cofactors. The oxidoreductase activity of complex I/NDH-1 requires the presence of a number of cofactors and iron−sulfur clusters in addition to the numerous polypeptides discussed above. A full list of required cofactors

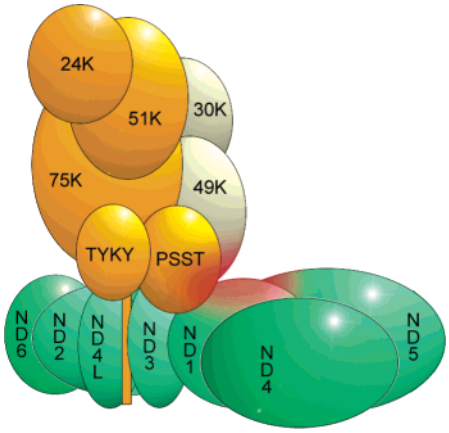

FIGURE 3: Speculative model of the subunit arrangement of NDH-1 based on available data. Mammalian complex I terminology is used. Peripheral subunits housing cofactors are shown in brown. Hydrophobic subunits are in green. The proposed "common" inhibitor-binding pocket area is shown in red. It should be noted that this subunit organization is tentative.

and their locations is provided in Table 1. The complex 1/NDH-1 also has two known substrates, NADH and Q. In terms of active sites for these substrates, the 51K/Nqo1 subunit has been identified as the NADH-binding subunit by direct photoaffinity labeling with [^{32}P]NADH (*31*, *32*) and photoaffinity NAD analogues. Unexpectedly, certain photoaffinity analogues of NADH also label the 24K/Nqo2 and 9K subunits, although to a lesser extent than the 51K subunit, suggesting that the latter two subunits may also contribute to the NADH binding. The second substrate, Q, is believed to play two roles in complex I/NDH-1 activity.

One role, played by endogenous Q, is as a cofactor of complex I/NDH-1. The other role, played by exogenous Q, is as a substrate for complex I/NDH-1. At the present time, little is known about the location or number of Q molecules involved in cofactor activity for complex I/NDH-1. However, data showing not only that EPR signals of the semiquinone (SUQ) are diminished by rotenone and piericidin A but also that two other SUQ species related to complex I are present in bovine SMP suggest that complex I may contain at least three endogenous Q molecules. Clues as to the location of the Q are available. For example, it has been reported that SUQ, spin coupled to iron−sulfur center N2, was found associated with coupling site 1 in oligomycin-treated bovine heart SMP under steady-state NADH oxidation (*33*). Because of this strong magnetic interaction between the N2 center and the SUQ, the SUQ is believed to be located in the proximity (8−11 Å) of this N2 center (*33*). Another approach to locating the Q-binding site involved the use of bioinformatics (*34*). On the basis of the known 3D structures of enzyme complexes bearing Q, the following sequence motifs of Q-binding sites were deduced: Lx_3Hx_2T or $(A/L)x_3Hx_2L$. These triad motifs are found to be present in complex I subunits ND4/Nqo13 and ND5/Nqo12. Although these motifs are not perfectly conserved among homologues in various organisms, these locations seem to provide a valuable working hypothesis. Yet another strategy which has been utilized to study substrate Q and its binding sites has been to examine structure−activity relationships of various Q analogues (*35*, *36*). As part of this study a wide variety of UQ2 and DB analogues were synthesized with the methoxy groups at the 2- and/or 3-positions of the quinone ring replaced by other bulky alkoxy groups such as ethoxy and butoxy moieties (*37*). These bulky quinones were then investigated with regard to the electron transfer activity they would support in bovine complex I and *Paracoccus* NDH-1. The data revealed that the UQ reduction site of bovine complex I is spacious enough to accommodate bulky exogenous forms of UQ. In contrast, bulky Q analogues are poor electron acceptors in the *Paracoccus* NDH-1, indicating that the NDH-1 Q-binding site is more specific than the bovine. These data are not surprising in light of a recent report showing that Q-binding sites exhibit significant organism specificity (*38*). Further studies comparing the UQ2 and DB analogues revealed that the substituent effects in the 2- and 3-positions of the quinone ring on the electron transfer activity with bovine complex I differed significantly between the UQ2 and DB series despite having the same total number of carbon atoms in the side chain. Finally, the inhibitory effect generally observed with UQ2 and attributed to its side chain was markedly diminished by structural modifications to the quinone ring moiety. Together, these results indicate that the side chain of Q plays a role in the redox reaction and that the Q ring and side-chain moieties contribute interdependently to the binding interaction between the enzyme complex and this substrate. Recently, it has been reported that Q-binding sites exhibit significant organism specificity (*38*). On the basis of the differences of properties, a promising drug, nafuredin, against a parasite (helminth) was discovered (*38*).

In addition to substrate-binding sites, complex I/NDH-1 is believed to harbor one noncovalently bound FMN and six to seven tetranuclear and two binuclear iron−sulfur clusters (*4*). Although the precise location of the FMN has not been identified, it seems likely that since FMN is believed to act as the primary oxidant of NADH, it will be located near the NADH-binding site. This hypothesis is supported by the fact that a subcomplex containing the *Paracoccus* Nqo1/51K plus Nqo2/24K subunits exhibits NADH dehydrogenase activity when reconstituted with FMN (*2*, *25*). In terms of iron−sulfur complexes, a typical complex I/NDH-1 contains eight iron−sulfur centers, which makes this enzyme complex the most elaborate iron−sulfur cluster protein known. These clusters are categorized as binuclear, [2Fe-2S], or tetranuclear, [4Fe-4S]. The [2Fe-2S] clusters are identified as centers N1a and N1b. The [4Fe-4S] clusters are centers N2, N3, N4, N5, and N6 (*2*). Certain bacterial NDH-1 enzymes contain an additional [4Fe-4S] cluster, center N1c, and are also anticipated to harbor a N4 center made up with 2[4Fe-4S] clusters (*39*, *40*). Centers N1a, N1b, N2, N3, and N4 are all present at a 1:1 ratio with respect to FMN. The concentration of center N5, however, has been reported as approximately one-fourth that of FMN (*41*). Nevertheless, the fact that N5 partially displays a $^3/_2$ spin makes this stoichiometry unlikely, suggesting instead that N5 is also present at 1 mol/mol of FMN (T. Yano et al., unpublished).

A number of studies designed to identify the locations of the [Fe-S] clusters have been conducted. Many of these studies have involved resolving the entire complex into various, smaller, pieces and identifying the cluster(s) remaining with each piece. In one such study the EPR signals of center N6 were observed both in a resolved subcomplex containing the Nqo9/TYKY subunit and in systems expressing this subunit (*20*, *42*). Similarly, the signals for N1c were found in expressed Nqo3/75k (*43*). Unfortunately, neither identification has been confirmed in situ.

In terms of the order of reduction of these cofactors, comparison of E_m values of individual iron−sulfur clusters suggests that N1a (−370 mV) is lower than FMN (−340 mV) while N1b, N3, N4, and N5 exhibit slightly higher, but similar values (−250 mV) and that N2 has the highest values (approximately −50 to −150 mV) (*44*). Of these centers, the E_m values of centers N1a and N2 have been reported to be dependent on pH (*44*). Recent voltammetric studies of center N1a showed that this pH dependency is correlated with ionic strength of the medium and disappeared in the milieu at high ionic strength (*45*). Therefore, it is anticipated that center N2 is the final center reduced and that it might be interacting with Q. Additional insight into the electron transfer between iron−sulfur clusters comes from genetic manipulation studies involving the construction of iron−sulfur cluster knockout mutants. Dupuis' group investigated the properties of center N6 using *Rhodobacter capsulatus* NDH-1 mutants (C74S and C67S) of subunit Nqo9/TYKY (*6*). Membranes from the C74S mutant were found to lack peripheral subunits Nqo1, 2, 4, and 5 (51K, 24K, 30K, and 49K). These investigators claimed that center N6 may play a critical role in the assembly process between the peripheral and membrane domains of NDH-1. In contrast, however, the C67S mutant not only displayed respiratory activity up to 43% of the wild type but, when characterized by EPR, showed evidence of N1, N2, N3, and N4 signals. In an independent study aimed at identifying the amino acid residues coordinating N2 in the Nqo6 subunit, Friedrich's group constructed mutants (C64A and C129A) of the Nqo6/

PSST subunit (*7*). Both of these mutants lost their N2 signals, suggesting that C69 and C129 participate in ligation of N2. However, Brandt's group reported that Nqo4/49K subunit mutants also lost their N2 signals (*46*). Furthermore, Videira's group reported that PSST/Nqo6 subunit mutants, modified at C101S and C102A, also lacked N2 signals (*47*). Similar characteristics were reported for N2 signals of bovine complex I (*48*). Together, these data suggest that N2 signals are extremely sensitive to their environment.

Inhibitors. Over the years the study of the mechanism of action and the structure−function relationships in complex I/NDH-1 has been aided considerably by the use of inhibitors of this enzyme complex. A variety of naturally occurring and synthetic chemicals are known which inhibit the enzyme activity of complex I/NDH-1 (*49*). These inhibitors are structurally diverse. Except for a few chemicals, including Rhein and diphenyleneiodium, that inhibit the complex at the level of NADH oxidation, most inhibitors of complex I/NDH-1 act at the level of electron transfer downstream of center N2. Because of the connection between enzyme active sites and inhibitor-binding sites, several attempts have been made to identify the subunits housing the inhibitor-binding site(s). For example, piericidin A and rotenone have been reported to inhibit complex I activity by binding at two sites in bovine complex I (*50*). The later studies involved the use of two rotenone-derived photoaffinity probes and isolated bovine complex I. The data revealed that the rotenone-binding site is housed in the ND1/Nqo8 subunit (*51*). Unfortunately, displacement experiments with other inhibitors were not performed. In similar experiments using a photoaffinity analogue of pyridaben, subunits ND1/Nqo8 and PSST/Nqo6 of bovine heart SMP were labeled in the presence of NADH (*52*). The labeling of the PSST subunit, but not the ND1 subunit, was correlated with inhibition of enzymatic activity and could be displaced by other inhibitors. In addition, this photoaffinity chemical specifically modified the PSST/Nqo6 subunit not only of complex I but of *Paracoccus* and *Thermus* membranes as well. These data indicate that the PSST/Nqo6 subunit is involved in pyridaben inhibition. In other recent studies it was shown that a photoaffinity analogue of fenpyroximate, which seems to inhibit complex I at approximately 1 mol/mol of complex I, labeled the ND5/Nqo12 subunit of bovine heart SMP (*53*). This labeling was stimulated by the presence of NADH and NADPH and was prevented by other inhibitors including rotenone and piericidin A. These results suggest the possibility of an inhibitor-binding pocket within the enzyme complex which may include the ND5, as well as a number of other subunits.

Another approach to studying structure/function relationships through complex I/NDH-1 inhibition is to construct inhibitor-insensitive mutants. For example, site-specific mutants of the Nqo4/49k subunit of *R. capsulatus* NDH-1 (V407M and D412E) exhibited significant resistance to piericidin A and rotenone but did not affect rolliniastatin-2 sensitivity (*54*). These data suggest that the segment of the Nqo4 subunit containing V407 and D412 may be involved in the proposed inhibitor-binding pocket. These data, together with other studies, indicate that subunits Nqo8/ND1, Nqo12/ND5, Nqo6/PSST, and Nqo4/49k may all form part of this inhibitor-binding pocket(s) (see area marked in red in Figure 3). The concept of a common inhibitor-binding region in this enzyme complex, however, is only speculative at this point since the possibility that these inhibitors prevent binding of other inhibitors by inducing a conformational change in NDH-1/complex I, rather than by occupying the same site, cannot be excluded. Clarification of the mechanism of action and the binding regions for these inhibitors requires the accumulation of more data using a variety of approaches.

Table 3: Properties of Accessory Subunits As Determined by Null Mutants of *N. crassa* Complex I

N. crassa subunit	bovine homologue	subunit location	effect on complex I assembly
40	39K	peripheral	no
29.9	B13	peripheral	yes
21.3a		peripheral	no
21.3b	B14.7	membrane	yes
21	AQDQ	peripheral	no
20.9		membrane	yes
20.8	PGIV	membrane	yes
19.3	PEST	peripheral	yes
12.3	PDSW	membrane	yes
ACP	SDAP	peripheral	yes
9.8	MWFE	membrane	unknown

Accessory Subunits. As described above, mitochondrial complex I has at least 32 more subunits than its bacterial counterpart. The fact that the bacterial NDH-1 performs the same function as complex I but with far fewer subunits raises the questions of what the extra complex I subunits are doing. These "extra" subunits were initially called "accessory" or "supernumerary". Their possible roles are discussed here.

One of the techniques that has proven the most useful in the study of these accessory subunits is gene manipulation. Using this technique, null mutants of several *N. crassa* accessory subunits have been constructed, and their effects on complex I were investigated (see Table 3) (*55, 56*). The results indicated that subunits 29.9/B13, 21.3b/B14.7, 20.9, 20.8/PGIV, 12.3/PDSW, and ACP/SDAP are all required for the assembly of complex I.

Surprisingly, the ACP/SDAP subunit described above as being necessary for assembly appears to play a dual role. Both mitochondria and bacteria contain an acyl-carrier protein (ACP) which requires phosphopantetheine as a cofactor. In the case of *N. crassa*, the mitochondrial ACP is a subunit of complex I and is located in the peripheral segment (*57*). In fact, it has been shown that *N. crassa* mitochondrial ACP-deletion mutants exhibit no complex I, while the bc_1 complex and cyt *c* oxidase were intact in this mutant (*58*). However, the mitochondrial ACP is also known as an enzyme which can mediate fatty acid synthesis, independent of the fatty acid synthase complex in the cytoplasm (*59*).

Additional studies conducted independently from those above have also suggested a possible role for the 39K accessory subunit in bovine complex I (40K in *N. crassa*). There have been a number of observations that point to the presence of NADH/NADPH-binding sites other than the substrate NADH-binding site. For example, complex I isolated from *N. crassa* contains tightly bound NADPH. Furthermore, experiments with null mutants suggest that this tightly bound NADPH of complex I is present in the *N. crassa* 40K subunit (*60*). Additional evidence in support of this role for the 39K (40K) subunit comes from work showing that the 39K subunit of bovine complex I can be

labeled by [^{32}P]NADPH (*32*). The NADPH-binding activity of this subunit may be involved in the speculated role of this subunit in intramitochondrial fatty acid synthesis. Furthermore, NADH and NADPH stimulated the labeling of bovine complex I by a photoaffinity analogue of fenpyroximate (*53*).

It has been reported that complex I exhibits two kinetically and structurally distinct forms (*61*). The two forms, one active and the other inactive, are interconvertible. Since this feature is not observed in bacterial NDH-1, it is anticipated that one or more of these accessory subunits might be involved in this activity. The 15K subunit of complex I is specifically labeled by the fluorescent SH modifier only when the complex is in its inactive form. Therefore, it is speculated that the 15K subunit may participate in the active/inactive transition of complex I (*62*).

Although the role played by the 18K subunit in complex I activity is far from clear, evidence does indicate that this role requires that the subunit be phosphorylated. Data supporting this understanding include a report that the 18K subunit (encoded by the *NDUFS4* gene) of mammalian complex I was phosphorylated by cAMP (*63*). In addition, phosphorylation of this subunit was observed in fibroblasts as well as in isolated mitochondria in the presence of cytosol, mitochondrial matrix fraction, and a cAMP-dependent protein kinase. Furthermore, it is speculated that Ca^{2+}-inhibited phosphatase in mitochondria acts to dephosphorylate the 18K phospho subunit. In fibroblast and myoblast, this phosphorylation is associated with potent stimulation of complex I respiratory activity of other NAD-involved substrates. Other evidence supporting both the critical role of the 18K subunit in complex I activity and the necessity that it be phosphorylated came from two patients having complex I defects and fatal neurological syndrome. Both patients exhibited mutations of the *NDUFS4* gene which codes for the 18K subunit. One showed destruction of the phosphorylation site of the 18K subunit. The other is a null mutant of this subunit.

The MWFE subunit is encoded by the *NDUFA1* gene and is a short protein composed of 70 amino acid residues (*64*). This subunit is imported into mitochondria and assembled into complex I without requiring proteolytic processing. A variety of mutants of this subunit in Chinese hamster cell lines have been characterized (*65*). Two of these mutations, a conservative substitution, R50K, and a short C-terminal deletion both make complex I completely inactive. In fact, in the absence of MWFE, complex I was not even detectable by techniques such as blue native gel electrophoresis. On the basis of these data it can be concluded that the MWFE subunit is essential for functional activity in mammalian complex I.

Although all of the necessary data for assigning roles to these accessory subunits has not yet been collected and, at this point, there are as many questions as answers, it is apparent that these "accessory subunits" cannot be ignored in understanding the functionality of complex I. Furthermore, a recent report by Hirst's group claiming that the GRIM-19 protein (the product of a cell death regulatory gene induced by interferon-β and retinoic acid) is also a subunit of complex I (*66*) suggests that the actual number and identity of all of the complex I accessory subunits are not yet known.

Energy Coupling. Complex I/NDH-1 is a proton pump. Available data suggest that approximately four protons are translocated for each NADH oxidized (*67*). This value was determined using membrane vesicles of mitochondria and bacteria (*Paracoccus*) bearing UQ. However, certain bacteria and mitochondria are known to contain various types of quinone other than UQ. For example, *Thermus* and adult parasites utilize MQ and RQ, respectively. This ability of complex I/NDH-1 to catalyze reduction of quinones with a wide variety of E_m values (MQ, −80 mV; RQ, −63 mV; PQ, +80 mV; UQ, +110 mV) results in a wide range of possible ΔE_m's. For example, when MQ is the electron acceptor in situ, the ΔE_m between NADH (−320 mV) and MQ is expected to be 240 mV, which is much lower than the ΔE_m between NADH and UQ (450 mV). This range of ΔE_m values raises an interesting question as to whether the number of protons pumped when MQ is reduced is smaller than when UQ is reduced. The answer, to date, remains unknown.

In addition to the uncertainty surrounding the stoichiometry of H^+ pumping, questions as to the mechanism of proton pumping also remain unanswered. There are, in principle, two types of models proposed for the mechanism of energy transduction in the NDH-1/complex I. The first type, direct coupling, assumes that the electron carriers are located in the transmembrane segment and are directly involved in the H^+ translocation. Enzymes belonging to this category include the bc_1 complex and the cyt *c* oxidase. Possible reaction mechanisms of complex I based on direct coupling have been extensively discussed in published reviews (*68*, *69*) and, therefore, will not be repeated in this article. The other type of model is based on indirect coupling. The essence of this model is that the catalytic sector, containing the electron carriers, and the H^+ pump sector of complex I are distinct entities and that energy transduction takes place through conformational coupling between the two sectors (*4*, *70*). Known examples of indirect coupling are the ATP synthase (*71*) and the nicotinamide adenine nucleotide transhydrogenase (*72*, *73*). The architecture of complex I makes it an excellent candidate for the indirect coupling mechanism. The first point supporting an indirect coupling mechanism is the fact that the FMN and all of the iron−sulfur clusters are located in the peripheral segment. This leaves Q as the only carrier in the membrane domain which, according to the direct coupling mechanism, would require that it alone would work as both the proton and electron carrier. The assignment of this role to Q seems unlikely, however, in light of the fact that complex I isolated from *Y. lipolytica* mitochondria contains only 0.2−0.4 mol of UQ-9/mol but still exhibits full activity upon reconstitution with phospholipids (*74*). Another factor arguing against a direct mechanism is the absence of redox carriers such as hemes in the membrane part of complex I, which makes this enzyme distinct from other respiratory complexes (Figure 1). In fact, complex I/NDH-1 shares common properties with those of other *indirect* coupling enzymes rather than with the known direct coupling enzymes (Table 4). For example, complex I/NDH-1 is a reversible enzyme which can catalyze both NADH−Q reductase (forward) and QH_2−NAD reductase (backward) reactions. In contrast, the bc_1 complex and cyt *c* oxidase catalyze only forward reactions. Furthermore, certain complex I/NDH-1 enzymes can translocate Na^+ as well as H^+ as demonstrated for *E. coli* and *Klebsiella pneumoniae* (*75*).

Table 4: Comparison of Complex I/NDH-1, bc_1 Complex, Cytochrome *c* Oxidase, and ATP Synthase Properties

properties	complex I/ NDH-1	bc_1 complex	cyt *c* oxidase	ATP synthase
catalysis	reversible	irreversible	irreversible	reversible
Na^+ translocation	yes	no	no	yes
decoupling effect of DCCD	no	yes	yes	no

To date, there has not been any report of a bc_1 complex or a cyt *c* oxidase capable of pumping Na^+. Other data include the fact that amiloride derivatives, inhibitors for Na^+/H^+ antiporters (*76*), inactivate energy-coupled electron transfer activity of bovine complex I but not of other complexes (*53*) and that DCCD inhibits both electron transfer and H^+ translocation in complex I/NDH-1 while in bc_1 complexes and cyt *c* oxidases, DCCD diminishes proton translocation without affecting electron transfer (decoupling). These results argue in favor of indirect coupling as the energy transfer mechanism of complex I/NDH-1.

Although the actual mechanism of proton pumping has not yet been elucidated, some data are available which may help to localize residues involved in this pumping. As described above, DCCD inhibits energy-transducing electron transfer of complex I/NDH-1 (*77*, *78*). This inhibitor also inhibits the activity of the ATP synthase. It has been known for some time that this inhibition is due to binding of the DCCD to a well-conserved carboxyl residue centered in a transmembrane helix of the ATP synthase subunit c and that this carboxyl group participates in the proton translocation of the membrane sector of the ATP synthase (*79*). Therefore, it was of interest to locate conserved carboxyl residues presumed to be located in the transmembrane segments of the seven NDH-1/complex I hydrophobic subunits (Nqo7/ND3, Nqo8/ND1, Nqo10/ND6, Nqo11/ND4L, Nqo12/ND5, Nqo13/ND4, and Nqo14/ND2). In an attempt to pursue this line of inquiry, all D and E residues predicted to be located in transmembrane helices were identified by using TMpred and TMHMM software. The extent to which these residues were conserved across species was then determined by comparison against known sequences of homologues of the corresponding subunits. This process left only eight conserved carboxyl residues predicted to be located in the transmembrane segments, two in Nqo7/ND3 (D74 and E76), two in Nqo11/ND4L (E37 and E73), and one in each of Nqo8/ND1 (E247), Nqo12/ND5 (E146), Nqo13/ND4 (E141), and Nqo14/ND2 (E134) (*Paracoccus* numbering). Topological studies of Nqo7/ND3 (*16*), Nqo11/ND4L (*17*), and Nqo10/ND6 (Kao et al., unpublished data) of the *Paracoccus* and for Nqo8/ND1 and Nqo12/ND5 in *Rhodobacter* NDH-1 (*80*, *81*) support the membrane localization of some of these carboxyl residues. Using the available data together with mutation experiments in *E. coli* NDH-1, it appears that D74 and E76 in Nqo7/ND3 (Di Bernardo et al., unpublished data) and E37 and E73 in Nqo11/ND4L (*82*) are essential for energy-transducing electron transfer of NDH-1 but E247 in Nqo8/ND1 is not significant (*83*). Interestingly, these mutants, which are all missing carboxyl residues in the center of at least one transmembrane segment, did not show any of the decoupling effects which have been reported for similar mutations in the bc_1 complex and cyt *c* oxidase (*84*, *85*). This information, again, points to an indirect coupling mechanism as an option for complex I/NDH-1.

If indeed complex I/NDH-1 undergoes conformational coupling, it is important to attempt an understanding at the molecular level of how this could work. It is probably far-fetched to imagine that conformational coupling in complex I/NDH-1 operates as a rotating motor as has been shown to be the case with ATP synthase (*71*, *86*). Structural information, although still considerably limited, would make this option unrealistic. However, it is possible that complex I/NDH-1 pumps H^+ or Na^+ in a manner similar to that of Ca^{2+}-ATPase. According to recent structural studies (*87*, *88*), the Ca^{2+}-ATPase in the sarcoplasmic reticulum translocates Ca^{2+} by undergoing a dynamic conformational change in the peripheral segment which induces a rearrangement (up−down movement) of its membrane domain helices. Coupled to this movement, two Ca^{2+} are translocated across the membrane. This enzyme resembles a manual water pump in which the peripheral (catalytic) domain works as a lever and the membrane domain functions as a piston.

Mechanisms similar to that of other ion pumps must also be considered. For example, the Na^+-dependent ATP synthase of *Propionigenium modestum* pumps H^+ in conjunction with ATP synthesis but pumps Li^+ or Na^+ as well (*89*). The subunit c of this ATP synthase apparently uses three amino acid residues to ligate Na^+ (Q32, E65, S66), two residues for Li^+ (E65, S66), and one residue for H^+ (E65). It would be interesting to clarify whether similar binding options for multiple ions are also present in the proximity of conserved and essential carboxyl residues located in the middle of the transmembrane segments in complex I/NDH-1.

Epilogue. As is clear from the growing number of complex I-related publications (*90*), significant progress in the area of complex I/NDH-1 research has been made in recent years and continues to be made.[3] Nevertheless, there are many questions which still remain with regard to the structure and function of this huge enzyme complex. One of the reasons for this lack of understanding is the lack of a high-resolution 3D structure for this enzyme. Many talented researchers have now become involved in this task. Thus it can be hoped that it will not be long before the structure becomes available. Furthermore, areas of complex I research continue to expand (e.g., accessory subunits, clinical aspects) and will surely shed more light on the working of this enzyme soon. The goal of this article has been to show readers how much is known about this enzyme, to point out areas where more data are clearly needed, and to draw more young investigators into this fascinating field of study.

ACKNOWLEDGMENT

Due to space constraints, we have been unable to cite all relevant contributions to this field. We apologize to our colleagues for this. We thank Drs. Byoung Boo Seo, Salvatore Di Bernardo, Eiko Nakamaru-Ogiso, Mou-Chieh Kao, and Isabel Velazquez for discussion.

REFERENCES

1. Carroll, J., Shannon, R. J., Fearnley, I. M., Walker, J. E., and Hirst, J. (2002) *J. Biol. Chem. 277*, 50311−50317.

[3] See the complex I home page at http://www.scripps.edu/mem/biochem/CI/ for updated publication lists.

2. Ohnishi, T., Sled, V. D., Yano, T., Yagi, T., Burbaev, D. S., and Vinogradov, A. D. (1998) *Biochim. Biophys. Acta 1365*, 301−308.
3. Moller, I. M. (2001) *Annu. Rev. Plant Physiol. Plant Mol. Biol. 52*, 561−591.
4. Yagi, T., Yano, T., Di Bernardo, S., and Matsuno-Yagi, A. (1998) *Biochim. Biophys. Acta 1364*, 125−133.
5. Yagi, T., Di Bernardo, S., Nakamaru-Ogiso, E., Kao, M.-C., Seo, B. B., and Matsuno-Yagi, A. (2003) in *Respiration in Archaea and Bacteria* (Zannoni, D., Ed.) Kluwer Publishings, Dordrecht.
6. Dupuis, A., Chevallet, M., Darrouzet, E., Duborjal, H., Lunardi, J., and Issartel, J. P. (1998) *Biochim. Biophys. Acta 1364*, 147−165.
7. Friedrich, T. (1998) *Biochim. Biophys. Acta 1364*, 134−146.
8. Leonard, K., Haiker, H., and Weiss, H. (1987) *J. Mol. Biol. 194*, 277−286.
9. Hofhaus, G., Weiss, H., and Leonard, K. (1991) *J. Mol. Biol. 221*, 1027−1043.
10. Guénebaut, V., Schlitt, A., Weiss, H., Leonard, K., and Friedrich, T. (1998) *J. Mol. Biol. 276*, 105−112.
11. Grigorieff, N. (1998) *J. Mol. Biol. 277*, 1033−1046.
12. Djafarzadeha, R., Kerschera, S., Zwickera, K., Radermacherb, M., Lindahlb, M., Schaggera, H., and Brandt, U. (2000) *Biochim. Biophys. Acta 1459*, 230−238.
13. Bottcher, B., Scheide, D., Hesterberg, M., Nagel-Steger, L., and Friedrich, T. (2002) *J. Biol. Chem. 277*, 17970−17977.
14. Sazanov, L. (2002) *12th EBEC Short Report*, 201.
15. Yano, T., and Yagi, T. (1999) *J. Biol. Chem. 274*, 28606−28611.
16. Di Bernardo, S., Yano, T., and Yagi, T. (2000) *Biochemistry 39*, 9411−9418.
17. Kao, M.-C., Di Bernardo, S., Matsuno-Yagi, A., and Yagi, T. (2002) *Biochemistry 41*, 4377−4384.
18. Takano, S., Yano, T., and Yagi, T. (1996) *Biochemistry 35*, 9120−9127.
19. Chomyn, A., Cleeter, M. W. J., Ragan, C. I., Riley, M., Doolittle, R. F., and Attardi, G. (1986) *Science 234*, 614−618.
20. Yano, T., Magnitsky, S., Sled, V. D., Ohnishi, T., and Yagi, T. (1999) *J. Biol. Chem. 274*, 28598−28605.
21. Di Bernardo, S., and Yagi, T. (2001) *FEBS Lett. 508*, 385−388.
22. Patel, S. D., and Ragan, C. I. (1988) *Biochem. J. 256*, 521−528.
23. Yamaguchi, M., and Hatefi, Y. (1993) *Biochemistry 32*, 1935−1939.
24. Braun, M., Bungert, S., and Friedrich, T. (1998) *Biochemistry 37*, 1861−1867.
25. Yano, T., Sled, V. D., Ohnishi, T., and Yagi, T. (1996) *J. Biol. Chem. 271*, 5907−5913.
26. Sazanov, L. A., and Walker, J. E. (2000) *J. Mol. Biol. 302*, 455−464.
27. Sazanov, L. A., Peak-Chew, S. Y., Fearnley, I. M., and Walker, J. E. (2000) *Biochemistry 39*, 7229−7235.
28. Hofhaus, G., and Attardi, G. (1993) *EMBO J. 12*, 3043−3048.
29. Hofhaus, G., and Attardi, G. (1995) *Mol. Cell. Biol. 15*, 964−974.
30. Bai, Y. D., and Attardi, G. (1998) *EMBO J. 17*, 4848−4858.
31. Yagi, T., and Dinh, T. M. (1990) *Biochemistry 29*, 5515−5520.
32. Yamaguchi, M., Belogrudov, G. I., Matsuno-Yagi, A., and Hatefi, Y. (2000) *Eur. J. Biochem. 267*, 329−336.
33. Magnitsky, S., Toulokhonova, L., Yano, T., Sled, V. D., Hagerhall, C., Grivennikova, V. G., Burbaev, D. S., Vinogradov, A. D., and Ohnishi, T. (2002) *J. Bioenerg. Biomembr. 34*, 193−208.
34. Fisher, N., and Rich, P. R. (2000) *J. Mol. Biol. 296*, 1153−1162.
35. Miyoshi, H. (1998) *Biochim. Biophys. Acta 1364*, 236−244.
36. Miyoshi, H. (2001) *J. Bioenerg. Biomembr. 33*, 223−231.
37. Ohshima, M., Miyoshi, H., Sakamoto, K., Takegami, K., Iwata, J., Kuwabara, K., Iwamura, H., and Yagi, T. (1998) *Biochemistry 37*, 6436−6445.
38. Omura, S., Miyadera, H., Ui, H., Shiomi, K., Yamaguchi, Y., Masuma, R., Nagamitsu, T., Takano, D., Sunazuka, T., Harder, A., Kolbl, H., Namikoshi, M., Miyoshi, H., Sakamoto, K., and Kita, K. (2001) *Proc. Natl. Acad. Sci. U.S.A. 98*, 60−62.
39. Finel, M. (1998) *Trends Biochem. Sci. 23*, 412−413.
40. Yano, T., Chu, S. S., Sled′, V. D., Ohnishi, T., and Yagi, T. (1997) *J. Biol. Chem. 272*, 4201−4211.
41. Ingledew, W. J., and Ohnishi, T. (1980) *Biochem. J. 186*, 111−117.
42. Rasmussen, T., Scheide, D., Brors, B., Kintscher, L., Weiss, H., and Friedrich, T. (2001) *Biochemistry 40*, 6124−6131.
43. Nakamaru-Ogiso, E., Yano, T., Ohnishi, T., and Yagi, T. (2002) *J. Biol. Chem. 277*, 1680−1688.
44. Ohnishi, T. (1998) *Biochim. Biophys. Acta 1364*, 186−206.
45. Zu, Y., Di Bernardo, S., Yagi, T., and Hirst, J. (2002) *Biochemistry 41*, 10056−10069.
46. Kashani-Poor, N., Zwicker, K., Kerscher, S., and Brandt, U. (2001) *J. Biol. Chem. 276*, 24082−24087.
47. Duarte, M., Populo, H., Videira, A., Friedrich, T., and Schulte, U. (2002) *Biochem. J. 364*, 833−839.
48. Ohnishi, T., and Salerno, J. C. (1982) *Iron−Sulfur Proteins 4*, 285−327.
49. Degli Esposti, M. (1998) *Biochim. Biophys. Acta 1364*, 222−235.
50. Singer, T. P. and Ramsay, R. R. (1994) in *Molecular Mechanisms in Bioenergetics* (Ernster, L., Ed.) pp 145−162, Elsevier Science Publishers, BV, Amsterdam.
51. Earley, F. G. P., Patel, S. D., Ragan, C. I., and Attardi, G. (1987) *FEBS Lett. 219*, 108−113.
52. Schuler, F., Yano, T., Di Bernardo, S., Yagi, T., Yankovskaya, V., Singer, T. P., and Casida, J. E. (1999) *Proc. Natl. Acad. Sci. U.S.A. 96*, 4149−4153.
53. Nakamaru-Ogiso, E., Sakamoto, K., Matsuno-Yagi, A., Miyoshi, H., and Yagi, T. (2002) *12th EBEC Short Report*, 206.
54. Darrouzet, E., Issartel, J. P., Lunardi, J., and Dupuis, A. (1998) *FEBS Lett. 431*, 34−38.
55. Videira, A., and Duarte, M. (2001) *J. Bioenerg. Biomembr. 33*, 197−203.
56. Videira, A. (1998) *Biochim. Biophys. Acta 1364*, 89−100.
57. Zensen, R., Husmann, H., Schneider, R., Peine, T., and Weiss, H. (1992) *FEBS Lett. 310*, 179−181.
58. Schneider, R., Massow, M., Lisowsky, T., and Weiss, H. (1995) *Curr. Genet. 29*, 10−17.
59. Mikolajczyk, S., and Brody, S. (1990) *Eur. J. Biochem. 187*, 431−437.
60. Schulte, U., Haupt, V., Abelmann, A., Fecke, W., Brors, B., Rasmussen, T., Friedrich, T., and Weiss, H. (1999) *J. Mol. Biol. 292*, 569−580.
61. Vinogradov, A. D., and Grivennikova, V. G. (2001) *IUBMB Life 52*, 129−134.
62. Grivennikova, V. G., Serebryanaya, D. V., Isakova, E. P., Belozerskaya, T. A., and Vinogradov, A. D. (2002) *Biochem. J.* (in press).
63. Papa, S., Sardanelli, A. M., Scacco, S., Petruzzella, V., Technikova-Dobrova, Z., Vergari, R., and Signorile, A. (2002) *J. Bioenerg. Biomembr. 34*, 1−10.
64. Au, H. C., Seo, B. B., Matsuno-Yagi, A., Yagi, T., and Scheffler, I. E. (1999) *Proc. Natl. Acad. Sci. U.S.A. 96*, 4354−4359.
65. Yadava, N., Potluri, P., Smith, E. N., Bisevac, A., and Scheffler, I. E. (2002) *J. Biol. Chem. 277*, 21221−21230.
66. Fearnley, I. M., Carroll, J., Shannon, R. J., Runswick, M. J., Walker, J. E., and Hirst, J. (2001) *J. Biol. Chem. 276*, 38345−38348.
67. Galkin, A. S., Grivennikova, V. G., and Vinogradov, A. D. (1999) *FEBS Lett. 451*, 157−161.
68. Dutton, P. L., Moser, C. C., Sled, V. D., Daldal, F., and Ohnishi, T. (1998) *Biochim. Biophys. Acta 1364*, 245−257.
69. Brandt, U. (1997) *Biochim. Biophys. Acta 1318*, 79−91.
70. Belogrudov, G. I., and Hatefi, Y. (1994) *Biochemistry 33*, 4571−4576.
71. Sambongi, Y., Iko, Y., Tanabe, M., Omote, H., Iwamoto-Kihara, A., Ueda, I., Yanagida, T., Wada, Y., and Futai, M. (1999) *Science 286*, 1722−1724.
72. Hatefi, Y., and Yamaguchi, M. (1996) *FASEB J. 10*, 444−452.
73. Jackson, J. B., White, S. A., Quirk, P. G., and Venning, J. D. (2002) *Biochemistry 41*, 4173−4185.
74. Drose, S., Zwicker, K., and Brandt, U. (2002) *Biochim. Biophys. Acta 1556*, 65−72.
75. Gemperli, A. C., Dimroth, P., and Steuber, J. (2002) *J. Biol. Chem. 277*, 33811−33817.
76. Wiebe, C. A., Dibattista, E. R., and Fliegel, L. (2001) *Biochem. J. 357*, 1−10.
77. Yagi, T. (1987) *Biochemistry 26*, 2822−2828.
78. Hassinen, I. E., and Vuokila, P. T. (1993) *Biochim. Biophys. Acta 1144*, 107−124.
79. Fillingame, R. H. (1997) *J. Exp. Biol. 200*, 217−224.
80. Roth, R., and Hagerhall, C. (2001) *Biochim. Biophys. Acta 1504*, 352−362.
81. Mathiesen, C., and Hagerhall, C. (2002) *12th EBEC Short Report*, 193.
82. Kervinen, M., Pätsi, J., Finel, M., and Hassinen, I. (2002) *12th EBEC Short Report*, 188.
83. Kurki, S., Zickermann, V., Kervinen, M., Hassinen, I., and Finel, M. (2000) *Biochemistry 39*, 13496−13502.
84. Casey, R. P., Thelen, M., and Azzi, A. (1980) *J. Biol. Chem. 255*, 3994−4000.
85. Wang, Y. D., Obungu, V., and Beattie, D. S. (1998) *Arch. Biochem. Biophys. 352*, 193−198.

86. Noji, H., Yasuda, R., Yoshida, M., and Kinosita, K., Jr. (1997) *Nature 386*, 299−302.
87. Toyoshima, C., Nakasako, M., Nomura, H., and Ogawa, H. (2001) *Tanpakushitsu Kakusan Koso 46*, 1374−1380.
88. Toyoshima, C., and Nomura, H. (2002) *Nature 418*, 605−611.
89. Kaim, G., Wehrle, F., Gerike, U., and Dimroth, P. (1997) *Biochemistry 36*, 9185−9194.
90. Matsuno-Yagi, A., and Yagi, T. (2001) *J. Bioenerg. Biomembr. 33*, 155−157.
91. Humphrey, W., Dalke, A., and Schulten, K. (1996) *J. Mol. Graphics 14*, 33−38.
92. Guénebaut, V., Vincentelli, R., Mills, D., Weiss, H., and Leonard, K. R. (1997) *J. Mol. Biol. 265*, 409−418.

BI027158B