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Review

The nuclear encoded subunits of complex I from bovine heart mitochondria

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Abstract

NADH:ubiquinone oxidoreductase (complex I) from bovine heart mitochondria is a complicated, multi-subunit, membrane-bound assembly. Recently, the subunit compositions of complex I and three of its subcomplexes have been reevaluated comprehensively. The subunits were fractionated by three independent methods, each based on a different property of the subunits. Forty-six different subunits, with a combined molecular mass of 980 kDa, were identified. The three subcomplexes, Iα, Iβ and Iλ, correlate with parts of the membrane extrinsic and membrane-bound domains of the complex. Therefore, the partitioning of subunits amongst these subcomplexes has provided information about their arrangement within the L-shaped structure. The sequences of 45 subunits of complex I have been determined. Seven of them are encoded by mitochondrial DNA, and 38 are products of the nuclear genome, imported into the mitochondrion from the cytoplasm. Post-translational modifications of many of the nuclear encoded subunits of complex I have been identified. The seven mitochondrially encoded subunits, and seven of the nuclear encoded subunits, are homologues of the 14 subunits found in prokaryotic complexes I. They are considered to be sufficient for energy transduction by complex I, and they are known as the core subunits. The core subunits bind a flavin mononucleotide (FMN) at the active site for NADH oxidation, up to eight iron–sulfur clusters, and one or more ubiquinone molecules. The locations of some of the cofactors can be inferred from the sequences of the core subunits. The remaining 31 subunits of bovine complex I are the supernumerary subunits, which may be important either for the stability of the complex, or for its assembly. Sequence relationships suggest that some of them carry out reactions unrelated to the NADH:ubiquinone oxidoreductase activity of the complex.

Keywords: Complex I; NADH:ubiquinone oxidoreductase; Mitochondrion; Subunit composition; Electron-transport chain; Respiratory chain

1. Introduction

NADH:ubiquinone oxidoreductase (complex I) [1–5] catalyses the first step of the respiratory electron transport chain [6,7]. As depicted in Scheme 1, the oxidation of NADH provides two electrons for the reduction of ubiquinone to ubiquinol. They are transferred from NADH to the primary electron acceptor, a non-covalently bound flavin mononucleotide (FMN), and then via a series of iron–sulfur clusters to bound ubiquinone. In mitochondria, the transfer of two electrons is coupled to the translocation of four protons across the inner membrane [8,9], contributing to the proton motive force. Ubiquinol is reoxidised by the cytochrome bc_1 complex.

Complex I is a multi-subunit assembly with a characteristic L-shape. One arm is in the plane of the membrane and the other protrudes from it by about 100 Å into either the mitochondrial matrix or the bacterial cytoplasm [10,11]. This review will summarise our current knowledge of the subunit composition of the mammalian enzyme, and the contribution made by subcomplexes to our understanding of the distribution of subunits in the L-shaped structure. The analysis of the subunit composition of such a complicated enzyme as mammalian complex I is a challenging task. It depends upon the availability of highly pure preparations of the enzyme, with a reproducible

Abbreviations: ACP, acyl carrier protein; DDM, *n*-dodecyl-β-D-maltoside; EPR, electron paramagnetic resonance; ESI, electrospray ionisation; FMN, flavin mononucleotide; GRIM, gene associated with retinoid-IFN induced mortality; LDAO, lauryldimethylamine oxide; MS, mass spectrometry; SDR, short-chain dehydrogenase/reductase; TMH, transmembrane helix

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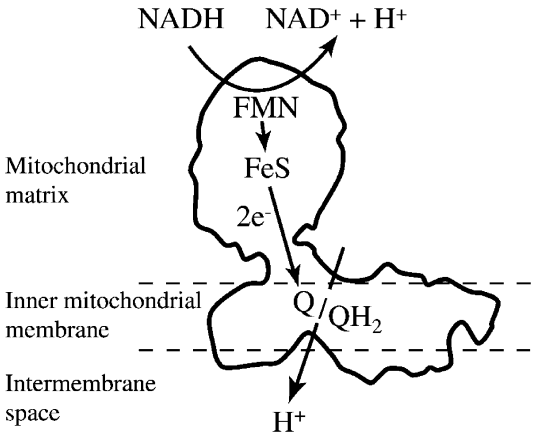


Scheme 1. The structure [10] and catalytic reaction of complex I. Two electrons from the oxidation of NADH, at the FMN active site, are passed along a chain of iron–sulfur clusters, to bound quinone.

cohort of subunits, and upon the power of modern techniques of protein chemistry and molecular biology to separate the subunits and to analyse their sequences. In addition to the complexity of the subunit composition of the mammalian assembly, various other practical considerations have hampered the analysis. They include the hydrophobicity of many subunits, and the significant number of subunits with modified N termini, which cannot be sequenced directly by Edman degradation. With mammalian complex I, the issue of deciding what is an authentic subunit is especially difficult, since mutation of the individual subunits is much less readily carried out than for complex I of eubacteria and lower eukaryotes.

The enzyme from bovine heart mitochondria is by far the best characterised eukaryotic complex I. Over the past 14 years, its subunit composition has been analysed extensively by Walker et al. [12]. Recently, in an attempt to finalise the definition of the mammalian enzyme, the subunit composition of complex I from bovine heart mitochondria was subjected to a comprehensive re-investigation [13–15]. This was aided by the independent re-analysis of several smaller subcomplexes, resolved from the intact complex with chaotropic detergents. In these analyses, 46 subunits have been detected in the bovine complex, and the sequences of 45 of them have been described [1,14,16]. Their combined molecular mass is 980 kDa. Seven of them, the 'ND-subunits', are encoded by the mitochondrial genome [17,18], and the rest are nuclear gene products that are imported from the cytoplasm into the organelle. This review focuses primarily on the nuclear encoded subunits of complex I.

The bovine enzyme has been adopted as a model for the human enzyme, and is crucial to our understanding of the role of complex I in human disease [3,19]. The nuclear genes for the human complex, identified by homology with the bovine sequences, are distributed over 18 of the 23 chromosomes (Table 1) [3], and there is high sequence identity between bovine and human subunits. Progress has also been made in determining the subunit compositions of complex I from *Neurospora crassa* [20,21], *Yarrowia lipolytica* [22], and the mitochondria of several plants [23,24], but their characterisation remains incomplete and so a comprehensive comparison of eukaryotic enzymes is not possible at present. *Saccharomyces cerevisiae* has no complex I.

The ND subunits and seven of the nuclear encoded subunits contain all the known redox cofactors of the enzyme, and its substrate binding sites. They have been defined as the 'minimal enzyme', or the 'core subunits' [1]. Other subunits have less well-understood roles and they are described as 'supernumerary' or 'peripheral'. Prokaryotic complexes I have a set of redox cofactors equivalent to those in the eukaryotic enzymes, but their subunit compositions are much simpler. Depending on the species, they consist of 13 or 14 subunits that are homologues of the subunits of the mitochondrial core complex [4,5] and the corresponding genes are organized into operons which facilitates their sequence analysis [25–27]. In this review, the sequences of common nuclear encoded subunits in complex I from the cow, man, *N. crassa*, *Paracoccus denitrificans*, *Thermus thermophilus*, *Rhodobacter capsulatus* and *Escherichia coli* are compared. Information contained within the sequences of the nuclear encoded core subunits, regarding the binding and location of the electron transfer cofactors and the substrates, is summarised. For the eukaryotic complexes, possible functions of some of the supernumerary subunits are discussed.

2. The nuclear encoded subunits of complex I from bovine heart mitochondria

The comprehensive picture of the subunit composition of bovine complex I described in this review has benefited from two approaches [15]. The first was to resolve the intact complex into several subcomplexes, with significantly simpler compositions, which are easier to analyse. The second was to employ three separate methods for subunit fractionation, which exploit different properties of the subunits, and then to analyse the proteins by mass spectrometric methods. Each separation procedure led to the identification of a hitherto undetected subunit in complex I: subunit B16.6 was identified from SDS-PAGE analysis [13], subunit B14.7 by 2D gel analysis, and subunit ESSS by reverse-phase HPLC [14].

An SDS-PAGE gel of bovine complex I is shown in Fig. 1, and two-dimensional gels (over two different pH ranges in the first dimension) are shown in Fig. 2. The positions of the subunits are marked on the figures. These figures present the consensus picture, constructed from many different experiments on complex I and its subcomplexes (see below). The analysis of the subunit composition of complex I has been described recently, in detail

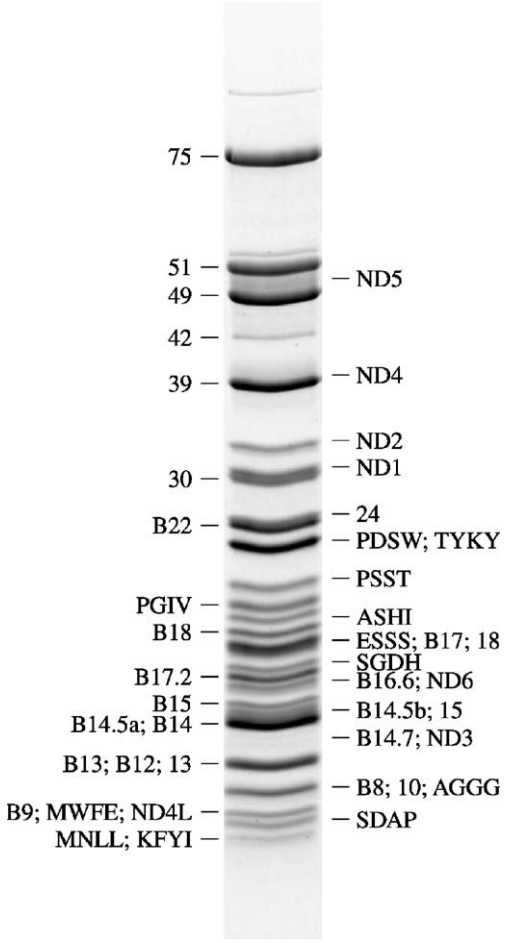


Fig. 1. Fractionation of the subunits of bovine complex I on a 1D gel. Subunits were resolved by SDS-PAGE on a 12–22% gradient gel and protein bands were analysed by peptide mass fingerprinting and tandem mass spectrometry (MS) [15]. Subunit identities are indicated at the side. The ND subunits and B14.7 are all hydrophobic proteins, therefore, they run as diffuse bands and do not stain well with Coomassie blue dye. The position of subunit ND6 was identified by an antipeptide antibody.

[15]. Forty-six different proteins have been identified in complex I from bovine heart mitochondria, and the sequences of 45 of them have been determined. The EMBL acquisition numbers and the molecular masses of the 38 sequenced, nuclear encoded subunits are given in Table 1. Assuming that complex I contains one copy of each subunit, its molecular mass is 980 kDa. Two recent studies using human heart mitochondria and complex I purified by immunoprecipitation each reported the identification of 42 of the 45 subunits [28,29]. Only subunit ND4L was not identified by either study.

Each of the subunits listed in Table 1 has been detected consistently in substantial amounts in complex I from bovine heart mitochondria, with two exceptions. As Fig. 1 shows, the 42-kDa subunit is consistently present in sub-stoichiometric quantities. This subunit is lost gradually during chromatography, suggesting that it is only loosely bound to the complex. Subunit MLRQ has been detected in only a minority of preparations, raising the question of whether it is a true subunit of complex I. The presence of subunit MLRQ correlated with contamination by cytochrome *c* oxidase subunit VIB (CoxVIB), and both subunit MLRQ and CoxVIB coelute with the cytochrome bc_1 complex during the purification of complex I. CoxVIB is a structurally characterised subunit of bovine cytochrome *c* oxidase and is involved in complex dimerisation [30], but it is lost upon the removal of cardiolipin [31]. It is possible that the presence of subunit MLRQ in complex I also depends on the presence of cardiolipin. Subunit MLRQ has been detected in human complex I [28]. The separation of intact protein complexes from human heart mitochondria by sucrose gradient centrifugation also led to the detection of subunit MLRQ, but it was distributed amongst a number of different fractions [29].

Complex I contains 38 sequenced subunits that are imported from the cytoplasm and 18 of them have mitochondrial import sequences that are removed post-translationally (see Table 2). The remaining 20 subunits, all with molecular masses below 22 kDa, lack processed import sequences, and therefore they are directed into the mitochondrion by information contained within the mature

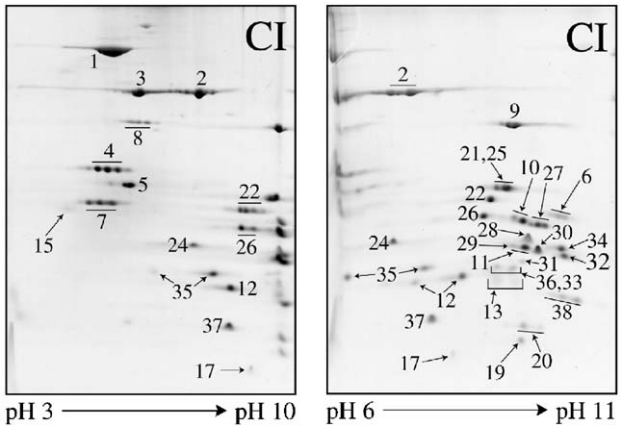


Fig. 2. Resolution of the subunits of complex I on 2D gels. First dimension: Immobilised pH gradient (IPG) 3–10 (left) or IPG 6–11 (right); second dimension: tricine SDS-PAGE (13%). Proteins were identified by peptide mass fingerprinting and by tandem MS (subunit KFYI was identified by peptide mass fingerprinting alone). Each figure is a summary of several experiments. A total of 34 of the 45 sequenced subunits of the intact complex was identified, and their positions are consistent with calculated isopotential points and molecular masses. The seven hydrophobic subunits ND1–ND6 and ND4L were not detected, nor were three of the subunits that belong to the hydrophobic fraction of the complex (AGGG, ESSS and SDAP) or subunit MLRQ. The absence of these subunits illustrates the well-known unsuitability of 2D-gels for analysis of membrane proteins. The key to the labelling corresponds to the order of the subunits in the tables: (1) 75 kDa; (2) 51 kDa; (3) 49 kDa; (4) 30 kDa; (5) 24 kDa; (6) PSST; (7) TYKY; (8) 42 kDa; (9) 39 kDa; (10) 18 kDa; (11) 15 kDa; (12) 13 kDa; (13) 10 kDa; (15) ASHI; (17) KFYI; (19) MNLL; (20) MWFE; (21) PDSW; (22) PGIV; (24) SGDH; (25) B22; (26) B18; (27) B17.2; (28) B17; (29) B16.6; (30) B15; (31) B14.7; (32) B14.5a; (33) B14.5b; (34) B14; (35) B13; (36) B12; (37) B9; (38) B8.

Table 1
The nuclear encoded subunits of complex I from bovine heart mitochondria and their homologues in other species

Bovine subunit	Acquisition number	Sequence reference	Mass (kDa)	Human [104]	Human chromosome	*N. crassa* [21]	*Tt, Pd*	*Ec, Rc*
75 kDa	P15690	[105]	77.0	NDUFS1	2q33–q34	78	NQO3	NUOG
51 kDa	P25708[a]	[39,106]	48.5	NDUFV1	11q13	51	NQO1	NUOF
49 kDa	P17694	[107]	49.2	NDUFS2	1q23	49	NQO4	NUOCD
30 kDa	P23709	[108]	26.4	NDUFS3	11p11.11	30.4	NQO5	NUOCD
24 kDa	P04394	[109,110]	23.8	NDUFV2	18p11.31–p11.2	24	NQO2	NUOE
PSST	P42026	[111]	20.1	NDUFS7	19p13	19.3	NQO6	NUOB
TYKY	P42028	[56]	20.2	NDUFS8	11q13	21.3c	NQO9	NUOI
42 kDa	P34942[a]	[112]	36.7	NDUFA10	2q37.3			
39 kDa	P34943	[112]	39.1	NDUFA9	12p13.3	40		
18 kDa	Q02375	[16]	15.3	NDUFS4	5q11.1	21		
15 kDa	Q02379	[16]	12.5	NDUFS5	1p34.2–p33			
13 kDa	P23934	[16,113]	10.5	NDUFS6	5p15.33			
10 kDa	P25712	[114,115]	8.4	NDUFV3	21q22.3			
AGGG	Q02374	[16]	8.5	NDUFB2	7q34			
ASHI	Q02372	[16]	18.7	NDUFB8	10q23.2–q23.33			
ESSS	CAD52868	[14]	14.5	P17.3[b]	Xp11.23			
KFYI	Q02376	[16]	5.8	NDUFC1	4q28.2–q31.1			
MLRQ	Q01321	[16]	9.3	NDUFA4	7p21.3			
MNLL	Q02378	[16]	7.0	NDUFB1	14q32.12			
MWFE	Q02377	[16]	8.1	NDUFA1	Xq24	9.8		
PDSW	Q02373	[16]	20.8	NDUFB10	16p13.3	12.3		
PGIV	P42029	[116]	20.0	NDUFA8	9q33.2–q34.11	20.8		
SDAP	P52505	[81]	10.7	NDUFAB1	16p12.3	ACP		
SGDH	Q02380	[16]	16.7	NDUFB5	3q27.1			
B22	Q02369	[16]	21.7	NDUFB9	8q13.3			
B18	Q02368	[16]	16.5	NDUFB7	19p13.12–p13.11			
B17.2	O97725	[117]	17.1	DAP13[b]	12q21.33			
B17	Q02367	[16]	15.4	NDUFB6	9p13.3			
B16.6	Q95KV7	[13]	16.6	GRIM19[b]	19p13.2			
B15	P48305	[16]	15.1	NDUFB4	3q13.33			
B14.7	CAD52867	[14]	14.8	NDUFA11	19p13.3	21.3b		
B14.5a	Q05752	[118]	12.6	NDUFA7	19p13.2			
B14.5b	Q02827	[118]	14.1[c]	NDUFC2	11q13.3			
B14	Q02366	[16]	15.0	NDUFA6	22q13.2–q13.31	14.8		
B13	P23935	[16]	13.2	NDUFA5	7q32	29.9		
B12	Q02365	[16]	11.1[c]	NDUFB3	2q31.3			
B9	Q02371	[16]	9.3	NDUFA3	19q13.42	9.3		
B8	Q02370	[16]	11.0	NDUFA2	5q31	10.5		

Human chromosome loci were obtained by using LocusLink at www.ncbi.nlm.nih.gov/LocusLink/list.cgi and were updated at the time of manuscript submission.

[a] For both the 51- and the 42-kDa subunits, two sequences have been determined, each differing in a single amino acid. In the 51-kDa subunit, residue 393 has been determined as cysteine [39] and tryptophan [106]. ESI-MS, peptide mass fingerprinting, and tandem MS sequencing experiments support tryptophan [15]. By sequencing the cDNA of the 42-kDa subunit, evidence has been found for both asparagine [112] and lysine (M.S. Sharpley and J. Hirst, unpublished data) at position 255. The ESI-MS experiments support lysine [15]. In both cases, the available evidence is consistent with the presence of polymorphisms in the bovine population.

[b] The gene names for human subunits ESSS, B17.2 and B16.6 were assigned independently of their identification as complex I subunits.

[c] The masses of the acetylated forms of the partially acetylated subunits B14.5b and B12 are reported.

protein. Four of these subunits retain their translation-initiator methionine, and in the other 16 subunits, it is removed post-translationally. The removal or retention of the methionine is largely consistent with the rule that methionine is cleaved from penultimate residues with small radii of gyration (Gly, Ala, Ser, Cys, Thr, Pro or Val) [32]. Subunit MNLL is the only exception. The cDNA sequence encodes for the N-terminal sequence MMNLL, but the predominant form of the mature protein has the N-terminal sequence MNLL. The N termini of the majority of the subunits lacking processed import sequences are acetylated (see Table 2), or partially acetylated (B14.5b and B12). The α-amino groups of six subunits (15 kDa, MLRQ, MNLL, MWFE, PDSW, PGIV, see Table 2) are unmodified. These subunits have either Met or Pro at the N terminus, whereas the acetylated subunits are dominated by Ala or Ser at the N terminus, the two most commonly acetylated residues [32]. The N terminus of subunit B18 is myristoylated [16] consistent with the N-terminal Gly residue, and Ala at positions 2 and 5 [33]. These N-terminal modifications have been identified largely by electrospray ionisation (ESI)-MS measurements of the intact masses of the mature subunits,

Table 2
Amino-terminal modifications of the nuclear encoded subunits of bovine complex I

Subunit	Modification	Mature N-terminal sequence
75 kDa	Δ1–23	TATAASNLIE
51 kDa	Δ1–20	SGDTTAPKKT
49 kDa[a]	Δ1–33	ARQWQPDVEW
30 kDa	Δ1–38	ESSAADTRPT
24 kDa	Δ1–32	GAGGALFVHR
PSST	Δ1–37	PSSTQPAVSQ
TYKY	Δ1–36	TYKYVNLREP
42 kDa	Δ1–23	LQYGPLAYIL
39 kDa	Δ1–35	LHHAVIPHGK
18 kDa	Δ1–42	AQDQTRDTQL
15 kDa	− Met	PFFDVQKRLG
13 kDa	Δ1–28	GVRTSPTGEK
10 kDa	Δ1–34	SAESGKDEKG
AGGG	Δ1–36	AGGGAHIEPR
ASHI	Δ1–28	ASHITKDMLP
ESSS[a]	Δ1–29	ESSSSRAVIA
KFYI	Δ1–27	KFYIQEPPHG
MLRQ	–	MLRQIIGQAK
MNLL[b]	− Met	MNLLQVVRDH
MWFE	–	MWFEVLPGIA
PDSW	− Met	PDSWDKDVYP
PGIV	− Met	PGIVELPSLE
SDAP[a]	Δ1–68	SDAPPLTLEG
SGDH	Δ1–46	SGDHGKRLFI
B22	− Met + Ac	Ac-AFLSSGAYLT
B18	− Met + Myr	Myr-GAHLARRYLG
B17.2	+ Ac	Ac-MELLQVLKRG
B17	− Met + Ac	Ac-SGYTPEEKLR
B16.6	− Met + Ac	Ac-AASKVKQDMP
B15	− Met + Ac	Ac-SFPKYEASRL
B14.7	− Met + Ac	Ac-AKTVLRQYWD
B14.5a	− Met + Ac	Ac-ASATRFIQWL
B14.5b	+ Ac (partial)	(Ac)-MMTGRQGRAT
B14	− Met + Ac	Ac-AASGLRQAAV
B13	− Met + Ac	Ac-AGLLKKTTGL
B12	− Met + Ac (partial)	(Ac)-AHGHGHEHGP
B9	− Met + Ac	Ac-AERVAAFLKN
B8	− Met + Ac	Ac-AAAAAIRGVR

− Met: N-terminal methionine has been removed post-translationally; + Ac: N-terminal residue is acetylated; + Myr: N-terminal residue is myristoylated. Δ*a*–*b*: known mitochondrial import sequence (residues *a* to *b*) has been removed.

[a] Coding sequences upstream of the mature bovine proteins are not known. However, the cDNA sequences for human homologues code for predicted mitochondrial import sequences which are removed post-translationally from the protein.

[b] The sequence MMNLL is encoded by the cDNA sequence but the N terminus of the mature protein is predominantly MNLL [15].

and by comparison of these values with masses calculated from sequences encoded in cDNAs [15].

3. Subcomplexes Iα, Iβ and Iλ

Subcomplexes Iα and Iβ are prepared by incubating complex I with the chaotropic detergent lauryldimethylamine oxide (LDAO), followed by separation by ion-exchange chromatography on a Q-Sepharose HP column

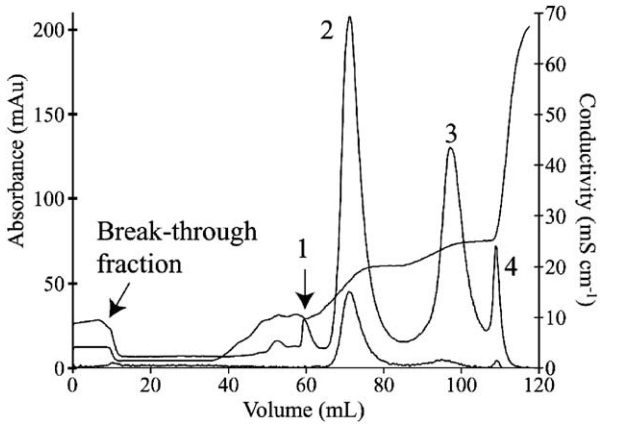


Fig. 3. Purification of subcomplexes Iα (peak 2) and Iβ (peak 3). The subcomplexes were resolved from complex I with LDAO and separated by ion-exchange chromatography on a Q-Sepharose HP column [14]. The eluate was monitored at 280 nm (upper trace), and also at 420 nm (lower trace) to detect the FMN and the iron–sulfur clusters. The composition of the breakthrough fraction and of peak 1 is discussed in the text; peak 4 contained mainly subcomplex Iβ, contaminated with some subunits of subcomplex Iα (data not shown).

[14,34]. By immediately exchanging the purified subcomplexes into *n*-dodecyl-β-D-maltoside (DDM), their further dissociation is prevented, so that each subcomplex described

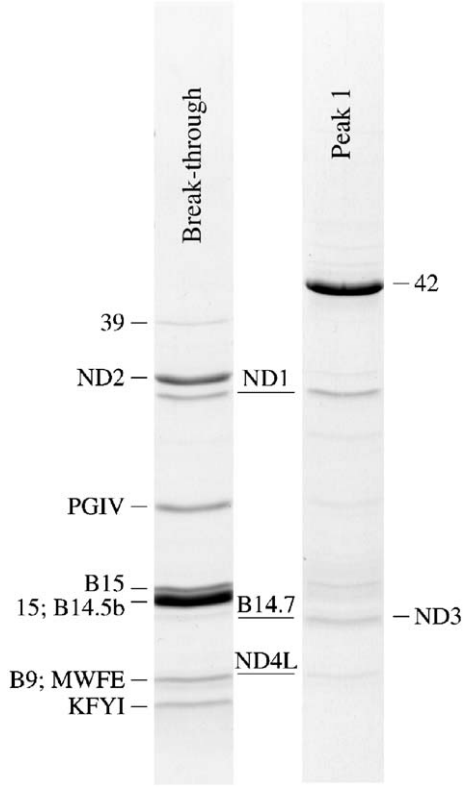


Fig. 4. Subunit composition of the breakthrough fraction and peak 1, from the separation of subcomplexes Iα and Iβ. The proteins were analysed by SDS-PAGE on 12–22% gradient gels and protein bands were identified by peptide mass fingerprinting and tandem MS. Subunit identities are indicated at the side.

Table 3
The subunit compositions of the three defined subcomplexes of bovine complex I, subcomplexes Iα, Iλ and Iβ

Subcomplex		
Iα[a]	Iλ[a]	Iβ[a]
75 kDa	75 kDa	AGGG[b]
51 kDa	51 kDa	ASHI
49 kDa	49 kDa	ESSS[b]
30 kDa	30 kDa	MNLL
24 kDa	24 kDa	PDSW
PSST	PSST	SDAP[b,c]
TYKY	TYKY	SGDH
18 kDa	18 kDa	B22
13 kDa	13 kDa	B18
10 kDa	10 kDa	B17
B17.2	B17.2	(B15[c])
B16.6	B16.6	(B14.5b[d])
B14.7[e]	(B14.7[d,e])	B12
B14.5a	B14.5a	ND4[b,e]
B13	B13	ND5[b,e]
B8	B8	
(42 kDa[e])		
39 kDa		
15 kDa[d]		
MWFE		
PGIV		
SDAP[b,c,e]		
(B15[c])		
B14		
B9		
ND6[b,e]		

Parentheses indicate a consensus that the subunit is present in minor amounts.

[a] Previous analyses of subcomplexes Iα, Iλ and Iβ have been reported [34–36].

[b] Not detected by 2D gels.

[c] Present in both Iα and Iβ, in substoichiometric amounts.

[d] Not detected by 1D gels.

[e] Not detected by reverse-phase HPLC.

here is stable. In each case, their stability was confirmed by gel-filtration chromatography. In contrast, subcomplexes Iα and Iβ retained in a high concentration of LDAO break down further into smaller subcomplexes. Although subcomplex Iα dissociates to produce subcomplex Iλ [35], the subcomplex Iλ described here was produced by a different procedure and purified by sucrose gradient centrifugation [13,36].

The chromatographic separation of subcomplexes Iα and Iβ is shown in Fig. 3. Two fractions elute from the column before the two subcomplexes and their subunit compositions are presented in Fig. 4. The weakly bound 42-kDa subunit is recovered separately in peak 1, in an almost homogeneous state. The breakthrough fraction is similar to a fraction characterised previously as subcomplex Iγ [35] but, in contrast to the results discussed here, it was observed previously that the 42-kDa subunit eluted with the breakthrough proteins. Therefore, it was classified as being part of subcomplex Iγ. This difference may result from the higher ionic strength of the solution used to resolve the subcomplexes. However, the dissociation of the 42-kDa subunit casts doubt on the integrity of subcomplex Iγ; subcomplex Iγ can also be separated into two smaller fragments by gel-filtration chromatography [35]. Furthermore, most of the subunits detected in the gels shown in Fig. 4 were found also in subcomplex Iα or Iβ (see Table 3). Only subunits KFYI, ND1, ND2, ND3 are ND4L are found uniquely in the two pre-fractions, and several of them are distributed between the two. This finding suggests that the breakthrough proteins and peak 1 contain subunits which 'splinter' away from both subcomplexes during fractionation, and

Table 4
Predicted transmembrane helices in complex I from bovine heart mitochondria

Subunit	Predicted TMHs	Subcomplex location
75 kDa	0	Iλ and Iα
51 kDa	0	Iλ and Iα
49 kDa	0	Iλ and Iα
30 kDa	0	Iλ and Iα
24 kDa	0	Iλ and Iα
PSST	2/0[a]	Iλ and Iα
TYKY	0	Iλ and Iα
42 kDa	0	Iα
39 kDa	3/0[a]	Iα
18 kDa	0	Iλ and Iα
15 kDa	0	Iα
13 kDa	0	Iλ and Iα
10 kDa	0	Iλ and Iα
AGGG	1/0[b]	Iβ
ASHI	1	Iβ
ESSS	1	Iβ
KFYI	1	–
MLRQ	1	–
MNLL	0/1[b]	Iβ
MWFE	1	Iα
PDSW	0	Iβ
PGIV	0	Iα
SDAP	0	Iα and Iβ
SGDH	1	Iβ
B22	0	Iβ
B18	0	Iβ
B17.2	0	Iλ and Iα
B17	1	Iβ
B16.6	1	Iλ and Iα
B15	1	Iα
B14.7	3/2[b]	Iλ and Iα
B14.5a	0	Iλ and Iα
B14.5b	1	Iβ
B14	0	Iα
B13	0	Iλ and Iα
B12	1	Iβ
B9	1	Iα
B8	0	Iλ and Iα

Locations of the subunits within the subcomplexes are taken from Table 3. The predictions of TMHs from two hidden Markov models, HMMTOP (http://www.enzim.hu/hmmtop/ [119]) and TMHMM (http://www.cbs.dtu.dk/services/TMHMM/ [120]), were compared and agree in the majority of cases.

[a] Despite the predictions of HMMTOP for the PSST and 39 kDa subunits (two and three transmembrane helices, respectively) it is unlikely that either of these subunits contains a TMH.

[b] The prediction of HMMTOP is presented first, followed by the prediction from TMHMM.

therefore, these proteins may not necessarily constitute an integral subcomplex. Many of the proteins in the breakthrough fraction and peak 1 are hydrophobic proteins (see Table 4) and therefore they are likely to have formed part of the membrane arm of complex I [35].

Analysis of the subunit compositions of subcomplexes Iα, Iβ and Iλ has played an important role in defining the subunit composition of complex I [15]. Fig. 5 shows the analysis of subcomplexes Iα, Iβ and Iλ by 1D SDS-PAGE. The positions of the subunits are marked at the sides of the gels; they were identified predominantly by tryptic peptide mass fingerprinting and tandem mass spectrometry. Fig. 6 shows the fractionation of the subcomplexes by 2D gel electrophoresis; again the subunits were identified by tryptic peptide mass fingerprinting and tandem mass spectrometry. Fig. 7 shows the separation of the subunits of the subcomplexes by reverse-phase HPLC. In this case, the subunits in each peak were identified by either ESI-MS measurements of the subunit mass, or by SDS-PAGE and mass spectrometric analysis. Despite many attempts, it has not proved to be possible to obtain any sequence information on an additional protein with a mass of 10,566 (±2) Da, the proposed 46th subunit of complex I. It has been observed to coelute with subunit

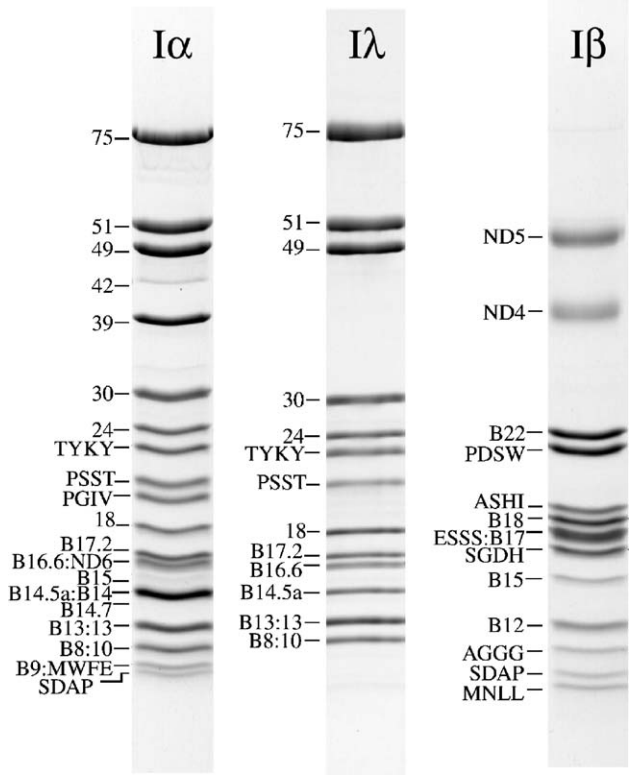


Fig. 5. Fractionation of the subunits of the subcomplexes of bovine complex I on 1D gels. Subunits were resolved by SDS-PAGE on 12–22% gradient gels and protein bands were identified by peptide mass fingerprinting and tandem MS. The position of subunit ND6 was identified by an antipeptide antibody. Subunit identities are indicated at the side.

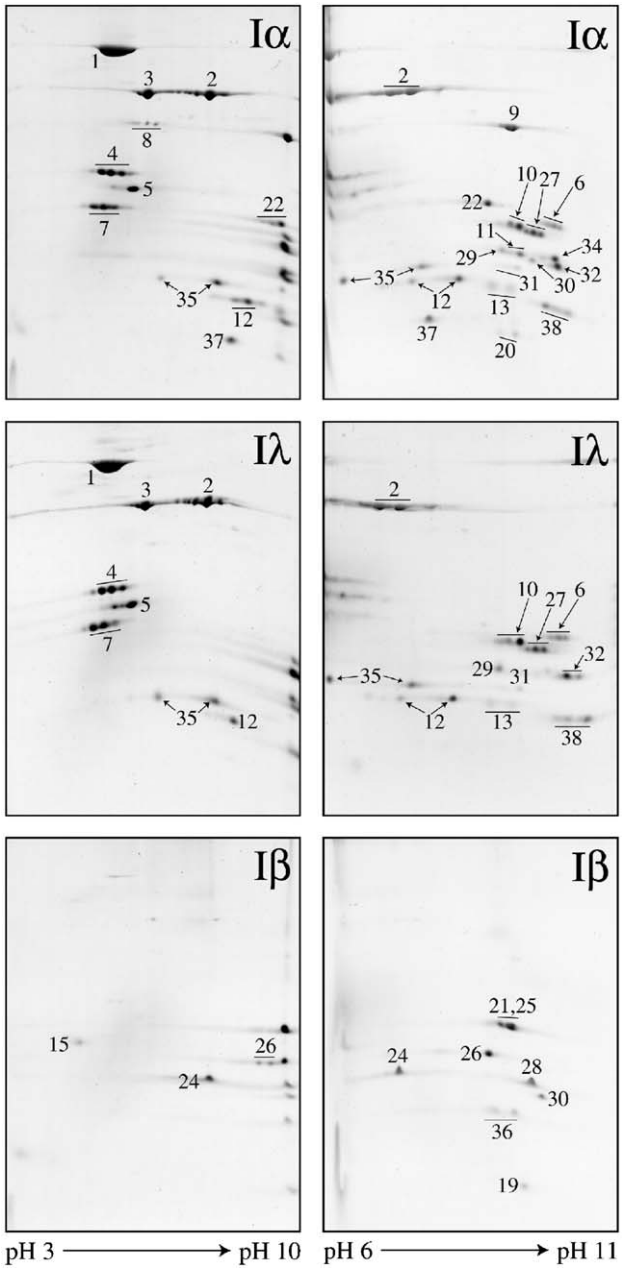


Fig. 6. Resolution of the subunits of the subcomplexes of bovine complex I on 2D gels. First dimension: Immobilised pH gradient (IPG) 3–10 (left) or IPG 6–11 (right); second dimension: tricine SDS-PAGE (13%). Proteins were identified by peptide mass fingerprinting and tandem MS. Each figure is a summary of several experiments. The key to the subunit identification is given in the legend to Fig. 2.

SGDH (see Fig. 7) in numerous independent analyses of subcomplex Iβ.

4. The topography of complex I

The subunit compositions of subcomplexes Iα, Iλ and Iβ are summarised in Table 3. It is clear from Table 3, and by comparison of the data in Figs. 5–7, that subcomplex Iλ is a

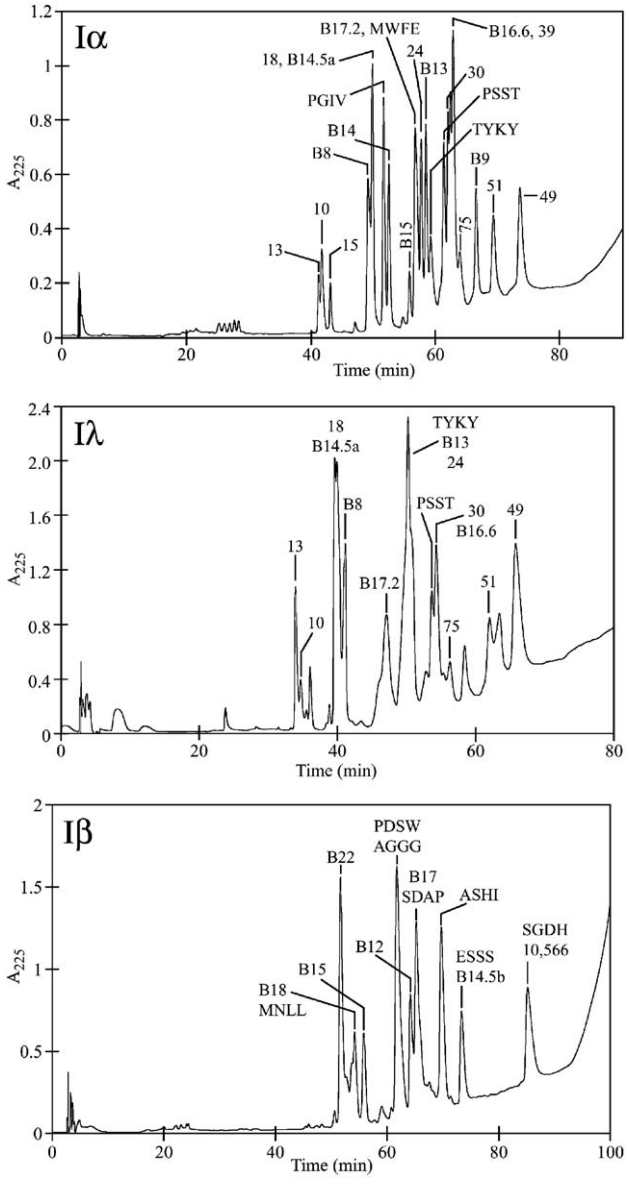
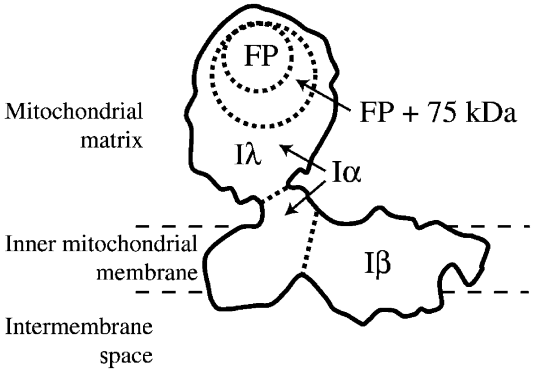


Fig. 7. Separation of the subunits of the subcomplexes of bovine complex I by reverse-phase HPLC. An Aquapore RP-300 column (100 × 2.1 mm i.d.) was employed at a flow rate of 100 μl min^{-1}. Proteins were applied in 6 M guanidine–HCl and 0.1% trifluoroacetic acid and eluted with a linear gradient of acetonitrile. The eluate was monitored at 225 nm and the elution positions of the components are indicated. The most hydrophobic subunits of complex I (ND1–ND6, ND4L and B14.7) were not recovered from the HPLC column.

fragment of subcomplex Iα. Similarly, Table 3 and Figs. 5–7 show that subcomplexes Iα and Iβ represent essentially non-overlapping subsets of the subunits of complex I, which together account for most, but not all, of its subunits. Of the 39 subunits encoded by the nuclear genome, only two (KFYI and MLRQ) have not been detected in at least one of the three subcomplexes.

Subunits ND1–6 and ND4L all contain a number of transmembrane helices and they are integral membrane proteins. A number of the nuclear encoded subunits of complex I from bovine heart mitochondria also contain predicted transmembrane helices (see Table 4). The 15 subunits of subcomplex Iλ which are present in apparently stoichiometric amounts contain only one transmembrane helix, in subunit B16.6 [13]. Subunit B14.7, which is present in only trace amounts, is also predicted to contain two to three transmembrane helices (TMHs). These hydrophobic regions probably explain the tendency of subcomplex Iλ to aggregate in the absence of detergent. The subunits of subcomplex Iλ are predominantly hydrophilic and therefore it constitutes the globular arm of the complex, which protrudes into the mitochondrial matrix. Subcomplex Iλ includes the seven core subunits which are known to bind the FMN and ligate all of the iron–sulfur clusters (see below). In addition to the 15 subunits present in subcomplex Iλ, subcomplex Iα contains a further 8 subunits, in apparently stoichiometric amounts (Table 3). These subunits contain 9–10 TMHs (including the 5 TMHs predicted for ND6, see Table 4), therefore, it is likely that the additional domain of subcomplex Iα is embedded in the inner membrane. Subcomplex Iβ contains 12 subunits in apparently stoichiometric amounts (Table 3) that are predicted to contain a total of approximately 36 TMHs (including 30 TMHs predicted for ND4 and ND5, Table 4). Therefore, subcomplex Iβ is the most hydrophobic of the three subcomplexes, and it constitutes a major part of the membrane arm of complex I. The arrangement of subcomplexes Iα, Iβ and Iλ within complex I is shown in Scheme 2.

Several other fragments of complex I are also known and they add to the overall picture (see Scheme 2). Bovine complex I has also been resolved with chaotropic anions such as perchlorate. This treatment produced three fractions which were separated by ammonium sulfate precipitation: they are known as the flavoprotein (FP), iron–protein (IP) and hydrophobic-protein (HP) fractions [37]. FP is a well-defined and catalytically active subcomplex, comprised of the 51-, 24- and 10-kDa subunits,



Scheme 2. Structural relationships amongst the subcomplexes of complex I. FP is the flavoprotein fragment of bovine complex I, FP + 75 kDa is the *E. coli* NuoE, F and G fragment, and subcomplexes Iα, Iβ and Iλ are as described in the text. The location of the subunits lost from both subcomplexes Iα and Iβ is not known.

but it is not clear that the IP and HP fragments represent discrete subcomplexes of complex I. FP is the simplest subject for study of the complex I active site since it contains only the active-site FMN, the NADH binding site, one [4Fe–4S] (51 kDa subunit) and one [2Fe–2S] (24 kDa subunit) [38, 39]. An overexpressed fragment of complex I from *E. coli*, which consists of the NuoE, F and G subunits (corresponding to the 24-, 51- and 75-kDa subunits of bovine complex I) is also catalytically active [40] and it is likely that the structural relationship amongst these subunits is conserved in the mitochondrial enzyme.

5. The core subunits

The core subunits of complex I are the 14 subunits considered essential for catalysis of electron transfer from NADH to ubiquinone and for generation of the proton-motive force, and are the subunits conserved throughout all complexes I. Table 5 presents sequence identities of the bovine protein with the homologues from man, *N. crassa*, *P. denitrificans*, *T. thermophilus*, *R. capsulatus* and *E. coli*. In this review, only the seven core subunits which are encoded on the bovine nuclear genome (the 75-, 51-, 49-, 30-, 24-kDa, PSST and TYKY subunits) are discussed. These seven subunits are the most conserved of the eukaryotic subunits, and are considered to be sufficient to ligate all the redox cofactors of complex I (except ubiquinone). Average sequence identities are 96%, 65%, 54%, 36%, 56% and 33% for the core subunits of man, *N. crassa*, *P. denitrificans*, *T. thermophilus*, *R. capsulatus* and *E. coli*, respectively. *E. coli* complex I is the most distant from the bovine complex and this observation may be related to recent proposals that *E. coli* complex I translocates sodium ions rather than protons [41,42].

5.1. The 24- and 51-kDa subunits

The 24-kDa subunit houses one [2Fe–2S] cluster, ligated by four conserved cysteine residues, Cys(103)-X_4-Cys-X_{35}-Cys-X_3-Cys [43]. It is a member of the *Clostridium pasteurianum* class of [2Fe–2S] ferredoxins, and is homologous to the [2Fe–2S] ferredoxin from *Aquifex aeolicus*, in which the cluster is bound at the tip of a thioredoxin-type fold [44]. The 24-kDa subunit [2Fe–2S] cluster is likely to be responsible for electron paramagnetic resonance (EPR) signal N1a [45].

The 51-kDa subunit is proposed to be adjacent to the 24-kDa subunit for the following reasons: (i) fractionation of the bovine enzyme by perchlorate produces the FP subcomplex, containing the 51-, 24- and 10-kDa subunits [37]; (ii) HoxF, the α-subunit of the NAD-reducing hydrogenases typified by that of *Ralstonia* (formerly *Alcaligenes*) *eutrophus*, is homologous to a fusion of the 51- and 24-kDa subunits [39]; (iii) co-expression of the 51- and 24-kDa subunits produces a catalytically active FP-type enzyme [46]. Photoaffinity analogues of NAD^+ react with the 51-kDa subunit [47], and the FP subcomplex contains one FMN per complex and catalyses the oxidation of NADH. Therefore, the 51-kDa subunit contains the non-covalently bound active-site FMN and the NADH binding site. Although the 51-kDa subunit is not evidently related to any protein of known structure, sequence analysis suggests that

Table 5
Sequence identities between the nuclear encoded subunits of complex I from bovine heart mitochondria and other species

	Sequence identity (%)					
	Human	*N. crassa*[a]	*P. denitrificans*	*T. thermophilus*	*R. capsulatus*	*E. coli*
75 kDa	97	55	46	27	44	24
51 kDa	98	74	62	43	65	40
49 kDa	96	66	57	42	57	36[b]
30 kDa	95	55	43	24	45	26[b]
24 kDa	99	62	41	29	45	25
PSST	93	71	65	49	69	46
TYKY	96	72	67	36	69	35
42 kDa	81	–				
39 kDa	80	32				
18 kDa	95	37				
15 kDa	74	–				
13 kDa	90	–				
10 kDa	87	–				
AGGG	90	–				
ASHI	85	–				
ESSS	86	–				
KFYI	82	–				
MLRQ	88	–				
MNLL	82	–				
MWFE	80	30				
PDSW	79	13				
PGIV	88	29				
SDAP	98	40				
SGDH	85	–				
B22	91	–				
B18	75	–				
B17.2	90	–				
B17	77	–				
B16.6	83	–				
B15	73	–				
B14.7	72	23				
B14.5a	88	–				
B14.5b	73	–				
B14	91	28				
B13	87	42				
B12	82	–				
B9	83	24				
B8	94	34				

All values are percentage identities based on the length of the bovine protein. Therefore, values are not prejudiced against homologues of significantly different lengths. Homologues to two mammalian subunits may be fused to form one subunit in *N. crassa* complex I.

[a] The composition of *N. crassa* complex I used here is based on a recent review [21]; only sequences published in protein sequence databases, and determined from the protein composition of the *N. crassa* complex I are included.

[b] In *E. coli*, the homologues of the 49- and 30-kDa subunits are fused to give a single sequence (NuoCD).

the ADP-binding pocket of the NADH-binding site is formed by residues 61–99, and that the highly conserved glycine-rich sequence (residues 180–234) is involved in binding the FMN [1]. The 51-kDa protein also contains a [4Fe–4S] cluster. The bovine protein has 12 cysteine residues, but only 5 of them are conserved. The first conserved cysteine is separated from the others by 172 residues, and lies within the proposed FMN binding site. However, the four remaining conserved residues form a typical [4Fe–4S] cluster ligation motif, Cys(359)-X_2-Cys-X_2-Cys-X_{39}-Cys (though the final proline is absent) [48,49] and this [4Fe–4S] cluster is proposed to produce EPR signal N3 [46,50,51].

5.2. The 75-kDa subunit

The bovine 75-kDa subunit contains 17 cysteines, and 11 of them are conserved throughout the six species in Table 5, and also in HoxU (the γ-subunit) of the NAD-reducing hydrogenases, such as that of *R. eutrophus* [39,52]. These cysteines, all found in the N-terminal domain of the protein, almost certainly ligate one [2Fe–2S] cluster and one [4Fe–4S] cluster [53]. The [4Fe–4S] cluster is ligated by the canonical motif Cys(153)-X_2-Cys-X_2-Cys-X_{43}-Cys-Pro [48,49], and has been assigned to EPR signal N4. The [2Fe–2S] cluster is responsible for EPR signal N1b. Evidence for a second [4Fe–4S] cluster, corresponding to the rapidly relaxing EPR signal N5, has been presented [54]. It is likely that this cluster is coordinated by three cysteine residues and one conserved histidine residue. The ligands to the [2Fe–2S] cluster and the second [4Fe–4S] cluster are unclear, since the potential residues [Cys(41)-X_{10}-Cys-X_2-Cys-X_{13}-Cys-X_{31}-His-X_3-Cys-Pro-X-Cys-X_5-Cys] do not contain any canonical binding motifs. Suggestions have been made for two binding motifs, based on the homology of the 75-kDa subunit to the *C. pasteurianum* Fe-only hydrogenase [54]. An additional motif is conserved in the homologues from *T. thermophilus* and *E. coli* (Cys-X_2-Cys-X_3-Cys-X_{27}-Cys, though again without the expected final proline), and is likely to coordinate an additional [4Fe–4S] cluster in these species [55]. The 75-kDa subunit is probably associated structurally with the 51- and 24-kDa subunits. They are homologous to the α (51 and 24 kDa) and γ (75 kDa) subunits of the NAD-reducing hydrogenases [39], and the NuoE, F and G subunits of *E. coli* complex I can be co-expressed to form a soluble and catalytically active subcomplex [40].

5.3. Subunit TYKY

The eight conserved cysteines of the TYKY subunit form a canonical 8Fe–ferredoxin motif, ligating two [4Fe–4S] clusters (Cys(77)*-X_2-Cys*-X_2-Cys*-X_3-Cys-Pro-X_{27}-Cys-X_2-Cys-X_2-Cys-X_3-Cys*-Pro, asterisks distinguish the two clusters) [48,49,56]. Overexpression of the NQO9 subunit of complex I from *P. denitrificans* has provided experimental support for this prediction [57], and although EPR studies of complex I generally only resolve signals from four [4Fe–4S] clusters, N2 (see below) and N3–5 [51,58] two further signals (N6a and 6b) have been ascribed recently to the clusters of the TYKY subunit [59].

5.4. The PSST and 49-kDa subunits

The PSST subunit contains four conserved cysteines in the motif Cys-Cys-X_{63}-Cys-X_{29}-Cys-Pro. This motif does not resemble any known cluster binding motif, and there are no known examples of two adjacent cysteines ligating the same cluster [48,49]. Therefore, an iron–sulfur cluster in PSST might either recruit a cysteine from another subunit, or adopt a non-cysteine ligand. A [4Fe–4S] cluster, known from its EPR signal as cluster N2, has the highest observed redox potential amongst the complex I clusters, and is also pH dependent [51,58]. Cluster N2 is proposed to donate electrons to ubiquinone [51,60], and is likely to be ligated by either subunit TYKY or subunit PSST [61–63]. In the latter case, the fourth ligand has been suggested to be provided by the 49-kDa subunit [64]. The PSST and 49-kDa subunits are homologous to the large and small subunits of the NiFe hydrogenases, respectively [65]. Therefore, they are likely to be intimately associated in complex I. Both the 49-kDa subunit, and the 49-kDa–PSST pair, have been suggested to be involved in binding of the hydrophilic ubiquinone headgroup [61,64], further indicating that the 49-kDa subunit may play a key role in the energy transduction mechanism of complex I.

Therefore, specific functions have been proposed for six of the seven nuclear encoded core subunits. Very little is known about the seventh protein, the 30-kDa subunit. Its sequence is not related to any protein of known structure, and it is not proposed to bind any redox cofactors. The proposed functions and cofactors of the nuclear encoded core subunits are summarised in Table 6.

Table 6
The electron-transport cofactors of bovine complex I and their subunit locations

	Cofactors	EPR Signal	Function
75 kDa	[2Fe–2S], [4Fe–4S]	N1b, N4	Electron transport
	[4Fe–4S]	N5	
51 kDa	FMN		NADH binding and reduction
	[4Fe–4S]	N3	Electron transport
49 kDa	–	–	Ubiquinone binding? Ligand to N2?
24 kDa	[2Fe–2S]	N1a	Electron transport
PSST	[4Fe–4S]?	N2?	Electron transport Ubiquinone binding?
TYKY	2 × [4Fe–4S]	N2? N6a? N6b?	Electron transport

6. The supernumerary subunits

Sequences have been determined for 38 nuclear encoded subunits of complex I from bovine mitochondria, and therefore, 31 subunits are not in the core of complex I. They are known as the supernumerary or peripheral subunits of complex I. Table 5 presents the sequence identities of the supernumerary subunits of bovine complex I with the human and *N. crassa* homologues. The average sequence identities are 84% and 30%, for the human and *N. crassa* proteins, respectively, significantly lower than the values of 96% and 65% for the nuclear encoded core subunits.

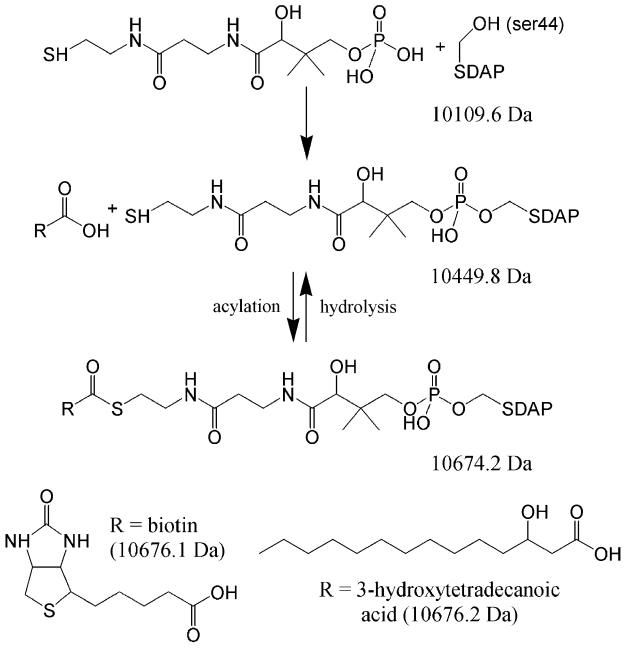
The functions of the supernumerary subunits of complex I are unclear. In mitochondria, the cytochrome bc_1 complex and cytochrome *c* oxidase also possess a number of supernumerary subunits. The bovine enzymes have 11 and 13 subunits [30,67], whereas their equivalents in *P. denitrificans* have only 3 and 4 subunits, respectively [66,67]. This suggests that supernumerary subunits may have general roles, including protecting the complexes against oxidative stress or improving their structural stability. However, they may also play specific roles in regulating activity, or in the assembly of the complexes. In cytochrome *c* oxidase, the intra-mitochondrial ATP/ADP ratio regulates turnover by binding to the matrix domain of supernumerary subunit IV [68]. In the cytochrome bc_1 complex in plants, supernumerary subunits constitute the mitochondrial processing peptidase (MPP) [69]. In the bovine enzyme, the corresponding subunits retain this activity, but process only the precursor to the Rieske protein, the cleaved import presequence being retained in the MPP active site as subunit 9 [70,71]. In complex I, a number of the supernumerary subunits show some homology to an enzyme or protein of alternative function, although in the majority of cases, this is not supported by any experimental evidence. The discussion here is restricted to subunits for which there is some experimental support for an alternative function within the mitochondrial complex, and to proteins that have been shown to have other cellular roles.

6.1. The 39-kDa subunit

The sequence of the 39-kDa subunit has a nucleotide-binding motif between residues 19 and 49 [1,72]. Although there is experimental evidence, from photoaffinity labelling and proteolysis studies, to suggest that the 39-kDa subunit may bind either NADH or NADPH, the specificity and the conditions under which nucleotide binding occurs remain unclear [73,74]. Disruption of the gene for the 39-kDa homologue in *N. crassa* resulted in the absence of the tightly bound NADPH observed in the wild-type enzyme [75]. In addition, sequence comparisons suggest that the 39-kDa subunit is related to the short-chain dehydrogenase/reductases (SDRs) [72,76]. They use NAD(H) or NADP(H) as a cofactor and they include steroid and cholesterol dehydrogenases, dihydroflavanol reductases and nucleotide sugar epimerases [77,78]. At present, it is not clear how this sequence similarity should be interpreted. One suggestion is that the 39-kDa subunit has a role in a biosynthetic pathway, separate from the energy transducing activity of complex I. Since examples of interactions between SDRs and acyl carrier proteins (ACPs) are known, one hypothesis is that it forms a 'biosynthetic module' with the acyl-carrier protein SDAP (see below). For example, the structurally characterised β-keto acyl carrier protein reductase of *Brassica napus* belongs to the SDR family [79]. Alternatively, the 39-kDa subunit has been proposed to be involved in the biosynthesis of cofactor 'X', which has been reported to be present in complex I from *N. crassa* [75,80]. At present, there is little experimental evidence to support either of these proposals.

6.2. The acyl-carrier protein, subunit SDAP

The complexes I from bovine mitochondria and *N. crassa* contain a subunit which is closely related to the ACPs involved in fatty acid biosynthesis in bacteria and chloroplasts [81,82]. In each case, a covalently attached pantetheine-4′-phosphate group, modified by an acyl group, has been detected (see Scheme 3). However, there is no clear consensus on the identity of the acyl group. Initially, the complex I ACP from *N. crassa* was proposed to be modified by 3-hydroxytetradecanoate [83], whereas a different (but unassigned) acyl group was observed in the bovine homologue [81]. As discussed previously [15], this mass has now been shown to be partly due to modification by β-mercaptoethanol at a separate site, and the mass now



Scheme 3. The post-translational modification of the SDAP subunit and the mass of each state of modification.

ascribed to the bovine acyl group matches that of 3-hydroxytetradecanoate. However, separate experiments have identified a further range of acyl groups bound to the *N. crassa* complex I ACP. Incubation with ^{14}C-labelled malonate led to the detection of hexanoic, caprylic, decanoic and lauric acids, as well as hydroxymyristic and myristic acid; uptake and release of the ^{14}C label from the ACP was demonstrated [84]. Gas–liquid chromatography detected saturated C_6 to C_{18} fatty acids and oleic acid [85]. Therefore, a role for the complex I ACP in mitochondrial de novo fatty acid synthesis, elongation, or desaturation, was suggested. In this context, it is possible that the 3-hydroxytetradecanoate acyl group is a dead-end product, or that it is formed when complex I is isolated. Disruption of the gene for the ACP subunit of *N. crassa* complex I resulted in incorrect assembly of complex I, but also in a 4-fold increase of the lysophospholipid content of the mitochondrial membranes [86,87]. Therefore, a possible role for the mitochondrial ACP is to recycle lysophospholipids, formed from lipid hydroperoxides by a phospholipase, by using an ACP-bound fatty-acid for reacylation. Alternatively, the mitochondrial ACPs have been suggested to be involved in the biosynthesis of lipoic acid, or to donate lipoic acid to proteins such as the pyruvate dehydrogenase complex [88–91].

6.3. The 18-kDa subunit

Papa et al. [92] have suggested that the 18-kDa subunit of complex I is phosphorylated by a mitochondrial cAMP-dependent protein kinase, at a canonical RVS site (position 129–131 in the mature protein), to bring about a stimulation of the catalytic rate. A 5-bp duplication which destroys the phosphorylation site has been identified in a human patient with fatal neurological syndrome. Therefore, an important role for the 18-kDa subunit in complex I regulation and activity has been proposed. This work has been reviewed recently by Papa et al. [92].

6.4. Subunit MWFE

The MWFE subunit is one of the two X-linked subunits of complex I (Table 1). It has one predicted TMH (Table 4) and is found in subcomplex Iα but not subcomplex Iλ (Table 3); therefore, it is likely to form part of the membrane-bound domain of the complex. MWFE mutants have been identified from respiration-deficient Chinese hamster cell lines, and have a deleterious effect on complex I activity [93]. In particular, the R50K mutation results in severe disruption of the normal complex I activity [94] and the segment between amino acids 39 and 46 appears to be critical for species-specific compatibility [95]. Therefore, the MWFE subunit is essential for formation of an active complex I. It is still unclear, however, whether this loss of complex I activity results from a decrease in the turnover rate, or from disruption of the enzyme assembly.

6.5. Subunit B16.6

The sequence of bovine complex I subunit B16.6 is 83% identical to the sequence of human GRIM-19 (Gene associated with Retinoid-IFN induced Mortality) [96], and each have 143 amino acids [13]. Therefore, it is very likely that the two proteins are direct homologues of one another. GRIM-19 was originally identified in studies of breast carcinoma cells, and is thought to be part of the interferon-β and all-*trans*-retinoic acid pathway of cell-death induction [96,97]. Viral interferon regulatory factor 1 (vIRF1) from Karposi's sarcoma-associated herpes virus has been found to bind to GRIM-19 and to co-localise with it. The vIRF1 protein thereby deregulates GRIM-19-induced apoptosis and inhibits interferon-β and all-*trans*-retinoic acid-induced cell death [98]. A second viral protein from human papillomavirus also binds GRIM-19, suggesting that it may be a general target of viral proteins. Recently, GRIM-19 was also shown to interact with Stat3, and to repress its transcriptional activity and target gene expression, suggesting that GRIM-19 is a novel negative regulator of Stat3 [99].

Confocal microscopy studies on cancer cell lines indicated initially that GRIM-19 is primarily a nuclear protein, though some 'punctate' staining of the cytoplasm was observed also [96,100]. However, GRIM-19 has been identified unambiguously as a subunit of mitochondrial complex I [13, 28] and in human mitochondria [29], and later studies using confocal microscopy also indicated that GRIM-19 is primarily a mitochondrial protein [99]. Therefore, there are two possibilities: either the same protein, GRIM-19/B16.6, is involved in two independent cellular processes, or the two observed locations point to a further link between mitochondria and apoptosis. Mitochondrial proteins can be exported to other cellular compartments [101]: for example, endonuclease G is released from mitochondria during apoptosis and translocated to the nucleus [102]. Finally, a possible link may be provided by the synthetic retinoid, *N*-(4-hydroxyphenyl)retinamide (4HPR), a promoter of apoptosis in tumour cell lines. It is thought to act by increasing the mitochondrial production of reactive oxygen species, possibly via interaction with a quinone binding site in either complex I or the cytochrome bc_1 complex [103].

7. Concluding remarks

Mitochondrial complex I is one of the most complicated enzymes known. It is likely to contain 46 different subunits, with a combined mass of 980 kDa. It is membrane bound, and contains an active site flavin mononucleotide, up to eight iron–sulfur clusters, and one or more bound quinones. The subunit composition of complex I from bovine heart mitochondria has now been established, and the primary sequences of 45 of the subunits have been determined. This represents the first step in the process of determining the structure of complex I, and in understanding its mechanism

of energy transduction. The roles of the numerous supernumerary subunits, especially those with sequences which suggest they have properties distinct from the electron and proton transfer activities of complex I, demand further investigation. Complex I from bovine heart mitochondria continues to provide a template for the human enzyme, which has an increasing medical importance as neuromuscular diseases are linked to mutations in both the mitochondrially and nuclear encoded subunits. Therefore, complex I from bovine heart mitochondria will continue to contribute significantly to future studies of the structure, function, and biomedical relevance of complex I.

Acknowledgements

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References

[1] J.E. Walker, The NADH:ubiquinone oxidoreductase (complex I) of respiratory chains, Q. Rev. Biophys. 25 (1992) 253–324.
[2] H. Weiss, T. Friedrich, G. Hofhaus, D. Preis, The respiratory-chain NADH dehydrogenase (complex I) of mitochondria, Eur. J. Biochem. 197 (1991) 563–576.
[3] J. Smeitink, R. Sengers, F. Trijbels, L. van den Heuvel, Human NADH:ubiquinone oxidoreductase, J. Bioenerg. Biomembr. 33 (2001) 259–266.
[4] T. Yagi, T. Yano, S. DiBernardo, A. Matsuno-Yagi, Procaryotic complex I (NDH-1), an overview, Biochim. Biophys. Acta 1364 (1998) 125–133.
[5] T. Friedrich, The NADH:ubiquinone oxidoreductase (complex I) from *Escherichia coli*, Biochim. Biophys. Acta 1364 (1998) 134–146.
[6] M. Saraste, Oxidative phosphorylation at the *fin de siècle*, Science 283 (1999) 1488–1493.
[7] B.E. Schultz, S.I. Chan, Structures and proton-pumping strategies of mitochondrial respiratory enzymes, Annu. Rev. Biophys. Biomol. Struct. 30 (2001) 23–65.
[8] M. Wikström, Two protons are pumped from the mitochondrial matrix per electron transferred between NADH and ubiquinone, FEBS Lett. 169 (1984) 300–304.
[9] G.C. Brown, M.D. Brand, Proton/electron stoichiometry of mitochondrial complex I estimated from the equilibrium thermodynamic force ratio, Biochem. J. 252 (1988) 473–479.
[10] N. Grigorieff, Structure of the respiratory NADH:ubiquinone oxidoreductase (complex I), Curr. Opin. Struct. Biol. 9 (1999) 476–483.
[11] V. Guénebaut, A. Schlitt, H. Weiss, K. Leonard, T. Friedrich, Consistent structure between bacterial and mitochondrial NADH:ubiquinone oxidoreductase (complex I), J. Mol. Biol. 276 (1998) 105–112.
[12] J.E. Walker, J.M. Skehel, S.K. Buchanan, Structural analysis of NADH:ubiquinone oxidoreductase from bovine heart mitochondria, Methods Enzymol. 260 (1995) 14–34.
[13] I.M. Fearnley, J. Carroll, R.J. Shannon, M.J. Runswick, J.E. Walker, J. Hirst, GRIM-19, a cell death regulatory gene product, is a subunit of bovine mitochondrial NADH:ubiquinone oxidoreductase (complex I), J. Biol. Chem. 276 (2001) 38345–38348.
[14] J. Carroll, R.J. Shannon, I.M. Fearnley, J.E. Walker, J. Hirst, Definition of the nuclear encoded protein composition of bovine heart mitochondrial complex I: identification of two new subunits, J. Biol. Chem. 277 (2002) 50311–50317.
[15] J. Carroll, I.M. Fearnley, R.J. Shannon, J. Hirst, J.E. Walker, Analysis of the subunit composition of complex I from bovine heart mitochondria, Mol. Cell. Proteomics 2 (2003) 117–126.
[16] J.E. Walker, J.M. Arizmendi, A. Dupuis, I.M. Fearnley, M. Finel, S.M. Medd, S.J. Pilkington, M.J. Runswick, J.M. Skehel, Sequences of 20 subunits of NADH:ubiquinone oxidoreductase from bovine heart mitochondria, J. Mol. Biol. 226 (1992) 1051–1072.
[17] A. Chomyn, P. Mariottini, M.W.J. Cleeter, C.I. Ragan, A. Matsuno-Yagi, Y. Hatefi, R.F. Doolittle, G. Attardi, Six unidentified reading frames of human mitochondrial DNA encode components of the respiratory-chain NADH dehydrogenase, Nature 314 (1985) 592–597.
[18] A. Chomyn, M.W.J. Cleeter, C.I. Ragan, M. Riley, R.F. Doolittle, G. Attardi, URF6, last unidentified reading frame of human mtDNA, codes for an NADH dehydrogenase subunit, Science 234 (1986) 614–618.
[19] J. Smeitink, L. van den Heuvel, S. DiMauro, The genetics and pathology of oxidative phosphorylation, Nat. Rev. Genet. 2 (2001) 342–352.
[20] A. Videira, M. Duarte, On complex I and other NADH:ubiquinone reductases of *Neurospora crassa* mitochondria, J. Bioenerg. Biomembr. 33 (2001) 197–203.
[21] A. Videira, M. Duarte, From NADH to ubiquinone in *Neurospora* mitochondria, Biochim. Biophys. Acta 1555 (2002) 187–191.
[22] R. Djafarzadeh, S. Kerscher, K. Zwicker, M. Radermacher, M. Lindahl, H. Schägger, U. Brandt, Biophysical and structural characterization of proton-translocating NADH–dehydrogenase (complex I) from the strictly aerobic yeast *Yarrowia lipolytica*, Biochim. Biophys. Acta 1459 (2000) 230–238.
[23] A.G. Rasmusson, V. Heiser, E. Zabaleta, A. Brennicke, L. Grohmann, Physiological, biochemical and molecular aspects of mitochondrial complex I in plants, Biochim. Biophys. Acta 1364 (1998) 101–111.
[24] B. Combettes, J.-M. Grienenberger, Analysis of wheat mitochondrial complex I purified by a one-step immunoaffinity chromatography, Biochimie 81 (1999) 645–653.
[25] T. Yano, S.S. Chu, V.D. Sled, T. Ohnishi, T. Yagi, The proton-translocating NADH–quinone oxidoreductase (NDH-1) of thermophilic bacterium *Thermus thermophilus* HB-8, J. Biol. Chem. 272 (1997) 4201–4211.
[26] X. Xu, A. Matsuno-Yagi, T. Yagi, DNA sequencing of the seven remaining structural genes of the gene cluster encoding the energy-transducing NADH–quinone oxidoreductase of *Paracoccus denitrificans*, Biochemistry 32 (1993) 968–981.
[27] U. Weidner, S. Geier, A. Ptock, T. Friedrich, H. Leif, H. Weiss, The gene locus of the proton-translocating NADH:ubiquinone oxidoreductase in *Escherichia coli*, J. Mol. Biol. 233 (1993) 109–122.
[28] J. Murray, B. Zhang, S.W. Taylor, D. Oglesbee, E. Fahy, M.F. Marusich, S.S. Ghosh, R.A. Capaldi, The subunit composition of the human NADH dehydrogenase obtained by rapid one-step immunopurification, J. Biol. Chem. 278 (2003) 13619–13622.
[29] S.W. Taylor, E. Fahy, B. Zhang, G.M. Glenn, D.E. Warnock, S. Wiley, A.N. Murphy, S.P. Gaucher, R.A. Capaldi, B.W. Gibson, S.S. Ghosh, Characterization of the human heart mitochondrial proteome, Nat. Biotechnol. 21 (2003) 281–286.
[30] T. Tsukihara, H. Aoyama, E. Yamashita, T. Tomizaki, H. Yamaguchi, K. Shinzawa-Itoh, R. Nakashima, R. Yaono, S. Yoshikawa, The whole structure of the 13-subunit oxidized cytochrome *c* oxidase at 2.8 Å, Science 272 (1996) 1136–1144.
[31] E. Sedlák, N.C. Robinson, Phospholipase A_2 digestion of cardiolipin bound to bovine cytochrome *c* oxidase alters both activity and quaternary structure, Biochemistry 38 (1999) 14966–14972.
[32] B. Polevoda, F. Sherman, N^{α}-terminal acetylation of eukaryotic proteins, J. Biol. Chem. 275 (2000) 36479–36482.
[33] T.A. Farazi, G. Waksman, J.I. Gordon, The biology and enzymol-

ogy of protein N-myristoylation, J. Biol. Chem. 276 (2001) 39501–39504.
[34] M. Finel, J.M. Skehel, S.P.J. Albracht, I.M. Fearnley, J.E. Walker, Resolution of NADH:ubiquinone oxidoreductase from bovine heart mitochondria into two subcomplexes, one of which contains the redox centres of the enzyme, Biochemistry 31 (1992) 11425–11434.
[35] L.A. Sazanov, S.Y. Peak-Chew, I.M. Fearnley, J.E. Walker, Resolution of the membrane domain of bovine complex I into subcomplexes: implications for the structural organization of the enzyme, Biochemistry 39 (2000) 7229–7235.
[36] M. Finel, A.S. Majander, J. Tyynelä, A.M.P. De Jong, S.P.J. Albracht, M. Wikström, Isolation and characterisation of subcomplexes of the mitochondrial NADH:ubiquinone oxidoreductase (complex I), Eur. J. Biochem. 226 (1994) 237–242.
[37] Y.M. Galante, Y. Hatefi, Purification and molecular and enzymic properties of mitochondrial NADH dehydrogenase, Arch. Biochem. Biophys. 192 (1979) 559–568.
[38] C.I. Ragan, Y.M. Galante, Y. Hatefi, T. Ohnishi, Resolution of mitochondrial NADH dehydrogenase and isolation of two iron–sulfur proteins, Biochemistry 21 (1982) 590–594.
[39] S.J. Pilkington, J.M. Skehel, R.B. Gennis, J.E. Walker, Relationship between mitochondrial NADH–ubiquinone reductase and a bacterial NAD-reducing hydrogenase, Biochemistry 30 (1991) 2166–2175.
[40] M. Braun, S. Bungert, T. Friedrich, Characterization of the overproduced NADH dehydrogenase fragment of the NADH:ubiquinone oxidoreductase (complex I) from *Escherichia coli*, Biochemistry 37 (1998) 1861–1867.
[41] A.C. Gemperli, P. Dimroth, J. Steuber, Sodium ion cycling mediates energy coupling between complex I and ATP synthase, Proc. Natl. Acad. Sci. U. S. A. 100 (2003) 839–844.
[42] J. Hirst, The dichotomy of complex I: a sodium ion pump or a proton pump, Proc. Natl. Acad. Sci. U. S. A. 100 (2003) 773–775.
[43] T. Yano, V.D. Sled, T. Ohnishi, T. Yagi, Identification of amino acid residues associated with the [2Fe–2S] cluster of the 25 kDa (NQO2) subunit of the proton-translocating NADH–quinone oxidoreductase of *Paracoccus denitrificans*, FEBS Lett. 354 (1994) 160–164.
[44] J. Meyer, Ferredoxins of the third kind, FEBS Lett. 509 (2001) 1–5.
[45] Y. Zu, S. Di Bernardo, T. Yagi, J. Hirst, Redox properties of the [2Fe–2S] center in the 24 kDa (NQO2) subunit of NADH: ubiquinone oxidoreductase (complex I), Biochemistry 41 (2002) 10056–10069.
[46] T. Yano, V.D. Sled, T. Ohnishi, T. Yagi, Expression and characterization of the flavoprotein subcomplex composed of 50-kDa (NQO1) and 25-kDa (NQO2) subunits of the proton-translocating NADH–quinone oxidoreductase of *Paracoccus denitrificans*, J. Biol. Chem. 271 (1996) 5907–5913.
[47] S. Chen, R.J. Guillory, Studies on the interaction of arylazido-β-alanyl NAD^+ with the mitochondrial NADH dehydrogenase, J. Biol. Chem. 256 (1981) 8318–8323.
[48] M.K. Johnson, Iron–sulfur proteins, in: R.B. King (Ed.), Encyclopaedia of Inorganic Chemistry, Wiley, Chichester, 1994, pp. 1896–1915.
[49] H. Matsubara, K. Saeki, Structural and functional diversity of ferredoxins and related proteins, Adv. Inorg. Chem. 38 (1992) 223–280.
[50] V.D. Sled, N.I. Rudnitzky, Y. Hatefi, T. Ohnishi, Thermodynamic analysis of flavin in mitochondrial NADH:ubiquinone oxidoreductase (complex I), Biochemistry 33 (1994) 10069–10075.
[51] T. Ohnishi, Iron–sulfur clusters/semiquinones in complex I, Biochim. Biophys. Acta 1364 (1998) 186–206.
[52] D. Preis, U. Weidner, C. Conzen, J.E. Azevedo, U. Nehls, D. Röhlen, J. Van der Pas, U. Sackmann, R. Schneider, S. Werner, H. Weiss, Primary structures of two subunits of NADH:ubiquinone reductase from *Neurospora crassa* concerned with NADH-oxidation. Relationship to a soluble NAD-reducing hydrogenase of *Alcaligenes eutrophus*, Biochim. Biophys. Acta 1090 (1991) 133–138.
[53] T. Yano, T. Yagi, V.D. Sled, T. Ohnishi, Expression and characterization of the 66-kilodalton (NQO3) iron–sulfur subunit of the proton-translocating NADH–quinone oxidoreductase of *Paracoccus denitrificans*, J. Biol. Chem. 270 (1995) 18264–18270.
[54] T. Yano, J. Sklar, E. Nakamaru-Ogiso, Y. Takahashi, T. Yagi, T. Ohnishi, Characterization of cluster N5 as a fast-relaxing [4Fe–4S] cluster in the Nqo3 subunit of the proton-translocating NADH–ubiquinone oxidoreductase from *Paracoccus denitrificans*, J. Biol. Chem. 278 (2003) 15514–15522.
[55] E. Nakamaru-Ogiso, T. Yano, T. Ohnishi, T. Yagi, Characterization of the iron–sulfur cluster coordinated by a cysteine cluster motif ($CXXCXXXCX_{27}C$) in the Nqo3 subunit in the proton-translocating NADH–quinone oxidoreductase (NDH-1) of *Thermus thermophilus* HB-8, J. Biol. Chem. 277 (2002) 1680–1688.
[56] A. Dupuis, J.M. Skehel, J.E. Walker, A homologue of a nuclear-coded iron–sulfur protein subunit of bovine mitochondrial complex I is encoded in chloroplast genomes, Biochemistry 30 (1991) 2954–2960.
[57] T. Yano, S. Magnitsky, V.D. Sled, T. Ohnishi, T. Yagi, Characterization of the putative 2 × [4Fe–4S]-binding NQO9 subunit of the proton-translocating NADH–quinone oxidoreductase (NDH-1) of *Paracoccus denitrificans*, J. Biol. Chem. 274 (1999) 28598–28605.
[58] W.J. Ingledew, T. Ohnishi, An analysis of some thermodynamic properties of iron–sulphur centres in site I of mitochondria, Biochem. J. 186 (1980) 111–117.
[59] T. Rasmussen, D. Scheide, B. Brors, L. Kintscher, H. Weiss, T. Friedrich, Identification of two tetranuclear FeS clusters on the ferredoxin-type subunit of NADH:ubiquinone oxidoreductase (complex I), Biochemistry 40 (2001) 6124–6131.
[60] S. Magnitsky, L. Toulokhonova, T. Yano, V.D. Sled, C. Hägerhäll, V.G. Grivennikova, D.S. Burbaev, A.D. Vinogradov, T. Ohnishi, EPR characterization of ubisemiquinones and iron–sulfur cluster N2, central components of the energy coupling in the NADH–ubiquinone oxidoreductase (complex I) in situ, J. Bioenerg. Biomembr. 34 (2002) 193–208.
[61] A. Dupuis, I. Prieur, J. Lunardi, Toward a characterization of the connecting module of complex I, J. Bioenerg. Biomembr. 33 (2001) 159–168.
[62] F. Schuler, T. Yano, S. Di Bernardo, T. Yagi, V. Yankovskaya, T.P. Singer, J.E. Casida, NADH–quinone oxidoreductase: PSST subunit couples electron transfer from iron–sulfur cluster N2 to quinone, Proc. Natl. Acad. Sci. U. S. A. 96 (1999) 4149–4153.
[63] P. Ahlers, K. Zwicker, S. Kerscher, U. Brandt, Function of conserved acidic residues in the PSST homologue of complex I (NADH:ubiquinone oxidoreductase) from *Yarrowia lipolytica*, J. Biol. Chem. 275 (2000) 23577–23582.
[64] N. Kashani-Poor, K. Zwicker, S. Kerscher, U. Brandt, A central functional role for the 49-kDa subunit within the catalytic core of mitochondrial complex I, J. Biol. Chem. 276 (2001) 24082–24087.
[65] S.P.J. Albracht, Intimate relationships of the large and small subunits of all nickel hydrogenases with two nuclear-encoded subunits of mitochondrial NADH:ubiquinone oxidoreductase, Biochim. Biophys. Acta 1144 (1993) 221–224.
[66] S. Iwata, C. Ostermeier, B. Ludwig, H. Michel, Structure at 2.8 Å resolution of cytochrome *c* oxidase from *Paracoccus denitrificans*, Nature 376 (1995) 660–669.
[67] E.A. Berry, M. Guergova-Kuras, L.-S. Huang, A.R. Crofts, Structure and function of cytochrome *bc* complexes, Annu. Rev. Biochem. 69 (2000) 1005–1075.
[68] B. Kadenbach, S. Arnold, A second mechanism of respiratory control, FEBS Lett. 447 (1999) 131–134.
[69] M. Emmermann, H.-P. Braun, M. Arretz, U.K. Schmitz, Characterization of the bifunctional cytochrome *c* reductase-processing peptidase complex from potato mitochondria, J. Biol. Chem. 268 (1993) 18936–18942.
[70] U. Brandt, L. Yu, C.-A. Yu, B.L. Trumpower, The mitochondrial targeting presequence of the Rieske iron–sulfur protein is processed

in a single step after insertion into the cytochrome bc_1 complex in mammals and retained as a subunit in the complex, J. Biol. Chem. 268 (1993) 8387–8390.
[71] K. Deng, S.K. Shenoy, S.-C. Tso, L. Yu, C.-A. Yu, Reconstitution of mitochondrial processing peptidase from the core proteins (subunits I and II) of bovine heart mitochondrial cytochrome bc_1 complex, J. Biol. Chem. 276 (2001) 6499–6505.
[72] I.M. Fearnley, J.E. Walker, Conservation of sequences of subunits of mitochondrial complex I and their relationships with other proteins, Biochim. Biophys. Acta 1140 (1992) 105–134.
[73] M. Yamaguchi, G.I. Belogrudov, Y. Hatefi, Mitochondrial NADH–ubiquinone oxidoreductase (complex I)—effects of substrates on the fragmentation of subunits by trypsin, J. Biol. Chem. 273 (1998) 8094–8098.
[74] M. Yamaguchi, G.I. Belogrudov, A. Matsuno-Yagi, Y. Hatefi, The multiple nicotinamide nucleotide-binding subunits of bovine heart mitochondrial NADH:ubiquinone oxidoreductase (complex I), Eur. J. Biochem. 267 (2000) 329–336.
[75] U. Schulte, V. Haupt, K. Abelmann, W. Fecke, B. Brors, T. Rasmussen, T. Friedrich, H. Weiss, A reductase/isomerase subunit of mitochondrial NADH:ubiquinone oxidoreductase (complex I) carries an NADPH and is involved in the biogenesis of the complex, J. Mol. Biol. 292 (1999) 569–580.
[76] M.E. Baker, W.N. Grundy, C.P. Elkan, A common ancestor for a subunit in the mitochondrial proton-translocating NADH:ubiquinone oxidoreductase (complex I) and short-chain dehydrogenases/reductases, Cell. Mol. Life Sci. 55 (1999) 450–455.
[77] H. Jörnvall, B. Persson, M. Krook, S. Atrian, R. Gonzàlez-Duarte, J. Jeffrey, D. Ghosh, Short-chain dehydrogenases/reductases (SDR), Biochemistry 34 (1995) 6003–6013.
[78] H. Jörnvall, J.-O. Höög, B. Persson, SDR and MDR: completed genome sequences show these protein families to be large, of old origin and of complex nature, FEBS Lett. 445 (1999) 261–264.
[79] M. Fisher, J.T.M. Kroon, W. Martindale, A.R. Stuitje, A.R. Slabas, J.B. Rafferty, The X-ray structure of *Brassica napus* β-keto acyl carrier protein reductase and its implications for substrate binding and catalysis, Structure 8 (2000) 339–347.
[80] T. Friedrich, B. Brors, P. Hellwig, L. Kintscher, T. Rasmussen, D. Scheide, U. Schulte, W. Mäntele, H. Weiss, Characterization of two novel redox groups in the respiratory NADH:ubiquinone oxidoreductase (complex I), Biochim. Biophys. Acta 1459 (2000) 305–309.
[81] M.J. Runswick, I.M. Fearnley, J.M. Skehel, J.E. Walker, Presence of an acyl carrier protein in NADH:ubiquinone oxidoreductase from bovine heart mitochondria, FEBS Lett. 286 (1991) 121–124.
[82] U. Sackmann, R. Zensen, D. Röhlen, U. Jahnke, H. Weiss, The acyl-carrier protein in *Neurospora crassa* mitochondria is a subunit of NADH:ubiquinone reductase (complex I), Eur. J. Biochem. 200 (1991) 463–469.
[83] S. Brody, S. Mikolajczyk, *Neurospora* mitochondria contain an acyl-carrier protein, Eur. J. Biochem. 173 (1988) 353–359.
[84] S. Mikolajczyk, S. Brody, De novo fatty acid synthesis mediated by acyl-carrier protein in *Neurospora crassa* mitochondria, Eur. J. Biochem. 187 (1990) 431–437.
[85] R. Zensen, H. Husmann, R. Schneider, T. Peine, H. Weiss, De novo synthesis and desaturation of fatty acids at the mitochondrial acyl-carrier protein, a subunit of NADH:ubiquinone oxidoreductase in *Neurospora crassa*, FEBS Lett. 310 (1992) 179–181.
[86] R. Schneider, M. Massow, T. Lisowsky, H. Weiss, Different respiratory-defective phenotypes of *Neurospora crassa* and *Saccharomyces cerevisiae* after inactivation of the gene encoding the mitochondrial acyl carrier protein, Curr. Genet. 29 (1995) 10–17.
[87] R. Schneider, B. Brors, M. Massow, H. Weiss, Mitochondrial fatty acid synthesis: a relic of endosymbiontic origin and a specialized means for respiration, FEBS Lett. 407 (1997) 249–252.
[88] S. Brody, C. Oh, U. Hoja, E. Schweizer, Mitochondrial acyl carrier protein is involved in lipoic acid synthesis in *Saccharomyces cerevisiae*, FEBS Lett. 408 (1997) 217–220.
[89] S.W. Jordan, J.E. Cronan, A new metabolic link. The acyl carrier protein of lipid synthesis donates lipoic acid to the pyruvate dehydrogenase complex in *Escherichia coli* and mitochondria, J. Biol. Chem. 272 (1997) 17903–17906.
[90] H. Wada, D. Shintani, J. Ohlrogge, Why do mitochondria synthesize fatty acids? Evidence for involvement in lipoic acid production, Proc. Natl. Acad. Sci. U. S. A. 94 (1997) 1591–1596.
[91] V. Gueguen, D. Macherel, M. Jaquinod, R. Douce, J. Bourguignon, Fatty acid and lipoic acid biosynthesis in higher plant mitochondria, J. Biol. Chem. 275 (2000) 5016–5025.
[92] S. Papa, A.M. Sardanelli, S. Scacco, V. Petruzzella, Z. Technikova-Dobrova, R. Vergari, A. Signorile, The NADH:ubiquinone oxidoreductase (complex I) of the mammalian respiratory chain and the cAMP cascade, J. Bioenerg. Biomembr. 34 (2002) 1–10.
[93] H.C. Au, B.B. Seo, A. Matsuno-Yagi, T. Yagi, I.E. Scheffler, The NDUFA1 gene product (MWFE protein) is essential for activity of complex I in mammalian mitochondria, Proc. Natl. Acad. Sci. U. S. A. 96 (1999) 4354–4359.
[94] I.E. Scheffler, N. Yadava, Molecular genetics of the mammalian NADH–ubiquinone oxidoreductase, J. Bioenerg. Biomembr. 33 (2001) 243–250.
[95] N. Yadava, P. Potluri, E.N. Smith, A. Bisevac, I.E. Scheffler, Species-specific and mutant MWFE proteins, J. Biol. Chem. 277 (2002) 21221–21230.
[96] J.E. Angell, D.J. Lindner, P.S. Shapiro, E.R. Hofmann, D.V. Kalvakolanu, Identification of GRIM-19, a novel cell death-regulatory gene induced by the interferon-β and retinoic acid combination, using a genetic approach, J. Biol. Chem. 275 (2000) 33416–33426.
[97] N.V. Chidambaram, J.E. Angell, W. Ling, E.R. Hofmann, D.V. Kalvakolanu, Chromosomal localization of human GRIM-19, a novel IFN-β and retinoic acid-activated regulator of cell death, J. Interferon Cytokine Res. 20 (2000) 661–665.
[98] T. Seo, D. Lee, Y.S. Shim, J.E. Angell, N.V. Chidambaram, D.V. Kalvakolanu, J. Choe, Viral interferon regulatory factor 1 of Kaposi's sarcoma-associated herpesvirus interacts with a cell death regulator, GRIM-19, and inhibits interferon/retinoic acid-induced cell death, J. Virol. 76 (2002) 8789–8807.
[99] C. Lufei, J. Ma, G. Huang, T. Zhang, V. Novotny-Diermayr, C.T. Ong, X. Cao, GRIM-19, a death-regulatory gene product, suppresses Stat3 activity via functional interaction, EMBO J. 22 (2003) 1325–1335.
[100] J. Hu, J.E. Angell, J. Zhang, X. Ma, T. Seo, A. Raha, J. Hayashi, J. Choe, D.V. Kalvakolanu, Characterization of monoclonal antibodies against GRIM-19, a novel IFN-β and retinoic acid-activated regulator of cell death, J. Interferon Cytokine Res. 22 (2002) 1017–1026.
[101] B.J. Soltys, R.S. Gupta, Mitochondrial-matrix proteins at unexpected locations: are they exported? Trends Biochem. Sci. 24 (1999) 174–177.
[102] L.Y. Li, X. Luo, X. Wang, Endonuclease G is an apoptotic DNase when released from mitochondria, Nature 412 (2001) 95–99.
[103] N. Hail, R. Lotan, Mitochondrial respiration is uniquely associated with the prooxidant and apoptotic effects of *N*-(4-hydroxyphenyl)-retinamide, J. Biol. Chem. 276 (2001) 45614–45621.
[104] J.L.C.M. Loeffen, R.H. Triepels, L.P. van den Heuvel, M. Schuelke, C.A.F. Buskens, R.J.P. Smeets, F. Trijbels, J. Smeitink, cDNA of eight nuclear encoded subunits of NADH:ubiquinone oxidoreductase: human complex I cDNA characterization completed, Biochem. Biophys. Res. Commun. 253 (1998) 415–422.
[105] M.J. Runswick, R.B. Gennis, I.M. Fearnley, J.E. Walker, Mitochondrial NADH:ubiquinone reductase: complementary DNA sequence of the import precursor of the bovine 75-kDa subunit, Biochemistry 28 (1989) 9452–9459.
[106] S.D. Patel, R. Aebersold, G. Attardi, cDNA-derived amino acid sequence of the NADH-binding 51-kDa subunit of the bovine respiratory NADH dehydrogenase reveals striking similarities to a bac-

terial NAD^+-reducing hydrogenase, Proc. Natl. Acad. Sci. U. S. A. 88 (1991) 4225–4229.
[107] I.M. Fearnley, M.J. Runswick, J.E. Walker, A homologue of the nuclear coded 49 kd subunit of bovine mitochondrial NADH–ubiquinone reductase is coded in chloroplast DNA, EMBO J. 8 (1989) 665–672.
[108] S.J. Pilkington, J.M. Skehel, J.E. Walker, The 30-kilodalton subunit of bovine mitochondrial complex I is homologous to a protein coded in chloroplast DNA, Biochemistry 30 (1991) 1901–1908.
[109] S.J. Pilkington, J.E. Walker, Mitochondrial NADH–ubiquinone reductase: complementary DNA sequences of import precursors of the bovine and human 24-kDa subunit, Biochemistry 28 (1989) 3257–3264.
[110] A. Chomyn, S.S.-A. Tsai Lai, cDNA of the 24 kDa subunit of the bovine respiratory chain NADH dehydrogenase: high sequence conservation in mammals and tissue-specific and growth-dependent expression, Curr. Genet. 16 (1989) 117–125.
[111] J.M. Arizmendi, M.J. Runswick, J.M. Skehel, J.E. Walker, NADH:ubiquinone oxidoreductase from bovine heart mitochondria. A fourth nuclear encoded subunit with a homologue encoded in chloroplast genomes, FEBS Lett. 301 (1992) 237–242.
[112] I.M. Fearnley, M. Finel, J.M. Skehel, J.E. Walker, NADH:ubiquinone oxidoreductase from bovine heart mitochondria. cDNA sequences of the import precursors of the nuclear-encoded 39 kDa and 42 kDa subunits, Biochem. J. 278 (1991) 821–829.
[113] R. Masui, S. Wakabayashi, H. Matsubara, Y. Hatefi, The amino acid sequences of two 13 kDa polypeptides and partial amino acid sequence of 30 kDa polypeptide of complex I from bovine heart mitochondria: possible location of iron–sulfur clusters, J. Biochem. 109 (1991) 534–543.
[114] J.M. Skehel, S.J. Pilkington, M.J. Runswick, I.M. Fearnley, J.E. Walker, NADH:ubiquinone oxidoreductase from bovine heart mitochondria: complementary DNA sequence of the import precursor of the 10 kDa subunit of the flavoprotein fragment, FEBS Lett. 282 (1991) 135–138.
[115] R. Masui, S. Wakabayashi, H. Matsubara, Y. Hatefi, The amino acid sequence of the 9 kDa polypeptide and partial amino acid sequence of the 20 kDa polypeptide of mitochondrial NADH:ubiquinone oxidoreductase, J. Biochem. 110 (1991) 575–582.
[116] A. Dupuis, J.M. Skehel, J.E. Walker, NADH:ubiquinone oxidoreductase from bovine mitochondria; cDNA sequence of a 19 kDa cysteine-rich subunit, Biochem. J. 277 (1991) 11–15.
[117] J.M. Skehel, I.M. Fearnley, J.E. Walker, NADH:ubiquinone oxidoreductase from bovine heart mitochondria: sequence of a novel 17.2-kDa subunit, FEBS Lett. 438 (1998) 301–305.
[118] J.M. Arizmendi, J.M. Skehel, M.J. Runswick, I.M. Fearnley, J.E. Walker, Complementary DNA sequences of two 14.5 kDa subunits of NADH:ubiquinone oxidoreductase from bovine heart mitochondria; completion of the primary structure of the enzyme? FEBS Lett. 313 (1992) 80–84.
[119] G.E. Tusnády, I. Simon, Principles governing amino acid composition of integral membrane proteins: applications to topology prediction, J. Mol. Biol. 283 (1998) 489–506.
[120] A. Krogh, B. Larsson, G. von Heijne, E.L.L. Sonnhammer, Predicting transmembrane protein topology with a hidden Markov model: application to complete genomes, J. Mol. Biol. 305 (2001) 567–580.