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MINI-REVIEW

Complexity and Tissue Specificity of the Mitochondrial Respiratory Chain

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Abstract

There is a renewed interest in the structure and functioning of the mitochondrial respiratory chain with the realization that a number of genetic disorders result from defects in mitochondrial electron transfer. These so-called mitochondrial myopathies include diseases of muscle, heart, and brain. The respiratory chain can be fractionated into four large multipeptide complexes, an NADH ubiquinone reductase (complex I), succinate ubiquinone reductase (complex II), ubiquinol oxidoreductase (complex III), and cytochrome *c* oxidase (complex IV). Mitochondrial myopathies involving each of these complexes have been described. This review summarizes compositional and structural data on the respiratory chain proteins and describes the arrangement of these complexes in the mitochondrial inner membrane. This biochemical information is provided as a framework for the diagnosis and molecular characterization of mitochondrial diseases.

Key Words: Mitochondrial myopathies; electron transfer chain; tissue specificity; electron microscopy; protein sequences.

Introduction

For many years, study of the mitochondrial inner membrane was the province of the bioenergeticist interested in the mechanism of oxidative phosphorylation (reviewed in Wainio, 1970; Tzagoloff, 1982). The number of laboratories working on aspects of the structure of this membrane has expanded significantly since it became possible to examine the biogenesis of the mitochondrion and the targeting of proteins to this organelle (reviewed in Schatz and Butow, 1983).

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More recently, the structure of the mitochondrial inner membrane has become of interest to clinicians, geneticists, and biochemists working on mitochondrial myopathies, a group of diseases in which defective mitochondrial metabolism causes muscle weakness, exercise intolerance, and, in some cases, neurological, renal or cardiac dysfunction (reviewed in DiMauro *et al.*, 1985; Morgan-Hughes, 1982; Morgan-Hughes and Landon, 1983). This review is written with this latter group of workers in mind, and provides an overview of the structure of the mammalian mitochondrial inner membrane with particular focus on the composition of the respiratory chain complexes.

Many of the mitochondrial myopathies described so far involve complexes I, III and cytochrome *c* oxidase, and a knowledge of the structure of these multicomponent complexes is a necessary prerequisite to understanding of the molecular basis of defects responsible for these diseases. The sequences of the various subunits of complexes III and cytochrome *c* oxidase from beef heart are included as a reference source, as are data for the human enzyme where available.

General Organization of Respiratory Chain Components in the Mitochondrial Inner Membrane

It was shown more than 25 years ago that the mitochondrial electron transfer chain is organized into four large multipolypeptide complexes, an NADH ubiquinone reductase (complex I), succinate ubiquinone reductase (complex II), ubiquinol cytochrome *c* oxidoreductase (complex III), and cytochrome *c* oxidase (complex IV) (reviewed in Hatefi, 1976) (Fig. 1). These

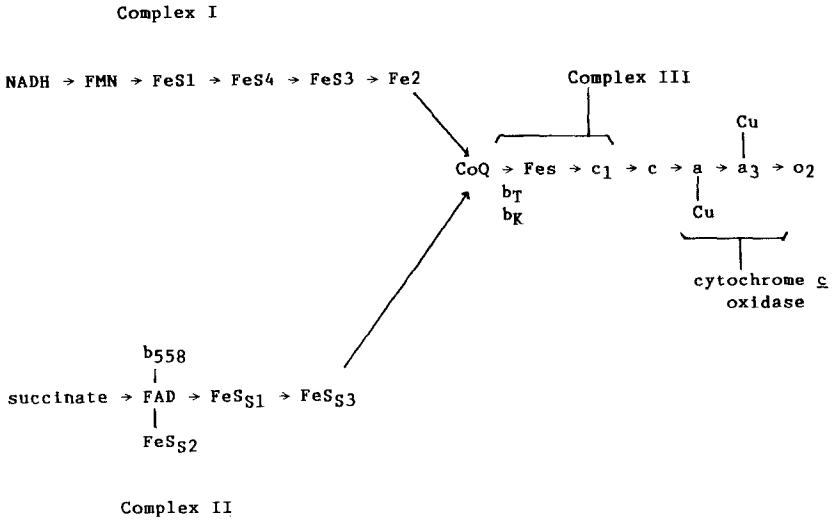


Fig. 1. Schematic of the mammalian mitochondrial respiratory chain.

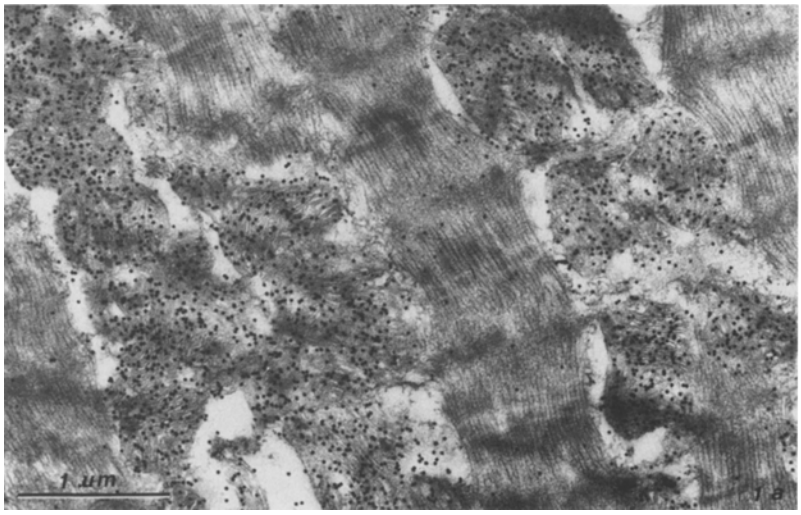


Fig. 2. Electron micrograph of thin sectioned beef heart tissue labeled for cytochrome *c* oxidase by antibody against the holoenzyme and protein A conjugated with colloidal gold [reproduced from Kim *et al.* (1987) with permission.]

four electron transfer complexes comprise most of the inner membrane of mitochondria from all mammalian tissues. Table I gives the relative amounts of these components in the inner membrane of beef heart mitochondria, along with the amounts of other major proteins including the ATP synthase and ADP-ATP translocase. The ratio of respiratory chain complexes based on these compositional data is 1 complex I; 2 complex II; 3 complex III; 6 cytochrome *c*; 6 cytochrome *c* oxidase.

Recently, the ratio of electron transfer complexes in bovine heart and human muscle mitochondria has been examined by immunoelectron microscopy, using antibodies generated to bovine proteins (Kim *et al.*, 1987). The labeling of bovine heart tissue with anti-cytochrome *c* oxidase antibodies conjugated with protein A colloidal gold is shown in Fig. 2. The cytochrome *c* oxidase molecules are seen to be randomly dispersed in the mitochondrial inner membrane. This immunolabeling method gave ratios of 0.6 : 3 : 6 for complex I, III, and IV, respectively, in both beef heart and human muscle mitochondria.

The monomer molecular weights of the respiratory chain complexes are listed in Table I. Both complex III and cytochrome *c* oxidase form stable dimers in detergent solution (Leonard *et al.*, 1981b; Robinson and Capaldi, 1977), and these complexes probably exist as dimers in the mitochondrial inner membrane (reviewed in Capaldi, 1982). The distribution of the redox components in the inner membrane is shown schematically in Fig. 3, with the

Table I. Protein Components of the Bovine Heart Mitochondrial Inner Membrane

Component	Concentration range (nmol/mg protein)	Reference	Molecular weight of monomer	Reference
Complex I	0.06–0.13	Smith *et al.*, 1980; Albracht *et al.*, 1979	700,000	Galante and Hatefi, 1979
Complex II	0.19	Vinogradov and King, 1979	200,000	Hatefi and Stiggel, 1976
Complex III	0.25–0.53	Smith *et al.*, 1980; Nelson and Mendel-Hartvig, 1977	300,000	von Jagow *et al.*, 1977
Cytochrome *c* oxidase	0.6–1.00	Azzone *et al.*, 1979; Vanneste, 1966	208,000	[a]
Cytochrome *c*	0.8–1.02	Vanneste, 1966; Vinogradov and King, 1979	12,000	Dickerson and Timkovich, 1975
ATP synthase	0.52–0.54	Bertina *et al.*, 1973; Slater, 1974	550,000	Kagawa *et al.*, 1976
ADP–ATP translocase	3.4–4.6	Vignais *et al.*, 1973; Klingenberg *et al.*, 1975	30,000	Klingenberg *et al.*, 1975
Transhydrogenase	0.05	Anderson *et al.*, 1981	100,000	Anderson *et al.*, 1981; Phelps and Hatefi, 1981
Ubiquinone	6–8	Vinogradov and King, 1979; Slater, 1974		
Phospholipid	440–587	Fleischer *et al.*, 1961; Krebs *et al.*, 1979		

[a] Calculated from the sequence data for the thirteen subunits of the enzyme.

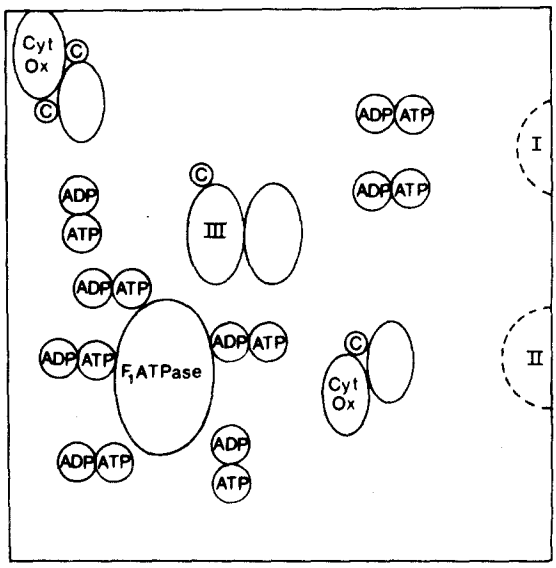


Fig. 3. Schematic of the density of the respiratory chain proteins in the mammalian mitochondrial inner membrane [reproduced from Capaldi (1982) with permission.]

individual complexes drawn roughly to scale. The area per complex III dimer is around 200,000 $Å^2$, as calculated by dividing the surface area of the inner membrane (2×10^6 cm^2/g protein) by the number of complex III dimers present (Table I). This gives an average distance between a complex I and a complex III of 310 Å and between a cytochrome *c* oxidase and a complex III of 225 Å. The diffusion of the small "mobile" carriers, ubiquinone and cytochrome *c*, is fast in/on the mitochondrial inner membrane [diffusion constant 10^{-8} cm^2/s (Kawato *et al.*, 1980)], and it is unlikely that intracomplex electron transfer is the rate-limiting step, even if the large respiratory chain complexes are hindered from diffusion by the highly viscous nature of the "protein solution" in the matrix (Capaldi, 1982).

Complex I (Brink *et al.*, 1987; Leonard *et al.*, 1987), complex III (Leonard *et al.*, 1981), and cytochrome *c* oxidase (Henderson *et al.*, 1977; Fuller *et al.*, 1979; Deatherage *et al.*, 1982; Kim *et al.*, 1985; Frey *et al.*, 1982) have each been obtained as two-dimensional crystals, suitable for analysis by electron microscopy and image analysis. The low-resolution structures of the three complexes obtained by this method (at 25 Å resolution), are used in Fig. 4 to generate a view perpendicular to the plane of the mitochondrial inner membrane. Complex I has most of its mass in the matrix space while cytochrome *c* oxidase extends most prominently from the cytoplasmic face of the inner membrane.

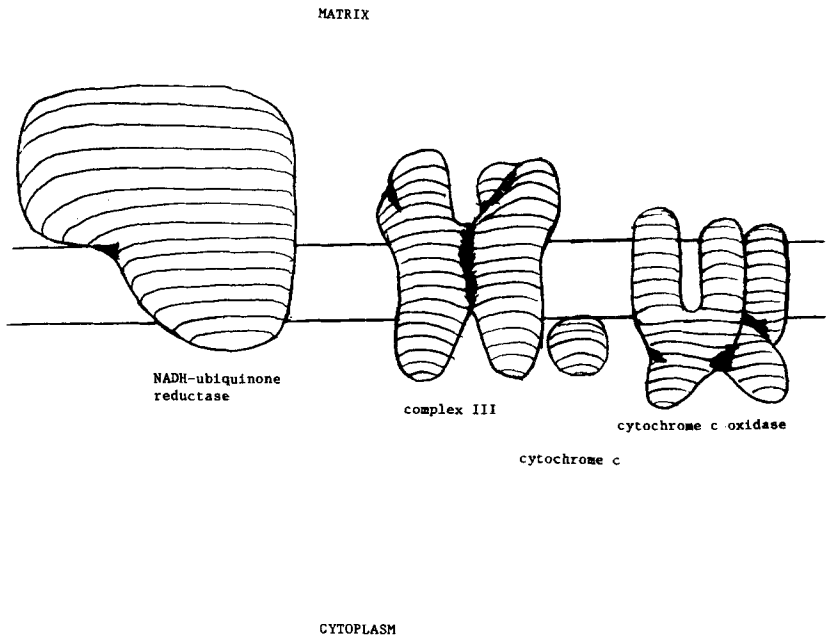


Fig. 4. Schematic of the electron transfer complexes spanning the mitochondrial inner membrane. The size and shape of each complex is based on low-resolution structural data obtained by electron microscopy and image analysis.

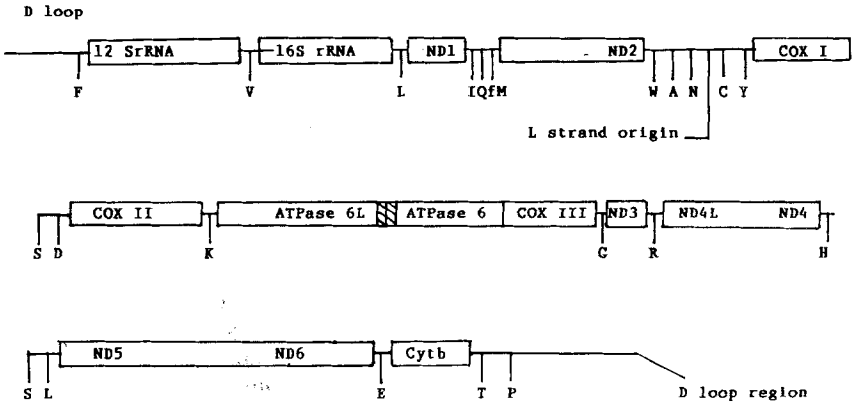


Fig. 5. Genomic map of beef mitochondrial DNA showing the locus of the complex I genes (ND), cytochrome *c* oxidase genes (COX), ATPase genes (ATPase), and the cytochrome *b* genes.

The Mitochondrial Genome

An important feature of the mitochondrion is the presence of DNA. The genetic contribution of the organelle to its own assembly is modest with only about 16,000 base pairs in the mtDNA of mammals (reviewed in Tzagoloff, 1982). The full sequence of the mitochondrial genome has been determined for several mammals including beef (Anderson *et al.*, 1982) and man (Anderson *et al.*, 1981). Figure 5 shows the organization of the bovine mitochondrial genome; the map of the human genome is identical. The gene map in Fig. 5 is shown in linear fashion for convenience, although the actual genome is circular. There are 13 open reading frames coding for 7 subunits of complex I (ND genes), 1 subunit of complex III (cyt *b* gene), 3 subunits of cytochrome *c* oxidase (COX genes), and 2 subunits of the ATP synthase (ATPase genes) (Tzagoloff, 1982; Anderson *et al.*, 1981, 1982; Chomyn *et al.*, 1985).

Mitochondrial DNA also codes for the 16S and 12S rRNA components of mitochondrial ribosomes as well as for 22 transfer RNA genes. The remaining components of the mitochondrial protein synthesizing machinery are made in the cytoplasm and imported into the mitochondrion (Schatz and Butow, 1983). Assembly of the mitochondrial inner membrane involves the insertion of mitochondrially coded subunits with the large number of nuclear coded subunits to form the respiratory chain complexes.

Subunit Structure of the Respiratory Chain Complexes

Recent studies indicate that complex I is composed of 25–28 subunits (Heron *et al.*, 1979; Ragan, 1980; Ragan *et al.*, 1982; Ise *et al.*, 1985); complex II, 5 subunits (Davis and Hatefi, 1971; Capaldi *et al.*, 1977; Capaldi, 1982); complex III, 11 subunits (Schagger *et al.*, 1986), and cytochrome *c* oxidase 13 subunits (Kadenbach and Merle, 1981; Capaldi *et al.*, 1986; Takamiya *et al.*, 1987), as shown in Figs. 6–8. Nine of the eleven subunits of beef heart complex III have been sequenced, and these sequences are listed in Table II. The sequences of all 13 different polypeptides of beef heart cytochrome *c* oxidase have been obtained, either from sequencing of mtDNA (for subunits MtI and III) or by direct sequencing of the isolated subunit (Tables III and IV).

The arrangement of subunits in both complex III and cytochrome *c* oxidase has been explored extensively, using chemical labeling with water-soluble as well as membrane-intercalated protein-modifying reagents (e.g., Gutweniger *et al.*, 1981; Gellerfors and Nelson, 1977; Beattie *et al.*, 1981; Bell *et al.*, 1979; Malatesta *et al.*, 1983), by crosslinking with bifunctional reagents (e.g, Smith and Capaldi, 1977; Briggs and Capaldi, 1977; Jarausch and Kadenbach, 1985), and with protease digestion experiments (e.g., Lorusso *et al.*, 1985; Zhang *et al.*, 1987). In complex III, the two core proteins (total $M_r \simeq 100{,}000$) and subunit VI (often incorrectly called the quinone-binding

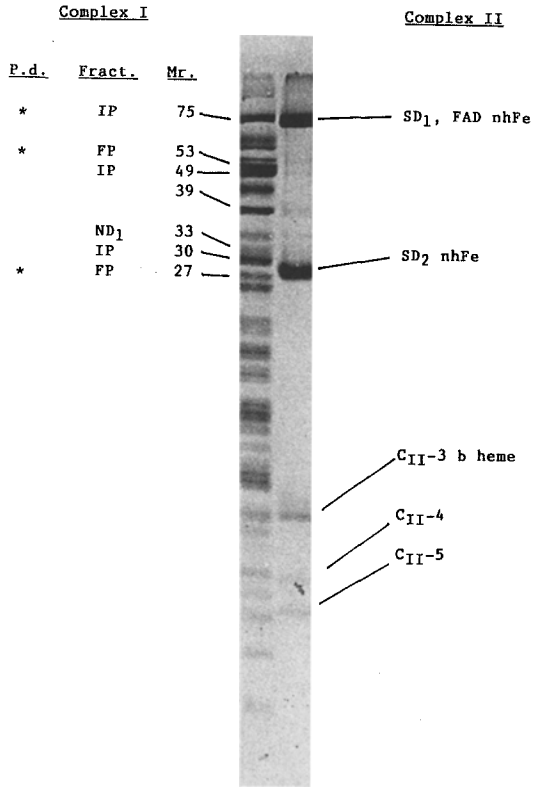


Fig. 6. Subunit structures of complexes I and II from beef heart mitochondria. IP and FP are iron proteins and flavin protein fractions. ND_1 is a mitochondrially coded subunit. Pd subunits with asterisk are present in *Parococcus denitrificans*.

protein) make up much of the M domain extending into the matrix of the mitochondrion. Cytochrome *b* and subunit VII make up much of the intermembrane domain while cytochrome c_1 and the nonheme iron protein contribute to the C (cytoplasm-facing) domain. In the cytochrome *c* oxidase complex, most of the subunits are transmembranous (Fig. 9) with subunit MtII involved in the high-affinity binding site for cytochrome *c* (Millett *et al.*, 1982; Bisson *et al.*, 1982) along with some of the smaller subunits.

Function of Subunits: Comparison of Prokaryote and Mammalian Respiratory Chain Complexes

There are considerably more subunits in the mammalian respiratory chain complexes than can act as apoproteins for the redox centers, raising the

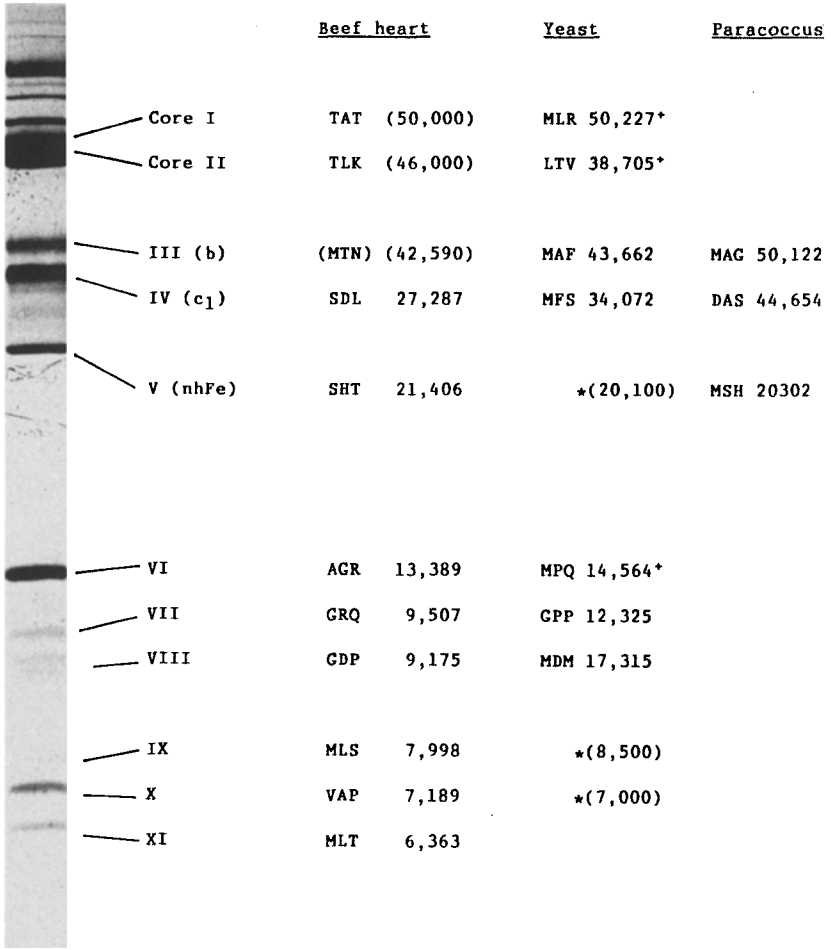


Fig. 7. Subunit structure of beef heart complex III with N terminal sequences and the molecular weights of each polypeptide listed along with the composition of the bc_1 segment of the respiratory chain from *Paracoccus denitrificans* and yeast. Amino terminal sequences for the yeast subunits were obtained from the DNA sequences as reported by Tzagoloff *et al.* (1986), Oudshoorn *et al.* (1987), Nobrega and Tzagoloff (1980), Sadler *et al.* (1984), De Haan *et al.* (1984), Van Loon *et al.* (1984), and Maarse and Grivell (1987). For beef sequences see Table II. Molecular weights in parenthesis are calculated from gene data.

question of the role(s) for these additional components. Studies of the respiratory chains of prokaryotes are relevant in this regard. *Paracoccus denitrificans* has a respiratory chain that is very similar to that of mammalian mitochondria in the number and spectral characteristics of the redox centers, as well as in overall functioning in oxidative phosphorylation (John and

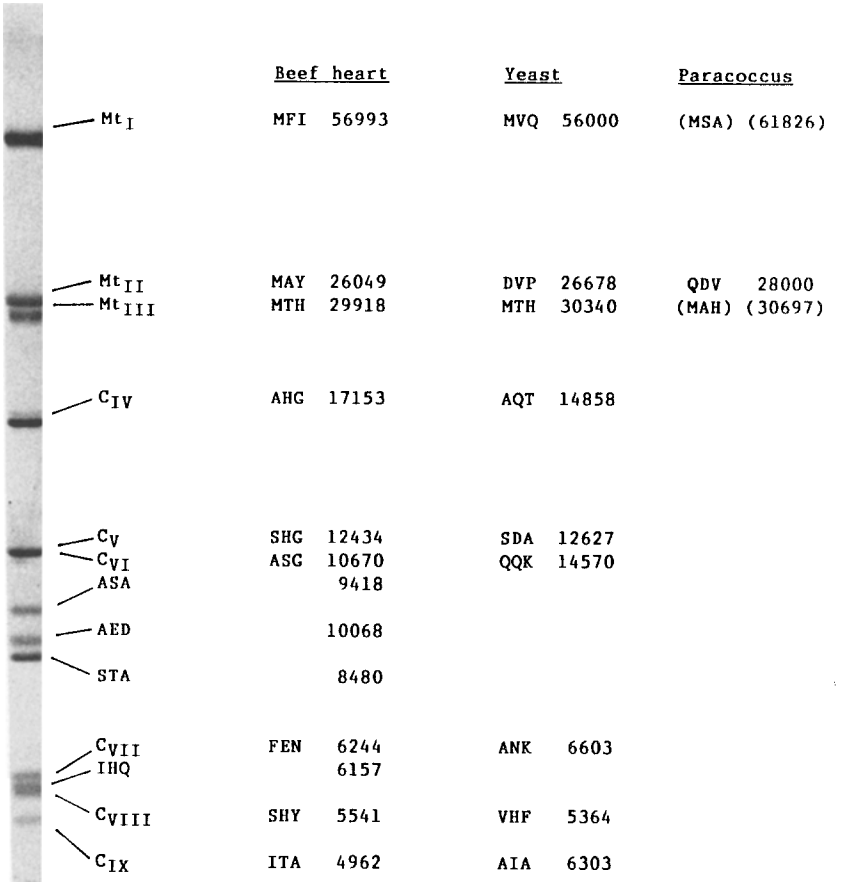


Fig. 8. Subunit structure of beef heart cytochrome *c* oxidase with N terminal sequences and the molecular weights of each polypeptide listed. The subunit structure of cytochrome *c* oxidase from *Paracoccus denitrificans* and yeast are given for reference. Amino terminal sequences for the yeast subunits were obtained from Bonitz *et al.* (1980), Coruzzi and Tzagoloff (1979), Pratje *et al.* (1983), Thalenfeld and Tzagoloff (1980), Power *et al.* (1984b), Maarse *et al.* (1984), Koerner *et al.* (1985), Gregor and Tsugita (1982), Power *et al.* (1984a), Wright *et al.* (1986), and Power *et al.* (1986). Beef sequences may be found in Tables III and IV.

Whatley, 1977; Poole, 1983; Ludwig, 1986). However, complexes I, III, and cytochrome *c* oxidase in this and other prokaryotes are much simpler in terms of subunit composition. For example, complex I in *Paracoccus denitrificans* contains flavin and at least four nonheme iron centers but only 8–10 subunits, compared with 25 or more in mammals (Yagi, 1986). Complex III from this and other bacteria contains three polypeptides only, which are highly homologous in amino acid sequence to cytochrome *b*, cytochrome c_1, and the

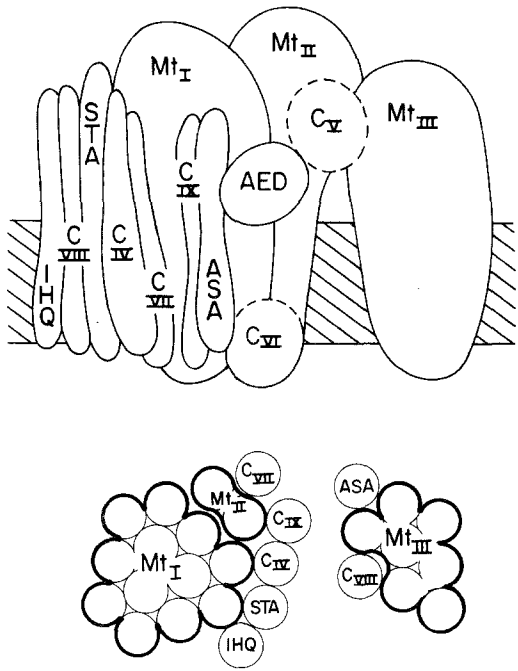


Fig. 9. Schematic of the topology of subunits in mammalian cytochrome *c* oxidase (adapted from Zhang *et al.*, 1987). This figure emphasizes the transmembrane nature of the subunits rather than near neighbor interactions. The distribution of subunits between the transmembrane domains remains to be determined, although subunits Mt_{III} and Mt_{I} are probably in different domains.

nonheme iron proteins (subunits III, IV, and V, respectively) of the mammalian complex (Yang and Trumpower, 1986; Kurowski and Ludwig, 1987). Likewise, the bacterial cytochrome *c* oxidase is composed of only 2 or 3 subunits (Ludwig and Schatz, 1980; Saraste *et al.*, 1986) with strong homology to the mitochondrially coded subunits of the mammalian complex (Raitio *et al.*, 1987).

Complexes I, III, and cytochrome *c* oxidase from *Paracoccus denitrificans* are efficient redox-linked proton pumps (Lawford, 1978; Ludwig *et al.*, 1983; Yang and Trumpower, 1986), implying that the function of any supernumery components is not in energy coupling *per se*. Instead, it has been suggested that these extra components of the eukaryotic respiratory chain are involved in regulation of oxidative phosphorylation. Huther and Kadenbach (1986) and Bisson and colleagues (Motecucco *et al.*, 1986; Bisson *et al.*, 1987) have each shown that the electron transfer activity of cytochrome *c* oxidase is sensitive to ATP concentration, with the high-affinity phase of this activity being lost at high ATP concentrations. Kadenbach (1986) has suggested that

Table II. Amino Acid Sequence of the Components of Beef Heart Complex III

Core protein I (Gonzalez-Halphen, unpublished)	1 TATYAQAL	QSVPETQVSQ				
Core protein II (Gonzalez-Halphen, unpublished)	1 TLKVAPKVKA	TEAPAGVPPH	PQDLEFRRLP	NG		
III cytochrome b (Anderson et al., 1982)	1 MTNIRKSHPL 51 LAMHYTSDTT 101 GLYYGSYTFL 151 SAIPYIGTNL 201 HETGSNNPTG 251 GDPDNYTPAN 301 LALIPLLHTS 351 GQLASVLYFL	MKIVNNAFID TAFSSVTHIC ETWNIGVILL VEWIWGGFSV ISSDVDKIPF PLNTPPHIKP KQRSMMFRPL	LPAPSNISSW RDVNYGWIIR LTVMATAFMG DKATLTRFFA HPYYTIKDIL EWYFLFAYAI SQCLFWALVA 379 LILVLMPTAG TIENKLLKW	WNFGSLLGIC YMHANGASMF YVLPWGQMSF FHFILPFIIM GALLLILALM LRSIPNKLGG DLLTLTWIGG	LILQILTGLF FICLYMHVGR WGATVITNLL AIAMVHLLFL LLVLFAPDLL VLALAFSILI QPVEHPYITI	50 100 150 200 250 300 350
IV cytochrome c_1 (Wakabayashi et al., 1982a)	1 SDLELHPPSY 51 LVGVCYTEDE 101 ARAANNGALP 151 PYFPGQAIGM 201 RKRMGLKMLL	PWSHRGLLSS AKALAEEVEV PDLSYIVRAR APPIYNEVLE MMGLLLPLVY	LDHTSIRRGF QDGPNEDGEM HGGEDYVFSL FDDGTPATMS AMKRHKWSVL	QVYKQVCSSC FMRPGKLSDY LTGYCEPPTG QVAKDVCTFL KSRKLAYRPP	HSMDYVAYRH FPKPYPNPEA VSLREGLYFN RWAAEPEHDH 241 K	50 100 150 200
V non-heme Fe protein (Schagger et al., 1987)	1 SHTDIKVPDF 51 AKNVVSQFVS 101 RTKKEIDQEA 151 GDFGGYYCPC	SDYRRPEVLD SMSASADVLA AVEVSQLRDP HGSHYDASGR	STKSSKESSE MAKIEIKLSD QHDLERVKKP IRKGPAPLNL	ARKGFSYLVT IPEGKNMAFK EWVILIGVCT EVPSYEFTSD	ATTTVGVAYA WRGKPLFVRH HLGCVPIANA 196 GMVIVG	50 100 150

there is a broader anion regulation of cytochrome *c* oxidase, and postulated that some of the supernumery subunits of cytochrome *c* oxidase are binding sites for regulatory hormones.

Tissue Specificity of the Respiratory Chain

Evidence is accumulating that there are tissue-specific isoenzyme forms of the respiratory chain complexes. This is expected if the respiratory chain is under physiological control as discussed above, and given the different constraints on electron transfer in different tissues.

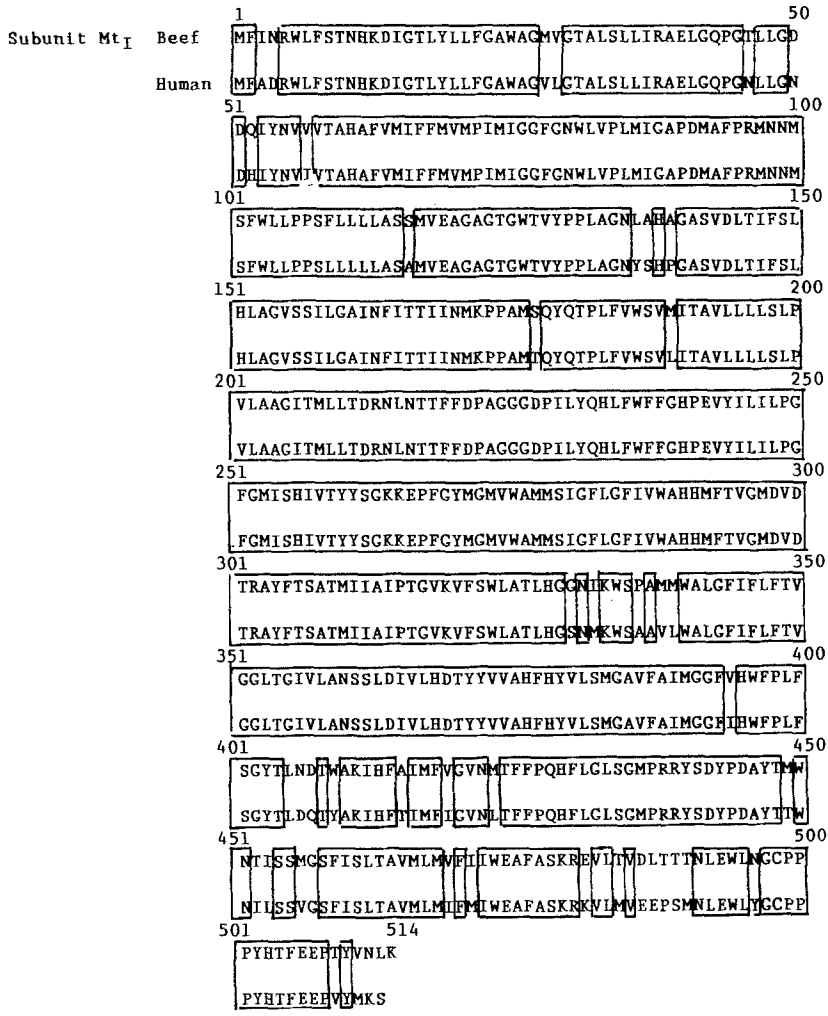
Table II. Continued.

	1				50
VI (Wakabayashi	AGRPAVSASS	RWLEGIRKWY	YNAAGFNKLG	LMRDDTIHEN	DDVKEAIRRL
et al., 1985)	51				100
	PENLYDDRVF	RIKRALDLSM	RQQILPKEQW	TKYEEDKSYL	EPYLKEVIRE
	101 110				
	RKEREEWAKK				
	1				50
VII (Borchart	GRQFGHLTRV	RHVITYSLSP	FEQRAFPHYF	SKGIPNVLRR	TRACILRVAP
et al., 1986)	51			81	
	PFVAFYLVYT	CGTQEFEKSK	RKNPAAYEND	R	
	1				50
VIII (Wakabayashi	GDPKEEEEEE	EELVDPLTTV	REQCEQLEKC	VKARERLELC	DERVSSRSQT
et al., 1982b)	51		78		
	EEDCTEELLD	FLHARDHCVA	HKLFNSLK		
	1				50
IX (Borchart	MLSVAARSGP	FAPVLSATSR	GVAGALRPLV	QAAVPATSES	PVLDLKRSVL
et al., 1985)	51		78		
	CRESLRGQAA	GRPLVASVSL	NVPASVRY		
	1				50
X (Schagger	VAPTLTARLY	SLLFRRTSTF	ALTIVVGALF	FERAFDQGAD	AIYEHINEGK
et al., 1983)	51	62			
	LWKHIKHKYE	NK			
	1				50
XI (Schagger	MLTRFLGPRY	RQLARNWVPT	AQLWGAVGAV	GLVSATDSRL	ILDWVPYING
et al., 1985)	51 56				
	KFKKDD				

There is indirect evidence of tissue specificity of complex I, complex III, and cytochrome *c* oxidase from studies of patients with mitochondrial myopathies. In many cases the defects in these complexes are present in one or relatively few tissues only (Bresolin *et al.*, 1985; Darley-Usmar *et al.*, 1982; DiMauro *et al.*, 1980, 1987; Kennaway *et al.*, 1987; Sengers *et al.*, 1984; Zeviani *et al.*, 1987b). The only direct evidence of tissue specificity is for cytochrome *c* oxidase. Figure 10 compares the subunit profile of beef heart and beef liver cytochrome *c* oxidase. It can be seen that three of the subunits of the enzyme have different migrations in the two tissue forms, as first reported by Kadenbach (1983). These are polypeptides labeled ASA, C_{VII}, and C_{IX}. The detection of isoenzyme forms of cytochrome *c* oxidase has been examined in rat tissues by Kuhn-Nentwig and Kadenbach (1985) using immunological techniques. All of the nuclear coded subunits appeared to show tissue specific as well as adult-fetal differences in immunological reactivity.

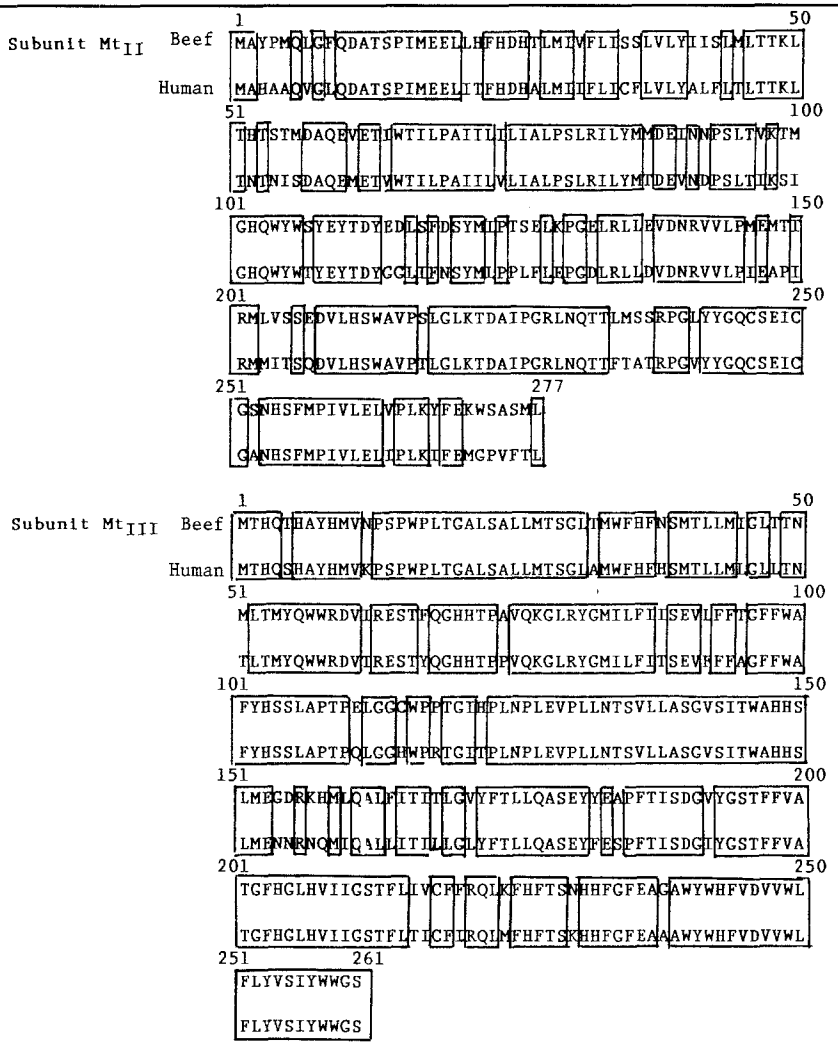
We have been studying the tissue specificity of bovine cytochrome *c* oxidase by amino acid sequencing of the various subunits. Table IV includes sequence data on ASA, C_{VII}, and C_{IX} from beef heart and beef liver, confirming that each is a tissue-specific subunit of the enzyme. N-terminal sequence data of the other nuclear coded subunits showed no differences between

Table III. Comparison of the Amino Acid Sequences of the Mitochondrially Coded Subunits of Beef and Human Cytochrome *c* Oxidase (Anderson *et al.*, 1981, 1982)



components of beef liver and beef heart cytochrome *c* oxidase. Subunit C_{IV} from beef liver and beef heart has been examined most extensively, using peptide mapping, and by sequencing of several of the CNBr fragments. No differences between the heart and liver forms of this subunit have been found. The isoelectric points of C_V and C_{VI} show no differences between heart and liver subunits and our evidence so far is that the isoenzyme forms of

Table III. Continued.



cytochrome *c* oxidase in these two tissues differ only in three of the ten nuclear coded genes.

Polypeptide Analysis of Human Mitochondria

Isolation of the respiratory chain complexes from human tissues has progressed slowly because of the restricted availability of tissue samples. Cytochrome *c* oxidase has been isolated from human placenta using a

Table IV. Sequences of the Nuclear-Coded Subunits of Beef Heart Cytochrome *c* Oxidase (Nomenclature from Takamiya *et al.*, 1987)

		1				50
Subunit C_{IV}		AHGSVVKSED	YALPSYVDRR	DYPLPDVAHV	KNLSASQKAL	KEKEKASWSS
(Sacher et al.,		51				100
1979)		LSIDEKVELY	RLKFKESFAE	MNRSTNEWKT	VVGAAMFFIG	TFALLLIWEK
		101				147
		HYVYGPIPHT	FEEEWVAKQT	KRMLDMKVAP	IQGFSAKWDY	DKNEWKK
		1				50
C_V (Tanaka et		SHGSHETDEE	FDARWVTYFN	KPDIDAWELR	KGMNTLVGYD	LVPEPKIIDA
al., 1979)		51				100
		ALRACRRLND	FASAVRILEV	VKDKAGPHKE	IYPYVIQELR	PTLNELGIST
		101 109				
		PEELGLDKV				
		1				50
C_{VI} (Brewald &		ASGGGVPTDE	EQATGLEREV	MLAARKGQDP	YNILAPKATS	GTKEDPNLVP
Buse, 1982)		51				98
		SITNKRIVGC	ICEEDNSTVI	WFWLHKGEAQ	RCPSCGTHYK	LVPHQAH
		1				50
C_{VII} Heart		FENRVAEKQK	LFQEDNGLPV	HLKGGATDNI	LYRVTMTLCL	GGTLYSLYCL
(Meinecke & Buse,						
(1986)						
Liver		FENKVPEKQK	LFQEDNGIPV			
(Yanamura, W.,						
unpublished)		51 56				
Heart		GHASKK				
		1				47
C_{VIII} (Buse &		SHYEEGPGKN	IPFSVENKWR	LLAMMTLFFG	SGFAAPFFIV	RHQLLKK
Steffens, 1978)						
		1				46
C_{IX} Heart		ITAKPAKTPT	SPKEQAIGLS	VTFLSFLLPA	GWVLYHLDNY	KKSSAA
(Meinecke et al.,						
1984)						
Liver		IHSKPPREQL	GTMEIAIGLT	SCFLD	MENY	KKRE
(Yanamura, W.,						
unpublished)						
		1				50
ASA Heart		ASAAKGDHGG	TGARTWRFLT	FGLALPSVAL	CTLNSWLHSG	HRERPAFIPY
(Meinecke & Buse,						
1985)						
Liver		SSGAHGEEG·	·SARM			
(Yanamura, W.,						
unpublished)						
		51			84	
Heart		HHLRIRTKPF	SWGDGNHTFF	HNPRVNPLPT	GYEK	
		1				30
AED (Steffens		AEDIQAKIKN	YQTAPFDSRF	PNQNQTRNCW	QNYLDFHRCE	KAMTAKGGDV
et al., 1979)		51			85	
		SVCEWYRRVY	KSLCPISWVS	TWDDRRAEGT	FPGKI	
		1				50
STA Erdwig &		STALAKPQMR	GLLARRLRFH	IVGAFMVSLG	FATFYKFAVA	EKRKKAYADF
Buse, 1985)		51		73		
		YRNYDSMKDF	EEMRKAGIFQ	SAK		
		1				50
IHQ (Takamiya, S.		IHQKRAPDFH	DKYGNAVLAS	GATFCVAVNV	YMATQIGIED	NPSPVGRVTP
et al.,		51 56				
unpublished)		KEWREQ				

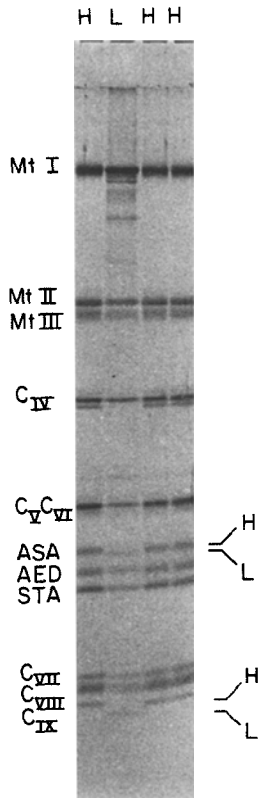


Fig. 10. A comparison of the subunit structures of beef heart and beef liver cytochrome *c* oxidase to show the altered migrations of ASA, C_{VII}, and C_{IX} (reproduced from Takamiya *et al.*, 1986).

large-scale preparative procedure (Hare *et al.*, 1980). More recently, Muijsers and colleagues have developed a small-scale procedure using HPLC to isolate cytochrome *c* oxidase from human tissues (Sinjorgo *et al.*, 1987a,b). These workers have provided preliminary gel electrophoretic evidence for tissue specificity of the human enzyme.

Based on the studies of the beef heart electron transfer complexes, the human mitochondrial respiratory chain must contain at least 60 different polypeptides and be coded by close to 100 genes, assuming that there are isoenzyme forms of each of the different complexes.

The extensive characterization of the beef heart respiratory chain has proved useful in studies of mitochondrial myopathies. We have found that most of the antibodies raised to beef heart enzymes react with human muscle and heart tissue (Darley-Usmar *et al.*, 1982; Kennaway *et al.*, 1987; Kim

et al., 1987; Takamiya *et al.*, 1986). However, it will be necessary to obtain antibodies to the human proteins, particular tissue-specific components, if defects in patients with mitochondrial myopathies are to be localized precisely. Also, identification of genetic alteration(s) will require cloning of the mitochondrial and nuclear genes for the respiratory chain polypeptides, as well as a full characterization of all nuclear gene products involved in the biosynthesis and assembly of the electron transfer complex. A start on this has already been made with the cloning of the genes for subunits C_{IV} and C_{IX} of human liver cytochrome *c* oxidase (Zeviani *et al.*, 1987a; Schon, E., personal communication).

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