Mitochondrial Copper Metabolism and Delivery to Cytochrome *c* Oxidase

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Summary

Metals are essential elements of all living organisms. Among them, copper is required for a multiplicity of functions including mitochondrial oxidative phosphorylation and protection against oxidative stress. Here we will focus on describing the pathways involved in the delivery of copper to cytochrome *c* oxidase (COX), a mitochondrial metalloenzyme acting as the terminal enzyme of the mitochondrial respiratory chain. The catalytic core of COX is formed by three mitochondrially-encoded subunits and contains three copper atoms. Two copper atoms bound to subunit 2 constitute the Cu_A site, the primary acceptor of electrons from ferrocytochrome *c*. The third copper, Cu_B, is associated with the high-spin heme a_3 group of subunit 1. Recent studies, mostly performed in the yeast *Saccharomyces cerevisiae*, have provided new clues about 1- the source of the copper used for COX metallation; 2- the roles of Sco1p and Cox11p, the proteins involved in the direct delivery of copper to the Cu_A and Cu_B sites, respectively; 3- the action mechanism of Cox17p, a copper chaperone that provides copper to Sco1p and Cox11p; 4- the existence of at least four Cox17p homologues carrying a similar twin CX_9C domain suggestive of metal binding, Cox19p, Cox23p, Pet191p and Cmc1p, that could be part of the same pathway; and 5- the presence of a disulfide relay system in the intermembrane space of mitochondria that mediates import of proteins with conserved cysteines motifs such as the CX_9C characteristic of Cox17p and its homologues. The different pathways are reviewed and discussed in the context of both mitochondrial COX assembly and copper homeostasis.

Keywords

Mitochondria; cytochrome oxidase; copper; metal homeostasis; superoxide dismutase

CYTOCHROME *C* OXIDASE: A MITOCHONDRIAL COPPER/HEME METALLOENZYME

Aerobic life depends on cellular copper homeostasis and distribution since this element is a critical component of enzymes involved in primary metabolism (1). Copper ions can undergo unique chemistry due to their ability to adopt distinct redox states, either oxidized [Cu(II)] or reduced [Cu(I)], and they serve as important catalytic cofactors in redox chemistry for proteins that carry out fundamental biological functions. A copper-containing metalloenzyme,

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mitochondrial cytochrome *c* oxidase (COX), is the final electron acceptor in the mitochondrial electron transport chain and is required for aerobic ATP production.

Over the last 15 years COX biogenesis has received significant attention because of its medical relevance. Defective COX biogenesis results in mitochondrial diseases frequently involving brain, skeletal muscle and heart (reviewed in (2-4)). In particular, mutations in genes required for COX copper metallation result in severe hepatopathies, encephalomyopathies, hypertrophic cardiomyopathy and muscular atrophy (5-9). Because most of the basic biological knowledge concerning mitochondrial copper homeostasis and insertion into COX derives from investigations in the yeast *Saccharomyces cerevisiae*, however, this review will primarily focus on these studies. Intracellular copper transport in mammals has been reviewed elsewhere (1, 10) but significant differences between yeast and human COX copper metallation will also be briefly discussed here.

Mitochondrial COX is a multi-subunit enzyme formed, depending on the eukaryotic organism, by 12 or more subunits (11) of dual genetic origin. The mitochondrial genome encodes the three catalytic core subunits which are also present in the bacterial enzyme (12), while the nucleus houses genes for nine other subunits that form a protective cage around the core. COX transverses the inner mitochondrial membrane with portions protruding into the intermembrane space and the matrix. The enzyme catalyzes electron transfer from cytochrome *c* to molecular oxygen. This reaction occurs concurrently with vectorial proton pumping from the matrix to the intermembrane space, thus contributing to the generation of a transmembrane proton gradient which is subsequently used by the mitochondrial ATP synthase to drive the synthesis of ATP.

COX is a heme/copper terminal oxidase which uses cytochrome *c* as an electron donor as mentioned above. The enzyme contains four redox-active metal centers which sequentially aid in reducing molecular oxygen to water: two iron centers, heme *a* and heme a_3 (also referred to as cytochromes *a* and a_3), and two copper centers, Cu_A and Cu_B. Electrons enter COX through a mixed valence dinuclear copper center, the Cu_A site, located in subunit 2. Cu_A transfers electrons to the low spin heme *a* located in subunit 1. From heme *a*, electrons are transferred intra-molecularly to the active site in subunit 1, composed of the high spin heme a_3 and Cu_B, where oxygen (and other ligands such as CO, NO and CN^-) binds. The mechanism of electron transfer through COX has been extensively studied and reviewed elsewhere (13-15). From an evolutionary point of view, the presence of the Cu_A center defines cytochrome *c* oxidase and distinguishes this enzyme from other terminal oxidases that use quinol instead of cytochrome *c* as the electron donor (13,16,17).

As a consequence of its central role in oxidative metabolism COX has been intensively studied by biochemical, genetic, spectroscopic, and crystallographic means (11,18,19). From these studies it is known that other metals such as zinc (in Cox4p) and magnesium (in the interface between Cox1p and Cox2p) are also bound to the enzyme, although the basis for this specific requirement is largely unknown. The zinc atom (Zn^{2+}), located in a non-catalytic subunit, could play a role in structural stability of the complex (20). The magnesium/manganese site (Mg^{2+}/ Mn^{2+}) is in close proximity to the H_2O exit channel and is thought to aid in the stability and release of H_2O produced by the reduction of O_2 (21). Interestingly, it has been proposed that insertion of Cu_B is likely to facilitate the stable formation of the Mg^{2+}/Mn^{2+} center (22), the two sites are approximately 13.5 Å apart, with imidazole side chains of ligands from each of these centers coming within 7 Å (11,19).

Copper metallation of mitochondrial subunits 1 and 2 is essential for the maturation and stability of these subunits. In the absence of the copper centers (as well as in the absence of the heme prosthetic groups) the proteins are rapidly degraded and the COX holoenzyme fails

to assemble (reviewed in (23,24)). Thus, copper is not only required for COX catalytic function but also for its biogenesis, assembly and stability. The following sections will focus on briefly describing the pathways involved in cellular copper uptake, delivery to mitochondria and insertion into the COX catalytic core subunits.

CELLULAR COPPER HOMEOSTASIS AND DELIVERY TO MITOCHONDRIA

Copper is a transition metal ion essential for the function of key metabolic enzymes as explained above. However, given its high redox reactivity copper can be the source of reactive oxygen species and extremely toxic to the cell. Therefore, a network of transporters strictly controls the trafficking of copper in living systems. Once within the cell, copper is either sequestered by metallothioneins (such as Cup1p and Crs5p), directed to the vacuole which is important for proper copper detoxification, or delivered to the sites of its utilization by specialized proteins called copper chaperones. An abundant amount of literature concerning the structure and function of these proteins is now available (recently reviewed in (1,25-27)).

Briefly, yeast cells acquire copper, depending on extracellular copper concentrations, through the high affinity specific transporters Ctr1p (28) and Ctr3p (29) or through low affinity permeases such as the iron transporter Fet4p (30) and the metal transporter Smf1p (31). Extracellular copper mostly exists in the oxidized form Cu(II), but it is reduced to the Cu(I) form upon entering the cell by the membrane integral cupric reductases Fre1p and Fre2p (32). Once within the cell, copper is bound to cytosolic copper chaperones. Copper binds Atx1p for specific delivery to the secretory pathway. In yeast, Ccc2p, a P-type ATPase located in the trans-Golgi network, accepts copper from Atx1p (33-35) and transfers it into the lumen of the trans-Golgi network where it is incorporated into the multicopper ferroxidase Fet3p in a manner facilitated by the Gef1p chloride channel (36,37). Active Fet3p forms a complex with the iron permease Ftr1p and both proteins translocate to the plasma membrane where they are responsible for high affinity iron uptake, thus connecting copper and iron metabolisms. In the cytosol, copper also binds Ccs1p which is required for the metallation and activation of the copper/zinc superoxide dismutase (Sod1p) (38). A small fraction of Sod1p and its metallochaperone, Ccs1p, localize to the intermembrane space of mitochondria (39). In both compartments the chaperone forms a Ccs1p–Sod1p heterodimer that facilitates copper transfer (40). Ccs1p-mediated copper insertion into Sod1p also catalyzes the formation of a critical disulfide bond in Sod1p which seems to be important for regulation of enzyme activity and for prevention of misfolding or aggregation (41). Alternative pathways for copper insertion into Sod1p have been proposed in mammals (42). Finally, copper also binds to Cox17p in the cytosol, a chaperone that has been proposed to have, similarly to Ccs1p, a dual location in the cytoplasm and in the mitochondrial intermembrane space. Mitochondrial Cox17p is necessary for copper delivery to cytochrome *c* oxidase (43) as discussed below.

Involvement of *COX17* in copper homeostasis was originally proposed because the respiratory deficient phenotype of *cox17* null mutant strains can be rescued by supplementation of copper to the media (43). It was proposed that *COX17* could be shuttling copper from the cytoplasm into the mitochondrial intermembrane space (43,44). This hypothesis has been challenged by the observation that while apo-Cox17p is predominantly a monomer with a simple hairpin structure, the protein-copper complex exists in a dimer/tetramer equilibrium (45) making its free passage though the outer membrane protein channel unlikely. In addition, it has been recently shown that the tethering of Cox17p to the inner mitochondrial membrane does not affect COX assembly (46). Two homologues of Cox17p, the small soluble proteins Cox19p and Cox23p, exhibit a cellular distribution similar to Cox17p when over-expressed and are required for COX assembly (47,48). As stated above for Cox17p, it is unlikely that these proteins will act as copper shuttles from the cytosol to the mitochondrial compartments. It was recently shown that Cox19p and Cox23p are not required for the import or accumulation of

copper in the mitochondrion (46). Mitochondria contain a matrix pool of copper bound by a low molecular weight non-proteinaceous ligand (46) which has been shown to be the copper source for metallation of COX and mitochondrial Sod1p (49). The ligand from the copper complex has been found in the cytoplasm and it has been suggested that it may recruit, in place of a copper chaperone, the copper that is translocated and stored within the mitochondrial matrix (49). Whatever the case, the identity of key proteins in the copper trafficking process to mitochondria remains elusive. Which is/are the predicted mitochondrial membrane copper permease/s and how is copper transported from the matrix pool to the copper chaperons located in the IMS are two obvious questions remaining.

COPPER DELIVERY TO COX

Mitochondrial copper homeostasis and delivery to cytochrome *c* oxidase appears to require the function of a growing number of gene products, some of them largely uncharacterized, listed in Table 1 (References (22,43-45,47,48,50-81)).

COX copper metallation involves the copper chaperone Cox17p, essential for COX assembly (43), a small (~8 kDa) hydrophilic protein containing a CCXC metal binding motif which binds copper ions (44). Cox17p also contains a twin CX_9C structural motif. While the first cysteine in the twin CX_9C motif is part of the CCXC copper binding motif, the remaining three conserved cysteines are not important for Cox17p copper-binding function (67). Cox17p transfers copper ions to two additional chaperones (63) that facilitate copper insertion into the COX Cu_A and Cu_B active sites, respectively Sco1p (55) and Cox11p (22,50) as depicted in Fig. 1A. These proteins are anchored to the mitochondrial inner membrane through a transmembrane α-helix and expose their copper binding sides in the IMS where copper transfer occurs (44,51). *In vitro* experiments have shown that a soluble truncated form of both Cox11p and Sco1p are able to bind copper (51,61). It is still not clear, however, how the transfer of copper occurs between Cox17p and these proteins because physical interactions among them have not been detected (63).

Cu_A site metallation

As stated above, the Cu_A site found in Cox2p is the ligand responsible for accepting electrons from cytochrome *c*. The two copper atoms in the Cu_A site are coordinated by a CX_3C motif as well as two histidines, one methionine and the backbone of a glutamine. Mutagenesis of any of these residues results in protein instability and the inability of yeast to grow on non-fermentable carbon sources (82). The metallochaperone for the formation of the Cu_A center is the product of *SCO1* (55) which was originally identified as essential for COX assembly in yeast (59) and subsequently as a multicopy suppressor of a *cox17* null mutant (43). Sco1p transfers copper from Cox17p to Cox2p and has been shown to directly interact with Cox2p (60). The protein contains a single transmembrane domain with a large soluble copper binding domain facing the intermembrane space. Sco1p has a metal binding thioredoxin-like CX_3C motif analogous to the copper binding motif of Cox2p and this motif is essential for its function as demonstrated by site-direct mutagenesis (62). NMR studies of the human Sco1p homologue both with Cu(I)-bound and Ni(II)-bound allowed Banci and coworkers to propose a model for the Sco1p-Cox2p copper transfer involving the formation of a transient Sco1p species carrying a disulfide moiety able to interact with a copper ion (83). Because the Cu_A center is formed by a Cu(I) ion and a Cu(II) ion, it remains to be elucidated if Sco1p mediates the transfer of both of the different valent ions or, alternatively, if two Cu(I) are inserted in Cox2p by Sco1p and the active site is successively oxidized. It was demonstrated that Sco1p is able to bind both Cu(I) and Cu(II) (61,84,85), although it is not clear if Sco1p receives both cations or only the monovalent copper Cu(I) from Cox17p (84). Although the experimental data could support a role for Sco1p in copper insertion, considering its structural similarity with the protein family of disulfide reductases it has been considered that it is more likely to be involved in the

reduction of cysteines in the Cox2p copper binding site (58). This hypothesis is supported by data from the NMR models mentioned above where Sco1p could function as a thioredoxin to reduce the cysteine residues in the Cu_A site of Cox2p (83). This reduction is necessary for the co-factor incorporation (56,86). Sco1p has the ability to form homodimeric complexes (60) which could facilitate the performance of both functions by the collaborative action of each monomer.

Interestingly, yeast *SCO1* has a highly conserved homologue, *SCO2* (87), with apparently non-overlapping functions. Deletion of *SCO2* does not affect COX assembly (55). Although *SCO2* over-expression does not suppress the COX assembly defect of a *sco1* null mutant strain it is able to partially rescue a *sco1* point mutant (55). Additionally, *SCO2* over-expression also suppresses *cox17* mutations, although less efficiently than *SCO1*, and only when the growth media is supplemented with copper (55). These data were interpreted to indicate that yeast Sco1p and Sco2p have overlapping but not identical functions (55).

It is noteworthy that humans also have two homologues of yeast Sco1p, named SCO1 and SCO2 (88), both of which are essential for COX assembly. Mutations in *SCO1* (5) and *SCO2* (6-8) actually result in severe mitochondrial disorders. Functional complementation studies have shown that expression of either human SCO1 or SCO2 does not complement a yeast strain carrying a null allele of *sco1* (89). Interestingly, the expression of a chimera with the N terminus of yeast and the C terminus (that contains the CX_3C copper binding domain) of human SCO1 is able to complement the yeast mutant, an ability that is lost when using the C terminus of SCO2 (89). The functional differences among the two yeast and human isoforms is explained by the fact that the two genes probably originated from a duplication that occurred separately in the two organisms (6).

In contrast with their yeast homologues, human SCO1 and SCO2 have been shown to perform independent, cooperative functions in the process of COX assembly (90). This is suggested by the fact that overexpression of each SCO protein in fibroblasts from patients with mutation in the other SCO protein results in a dominant negative phenotype (90). These results have suggested a model in which human COX17 delivers copper to SCO2 which in turn transfers it directly to the Cu_A site in COX subunit II in a reaction that is facilitated by SCO1 (90). Recently, SCO1 and SCO2 were shown to have additional regulatory roles in the maintenance of cellular copper homeostasis cooperating to regulate copper efflux under conditions of excessive cellular copper (91) and commented in (92). As stated above, the Cu_A site in COXII (as in yeast Cox2p) is of mixed valence. SCO2 could play a role in the formation of a heterodimer with SCO1 and this dimerization could be needed to create the Cu(II)-Cu(I) site (84).

Cu_B site metallation

The Cu_B site located in Cox1p is formed by one copper ion coordinated by three histidine ligands and present in close proximity to the heme *a* moiety. The metallochaperone for the formation of the Cu_B site of Cox1p is the product of *COX11* (22,50), as shown in the prokaryote *Rhodobacter sphaeroides* (22).

Similar to Sco1p, yeast Cox11p has one transmembrane domain and a soluble copper binding domain which faces the intermembrane space (51). Cox11p was shown to bind Cu(I) (50). The soluble C-terminal domain of Cox11p forms a dimer that coordinates one Cu(I) per monomer via three thiolate ligands. The two Cu(I) ions in the dimer exist in a binuclear cluster and appear to be ligated by three conserved cysteine residues (50). The mechanism of copper transfer to Cu_A remains to be elucidated.

Copper transfer from Cox11p to the Cox1p Cu_B site, deeply buried below the membrane surface, may occur co-translationally in nascent Cox1p polypeptides extruded across the inner membrane. This possibility is supported by the observation of a weak interaction of Cox11p with the mitochondrial ribosome (53). This interaction is probably mediated through a third partner because domain mapping of yeast Cox11p showed that the matrix domain of Cox11p is non-essential for its function (51).

Computer models suggest a direct interaction between Cox11p and Cox1p (93), an interaction that would serve not only for copper transfer to the Cu_B site but also to perform a second function by protecting an early Cox1p-hemeA maturation intermediate against oxidative damage (93).

COPPER TRAFFICKING IN THE INTERMEBRANE SPACE

How copper reaches Cox17p in the intermembrane space is still an open question. As mentioned above, the intermembrane space houses several other proteins, containing the twin CX_9C motif present in Cox17p, which are required for COX assembly. These proteins include Cox19p and Cox23p. The recombinant form of Cox19p was reported to bind copper (70), but the COX assembly defect of a *cox19* null mutant strain could not be rescued by copper supplementation to the media (47). Mutants of *cox23* also fail to assemble COX but their respiratory deficient phenotype is complemented by exogenous copper supplementation, albeit only with concomitant overexpression of *COX17* (48). Cox23p does not physically interact with Cox17p in a stable complex. Recent data showed that Cox23p is also required for mitochondrial copper homeostasis by functioning in a common pathway with Cox17p acting downstream of Cox23p (48). In this same line, Cox19p could participate in a different portion of the copper distribution pathway (48). At least two other uncharacterized small proteins residing in the intermembrane space also share some similarities with Cox17p and Cox19p. These proteins are Pet191p (71) and the product of the gene encoded in ORF YKL137W termed Cmc1p (CX_9C containing Mitochondrial protein required for full expression of COX) by our group. Both Pet191p (94) and Cmc1p (Horn and Barrientos, submitted for publication) are located in the intermembrane space but bound to the inner membrane. Like Cox17p and Cox19p, Pet191p and Cmc1p contain the CX_9C signature motif and their presence is required for full assembly of COX. Their copper binding ability is unknown at present, but it is conceivable that the CX_9C domains could confer this property to Pet191p and Cmc1p as well as to Cox23p.

An important remaining question in the field concerns the role of this relatively large number of different CX_9C-domain containing proteins. Their functions are certainly important in COX biogenesis and mitochondrial metabolism as suggested by the existence of putative homologues from yeast to human (2). In a hypothetical and simplistic way, depicted in Fig. 1B, we could envision copper transfer from the mitochondrial matrix pool across the inner membrane by a still uncharacterized transporter to one or both of the membrane bound small proteins. In a daisy chain transfer mechanism, copper would be subsequently transferred to the soluble Cox19p, Cox23p and ultimately to Cox17p for final delivery to Cox11p and Sco1p. Such a mechanism could appear slower and less efficient than a direct metallation of Cox17p from the copper pool. It could be useful, however, to facilitate and regulate the distribution of copper from the same matrix pool towards the two copper enzymes located in the intermembrane space, COX and Sod1p. Currently it is unknown how copper trafficking towards these enzymes is regulated. It is equally important to note that such a daisy chain transfer mechanism could be useful to accumulate a small bio-available copper pool in the intermembrane space ready to be directed towards Cox17p in a regulated fashion.

We speculate that at least two models could explain copper transfer through this pathway. A redox changing-based copper relay system for copper delivery from Cmc1p to the soluble copper chaperones Cox19p, Cox23p and Cox17p and subsequently to the COX copper site-specific Sco1p and Cox11p could exist. In support of this possibility, it has been shown Cox17p and Sco1p change redox states depending on copper binding (64,66,83,95,96). In the case of Cox17p, the yeast and mammalian homologues share six conserved cysteine residues which are involved in complex redox reactions as well as in metal binding and transfer (95). Cox17p exists in three oxidative states, each characterized by distinct metal-binding properties. Studies of mammalian COX17 have identified fully reduced COX17(0S-S) binding co-operatively to four Cu(I) atoms; COX17(2S-S), with two disulfide bridges, binding to one Cu(I) atom; and COX17(3S-S), with three disulfide bridges, which does not bind any metal ions (64). In the intermembrane space the protein is probably present mostly in the COX17(2S-S) form (66), a conformer that enables retention of the protein in the intermembrane space and is competent to transfer copper to SCO1 (97). Similar oxidative mechanisms of metal release by oxidation of cysteine residues is known to function in the metal release from zinc–thiolate clusters of metallothionein (98). Alternatively, or as a concurrent mechanism, one could speculate that a non-oxidative mechanism of copper transfer occurs, facilitated by the kinetic lability of metal–thiolate clusters. In the case that Cox19p, Cox23p and Cox17p had different, perhaps sequentially higher, copper–binding affinities, the resulting scenario could create a better regulated mechanism or pathway for copper delivery to COX. In this line, it is known that Cox17p has a high affinity for copper with a Kd of 13fM (64) while Cox19p has a lower affinity for copper as indicated by the inconsistent measurement of copper from purified Cox19p fractions (70).

BIOGENESIS OF COX COPPER CHAPERONES

Proteins are imported into mitochondria through several mechanisms extensively reviewed in Neupert and Herrmann (99). The CX_9C twin motifs present in Cox17p, Cox19p, Cox23p, Cmc1p and Pet191p are critical for the import of these proteins into the mitochondrial IMS through the recently described Mia40p pathway (73). After passage through the mitochondrial outer membrane TOM channel, these proteins are covalently trapped by Mia40p *via* disulfide bridges. Mia40p also contains cysteine residues which are oxidized by the sulfhydryl oxidase Erv1p, functioning as a disulfide relay system that catalyzes the import of proteins into the IMS by an oxidative folding mechanism (73). Once folded the protein is retained in the intermembrane space (76). The redox state of Cox17p, Cox19p and Cox23p is unknown but reduction of the cysteines could be required for metal binding as it has been recently proposed (64,66) suggesting the existence of a mechanism of cysteine reduction in the IMS after the oxidative folding.

Concerning the disulfide system, it was initially unclear how Erv1p itself is oxidized to became competent for new rounds of Mia40p oxidation. Interestingly, it has been recently reported that a connection exists between the disulfide relay import system and the mitochondrial respiratory chain consisting of electron transfer from Erv1p to molecular oxygen via an interaction with cytochrome *c* (75,80). Erv1p also utilizes molecular oxygen as an electron acceptor to generate hydrogen peroxide which is subsequently reduced to water by cytochrome *c* peroxidase (Ccp1). Oxidized Ccp1p is in turn reduced by the Erv1p-reduced cytochrome *c* (80). Cytochrome *c* efficiently oxidizes Mia40p in oxygen-limiting conditions (75). Although oxidized cytochrome *c* facilitates Mia40p oxidation, it was found to be non-essential in normoxic conditions suggesting the existence of additional electron acceptors for oxidizing reduced Erv1p (75).

CONCLUDING REMARKS AND OPEN QUESTIONS

The copper metallation of COX is an intricate process that requires the action of several metallochaperone complexes. The list of mitochondrial proteins that affect mitochondrial copper homeostasis and COX assembly is steadily growing. The continued search for protein candidates involved in these processes should allow for the identification of key proteins in the mitochondrial copper trafficking process, including putative copper permease/s located in the outer membrane, and a copper transporter located in the inner membrane to facilitate copper transport from the matrix pool to the intermembrane space. What is/are the role/s of the CX_9C containing proteins in COX assembly, how is the traffic of copper towards COX and Sod1p regulated, and how mitochondrial copper metabolism affects cellular copper homeostasis are some of the remaining questions to be answered.

Understanding these and other fundamental aspects of mitochondrial copper metabolism and COX assembly will aid in our understanding of the intricate process of building a polypeptidic enzyme crucial for aerobic production of energy. The biochemical and biological answers obtained will also contribute to a better understanding of human mitochondrial diseases resulting from mutations in the genes and pathways involved in copper homeostasis.

Acknowledgments

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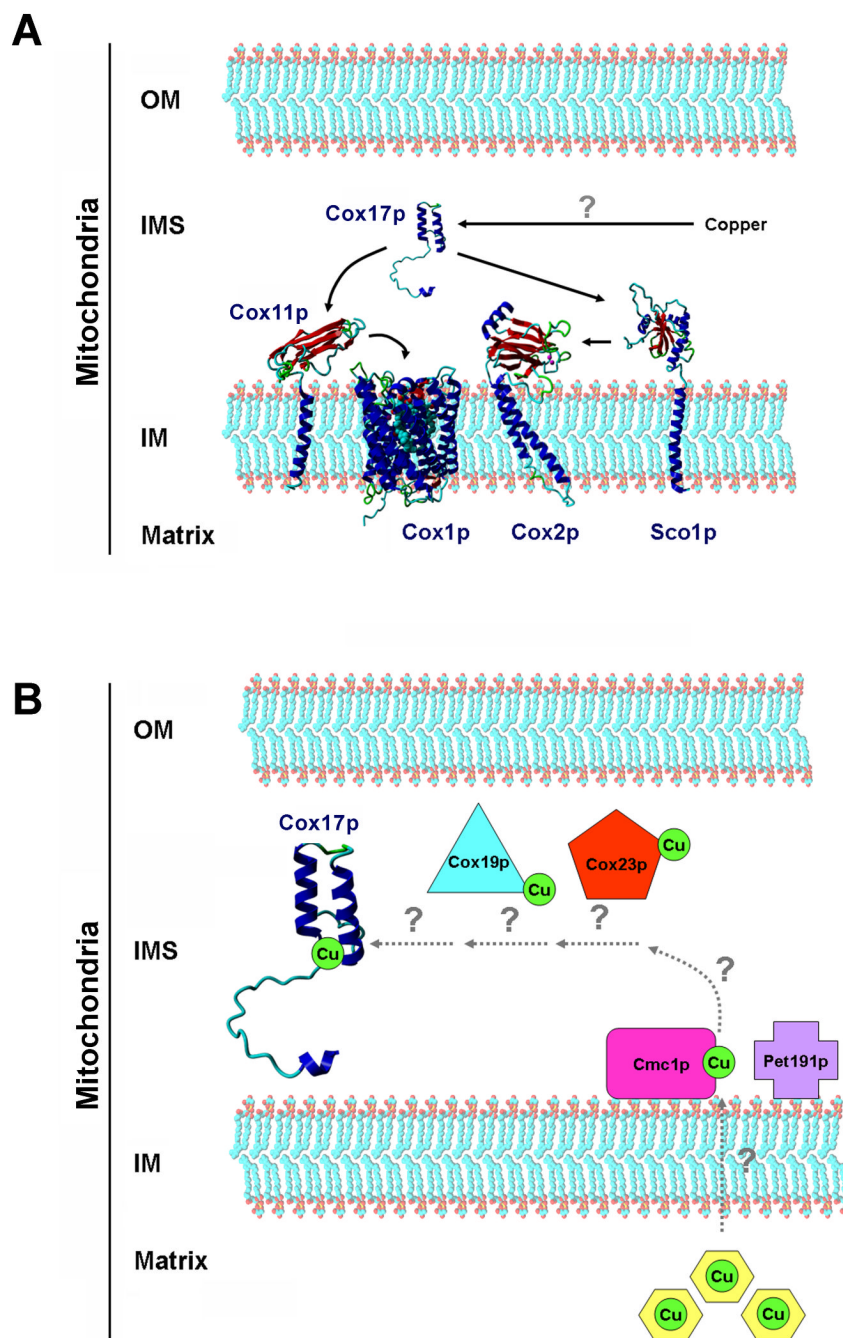
Figure 1.
Proteins proposed to be involved in mitochondrial copper metabolism and addition to COX subunits. (A) Copper insertion into the Cu_A and Cu_B sites in Cox2p and Cox1p, respectively. The soluble copper chaperone Cox17p transfers copper ions to two additional chaperones that facilitate copper insertion into the COX Cu_A and Cu_B active sites, respectively Sco1p and Cox11p. These proteins are anchored to the mitochondrial inner membrane through a transmembrane α-helix and expose their copper binding sides in the IMS, where copper transfer occurs. Solution structures of the apo- Cox17p, and of the globular intermembrane space domains of Sco1p and Cox11p and ligand-bound Cox1p and Cox2p structures were obtained from the protein data bank (PDB) website (http://www.rcsb.org/pdb/home/home.do) and

modified to prepare the figure. The transmembrane domains of Cox11p and Sco1p were artificially generated and used to represent the tethering of these proteins to the inner membrane. The YASARA molecular-graphics, -modeling and -simulation program, developed by Elmer Krieger, was used to generate a cartoon model of the different proteins. (B) Hypothetical role of the several CX_9C containing proteins in regulating copper transfer from a matrix copper pool, across the inner mitochondrial membrane through an uncharacterized transporter, towards Cox17p. The hypothetical model is explained in the text. A possible role of the CX_9C containing proteins in redox homeostasis within the intermembrane space is not depicted here. OM, outer membrane, IMS, intermembrane space, IM inner membrane. Black arrows indicate experimentally proved copper transfer while grey arrows indicate strictly hypothetical copper transfer.

Table 1

Genes encoding for mitochondrial proteins required for mitochondrial copper homeostasis and delivery to cytochrome *c* oxidase in the yeast *Saccharomyces cerevisiae*

GENE	FUNCTION	REFERENCES[*]
Copper transfer to Cox1p and Cox2p		
COX11	Stable formation of the Cu(B) center in COX subunit 1	(22,50-54)
SCO1	Transfer of copper to the Cu(A) center or reduction of cysteine residues in COX subunit 2.	(55-62)
Copper homeostasis in the intermembrane space		
COX17	Transfer of Copper to Cox11p and Sco1p	(43-45,63-69)
COX19	Copper metabolism in the intermembrane space?	(47,70)
COX23	Copper metabolism in the intermembrane space?	(48)
CMC1	Copper metabolism in the intermembrane space?	Horn and Barrientos, unpublished
PET191	*Uncharacterized*	(71)
Import of COX copper chaperones		
MIA40	Import of mitochondrial copper chaperons containing twin CX$_9$C motifs *via* a disulfide relay system	(72-78)
ERV1	Sulfhydryl oxidase involved in the reduction of Mia40p	(75,77,79-81)

[*] Due to space limitations, the list of references unfortunately does not include all the literature in the field but only some of the most relevant contributions