

Cytochrome oxidase in health and disease

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Abstract

Yeast and bovine cytochrome *c* oxidases (COX) are composed of 12 and 13 different polypeptides, respectively. In both cases, the three subunits constituting the catalytic core are encoded by mitochondrial DNA. The other subunits are all products of nuclear genes that are translated on cytoplasmic ribosomes and imported through different transport routes into mitochondria. Biogenesis of the functional complex depends on the expression of all the structural and more than two dozen COX-specific genes. The latter impinge on all aspects of the biogenesis process. Here we review the current state of information about the functions of the COX-specific gene products and of their relationship to human COX deficiencies. © 2002 Elsevier Science B.V. All rights reserved.

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1. Introduction

Cytochrome oxidase (COX) is the terminal complex of the mitochondrial respiratory chain. It is located in the mitochondrial inner membrane where it transfers electrons from ferrocytochrome *c* to molecular oxygen, the ultimate acceptor of all the reducing equivalents derived from the breakdown of sugars, amino acids, and fatty acids. This reaction is coupled to proton transfer from the matrix compartment to the intermembrane space, thereby contributing to the energy stored in the electrochemical gradient to be used for ATP synthesis. Mitochondrial COX, depending on the source, consists of a dozen or more subunit polypeptides (Yoshikawa et al., 1998). The three largest subunits forming the catalytic core of the enzyme are generally encoded in mitochondrial DNA. The other subunits, all products of nuclear genes, are translated on cytoplasmic ribosomes and transferred to mitochondria by means of different transport pathways. The functions of this set of proteins are not known but their absence in bacterial COX (Saraste, 1990) indicates that they are unlikely to be essential for the basic catalytic mechanisms of oxygen reduction and vectorial proton transfer. Even so, it is quite clear from studies of

yeast mutants that they are important in assembly and or stability of the holoenzyme (Table 1).

Over the years a great deal has been learned about the structure and catalytic mechanism of COX. Both areas have benefited enormously from the recent solutions of the atomic structures of mitochondrial (Yoshikawa et al., 1998, 2000) and bacterial cytochrome oxidases (Michel, 1999). The structure and arrangement of the subunits are shown in a simplified diagram in Fig. 1. Despite these advances, the manner in which the enzyme is assembled in mitochondria is poorly understood, in part because of extensive turnover of the hydrophobic core subunits in assembly-defective mutants. This is particularly true in yeast where the absence of partially assembly intermediates, when mitochondria are prevented from forming the fully active enzyme, has hampered the application of traditional biochemical tools to this problem.

In the face of these experimental constraints, a more profitable strategy has been to analyze mutants of *Saccharomyces cerevisiae* that are defective in COX assembly, the goal being to decipher the functions of the gene products responsible for this phenotype and, by this means, reconstruct the different steps of the assembly pathway. Screens of nuclear *pet* mutants have revealed a coterie of more than 30 genes that selectively affect expression of this respiratory complex in yeast (McEwen et al., 1986; Tzagoloff and Dieckmann, 1990). Of these, only eight code for subunits of the enzyme.

Abbreviations: COX, cytochrome *c* oxidase

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Table 1
Contacts of subunit polypeptides in bovine cytochrome oxidase and relationship to yeast subunits

Bovine	Yeast	Subunit contacts ^a	Contact region	Activity ^b	Assembly ^b
I	1 ^c	Subunit II (2) and III (3)	Membrane, intermembrane Membrane, intermembrane, matrix	–	–
II	2 ^c	Subunit I (1)	Membrane and intermembrane	–	–
III	3 ^c	Subunit I (1)	Membrane, intermembrane, matrix	–	–
IV	5a	Subunit I (1) and Va (6)	Membrane Matrix	–	–
Va	6	Subunits IV (5a) and VIc (7a)	Matrix	–	–
Vb	4	Subunits I (1) and III (3)	Matrix	–	–
VIb	9 ^d	Subunits II (2) and III (3)	Cytoplasmic	+ / =	+
VIa	10 ^d	Subunit III (3)	Membrane	+	+
VIc	7a	Subunits II (2) and Va (6)	Membrane	–	–
VIIa	7	Subunit III (3)	Membrane, matrix, intermembrane	–	–
VIIb			Intermembrane		
VIIc	8	Subunit I (1) and VIII		+	+
VIII		Subunit I	Matrix		

^a The contacts are based on the structure of the bovine enzyme reported by Tsukihara et al. (1996). The roman numerals refer to the bovine subunits. The corresponding yeast subunit is indicated in parentheses.

^b Catalytic activity or assembly of the yeast enzyme.

^c Only the contacts with other core subunits are indicated (subunits I, II, and III).

^d Subunits 9 and 10 are encoded by *COX12* and *COX13*, respectively.

The products of the remaining two dozen genes intercede at all stages of the assembly process, beginning with processing of the mitochondrially encoded mRNAs (Seraphin et al., 1988, 1989), translation of the core subunits (Mulero and Fox, 1993; Costanzo and Fox, 1995), insertion of these hydrophobic proteins into the inner membrane (Hell et al., 1997, 2001), and maturation of the catalytic subunits through

addition of the heme (Barros et al., 2001) and metal prosthetic groups (Glerum et al., 1996a,b). From these studies has emerged a rough outline of the assembly process whose details will be filled in as more information becomes available about the functions of still other COX-specific and other related genes (Fig. 2). Studies of the yeast mutants have also had an impact on identifying the genetic basis of human

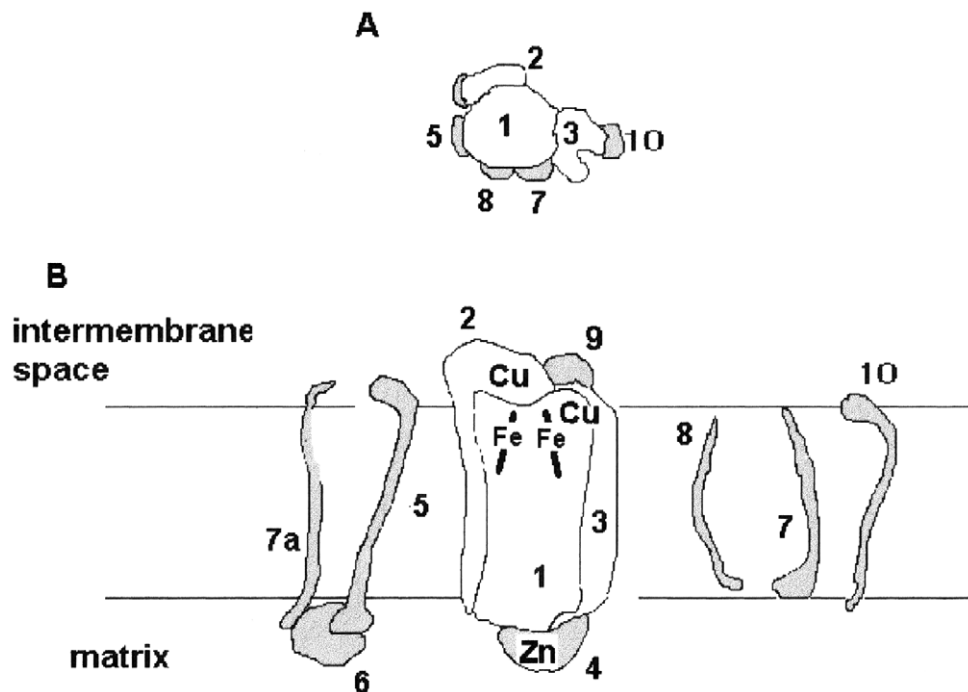


Fig. 1. Arrangement of COX subunits. The core subunits are depicted in white and the eight cytoplasmically synthesized subunits are in gray. In the frontal view (B) some of the subunits have been drawn separately to show the core structure. Their relationship to the core is more evident in the diagram showing the enzyme viewed from the top (A).

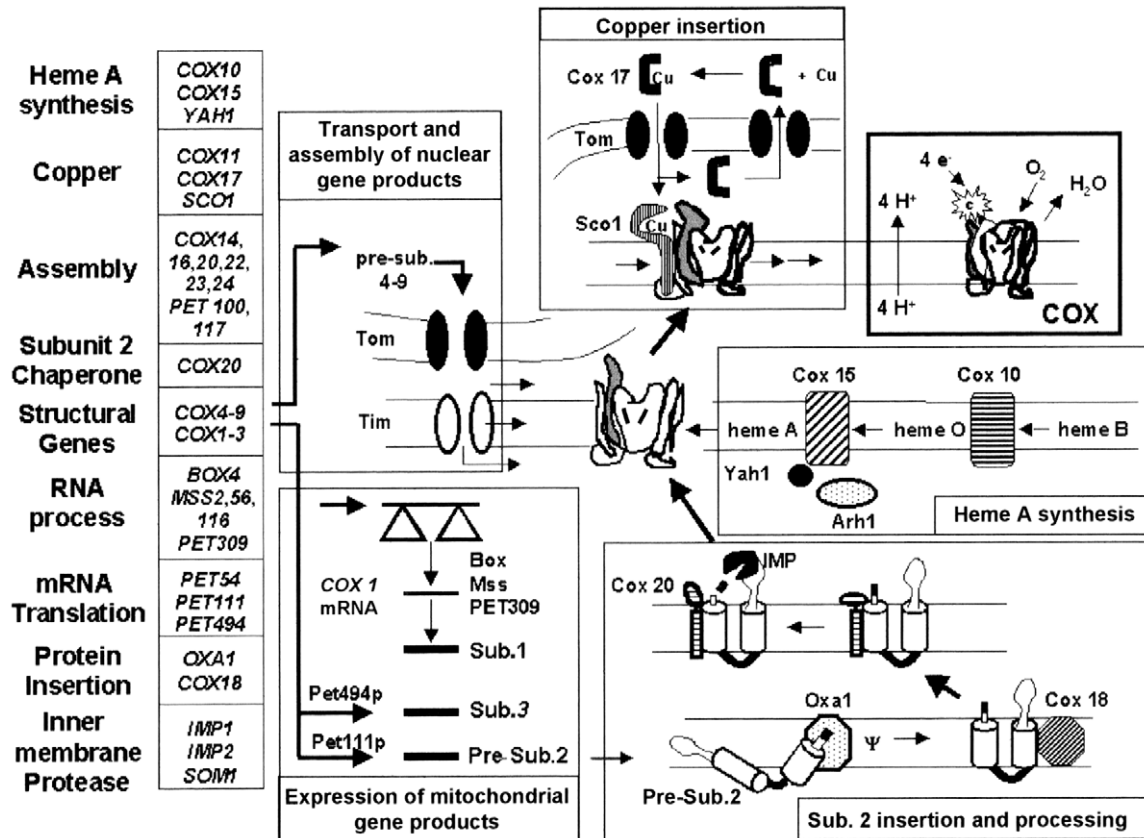


Fig. 2. Schematic depiction of different steps in COX assembly. Only some of the COX-specific genes are listed. Events catalyzed by functionally related gene products are boxed. In the box showing expression of mitochondrial products, the subunit 1 pre-messenger RNA is shown with only two introns because of space limitation. Subunit 2 is depicted with its two transmembrane helices in the box showing its insertion and processing. The mature subunit 2 is shown as a shaded subunit in the partially assembled and mature COX. The two bars in the central subunit 1 represent the two heme A groups. The Tom pore complex, depicted by the two solid oval shapes, is located in the outer membrane and the Tim pore complex, depicted by the open oval shapes, is located in the inner membrane.

diseases resulting from COX deficiency. The current status of what is known about the yeast and human genes that affect COX assembly will be briefly reviewed in this article.

2. Assembly of yeast cytochrome oxidase

2.1. Genes governing expression of the mitochondrially encoded core subunits

In *S. cerevisiae*, subunit 1 is encoded by the mitochondrial *COX1* gene that has multiple introns (Bonitz et al., 1980). Maturation of the subunit 1 pre-mRNA depends on proteins referred to as maturases whose genes are located in the introns (Lazowska et al., 1980; Levra-Juillet et al., 1989). In addition a number of nuclear gene products have been described that are also essential for maturation of the mRNA, although what they do precisely is not known (Seraphin et al., 1988, 1989). The maturation of the *COX1* transcript has not received much attention in recent years, probably because of the numerous processing intermediates contributing to the complexity of the problem. The *COX2* and *COX3* genes for subunits 2 and 3, respectively, have no

introns and the primary transcripts are individually transcribed and matured by the general mitochondrial transcription and 3' processing machinery. Nuclear genes also code for messenger-specific translational factors. Such factors interact with the 5' untranslated sequences of the subunit 2 and 3 mRNAs (Mulero and Fox, 1993; Costanzo and Fox, 1995). Two other nuclear genes (*PET309*, *MSS51*) have been implicated in the translation of subunit 1 (Decoster et al., 1990; Manthey and McEwen, 1995).

The untranslated leader sequences of the *COX2* and *COX3* mRNAs appear to be necessary for membrane insertion of the newly synthesized proteins, perhaps by tethering the mRNAs to the inner membrane (Sanchirico et al., 1998). This raises the intriguing possibility that translational factors may direct insertion of proteins destined to interact with each other to specific locations in the membrane. However, the fact that this group of proteins is not found outside of yeast tends to argue in favor of a function particular to COX assembly in this organism.

2.2. Membrane insertion of subunit 2

This protein consists of two transmembrane segments, a

short amino-terminal domain and a large hydrophilic carboxyl-terminal domain that houses the two copper atoms of the CuA site. The amino- and carboxyl-terminal domains are both exposed on the cytoplasmic side of the inner membrane (Fig. 1). Subunit 2 is one of two mitochondrially encoded proteins that are synthesized as precursors with cleavable amino terminal extensions. Membrane insertion of subunit 2 precursor from its site of synthesis in the matrix is accompanied by export of the amino- and carboxyl-domains to the intermembrane space. This requires the products of the nuclear *OXAI* (Bonnefoy et al., 1994a) and *COX18* (Souza et al., 2000) genes. The first step involves an interaction of the precursor with Oxa1p and insertion of the first transmembrane segment into the phospholipid bilayer with concomitant transfer of the amino-terminal domain to the intermembrane compartment (Hell et al., 1997). This is followed by a subsequent Cox18p-dependent insertion of the second, more carboxyl-proximal transmembrane domain into the bilayer (A. Saracco and T. Fox, pers. commun.). Both export events require energy in the form of a membrane potential.

Proteolytic removal of the amino-terminal sequence from the subunit 2 precursor is catalyzed by the three-subunit Imp protease (Jan et al., 2000). This enzyme is located on the intermembrane side of the inner membrane and, therefore, can only act after export of the amino terminus. One of the properties of *oxal* (but not *cox18*) mutants is their failure to process the subunit 2 precursor. This phenotype is also displayed by *cox20* mutants. In this case, however, the processing block is not due to a lack of export of the amino terminal presequence or a defect in the protease. Instead the *COX20* product appears to function as a subunit 2-specific chaperone (Hell et al., 2000). This inner membrane protein interacts with the precursor to form a complex that is recognized as the proper substrate by the protease (Hell et al., 2000).

2.3. Formation of the catalytic core

Biogenesis of the membrane forms of subunits 1 and 3 is less well understood. Earlier studies indicated that insertion of the two proteins into the inner membrane requires the help of Oxa1p (Hell et al., 1998). This is also supported by more recent evidence of a physical interaction between Oxa1p and newly synthesized but unassembled subunits 1 and 3 (Hell et al., 2001). Even though information about these hydrophobic components is scant, it is not unreasonable to think that their interaction with each other and with subunit 2 to form a core complex can occur independent of the subunits synthesized in the cytosol. This is already implicit from evidence of bacterial cytochrome oxidases that have the same core structure as the mitochondrial enzyme and assemble into a stable complex even though the other subunits are absent. The existence of contact interfaces between each of the three core subunits also makes it unlikely that the subunits synthesized in the cytosol contribute significantly to the stability of the

core complex. Based on the structure of the bovine COX (Tsukihara et al., 1996), only subunits Vb (yeast subunit 4), which makes contact with both subunits I and III, and subunit VIb (yeast subunit 6b), which contacts subunits II and III, could influence the stability of the core complex (Table 1). The core subunits have the hallmarks of a protective shield that surrounds and caps a good portion of the exposed surfaces of the enzymatic core (Fig. 1). This is not to say, however, that there needs to be an obligatory order of subunit interaction or that interactions cannot occur between some subunits prior to formation of the core. For example, an intermediate of subunits I and IV has been detected in human mitochondria (Nijtmans et al., 1998).

2.4. Biosynthesis of heme A

Heme A is a unique heme compound present only in cytochrome oxidase. The two heme A groups of cytochrome oxidase are non-covalently bound to subunit 1; they contribute to the low-spin heme of cytochrome *a* and the high-spin heme of cytochrome *a*₃ (Saraste, 1990). Heme A differs from protoheme (heme B) at carbons C2 and C8 of the porphyrin ring. It has a farnesyl instead of a vinyl group at C2, and a formyl instead of a methyl group at C8 (Caughey et al., 1975). The first step in heme A biosynthesis is a farnesylation of the vinyl at C2 of protoheme (Saiki et al., 1993). In yeast, this reaction is catalyzed by a farnesyl transferase encoded by the *COX10* to produce heme O (Tzagoloff et al., 1993). Heme O can function as a prosthetic group in some bacterial (Puustinen and Wikstrom, 1991) but not mitochondrial cytochrome oxidases. The further conversion of heme O to heme A probably involves a monooxygenase-catalyzed hydroxylation of the methyl group at carbon position 8. The resultant alcohol would then be further oxidized to the aldehyde by a dehydrogenase.

The *ctaA* gene of *Bacillus subtilis* has been shown to be required for the conversion of heme O to heme A (Svensson et al., 1996). *Escherichia coli*, which normally has only heme O, is able to synthesize heme A when transformed with *ctaA* (Svensson et al., 1993). Purified CtaA protein has both protoheme and heme A associated with it, suggesting that it is likely to be a heme-dependent monooxygenase (Svensson and Hederstedt, 1994; Svensson et al., 1996). Remarkably, CtaA has no homology to other known P450 cytochromes. We recently proposed that *COX15* is the yeast homolog of *ctaA* (Barros et al., 2001). Cox15p exhibits some sequence similarity to the bacterial protein. More significantly, *cox15* mutants are COX-deficient and have no heme A, although they have low levels of heme O (Barros et al., 2001). This phenotype is different from *cox10* mutants that lack both heme A and heme O (Tzagoloff et al., 1993).

In *Schizosaccharomyces pombe* *COX15* is fused to *YAH1*, the structural gene for mitochondrial adrenodoxin (Barros and Nobrega, 1999). Yah1p, an essential protein in *S. cerevisiae*, has been shown to function in the assembly of iron-sulfur clusters (Lange et al., 2000). A fusion of the *S. cere-*

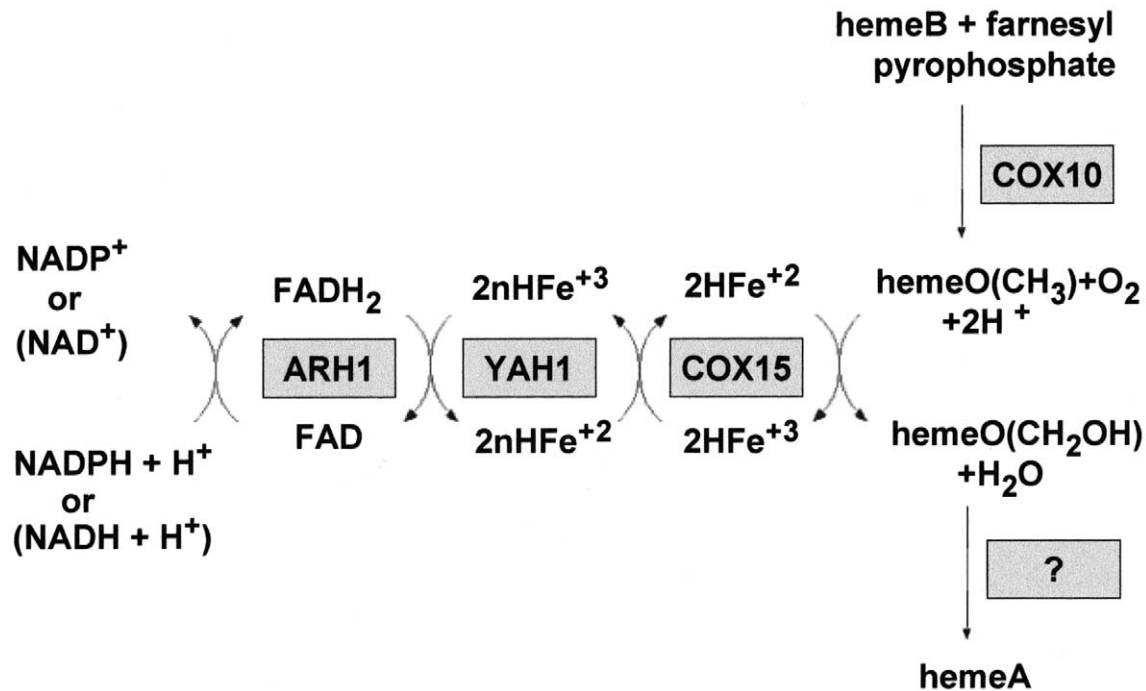


Fig. 3. Heme A biosynthetic pathway. The conversion of heme B to heme O is catalyzed by Cox10p, a farnesyl transferase. Arh1p (adrenodoxin reductase), Yah1p (adrenodoxin), and Cox15p jointly catalyze the hydroxylation of the methyl group at C8 of heme O producing an intermediate whose conversion to heme A is catalyzed by a still unidentified dehydrogenase. nHFe refers to the non-heme iron of adrenodoxin and hFe to the heme iron of Cox15p.

visiae *COX15* and *YAH1* genes, when introduced in single copy into chromosomal DNA, complements the respiratory defect of a *cox15* null mutant and the lethality of a *yah1* mutant, excluding any effect of the combined presence of the two proteins in a single polypeptide on their respective activities (Barros et al., 2001). These observations suggest that Cox15p in conjunction with Yah1p and adrenodoxin reductase encoded by *ARH1* (Manzella et al., 1998) function as a three component monooxygenase (Fig. 3).

2.5. The copper of the CuA and CuB sites

Mitochondrial cytochrome oxidase contains three coppers. Two copper atoms bound to subunit 2 constitute the CuA site, the primary acceptor of the electrons from ferrocycytochrome *c*. The third copper is associated with the high-spin heme A group of subunit 1. COX-deficient mutants of yeast have provided new clues about copper homeostasis in mitochondria. Three genes have been implicated in mitochondrial copper metabolism. *COX17* codes for a low molecular copper protein present in the cytosol and the mitochondrial intermembrane space. Mutations in this gene induce a COX deficiency that is partially rescued by inclusion of elevated concentrations of copper in the growth medium (Glerum et al., 1996a). Since the defect in *cox17* appears to be confined to COX, Cox17p targets copper specifically to mitochondria (Glerum et al., 1996a). The second protein, Sco1p, is an inner membrane protein facing the intermembrane space. Mutations in *SCO1* lead to a specific COX deficiency (Schulze and Rodel, 1988).

Subsequently, *SCO1* was shown to be a high copy suppressor of *cox17* (Glerum et al., 1996b). The genetic interaction of *SCO1* and *COX17* suggested that the two proteins function in a common pathway. Sco1p has a domain with sequence similarity to the copper-binding site of subunit 2. The functional importance of this region was demonstrated by the loss of Sco1p function when each of the cysteine residues in the presumed copper-binding CxxxC motif was changed to alanine by site-directed mutagenesis (Rentzsch et al., 1999). A role of Sco1p in subunit 2 maturation gains further support from antibody pull-down experiments demonstrating a complex of the two proteins (Lode et al., 2000). Sco1p was initially proposed to transfer copper from Cox17p to subunit 2 (Glerum et al., 1996a,b). More recently, however, an alternate function has been proposed for Sco1p based on its homology to disulfide reductases (Chinenov, 2000). According to this interpretation, Sco1p is more likely to be involved in reduction of the cysteines (copper ligands) in subunit 2 as a prerequisite for copper binding. Neither Sco1p mediated copper transfer, or disulfide reduction has been demonstrated directly. The *SCO2* gene of yeast is a highly conserved homolog of *SCO1* (Smits et al., 1994). The function of this gene is not known but in high copy, it also suppresses *cox17* mutations, although less efficiently than *SCO1* (Glerum et al., 1996b).

Cytochrome oxidase in *Rhodobacter sphaeroides* is an 'a₃' type enzyme with CuA and CuB sites. *COX11*, a gene essential for expression of COX in yeast (Tzagoloff et al., 1990), was first thought to function in heme A synthesis because of the low content of this heme in *cox11*

mutants (Tzagoloff et al., 1993). Recent studies of the *Rhodobacter* enzyme, however, indicate a role of Cox11p in the formation of the CuB and the Mg/Mn centers (Hiser et al., 2000). *Rhodobacter* COX purified from *cox11* mutants lacks the CuB center and is depleted in Mg, even though the CuA and heme A centers are present (Hiser et al., 2000).

2.6. Additional comments

It is obvious from this discussion that our knowledge of COX assembly is still very patchy. The temporal order of subunits interaction, the extent to which this is a protein-assisted process, and the timing of prosthetic group addition, are all questions that remain to be answered. Nor is it clear whether subunits are inserted at specific membrane sites or whether they find each other by lateral diffusion from different insertion sites. There are still a substantial number of COX-specific nuclear genes about which almost nothing is known except that they intervene late in the assembly pathway. Mutants defining this class of genes express all the COX components, import the nuclear products, but for unknown reasons are unable to complete assembly of the complex. Many existing gaps in the puzzle will be filled in once the functions of this class of genes are better understood.

3. Cytochrome oxidase deficiency and human diseases

3.1. Genetic and clinical heterogeneity of cytochrome deficiencies in humans

COX deficiency is the most frequent cause of respiratory chain defects in humans. Patients afflicted with this disease

present heterogeneous clinical phenotypes, including Leigh syndrome (Leigh, 1951), hepatic failure and encephalomyopathy (Table 2). Several factors probably contribute to the clinical heterogeneity. First, expression of the enzyme is affected by a large number of genes (Tables 1 and 3). Secondly, tissue-specific differences may exist in the cellular abundance of COX-related gene products. In the case of mutations in the mitochondrial genes, the phenotype will be determined by the percentage of mutated genomes in the mitochondrial population and tissue-specific differences in the threshold at which the biochemical lesions are manifested. As a consequence partial loss of function mutations are likely to be more severe in tissues or organs in which the concentration of the affected product is most limiting.

Mutations in the three maternally inherited genes *COXI*, *COXII* and *COXIII*, coding for the core subunits, have been reported in some patients. Paradoxically mutations in the nuclear genes for the other ten subunits have not yet been found, despite dedicated efforts of several laboratories to detect such lesions. While the reasons for this are not clear, it does suggest that the prevalent non-maternally transmitted mutations causing COX deficiency occur in non-structural genes.

3.2. COX-specific human and yeast homologs

Of the more than a dozen yeast genes governing different post-translational events in COX assembly, half are currently known to have human homologs (Table 3). This does not mean that other COX assembly genes may not also exist in mammalian genomes but have not yet been recognized because of their smaller size and less conserved

Table 2
Genetic and clinical heterogeneity of patients with COX deficiencies

Gene	Clinical features	Reference
<i>Mitochondrially encoded COX subunits</i>		
COXI	Sideroplastic anemia Motor neuro-like degeneration Multisystemic disorder	Gattermann et al., 1997 Comi et al., 1998 Bruno et al., 1999
COXII	Myoglobinuria Encephalomyopathy	Karadimas et al., 2000 Clark et al., 1999
COXIII	Myopathy MELAS Myoglobinuria Encephalomyopathy Leigh-like syndrome	Rahman et al., 1999 Manfredi et al., 1995 Keightley et al., 1996 Hanna et al., 1998 Tiranti et al., 2000
<i>Heme A biosynthesis</i>		
<i>COX10</i>	Ataxia, tubulopathy	Valnot et al., 2000b
<i>Copper metabolism and insertion</i>		
SCO1	Hepatic failure, encephalopathy	Valnot et al., 2000a
SCO2	Cardioencephalomyopathy	Papadopoulou et al., 1999
<i>Cox assembly</i>		
SURF1	Leigh's syndrome	Zhu et al., 1998 Tiranti et al., 1998

Table 3
Yeast COX-specific genes with human homologs

Yeast gene	Human gene	Function	Complementation of yeast mutant by human gene	Reference
<i>COX10</i>	<i>COX10</i>	Farnesylation of protoheme	Yes	Glerum and Tzagoloff, 1994
<i>COX17</i>	<i>COX17</i>	Delivery of copper to mitochondria	Yes	Amaravadi et al., 1997
<i>COX15</i>	<i>COX15</i>	Hydroxylation of heme O	Yes ^a	Tzagoloff, unpublished data
<i>SCO1</i>	<i>SCO2</i>	Transfer of copper to COX or reduction of cysteine residues in subunit 2	No ^b	Paret et al., 2000
<i>SCO2</i>	?	?	No ^c	Paret et al., 1999; Papadopoulou et al., 1999
<i>SHY1</i>	<i>SURF1</i>	COX assembly	No	Barrientos, unpublished data
<i>PET191</i>	<i>PET191</i>	?	?	
<i>OXA1</i> ^d	<i>OXA1</i>	Membrane insertion of COX subunits, Cytochrome <i>b</i> , and ATPase proteolipid	Yes	Bonnefoy et al., 1994b

^a Yeast *cox15* mutants transformed with the human gene give rise to respiratory competent clones after several days of incubation on selective YEPG medium.

^b Human *SCO1* does not complement yeast *sco1* mutants but a chimeric gene expressing the amino terminal half of the yeast and the carboxyl terminal half of the human *SCO1p* does.

^c This refers to complementation of the yeast *sco1* mutant.

^d Even though *OXA1* is not a COX-specific membrane insertion factor, it is included because the human gene is also functional in yeast.

sequences. Some of the human COX-specific homologs are able to complement the respective yeast mutants (Table 3). In some cases restoration of cytochrome oxidase and hence also growth on non-fermentable substrates is only partial. Nonetheless, rescue by the human genes elicits a sufficiently clear phenotype to allow human mutations to be tested in yeast.

3.3. Human *SCO1* and *SCO2* genes

Both the yeast and human genomes have two genes, *SCO1* and *SCO2*, coding for highly conserved proteins. Recently, a patient presenting hepatic failure and encephalopathy as a result of a cytochrome oxidase deficiency was shown to have compound heterozygous mutations in the *SCO1* gene located on chromosome 17 (Valnot et al., 2000a). The mutant allele inherited from the father was a 2 bp frameshift deletion resulting in a premature stop codon and a highly unstable mRNA. The maternally inherited mutation changed a highly conserved proline into a leucine (P174L). This proline, adjacent to the CxxxC copper-binding motif, is probably important for proper folding of this domain. Allotopically expressed human *SCO1* did not complement yeast *sco1* mutants (Paret et al., 1999). However, a chimera of the N-terminal half of *SCO1p* and the C-terminal half of the human *SCO1p* (but not *SCO2p*) containing the CxxxC sequence was able to substitute for the yeast gene (Paret et al., 1999). Taking advantage of this fact, the same chimeric protein containing the P174L mutation was also used to transform the yeast mutant. In this case, however, the COX deficiency of the mutant was not rescued, demonstrating the deleterious nature of the mutation in yeast and by inference humans also (Paret et al., 2000).

Mutations in *SCO2*, located on human chromosome 22, have been identified in patients presenting early fatal encephalomyopathy associated with cytochrome oxidase deficiencies, the most severe occurring in highly aerobic tissues such as muscle and heart (Papadopoulou et al., 1999). The three patients studied were compound heterozygotes, in two cases sharing the same nonsense and missense mutations, the latter resulting in a E140K substitution adjacent to the CxxxC motif of Papadopoulou et al. (1999). This mutation was also found in the third patient, who in addition had a second missense mutation in the more carboxyl proximal region of the protein.

The relationship of the human and yeast *SCO2* genes is not clear at present. As already mentioned, null mutations in yeast *SCO2* have no obvious effects on cytochrome oxidase and respiration. Moreover, *SCO2* does not suppress the COX defect of *sco1* mutants, perhaps because the two proteins differ in their substrate specificities (Glerum et al., 1996b). The presence of normal COX in yeast *sco2* mutants might be explained by the existence of a redundant function in yeast but not human cells. The discrepancy in the phenotypes of *sco2* mutations in yeast and human, therefore, need not necessarily indicate that the two genes have different functions.

Human *SCO1* and *COX10* are located in region 17p13.1 of chromosome 17. Homozygosity mapping of patients with cytochrome oxidase deficiencies uncovered another family with a mutation in this region (Valnot et al., 2000b). The clinical manifestation, however, which included ataxia and tubulopathy, was sufficiently different from the patient with the *SCO1p* defect to suggest that the mutation might be in *COX10*. The sequences of *COX10* in the patient disclosed a homozygous C to A transversion in exon 4, resulting in the replacement of a conserved lysine by an asparagine

3.4. Human *cox10* mutations

Human *SCO1* and *COX10* are located in region 17p13.1 of chromosome 17. Homozygosity mapping of patients with cytochrome oxidase deficiencies uncovered another family with a mutation in this region (Valnot et al., 2000b). The clinical manifestation, however, which included ataxia and tubulopathy, was sufficiently different from the patient with the *SCO1p* defect to suggest that the mutation might be in *COX10*. The sequences of *COX10* in the patient disclosed a homozygous C to A transversion in exon 4, resulting in the replacement of a conserved lysine by an asparagine

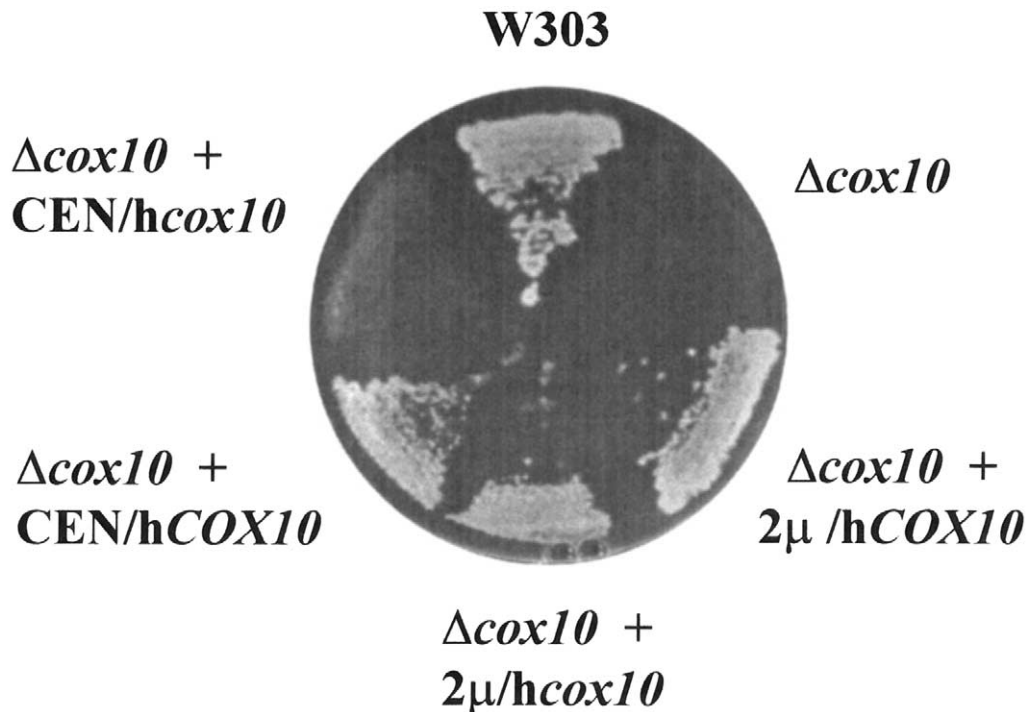


Fig. 4. Complementation of a yeast *cox10* null mutant by human *COX10*. The wild-type, mutant, and transformants were spread on rich glucose medium and following 1 day growth at 30°C were replicated on rich glycerol (YEPG). The photograph was taken after 1–2 days incubation of the YEPG plate at 30°C. W303, wild-type parent; Δcox10 , yeast *cox10* null mutant; $\Delta\text{cox10} + \text{CEN}/h\text{cox10}$, yeast *cox10* null mutant transformed with the human mutant gene in a CEN plasmid; $\Delta\text{cox10} + \text{CEN}/h\text{COX10}$, yeast *cox10* null mutant transformed with the human wild-type gene in a CEN plasmid; $\Delta\text{cox10} + 2\mu/h\text{cox10}$, yeast *cox10* null mutant transformed with the human mutant gene in a 2 μ high copy plasmid; $\Delta\text{cox10} + 2\mu/h\text{COX10}$, yeast *cox10* null mutant transformed with the human wild-type gene in a 2 μ high copy plasmid.

(N204K) in the protein. Both parents were heterozygous for the mutation and unaffected siblings were either heterozygous or homozygous for the wild-type allele.

To confirm that the N204K mutation in the heme farnesyl transferase was responsible for the COX deficiency, the human wild-type and mutant genes were tested for their ability to complement the respiratory defect of a yeast *cox10* null mutant. The two genes were cloned into (1) a high copy plasmid containing the yeast 2 μ origin of replication, (2) a low copy CEN plasmid, and (3) an integrative plasmid suitable for site-directed integration in chromosomal DNA. The six different plasmids were tested for their ability to complement a yeast *cox10* null mutant. Growth of the *cox10* mutant on glycerol, a non-fermentable carbon source, was restored by the wild-type but not the N204K mutant gene on the low copy plasmid (Fig. 4). Similar results were obtained when the two genes were integrated at the *leu2* locus of the host (data not shown). In contrast, both the wild-type and mutant genes complemented the mutant when introduced on the high copy plasmid. Since mitochondria of the patient had a low level of cytochrome oxidase (Valnot et al., 2000b), the N204K mutation must not completely abolish the farnesyl transferase activity, thereby accounting for the observed complementation when the mutant protein is overexpressed from high copy plasmid. The complementation data confirm that the COX deficiency

is caused by the point mutation in the *COX10* gene of chromosome 17.

3.5. Mutations in *SURF1*

A large proportion of patients with Leigh's syndrome, the most common disease associated with a COX deficiency, have recently been shown to have mutations in *SURF1*, the human homolog of yeast *SHY1* (Zhu et al., 1998; Tiranti et al., 1998). Patients suffering from this condition have severe neurological disorders characterized by bilaterally symmetrical necrotic lesions in subcortical regions of the brain (Leigh, 1951). Surf1p/Shy1p is a mitochondrial inner membrane protein essential for full expression of cytochrome oxidase. *SURF1* does not complement *shy1* mutants, even as a fusion gene expressing the carboxyl or amino terminal halves of the yeast gene (Barrientos, unpublished data). Nonetheless, the similarities in the amino acid sequences and the properties of mitochondria in *shy1* mutants and Leigh patients suggest that the two proteins are very likely to have the same function. What that function is precisely has not been established, but the protein is thought to be needed for assembly and/or maintenance of COX (Nijtmans et al., 2001). Unlike other COX-specific genes, mutations in *SHY1* do not completely abolish but merely reduce cytochrome oxidase levels to 10–20% of

wild-type (Mashkevich et al., 1997). The presence of a protein capable of substituting or compensating for Shy1p could explain the residual COX in mutants.

Revertants of *shy1* null mutants with nuclear extragenic suppressors have been isolated and found to have four to five times higher steady-state levels of COX, thereby allowing the mutant to respire and grow on non-fermentable carbon sources nearly as well as wild-type yeast (Barrientos and Tzagoloff, unpublished data). The suppressor gene has been cloned and identified to be *MSS51*, a gene previously shown to be involved in processing and translation of the mitochondrial *COX1* transcript (Decoster et al., 1990). It is noteworthy that no *MSS51* homolog has been reported in the human or other mammalian genomes.

Wild-type *MSS51* also rescues the respiratory defect of *shy1* mutants when present in more than one copy. The mutation(s) in the revertants probably increase the efficiency with which *MSS51* suppresses rather than creates a new activity (Barrientos, unpublished data). There are two mechanisms by which *MSS51* could be suppressing the defect in *shy1* mutants. If the phenotype of *shy1* mutants is the product of an altered expression of subunit I, *MSS51* could compensate for the defect by increasing the pool of the subunit available for assembly. Alternatively, *MSS51* may have another function related to some other aspect of subunit I biogenesis. In that event, Shy1p is likely to function at a step downstream of *COX1* expression.

3.6. Concluding comments

At present, only a small number of COX-specific genes have been correlated with human pathologies. This is likely to change as the search for the molecular basis of human mitochondrial diseases takes advantage of the genes already shown to be important in COX assembly. Continued studies of this process in yeast can be anticipated to further clarify the players and their functions and in so doing make the yeast paradigm even more useful in assessing the genotype–phenotype relationship in human COX deficiencies.

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