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Assembly of Cytochrome *c* Oxidase within the Mitochondrion

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ABSTRACT
Cytochrome *c* oxidase (CcO) is an oligomeric complex localized within the mitochondrial inner membrane. Assembly of the active oxidase complex requires the coordinate assembly of subunits synthesized in both the cytoplasm and the mitochondrion. In addition, assembly is dependent on the insertion of five types of cofactors, including two hemes, three copper ions, and one Zn, Mg, and Na ion. A series of accessory proteins are critical for synthesis of the heme A cofactor and insertion of the copper ions. This Account will focus on the steps in the coordinate assembly of CcO subunits, the formation of heme A, and the delivery and insertion of copper ions.

Introduction

Cytochrome *c* oxidase (CcO) is the terminal enzyme in the energy transducing respiratory chain of eukaryotes. Electrons culled from the oxidation of nutrients are channeled through the respiratory chain within the mitochondrion to CcO, where molecular oxygen is reduced to water. Mammalian CcO consists of a 13 subunit complex of 200 kDa which can also exist as a larger dimeric unit (Figure 1A). The assembly of CcO is complicated by three factors. First, three of the subunits (subunits 1, 2, and 3) are encoded by the mitochondrial genome and the remaining subunits encoded by the nuclear genome, so subunits synthesized in two compartments must be coordinately recruited to assembly sites. The mitochondrially encoded subunits represent 60% of the mass of the complex and are the core components necessary for electron transfer to oxygen and proton pumping.[1] In contrast, the 10 nuclear-encoded subunits are small polypeptides. Several of these small subunits consist merely of a single transmembrane helix (Figure 1A) that packs on the outside of the core complex.[2] Only a subset of the peripheral subunits show sequence conservation between animals and yeast; yet these subunits are functionally important. The nomenclature for the nuclear-encoded subunits differs between yeast and mammalian cells. In cases where differences exist, both designations will be presented for clarity.

The second complication of assembly is the complexity of the mitochondrial localization of CcO. The mitochondrion is an organelle with double-membrane-enclosed compartments. The inner membrane differs from the outer membrane in being highly convoluted, folding into tubular structures called cristae. Three internal spaces are created by the double-membrane structure. The volume enclosed within the inner membrane is the matrix, which represents about 80% of the total mitochondrial space.[3] The space between the inner (IM) and outer (OM) membranes is called the intermembrane space (IMS) and is interrupted by junction points in which the IM and OM are in contact. The IMS is very narrow and is separated from the third space, intracristae space, by tubular cristal junctions.[4,5] The bulk of the respiratory complexes exist within the cristae, and a significant number of the IM respiratory proteins protrude into the intracristal space.[6] Thus, assembly of functional CcO requires transport of the 10 nuclear-encoded subunits across both mitochondrial membranes. It is unclear whether the assembly of newly synthesized subunits occurs within the cristae or on the peripheral surface of the IM.

The third complication in the assembly of CcO lies in the number of cofactors necessary for function, including two heme moieties, three copper ions, and zinc, magnesium, and sodium ions (Figure 1B). Subunit 1 of CcO (Cox1) contains two heme cofactors, one that is involved in electron transfer and a second that interacts with a mononuclear copper site forming the heterobimetallic active site. Interestingly, the hemes contained within CcO are not typical hemes B; they are modified hemes known as heme A. Heme A differs from heme B in that a hydroxyethylfarnesyl group replaces one of the vinyl groups and a pyrrole methyl substituent is oxidized to a formyl group (Figure 2). The physiological importance of hydroxyethylfarnesylation may relate to protein packing. The farnesyl moiety of heme A is packed within the interior of an α-helical bundle and a segment of the heme A_3 farnesyl group is sandwiched between subunits 1 and 2.[2] Oxidation of the methyl to the formyl substituent may be important to modulate the redox potential of the heme.[7]

In addition to the Cu ion found at the bimetallic site in Cox1 (designated Cu_B), Cox2 contains two Cu ions in a binuclear, mixed valent center designated the Cu_A site (Figure 3). CcO is embedded within the IM with a portion of the molecule protruding into the IMS (37 Å) and a portion extending into the matrix (32 Å) (Figure 1).[2,8] The term IMS will be used to designate both the soluble space between the OM and IM and the soluble lumen of the intracristae tubules. The Cu_A site exists within the domain

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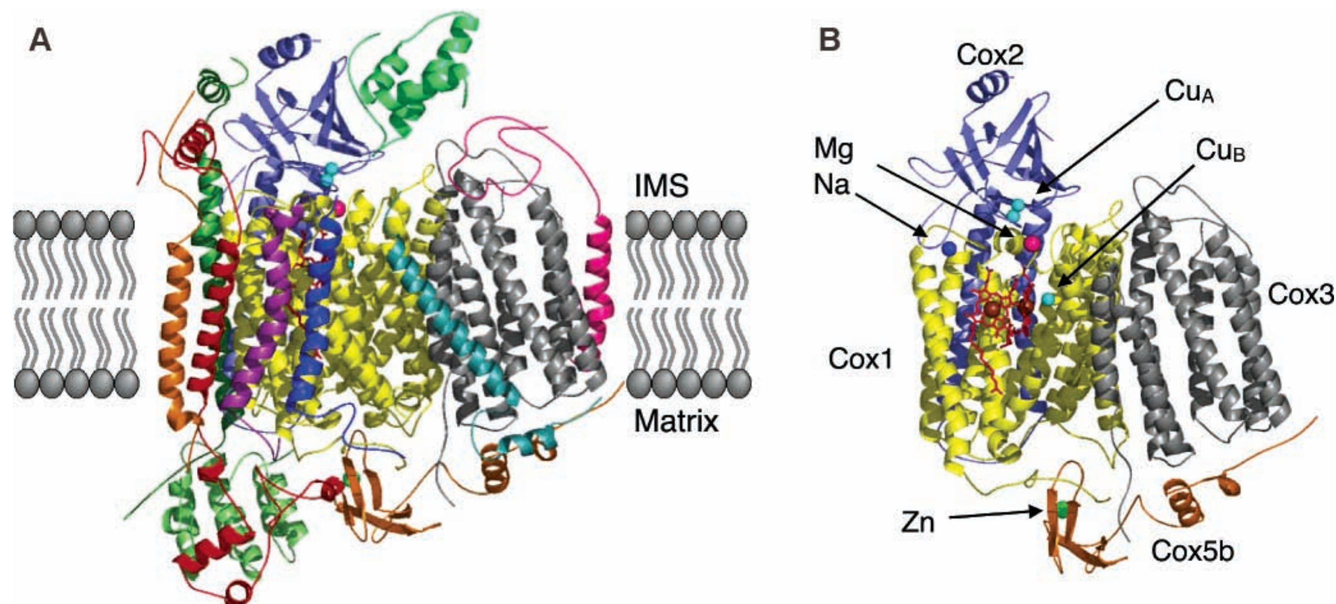


FIGURE 1. Panel A shows the structure of bovine CcO is shown with the 13 subunits colored differently.[2] The cofactors are largely obscured by the polypeptide chains. Panel B shows the cofactors with all the peripheral subunits removed except Cox5b, which contains the single Zn(II). To see the cofactors in Cox1, two helices of Cox1 were removed.

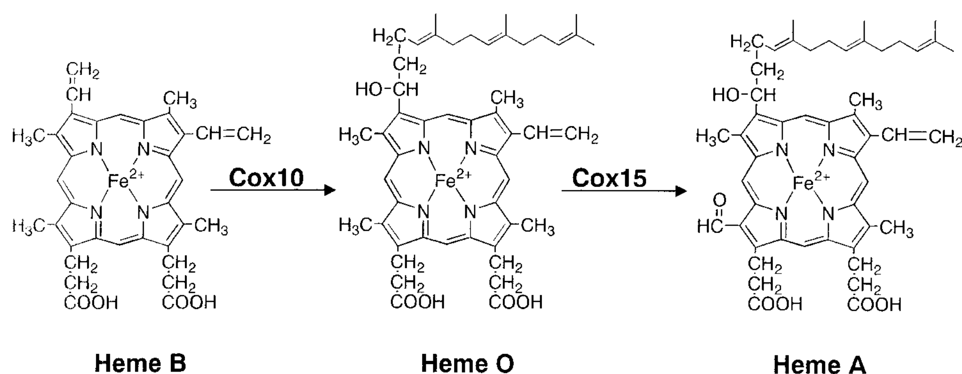


FIGURE 2. Heme A biosynthesis involves two successive reactions catalyzed by the Cox10 farnesyl transferase and Cox15 oxidase.

of Cox2 protruding into the IMS, whereas the heme A_3–Cu_B binuclear site is buried 13 Å below the membrane surface.[2] Cytochrome *c* docks with CcO interacting with the exposed Cox2 close to the Cu_A site.[9]

Although the remaining cofactors have been characterized structurally, less is known about their role in catalysis. The Mg ion is bound at the interface of subunits 1 and 2, close to the propionate groups of heme A_3, and shares a ligand with the binuclear Cu_A site.[2] The Zn ion is bound in bovine subunit 5b (yeast Cox4) (Figure 1B).[2] This subunit, along with subunit 5a (yeast Cox6), is localized on the matrix side of the IM. A Na ion is bound at a peripheral site in Cox1. A Ca ion can bind in the Na ion site, resulting in a perturbation in the heme A spectrum, although the significance of the Ca-induced spectral perturbation is unknown.[10,11]

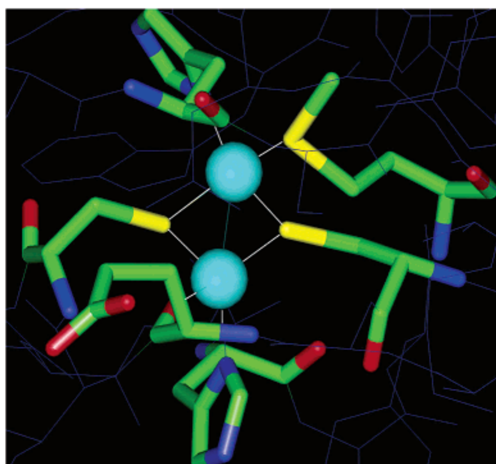
Cytochrome *c* Oxidase Assembly

Over 30 accessory proteins are necessary for the proper assembly of the enzyme complex.[12] The functional role of these accessory factors in the assembly of CcO will be reviewed with regard to (1) the assembly of subunits, (2) the formation and insertion of heme A, (3) the delivery and insertion of metal ions to the mitochondrion, and (4) final maturation of the complex. Each step will be discussed separately.

Assembly of CcO Subunits

The nuclear-encoded subunits must be imported into the mitochondrion for assembly of mature CcO. Many of these subunits are highly hydrophobic, so the translocation of these peptides may be protein-mediated. Several of these subunits can assemble into a stable complex within the mitochondrion in the absence of the mitochondrially encoded subunits. At least some of the nuclear-encoded subunits can form a complex independent of the core CcO subunits. These include yeast Cox4, Cox5a, Cox6, and Cox9.[13,14] Two of these (yeast Cox4 and Cox6) are extramembrane proteins that are stable as soluble proteins.[15]

Prior to the assembly of subunits, several processing steps are necessary for the core subunits. The three mitochondrial subunits are synthesized on ribosomes associated with the IM.[16] mRNAs for these subunits are recruited to the IM by a series of membrane proteins.[17]



Cox2 H-x$_{34}$-**C**xEx**C**-x$_3$-HxxxM

FIGURE 3. Structure of the mixed valent Cu_A site of CcO.[2] The ligands for the binuclear Cu site are shown in the sequence with the two bridging Cys residues in bold.

These proteins bind sequences in the 5′ UTR of the mRNA and may be important in the recruitment and positioning of ribosomes on the message. The Cox2 translational activator Pet111 is found only in fungi, so an unresolved question is whether biosynthesis of mammalian Cox2 requires similar translational activators.[16]

The processing and maturation of Cox2 in yeast has been investigated most thoroughly. Upon completion of translation of Cox2, two additional steps are necessary to get the protein in the proper juxtaposition within the IM. Cox2 is anchored in the IM by two transmembrane helices such that both the N-terminal 15 residues, and C-terminal 137 residues protrude into the IMS. The nascent Cox2 chain requires two translocation events to extrude the N- and C-terminal domains across the IM. Oxa1 and Cox18 appear to be the functional translocators for extrusion. Yeast Cox2 is synthesized as a precursor protein with an extension of 15 residues at the N-terminus. Once the N-terminus is extruded by Oxa1, the precursor segment is cleaved by the Imp1/Imp2 protease within the IMS.[18,19] This cleavage is dependent on the presence of Cox20, which functions as a protein chaperone facilitating proteolysis.[20] In the absence of cleavage, Cox2 fails to assemble into the CcO complex. The C-terminal domain of Cox2 is extruded through the IM by three proteins, including Cox18.[21] Mammalian Cox2 is not synthesized as a precursor, so the ImpI/Imp2/Cox20-dependent cleavage step is essential only in fungi. In contrast, the two translocation events for Cox2 are important in mammalian CcO assembly, and the Oxa1 and Cox18 translocases are conserved in eukaryotes.

Preliminary work investigating the assembly process of CcO has shown that mammalian Cox4 associates with Cox1 as an early intermediate in assembly,[22] perhaps due to the tight packing of the transmembrane helix of bovine Cox4 against Cox1 (Cox4 is colored red in Figure 1). Subunits 6a and 7a appear to be added last.[22] No accessory proteins have yet been identified that function in the assembly of subunits. The dissection of the assembly pathway is complicated by the fact that deletions of some yeast nuclear CcO subunit genes and many accessory factor genes result in a destabilized CcO complex.[23]

Biosynthesis of CcO is believed to occur primarily along the inner boundary of the IM, as mitochondrial ribosomes are concentrated on this segment of the IM and along the cristal IM.[24] The biosynthesis of CcO on the inner boundary places the assembly point near sites of import of nuclear-encoded polypeptides as they are extruded through the TOM/TIM complex. It is unclear how the nuclear-encoded subunits are partitioned into intermediate complexes competent for packaging onto the core subunits. If assembly occurs completely in the inner boundary IM, the complex may be translocated through the cristal junction.

Heme A Formation and Insertion

Only a small amount of the heme B produced by the cell is converted to heme A. The first step in the formation of heme A is the conversion of heme B into heme O by Cox10, a mitochondrial membrane-associated farneysl transferase (Figure 2).[25] The C2 vinyl group on pyrrole ring A undergoes stereospecific nucleophilic addition to be converted to a hydroxyethyfarnesyl group. Cox10 requires no partners in the formation of heme O, as overexpression of *COX10* in yeast effects an increase in the heme O content.[26] The second step in heme A formation is the oxidation of the C8 pyrrole methyl moiety to an aldehyde. The oxidation appears to proceed by two successive monooxygenase steps[27] catalyzed by the Cox15 heme A synthase in conjunction with ferredoxin (Yah1) and ferredoxin reductase (Arh1).[28] The first indication that ferredoxin was an important component of the hydroxylation reaction was the demonstration that Cox15 in *Schizosaccharomyces pombe* was fused to Yah1.[29] The monooxygenase reactions require reducing equivalents that are presumably supplied by ferredoxin.

The insertion of heme A into Cox1 has not been characterized. The two heme A moieties bind in hydrophobic pockets formed within the 12 transmembrane helices of Cox1. Given their placement within the IM bilayer, the insertion of hemes A and A_3 is likely to occur concomitantly with the translation of Cox1 rather than after assembly of the core complex. A reasonable model of heme A insertion is that Cox10 and Cox15 form a complex, allowing heme O formed by Cox10 to be efficiently channeled to the active site of Cox15 for the oxidation reaction. Heme A formed by Cox15 may then be inserted into Cox1 chains emerging from the mitochondrial ribosomes. Alternatively, heme A may be inserted into Cox1 after formation of its early intermediate complex with yeast Cox5a (bovine Cox4). Cox5a contains a 70-residue domain that protrudes into the matrix, so this domain may be important in recruitment of Cox10/Cox15 for heme A insertion. Heme A insertion is likely to occur in Cox1 prior to addition of Cox2 as the farnesyl group of heme A_3 is packed between the two subunits.

Synthesis and insertion of heme A appear to be regulated. The conversion of heme O to heme A appears to be coupled to a step in CcO assembly, since cells lacking one of a variety of accessory factors fail to accumulate heme A.[26] This observation suggests regulation of heme A formation or rapid turnover of heme A not incorporated into Cox1. The low levels of heme A in these accessory factor mutants is not likely due to turnover of unbound heme A, since overexpression of Cox15 and ferredoxin results in a dramatic increase in heme A content.[28] Alternatively, heme A synthesis may be modulated by the assembly process. If assembly is blocked due to a missing subunit or cofactor, an unassembled component may inhibit heme A synthesis.

Delivery of Metal Ions to the Mitochondrion and Insertion into Cox1 and Cox2

Copper, zinc, and magnesium ions are necessary for CcO function. Little is known about the delivery of Zn and Mg ions to the mitochondrion or whether specific accessory factors are necessary for the insertion of these ions into CcO. More information is available concerning copper delivery and insertion. Three yeast proteins (Cox11, Cox17, and Sco1) have been implicated in the copper ion loading of CcO. Cox17 has been implicated as the key mitochondrial Cu ion shuttle, whereas Cox11 and Sco1 function downstream, presumably in the Cu insertion reaction into Cox1 and Cox2. These proteins will be separately discussed.

Cox17 was first identified as part of a genetic screen for mutants defective in CcO activity.[30] Yeast lacking Cox17 are respiratory deficient and unable to grow on nonfermentable carbon sources. These phenotypes are reversed by the addition of 0.4% copper salts to cells, making the *cox17*Δ mutation the only one suppressed by exogenous copper.[30] This effect is consistent with Cox17 functioning in copper delivery to CcO. Since *cox17*Δ cells contain a functional Cu,Zn-superoxide dismutase, the role of Cox17 is likely specific for CcO and/or the mitochondrion. As with other assembly factor mutants, *cox17*Δ cells are deficient in heme A.[30] Mitochondria isolated from *cox17*Δ cells contain both mitochondrial and nuclear oxidase subunits, although Cox2 levels are low.

Yeast Cox17 is a conserved, hydrophilic protein of 69 residues present in both the cytoplasm and mitochondrial IMS.[31] Three of the seven cysteines are present in a CCxC sequence motif that is important for Cu(I) binding.[32] The protein, purified as a recombinant molecule in *E. coli*, binds 3 Cu(I) ions per monomer in a polycuprous−thiolate cluster, as shown by X-ray absorption spectroscopy.[33] The Cu(I) cluster(s) exhibit predominantly trigonal Cu(I) coordination by cysteinyl thiolates and appear to resemble synthetic Cu(I)−thiolate clusters that have been crystallographically defined.[33,34] Cox17 exists in a dimer/tetramer equilibrium with a K_D of 20 μM.[33] The apo-Cox17 polypeptide is predominantly monomeric, so it is predicted that the polycuprous cluster forms at the dimer interface. The tetrameric species may be a dimer of dimers. Mutation of any of the three Cys residues in the CCxC sequence motif results in loss of function of the protein, even though each mutant is still able to bind Cu(I) and localize within the mitochondrion.[32] However, these mutants fail to form tetramers, suggesting that oligomerization is functionally important. Cox17 proteins containing a double Cys > Ser mutation in this CCxC motif fail to bind Cu(I) yet still localize within the IMS.[32] Thus, mitochondrial localization is independent of Cu(I) binding.

Cox17 appears to have two functional domains. Cox17 lacks a classical N-terminal target sequence that directs mitochondrial import. Rather, mitochondrial uptake appears to be conferred by a C-terminal motif. Within this segment, Cox17 contains three conserved, C-terminal Cys residues that are not important in Cu(I) binding.[32] This segment of the protein is likely important in mitochondrial import as mutations in this segment attenuate mitochondrial uptake.

The dual localization of Cox17 led to the suggestion that Cox17 ferries Cu(I) ions across the outer mitochondrial membrane. However, *cox17*Δ cells are not deficient in mitochondrial Cu (Cobine and Winge, unpublished observation), suggesting that Cox17 is not the primary mitochondrial copper shuttle. Cox17 may be the Cu(I) donor to CcO, but instead of shuttling Cu(I) to the mitochondrion, Cox17 may receive its Cu(I) within the IMS. The Cu,Zn superoxide dismutase is also localized in the IMS.[35] Sod1 is taken up into the IMS as an apo-protein, and this uptake is dependent on the Sod1 metallochaperone, CCS, that metalates Sod1.[35,36] Thus, CCS and Cox17 may both be metalated in the IMS.

A homologue of Cox17 was recently found to be important in CcO assembly.[37] Cox19 shares sequence similarity with the C-terminal segment of Cox17 but lacks the Cu(I) binding CCxC motif. Like Cox17, Cox19 exhibits dual localization in the cytoplasm and IMS.[37] Yeast cells lacking Cox19 are respiratory deficient, but this phenotype is not reversed by the addition of exogenous copper salts. The fact that Cox19 lacks the Cu(I) binding residues of Cox17 suggests that Cox19 performs a function that is independent of Cu(I) binding. The conserved C-terminal Cys residues may be important in mitochondrial uptake and/or a function within the IMS.

Cox11 and Sco1 appear to be co-metallochaperones assisting Cox17 in the metalation of CcO (Figure 4). Sco1 was first implicated in Cu delivery to CcO by the observation that the respiratory deficient phenotype of a *cox17−1* strain could be suppressed by overexpression of *SCO1* or a related gene designated *SCO2*.[38] Sco1 and Sco2 are highly similar proteins associated with the mitochondrial IM.[38,39] Both proteins contain a single transmembrane helix in the N-terminal segment of the proteins. The function of Sco1 is dependent on its transmembrane helix. Targeting of Sco1 to the mitochondrial matrix or to the IMS as a soluble protein fails to yield a functional molecule.[40] The phenotypes associated with the two genes are distinct. *sco1*Δ cells are respiratory deficient, with diminished CcO activity, undetectable heme A, and low levels of CcO subunits1 and 2.[41,42] In contrast, *sco2*Δ cells lack an obvious pheno-

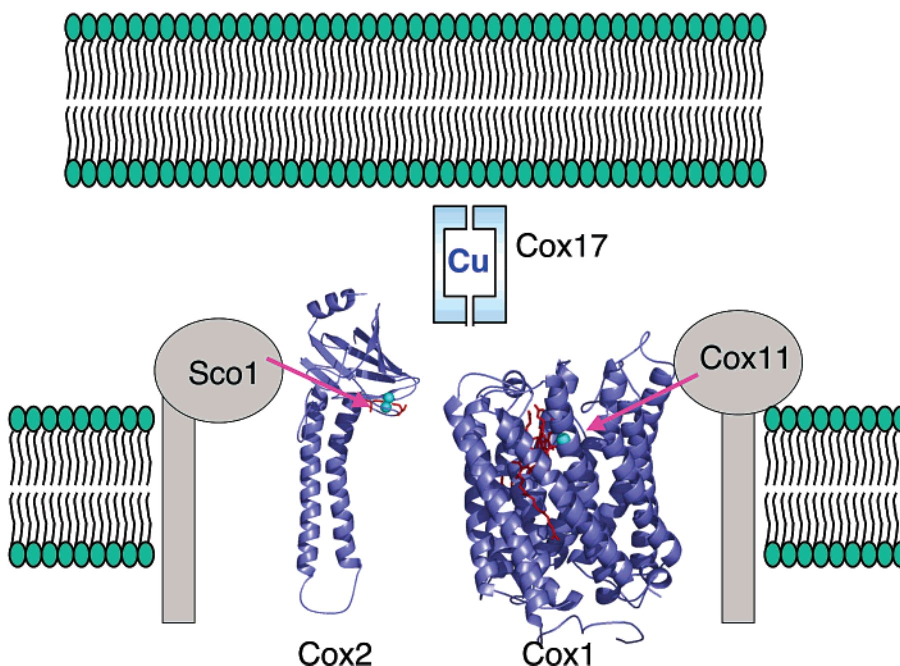


FIGURE 4. Scheme of Cu metalation of Cox1 and Cox2 mediated by Cox11 and Sco1, respectively. The original model suggesting that Cox17 shuttled Cu(I) ions across the mitochondrial OM may not be accurate. Cox17 is postulated to transfer Cu(I) to Sco1 prior to the transfer to the Cu_A site in Cox2 and to transfer Cu(I) to Cox11 prior to transfer to the buried Cu_B site in Cox1.

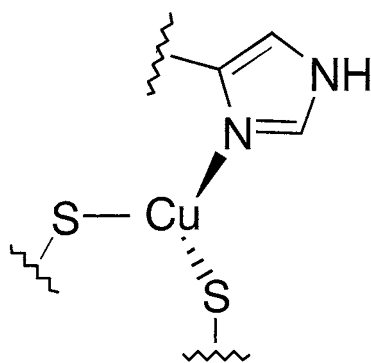


FIGURE 5. Proposed Cu(I) coordination in Sco1. The 2 Cys and single His ligands exist within aCxxxC$-$x$_{86}$$-$H sequence.

type.[43] Sco2 overexpression only partially restores respiratory growth in *cox17*Δ cells, and this reversal requires addition of Cu salts to the growth medium.[38] High levels of Sco2 fail to overcome the respiratory defects of *sco1*Δ cells.[38] On the basis of this evidence, Sco1 is proposed to mediate the transfer Cu(I) from Cox17 to Cox2. The role of Sco2 in yeast remains unresolved.

The function of Sco1 in metalation of CcO appears to involve a Cu(I)-binding intermediate. Sco1 contains a conserved CxxxC sequence motif that is essential in Cu(I) binding and in vivo function.[44,45] X-ray absorption spectroscopy suggests that the Cu(I) is ligated via three ligands.[44] The two Cys residues in a conserved motif, CxxxC and a conserved histidine are involved in Cu(I) ligation (Figure 5). Mutation of any one of these conserved residues in Sco1 abrogates function, resulting in a nonfunctional CcO complex. Thus, the function of Sco1 correlates with Cu(I) binding. Human cells have two Sco molecules and both are required for viability. Mutations in either h*SCO1* or h*SCO2* lead to decreased CcO activity and early death.[46−48] The missense mutations identified in *sco1* and *sco2* patients are located near the conserved CxxxC and essential His residues, suggesting that the loss of function in both proteins is due to diminished copper binding.

Sco1 appears to be important for formation of the Cu_A site in Cox2. Yeast Sco1 was shown to interact with Cox2.[49] The Cu_A site in Cox2, which is formed within a ten-stranded β-barrel, resembles a type I copper site. The two Cu ions are bridged by two cysteine residues within a CxxxC motif, similar to part of the Cu-binding site in Sco1. Although the structure of Sco1 is unknown, Sco1 is highly homologous to the peroxiredoxin family of proteins.[50,51] Modeling of Sco1 on the coordinates of rat 2-Cys peroxiredoxin reveals an exposed β-hairpin loop containing the two essential Sco1 Cys residues involved in Cu(I) coordination (Figure 6). We predict that the single Cu(I) ion coordinated to Sco1 is solvent-exposed and poised for a ligand exchange transfer reaction. Consistent with this prediction, the yeast CuSco1 complex lacks luminescence despite its Cu(I) valence state, suggesting that it is solvent exposed.

If Sco proteins are primarily important in Cu(I) insertion into Cox2, then how is Cu provided to Cox1? This pathway appears to require another mitochondrial IM protein, Cox11 (Figure 4).[13] *S. cerevisiae* lacking Cox11 have impaired CcO activity and have lower levels of Cox1.[13] The critical observation suggesting a role for Cox11 in Cu_B site formation was the demonstration that CcO isolated from *R. sphaeroides cox11*Δ cells lacked Cu_B but contained both hemes; however, heme A_3 showed an altered environment by EPR spectroscopy.[52] Formation of Cu_A, subunit assembly, and heme synthesis was not affected by the absence of Cox11. Thus, the absence of Cox11 appears to

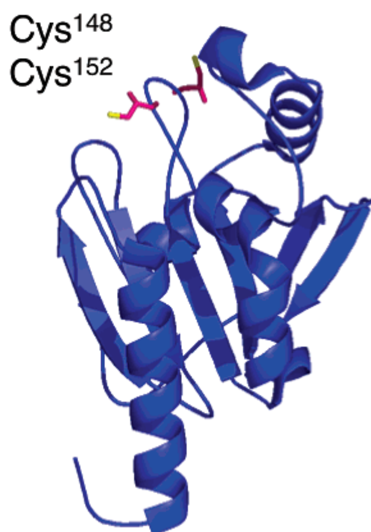


FIGURE 6. The structure of peroxiredoxin is shown.[46] Sco1 modeled onto the peroxiredoxin coordinates reveals a similar tertiary fold, with Sco1's two critical Cys residues in an exposed β loop. The side chains of the modeled Cys residues are shown. The third Cu(I) ligand, His[239], is predicted to exist on the adjacent β hairpin loop.

preclude Cu_B site formation without affecting binding of other cofactors.

Cox11 resembles Sco1, with a single transmembrane helix just downstream of the N-terminal mitochondrial targeting sequence. The C-terminal domain of 189 residues protrudes into the IMS and, as with the soluble C-terminal domain of Sco1, binds a single Cu(I) ion.[53] Three conserved Cys residues are ligands for the Cu(I) ion. Mutation of any of these Cys residues reduces Cu(I) binding and confers respiratory incompetence and reduced CcO activity.[53] Thus, the residues important for Cu(I)-binding correlate with in vivo function, suggesting that Cu(I)-binding is important in Cox11 function. The soluble C-terminal Cox11 domain exists as a dimer with the Cu(I) ions in the monomer in close proximity. The two Cu(I) ions are separated by 2.7 Å—a distance similar to the separation of Cu(I) ions in several polycopper thiolate clusters.[54]

If Cox11 is the co-metallochaperone for the Cu_B site, an unresolved question is how Cox11 transfers a Cu(I) ion to the site that is buried 13 Å below the membrane surface. One of the three Cys ligands is immediately adjacent to the transmembrane segment of Cox11, suggesting that the Cu(I) binding site is close to the bilayer on the IMS side of the IM. One clue to the mechanism of Cu transfer from Cox11 to Cox1 is the observation that Cox11 in *S. pombe* exists as a fusion protein with an N-terminal extension of 530 residues. That sequence is highly similar to the *S. cerevisiae* protein Rsm22, which is associated with the mitochondrial small ribosomal subunit.[55] One postulate is that Cox11 interacts with Rsm22 in yeast and this interaction permits Cox11 to insert Cu(I) in a cotranslational manner. Since Rsm22 is a matrix protein and Cox11 is a transmembrane protein with only 39 residues protruding into the matrix, an interface for the interaction between the two proteins may be limited to the small N-terminal Cox11 matrix peptide. Rsm22 has homologues only in fungal species, so the question remains whether this putative fungal interaction is functionally important in animal cells.

Curiously, two of the proteins important for CcO metalation bind Cu(I) in polycopper centers. The significance of polycuprous clusters in the assembly pathway remains unclear. The presence of a polycopper cluster in Cox17 as the initial Cu(I) donor within the IMS may be significant for two reasons. First, a polycopper cluster ensures that metal transfer is Cu-specific. Mononuclear metal sites are less able to discriminate between metal ions than polymetallic sites. Second, a polycopper cluster may be significant in providing a reservoir of Cu(I) ready for CcO assembly. The cluster in Cox11 forms with the Cu(I) site of each monomer in close juxtaposition. As mentioned, CcO can exist as a dimeric complex, so the oligomeric accessory factors may relate to the dimeric nature of CcO. Alternatively, if Sco1 and Cox11 metalate Cox2 and Cox1, respectively, in a cotranslational manner, the dimeric state of Cox11 may protect the Cu(I) until a time when transfer to Cox1 is required. The actual Cu(I) transfer may occur through a transient heterodimer of Sco1 and Cox2 or Cox11 and Cox1. If Cu(I) transfer occurs in a heterodimeric complex, the reaction would be analogous to the Cu(I) transfer between CuCCS and apo-Sod1. CCS and Sod1 exist as dimers; yet a heterodimer forms and appears to be functional in Cu(I) transfer reaction.[56]

Final Maturation of CcO

Upon assembly of subunits and insertion of cofactors, two additional steps are essential for fully functional CcO. First, a posttranslational modification occurs in which Tyr[244] forms a covalent link with the Cu_B-ligating His[240].[57,58] The cross-link appears to fix the Cu_B ion in a precise position relative to heme A_3.[59] Second, CcO assembles into a supracomplex involving complex III (ubiquinol-cytochrome *c* reductase).[60] This supracomplex requires cardiolipin, a phospholipid found only tightly associated with various IM protein complexes.[61] Purified CcO tightly binds 2 mol of cardiolipin; removal of this lipid results in the dissociation of the two subunits (6a and 6b) that constitute the bulk of the dimeric interface.[62] This may be functionally significant, as the oligomeric state of CcO and the other respiratory complexes appears important in efficient electron transfer and proton pumping.[63]

Summary

The identification of accessory proteins involved in heme A biosynthesis and Cu metalation of CcO provides an exciting chance to elucidate the mechanism of assembly of this respiratory complex. The assembly of respiratory complexes I, II, and III is also expected to involve numerous accessory proteins, so these processes present clear opportunities for future studies.

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