Building the Cu_A site of cytochrome *c* oxidase: A complicated, redox-dependent process driven by a surprisingly large complement of accessory proteins

Published, Papers in Press, September 29, 2017, DOI 10.1074/jbc.R117.816132

Kimberly A. Jett and Scot C. Leary[1]

From the Department of Biochemistry, University of Saskatchewan, Saskatoon, Saskatchewan S7N 5E5, Canada

Edited by F. Peter Guengerich

Cytochrome *c* oxidase (COX) was initially purified more than 70 years ago. A tremendous amount of insight into its structure and function has since been gleaned from biochemical, biophysical, genetic, and molecular studies. As a result, we now appreciate that COX relies on its redox-active metal centers (heme *a* and a_3, Cu_A and Cu_B) to reduce oxygen and pump protons in a reaction essential for most eukaryotic life. Questions persist, however, about how individual structural subunits are assembled into a functional holoenzyme. Here, we focus on what is known and what remains to be learned about the accessory proteins that facilitate Cu_A site maturation.

Cytochrome *c* oxidase is a multisubunit enzyme of dual genetic origin

COX[2] is a member of the A1 subgroup of a diverse superfamily of heme-copper oxidases. Embedded in the inner mitochondrial membrane, it is a multimeric protein complex composed of structural subunits that are encoded by two distinct genomes. The three largest of these, COX1–3, are mitochondrially encoded and form the catalytic core of the enzyme. COX1 contains the two heme (*a* and a_3) moieties and a mononuclear Cu_B center, all of which are buried within the lipid bilayer in the fully assembled holoenzyme. COX2 harbors a mixed valence, binuclear Cu_A site within a cupredoxin fold that is localized to the intermembrane space (IMS) and is solvent-exposed. The Cu_A site accepts electrons from cytochrome *c*, and subsequent electron transfer steps to the heme *a* and then the heme a_3–Cu_B metal centers of COX1 ultimately allow COX to convert molecular oxygen to water. Four protons are pumped across the membrane during each catalytic cycle and contribute to the electrochemical gradient that is required for aerobic ATP production. The catalytic core is surrounded by a variable number of nuclear-encoded structural subunits (8 in yeast and 11 in humans), which function collectively to stabilize the holoenzyme, provide sites for the allosteric modulation of its catalytic activity, and facilitate its organization into higher order structures termed supercomplexes or respirasomes (1, 2). High resolution structures of mammalian COX (3) and of eight mammalian respirasomes that contain COX (4) have been invaluable to advancing our understanding of how inter-subunit interactions impinge upon enzyme activity and dimerization and the integration of COX into higher order structures.

COX is assembled in a modular fashion

COX assembly is a very complex process that requires the stoichiometric expression of its nuclear- and mitochondrially-encoded subunits and the ordered incorporation of its heme and copper prosthetic groups. The basic blueprint that defines the steps and mechanisms that facilitate COX assembly within the inner mitochondrial membrane has been derived almost exclusively from studies of mutant yeast strains and cell lines from patients who presented with an isolated COX deficiency. Elegant work done by Nitjmans *et al.* (5) in the late 1990s established that the individual structural subunits of COX are matured and assembled in modules, with the formation of three assembly intermediates (S1–S3) preceding the biogenesis of the mature holoenzyme (S4). Subsequent studies identified the existence of unique maturation modules specific to COX1, COX2, and COX3 (6, 7) and challenged the exact subunit composition of individual modules and the order in which they are added to the assembling holoenzyme (8). However, the original concept that COX is built using a modular blueprint remains intact.

Maturation of individual modules and progression through the various stages of holoenzyme assembly requires a surprisingly large number of accessory proteins, termed COX assembly factors. Pioneering studies of yeast nuclear petite (*PET*) mutant collections identified more than 30 different complementation groups that encode for gene products with unique functions in holoenzyme biogenesis (9, 10), many of which are conserved in humans (11). At least six of these complementation groups encode for accessory proteins crucial to the maturation of COX2 and the metallation of its Cu_A site (Table 1). Additionally, evolutionarily conserved COX assembly factors with poorly characterized functions like COA5 (12, 13) and CMC2 (14, 15) may also function in this pathway. Pathogenic mutations in *COX2* (16–18), *COX20* (19, 20), *COA6* (21–23), *SCO1* (24–26), and *SCO2* (27, 28) all cause severe, early onset forms of fatal disease because of an isolated COX deficiency (Table 1). Characterization of human cell lines in which COX20

This is the fifth article in the Thematic Minireview series "Metals in Biology 2018: Copper homeostasis and utilization in redox enzymes." The authors declare that they have no conflicts of interest with the contents of this article.

[1] To whom correspondence should be addressed: Health Sciences Bldg., Rm. 4D10, University of Saskatchewan, Dept. of Biochemistry, 107 Wiggins Rd., Saskatoon, Saskatchewan S7N 5E5, Canada. Tel.: 306-966-4349; E-mail: scot.leary@usask.ca.

[2] The abbreviations used are: COX, cytochrome *c* oxidase; IMS, intermembrane space.

Table 1
COX2 and COX assembly factors required for polypeptide maturation and Cu_A site formation

Gene	Function	Clinical course(s) of disease
COX2	Catalytic core	Myopathy (16), encephalopathy (17), multisystem disorder (18)
OXA1L	N-terminal insertase?	NA[a] (33)
COX18	C-terminal insertase	NA (34)
COX20	Chaperone	Dystonia and ataxia (19)
COX17	IMS Cu trafficking	NA (35)
COA6	Cu_A site maturation	Hypertrophic cardiomyopathy (21, 22)
SCO1	Cu_A site maturation	Hypertrophic cardiomyopathy and encephalopathy (24), hepatopathy (25), encephalopathy (26)
SCO2	Cu_A site maturation	Hypertrophic cardiomyopathy and encephalopathy (27, 28)

[a] NA means not applicable.

(29), COA6 (23), SCO1 (30), or SCO2 (31, 32) function is impaired indicates stalling of COX assembly always occurs at the S2 stage, when fully matured COX2 is normally inserted into the assembling holoenzyme. Loss of function mutations in the remaining COX assembly factors with putative (OXA1L) or established (COX17 and COX18) roles in COX2 maturation have yet to be described in COX-deficient patients (Table 1), despite candidate gene screening (33, 34) and the advent of next generation sequencing technologies, suggesting that such variants may be embryonic lethal in humans. Consistent with this idea, *Cox17* deletion in the mouse results in a severe COX deficiency at embryonic day 6.5 and lethality shortly thereafter (35). The clinically diverse forms of fatal disease caused by mutations that compromise the function of any member of the COX2 assembly module (Table 1) means that there is great interest from a biomedical perspective in defining the precise roles of the individual COX assembly factors, and identifying how they collaborate with one another at various stages as COX2 matures. Such insight, once garnered, will be equally fascinating from a basic biological point of view, for it will clarify how each step in COX2 maturation ultimately leads to the metallation of its Cu_A site.

Co-translational insertion of apo-COX2 into the inner membrane is paired with the translocation of its N terminus into the IMS

COX2 is a polytopic protein with two transmembrane domains whose N and C termini protrude into the IMS. Because of its inherent hydrophobicity, COX2 insertion into the inner membrane is always coupled to mRNA translation. In yeast, a long 5′-untranslated leader sequence is recognized by the translational activator Pet111, which recruits *COX2* mRNA to specific ribosomes (Fig. 1*A*). Mammalian *COX2* mRNA is essentially devoid of a 5′-untranslated leader sequence, and searches for the ortholog of Pet111 based on amino acid identity have proved fruitless. However, it has long been postulated that mammalian mitochondrial mRNAs contain recognition motifs within their coding sequences that are similarly vital to their efficient translation, and this is now known to be true at least for COX1 (36), raising the intriguing possibility that a mammalian-specific COX2 translational activator remains to be discovered.

Co-translational insertion of yeast Cox2 into the membrane also requires Oxa1, a member of the conserved YidC/Alb3/Oxa1 family of insertases (37). The C terminus of Oxa1 interacts with the large ribosomal subunit in close proximity to the polypeptide exit tunnel where it promotes protein insertion into the inner membrane (38, 39). Oxa1 function in this regard is aided by Mba1, which acts to tether ribosomes to the inner membrane on the matrix side of the leaflet (Fig. 1*A*) (40). The human homolog of Oxa1, OXA1L, also physically interacts with mitochondrial ribosomes via its C-terminal tail (41, 42) and is able to restore COX assembly in an *OXA1* null yeast strain, arguing that Oxa1-dependent insertion of the N-terminal transmembrane domain of Cox2 and the simultaneous translocation of its N terminus into the IMS is an evolutionarily conserved function (Fig. 1*B*) (43). However, OXA1L knockdown in HEK293 cells leads to a diminution in the abundance of complexes I and V rather than COX (44). This has led to the suggestion that in mammals COX20 may be sufficient for insertion of the N-terminal transmembrane domain of COX2, given that its hydrophilic N terminus is shorter than that of yeast Cox2 and does not require proteolytic processing upon membrane insertion (45). Further studies of OXA1L are required to discriminate between these distinct mechanistic possibilities, but they are challenged by its broad function as an insertase of subunits of multiple complexes of oxidative phosphorylation.

COX20 stabilizes apo-COX2 in the inner membrane to protect it from degradation and promote the export of its C terminus into the IMS by COX18

Although the mechanisms that facilitate export of the N-terminal tail of mammalian COX2 remain unclear, recent studies emphasize that COX20 fulfills an essential, evolutionarily conserved role during the early stages of COX2 maturation (Figs. 1 and 2). COX20 contains two transmembrane domains with N and C termini that also protrude into the IMS (29) and serves as a chaperone, stabilizing the newly synthesized polypeptide and protecting it from degradation (29, 46). Consistent with this idea, deletion of *COX20* in either yeast (46) or human (29) cells results in rapid COX2 turnover. In yeast, Cox20 interacts with Cox2 and Mba1 at the ribosome (Fig. 1*A*) (47). Cox20 then presents the pre-protein to an inner membrane peptidase complex, which cleaves the N-terminal yeast leader sequence within the IMS to yield mature apo-Cox2 (Fig. 1*A*) (48).

Cleavage of the N-terminal leader sequence results in Mba1 dissociation from the Cox20–Cox2 complex (47) and the subsequent recruitment of Cox18, which promotes the insertion of the C-terminal transmembrane domain and concomitant export of the long soluble C-terminal tail of Cox2 (Fig. 2*A*) (49). In yeast, deletion of *COX18* stalls Cox2 maturation and leads to the accumulation of a species in which the N-terminal transmembrane domain is inserted into the membrane, and the leader sequence has been cleaved (50). Deletion of *COX18* in human cells similarly leads to the accumulation of a stalled

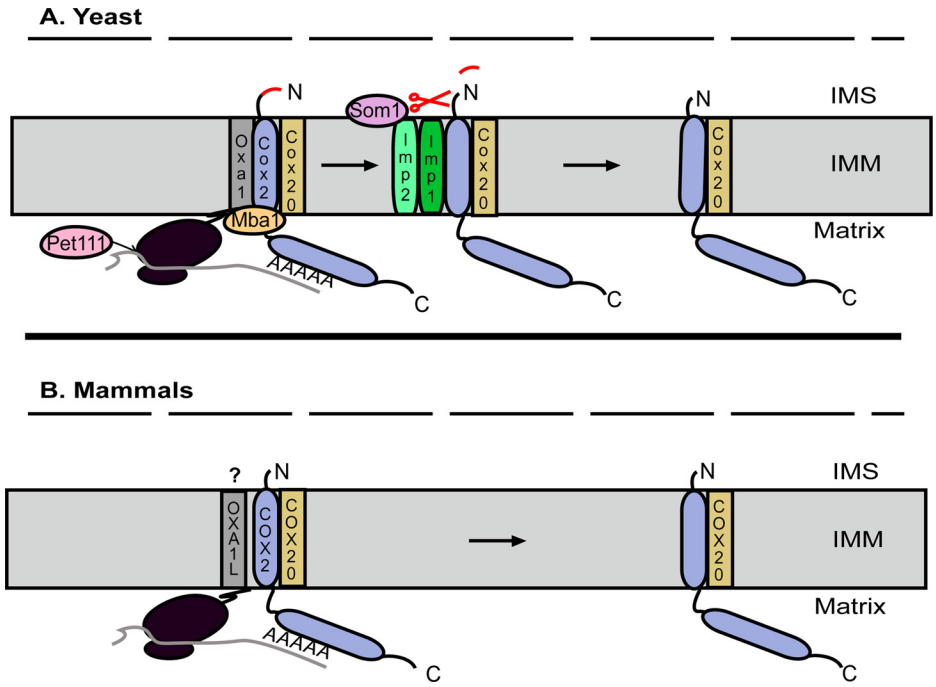

Figure 1. Co-translational insertion of COX2 and export of its N terminus. *A*, in yeast, Pet111 binds to Cox2 mRNA to stimulate its translation. Translation is coupled with polypeptide insertion into the inner mitochondrial membrane (*IMM*), in a process that is facilitated by both Oxa1 and Mba1. The C terminus of Oxa1 interacts with the large ribosomal subunit, and its interaction with Mba1 positions it near the exit tunnel where Oxa1 promotes Cox2 insertion and export of its N terminus into the IMS. Cox20 interacts with Mba1 and Cox2 to stabilize and prevent degradation of the pre-protein. The N terminus of the pre-protein is then proteolytically processed to yield mature Cox2 by a peptidase complex composed of Imp1, Imp2, and Som1. *B*, in mammals, the hydrophilic N terminus of COX2 is much shorter than that of yeast Cox2, and it does not require proteolytic processing upon membrane insertion. Although OXA1L physically interacts with mitochondrial ribosomes and functions as an insertase, questions remain about whether it promotes co-translational insertion of COX2 into the IMM or whether this function is fulfilled by COX20. The outer mitochondrial membrane is represented by the *dashed line* in both panels.

COX2–COX20 complex (45). Although COX18 belongs to the OXA1 protein family (50), COX2 is its only known substrate in both yeast and mammals (45). Yeast experiments in which Oxa1 was overexpressed in a *COX18* null strain failed to restore growth on a non-fermentable carbon source even though some export of the C-terminal tail of Cox2 was observed, emphasizing that Cox18 translocates the tail across the membrane and delivers it in a state competent for metallation of its Cu_A site (51). In yeast, Cox18 depends on a physical association with two additional factors, Mss2 and Pnt1 (50, 52), for its function, although it is thought that COX18 acts alone in mammals to promote insertion of the C-terminal transmembrane domain of COX2 and the export of its tail into the IMS (Fig. 2*B*) (45).

COA6, SCO1, and SCO2 form a metallochaperone module that interacts with the COX20–COX2 complex to metallate the Cu_A site

Following the export of its C-terminal tail into the IMS, COX2 remains bound to COX20 and is competent for Cu_A site metallation (29, 45). Physical interaction studies indicate that dissociation of COX18 from the COX20–COX2 complex coincides with the recruitment of a metallochaperone module composed of SCO1, SCO2, and COA6 (45). This trio of proteins then functions as a collective to deliver and insert copper into the Cu_A site (Fig. 3), the ligands for which are two conserved cysteines, two histidines, a glutamate, and a methionine contained within a cupredoxin fold (53).

Metallation of the Cu_A site of COX2 by its metallochaperone module requires a dedicated upstream copper donor within the IMS, and COX17 fulfills this role in both yeast and humans (Fig. 3). COX17 was the first member to be identified from a surprisingly large family of soluble proteins with twin CX_9C motifs localized to the IMS (54). The cysteinyl sulfurs of these twin CX_9C motifs are recognized by the MIA40–ERV1 import pathway within the IMS, which oxidizes them to form a pair of disulfide bonds that convert an intrinsically disordered protein into one with a helical hairpin structure (55–58). Mutagenesis studies in yeast revealed that two additional conserved cysteines at the N terminus of Cox17 are responsible for the coordination of Cu(I) ions (59, 60). Results from a comprehensive suite of functional genetic experiments argue that COX17 receives its copper from an evolutionarily conserved, labile Cu(I) pool that is housed in the matrix (61). Although Cobine and co-workers (62, 63) have provided some important insight into how Cu(I) is trafficked to and within mitochondria, the mechanisms that regulate IMS copper translocation and transfer to COX17 have yet to be identified. It is clear, however, that both yeast Cox17 and human COX17 transfer copper to both SCO1 and SCO2 (Fig. 3). Elegant work from Winge and co-workers (64) used *in vitro* and *in vivo* copper transfer studies to demonstrate that Cox17 requires a cysteine within one of its twin CX_9C motifs, Cys^{57}, to be competent to transfer copper to Sco1. Equally elegant work by Bertini and co-workers (65) used NMR to directly demonstrate that COX17 transfers Cu(I) to both SCO1 and SCO2 and that this copper

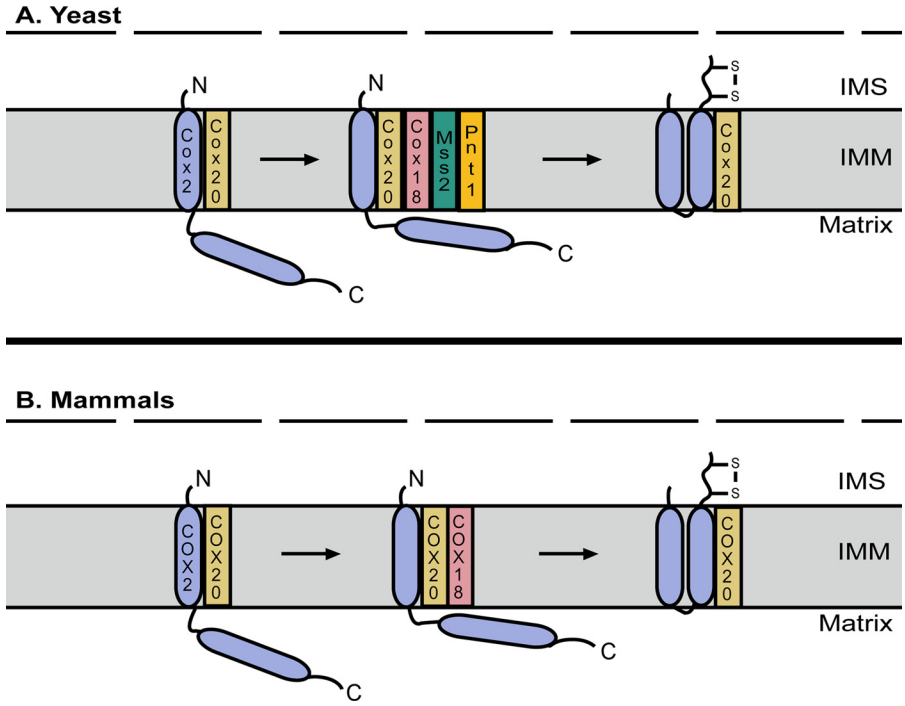

Figure 2. Insertion and export of the C terminus of COX2. *A*, in yeast, insertion and export of the C-terminal transmembrane domain of Cox2 requires recruitment of Cox18 to the Cox2–Cox20 complex. The insertase function of Cox18 then depends on its physical association with Mss2 and Pnt1. *B*, in mammals, COX18 is similarly recruited to the COX2–COX20 complex where it is thought to function alone to promote insertion of the C-terminal transmembrane domain of COX2 and export of its C terminus into the IMS. The outer mitochondrial membrane is represented by the *dashed line* in both panels. *IMM,* inner mitochondrial membrane.

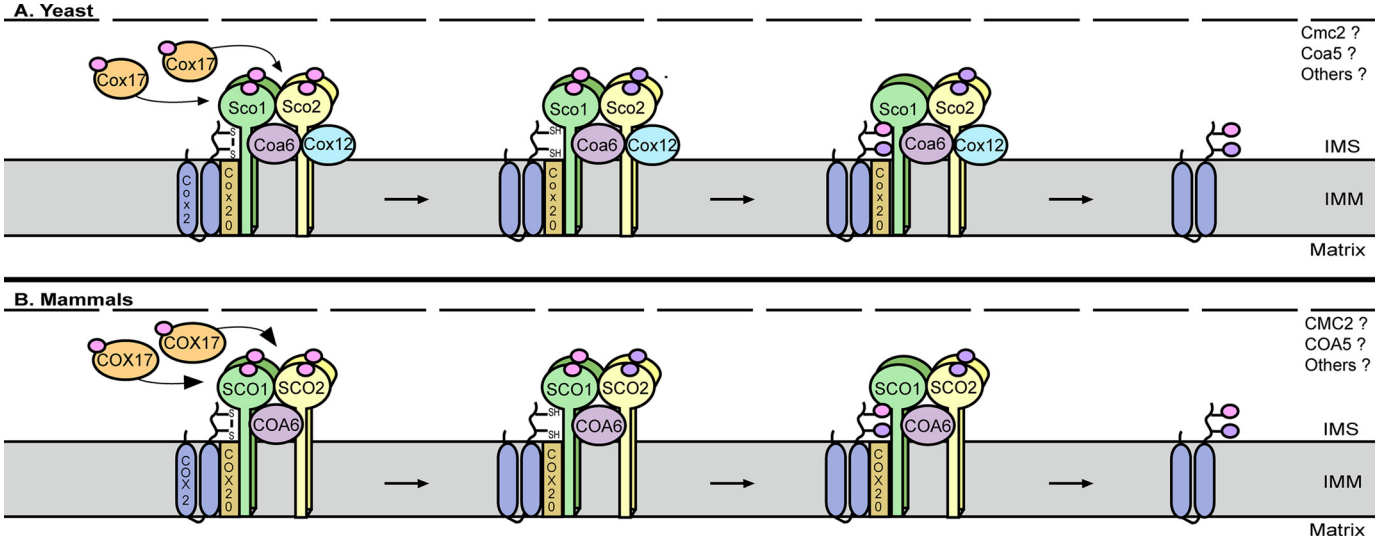

Figure 3. Cu_A site maturation of COX2. In yeast (*A*) and mammals (*B*), Cox17 and COX17, respectively, transfer Cu(I) ions to Sco1/SCO1 and Sco2/SCO2 to load the metallochaperone module. Because SCO1 and SCO2 are homodimers (30), this requires two successive rounds of COX17-dependent Cu(I) delivery to fully metallate each protein. *In vitro* studies support a model whereby copper-loaded SCO2 functions as a thiol-disulfide oxidoreductase to reduce the cysteinyl sulfurs of COX2 (66), in a reaction that converts Cu(I) (*pink circle*) to Cu(II) (*purple circle*) and requires COA6 function *in vivo*. In yeast, reduction of the cysteinyl sulfurs of Cox2 also requires Coa6 and Sco2, as well as Cox12 (69). Whether the human homolog of Cox12, COX6B, is similarly important for Cu_A site maturation is unclear. Upon priming of the cysteinyl sulfurs of Cox2/COX2, the Cu_A site is metallated by Sco1/SCO1. Whether other evolutionarily conserved COX assembly factors with poorly defined roles in COX assembly also participate in Cu_A site maturation remains unknown. The outer mitochondrial membrane is represented by the *dashed line* in both panels. *1MM,* inner mitochondrial membrane.

transfer step to SCO1 may be coupled to electron transfer, which serves to reduce the copper-binding site and promote ligand exchange.

SCO1 and SCO2 are paralogs that must be able to bind copper to promote Cu_A site maturation (30, 31, 66). Both proteins were originally identified in yeast as high copy suppressors of a *COX17* point mutant strain (54). Subsequent studies, however, found that only deletion of *SCO1* resulted in an inability to grow on a non-fermentable carbon source (67, 68), and until recently, the function of yeast Sco2 remained unknown (see below) (69). Both SCO proteins bind Cu(I) via a conserved *CXXXC* motif and a conserved histidine (70, 71) and rely on an additional conserved aspartate residue to coordinate Cu(II) (71). One copper atom is bound per monomer, and mutations that impair

Cu(I) or Cu(II) binding result in a non-functional SCO protein, arguing that this property is crucial to their roles in COX assembly (71). The importance of copper binding to SCO1 function is emphasized by two parallel studies that both found the pathogenic P174L substitution in SCO1 severely compromises COX17-dependent copper transfer (72, 73). Whether mutations in *SCO2* also impair copper transfer from COX17 remains unclear. However, the most common pathogenic variant of SCO2, SCO2 E140K, rescues the COX deficiency in *SCO2* patients when overexpressed, arguing that it is competent for metallation (31).

Although SCO1 and SCO2 are part of the same metallochaperone module, each protein physically interacts with COA6 (23, 69, 74) to fulfill a distinct function during the metallation of the Cu_A site of COX2 (Fig. 3) (30, 31, 66). Yeast studies were the first to show that Sco1 physically interacts with Cox2 (75) and map the functional interaction between the two proteins to a conserved sequence motif within loop 8 of Sco1 (76), which is solvent-exposed and undergoes structural rearrangement when Sco1 transitions from an apo- to a metallated conformer (77). Mutations in residues within this loop 8 motif do not affect physical interactions with Cox17 or copper binding, arguing that Sco1 uses distinct interfaces to interact with Cox17 and Cox2 (76). Pulse-chase labeling of mitochondrial translation products in human cells established that impaired SCO1 function did not affect COX2 synthesis but resulted in accelerated turnover of the newly synthesized protein (73). In contrast, COX2 synthesis is greatly reduced in *SCO2* patient cells, yet the residual protein that is made is much more stable than in control cells (31). These results led Shoubridge and co-workers (31) to propose that SCO2 acts upstream of SCO1 to stabilize newly synthesized COX2, and that the subsequent maturation of COX2 is contingent upon the formation of a ternary complex containing both SCO proteins and COX2. However, it is now clear that another small, soluble twin CX_9C motif-containing protein, COA6, is also an essential part of the metallochaperone module that associates with the COX20–COX2 complex to catalyze copper insertion into the Cu_A site of COX2 (Fig. 3) (23, 45, 69, 74, 78).

The fine mechanistic details of Cu_A site metallation in higher eukaryotes remain to be collected, but recent years have yielded a wealth of new information that brings much sharper focus to how the metallochaperone module functions as a unit. Elegant work from Vila and co-workers (66) has established that SCO1 and SCO2 are sufficient for Cu_A site maturation *in vitro*, with SCO2 functioning as a thiol-disulfide oxidoreductase to reduce the cysteinyl sulfurs of COX2 and SCO1 acting as the copper donor. However, in this system SCO2 performs its redox function in a copper-bound state rather than through a simple disulfide-exchange reaction (66), and questions remain with respect to the fate of its Cu(II) ion after SCO2 acts as an electron donor (Fig. 3). The cysteines of the *CXXXC* motif of SCO1 also exist as a mixed population composed of both oxidized disulfides and reduced thiols *in vivo* (26, 31), and how the cysteinyl sulfurs of SCO1 are oxidized to drive copper insertion into the Cu_A site in the IMS is also unclear. Excitingly, the findings from a flurry of recent studies strongly suggest that COA6 might in fact bridge these distinct aspects of SCO1 and SCO2 function to directly support Cu_A site maturation. COA6 is reported to be a copper-binding protein (23, 74), raising the intriguing possibility that it might transition from an apo- to a copper-loaded conformer upon SCO2-dependent reduction of the cysteinyl sulfurs of the Cu_A site. Consistent with this idea, human COA6 and SCO2 form a complex that is essential for COX2 maturation and COX assembly (74) and a yeast *COA6*/*SCO2* double mutant is less fit than the single mutants when grown on a non-fermentable carbon source, arguing that SCO2 and COA6 fulfill overlapping functions in Cu_A site metallation (69). Yeast Coa6 and human COA6 also physically interact with both Cox2/COX2 and Sco1/SCO1, and these interactions are crucial for the stability of newly synthesized polypeptide (23, 69).

Future directions

Our current understanding of mammalian COX2 maturation and Cu_A site metallation involves at least four distinct stages (Figs. 1–3). First, the N-terminal tail of COX2 is exported into the IMS as its N-terminal transmembrane domain is inserted into the inner membrane, in a translocation reaction that may involve OXA1L and/or COX20 but that has yet to be fully defined (1, 45). Second, COX20 associates with COX2 as it is co-translationally inserted into the membrane to stabilize the polypeptide and prevent its degradation. COX18 then promotes the simultaneous insertion of the C-terminal transmembrane domain of COX2 and translocation of its C-terminal tail into the IMS. Third, COX18 release from the COX20–COX2 complex coincides with the recruitment of the metallochaperone module composed of SCO1, SCO2, and COA6. Finally, the metallochaperone module functions collectively to reduce the cysteinyl sulfurs of COX2, with a SCO1 homodimer (30) inserting two copper ions into COX2 that it originally received from COX17 to form the Cu_A site. Whether COA6 oxidizes the *CXXXC* motif of SCO1 to promote insertion of copper into the Cu_A site, thereby priming its cysteinyl sulfurs to accept copper from SCO2, and whether this copper is in turn transferred to apo-SCO1 to prime SCO1 for a subsequent round of metallation are important, open, and exciting questions.

Despite the tremendous recent progress, many other questions remain with respect to the maturation of COX2 and the biogenesis of its Cu_A site. The first, and most obvious, question is whether the full complement of proteins that participates in this process has been identified. The twin CX_9C motif-containing family of proteins contains a surprisingly large number of soluble factors that are essential for COX assembly in yeast (14), and several of these, including Cmc1 (79), Cox19 (80), and Cox23 (81), are conserved in humans and have been shown to play a role in mitochondrial copper metabolism. However, recent studies indicate that all three of these factors fulfill important functions in delivering copper to COX1 rather than COX2 (82–84). Other family members with poorly understood but evolutionarily conserved roles in COX assembly, such as CMC2 and COA5, nonetheless remain and may facilitate Cu_A site maturation directly or indirectly by regulating IMS copper trafficking (Fig. 3). It is equally plausible that unrelated or novel mammalian-specific factors relevant to this process have yet to be identified.

A second critical question is whether the relative timing and composition of protein constituents that make up the various subassembly modules critical to COX2 maturation and Cu_A site formation are constant. For example, it remains to be seen whether the metallochaperone module is homogeneous in its composition or whether there is dynamic exchange of proteins in and out of this module as COX2 is matured. This is an important consideration because there is some discord in the literature with respect to the COX assembly factors that have been identified within this module using affinity purification methods (Ref. 26 *versus* Ref. 78). However, it is important to note that some of these discrepancies may be explained by inter-lab variation in methodology, which may favor the enrichment and therefore capture of one complex over another (*e.g.* ^{35}S-pulse-chase labeling protocols (45)). The mitochondrial proteome also differs across tissues (85, 86), and it is therefore worth considering the underexplored possibility that the constituents of the COX2 assembly module may in fact be specific to different cell types and/or species (11).

Other, more nuanced but equally important mechanistic questions also persist, independent of the complement of proteins that catalyzes Cu_A site maturation and its relative conservation across cell types. Why, for example, do both SCO proteins need to be able to coordinate Cu(I) and Cu(II), and how does this relate to the biogenesis of the mixed valence, binuclear Cu_A site? Redox functions have also been proposed for both human SCO1 (87) and SCO2 (31) based on *in vivo* and structural data, yet teasing apart the functional significance of their redox and copper-binding properties to Cu_A site maturation has yet to be realized. In fact, our general knowledge of redox homeostasis in the IMS and its impact on the redox state of cysteinyl sulfurs critical to protein function remains limited (88, 89). Beautiful work from Riemer and co-workers (88) has established that the IMS is not an oxidizing environment as was once thought and that its glutathione pool in fact exhibits the same redox potential as that of the cytosol. However, they have since demonstrated that thiol oxidation occurs within the reducing environment of the IMS because glutaredoxin levels are rate-limiting (89). The potential interplay between glutaredoxin and the metallochaperone module has obvious but as yet poorly understood consequences for maturation of the Cu_A site. More specifically, it remains unclear whether glutaredoxin abundance may be altered within the IMS and, if it can be, how this may impinge upon the redox state of cysteines within the *CXXXC* motif of SCO proteins and those within the twin CX_9C motifs of COA6 to modulate the function of the metallochaperone module. We impatiently await the mechanistic insight that will be afforded by future studies that tackle these big and small questions.

Acknowledgments—We recognize the outstanding contributions of those who have investigated bacterial aa_3-type oxidases and apologize for our inability to cite these and other unrelated studies due to space limitations. We thank Drs. Dennis Winge (University of Utah) and Paul Cobine (Auburn University, Auburn, AL) for critical feedback on an earlier version of this manuscript.

References

1. Timon-Gomez, A., Nyvltova, E., Abriata, L. A., Vila, A. J., Hosler, J., and Barrientos, A. (2017) Mitochondrial cytochrome *c* oxidase biogenesis: recent developments. *Semin. Cell Dev. Biol.* CrossRef Medline
2. Sinkler, C. A., Kalpage, H., Shay, J., Lee, I., Malek, M. H., Grossman, L. I., and Huttemann, M. (2017) Tissue- and condition-specific isoforms of mammalian cytochrome c oxidase subunits: from function to human disease. *Oxid. Med. Cell. Longev.* **2017,** CrossRef Medline
3. Tsukihara, T., Aoyama, H., Yamashita, E., Tomizaki, T., Yamaguchi, H., Shinzawa-Itoh, K., Nakashima, R., Yaono, R., and Yoshikawa, S. (1996) The whole structure of the 13-subunit oxidized cytochrome c oxidase at 2.8 A. *Science* **272,** 1136–1144 CrossRef Medline
4. Wu, M., Gu, J., Guo, R., Huang, Y., and Yang, M. (2016) Structure of mammalian respiratory supercomplex I1III2IV1. *Cell* **167,** 1598–1609.e1510 CrossRef Medline
5. Nijtmans, L. G., Taanman, J. W., Muijsers, A. O., Speijer, D., and Van den Bogert, C. (1998) Assembly of cytochrome-c oxidase in cultured human cells. *Eur. J. Biochem* **254,** 389–394 CrossRef Medline
6. Su, C.-H., McStay, G. P., and Tzagoloff, A. (2014) The Cox3p assembly module of yeast cytochrome oxidase. *Mol. Biol. Cell* **25,** 965–976 CrossRef Medline
7. Soto, I. C., Fontanesi, F., Liu, J., and Barrientos, A. (2012) Biogenesis and assembly of eukaryotic cytochrome c oxidase catalytic core. *Biochim. Biophys. Acta* **1817,** 883–897 CrossRef Medline
8. Vidoni, S., Harbour, M. E., Guerrero-Castillo, S., Signes, A., Ding, S., Fearnley, I. M., Taylor, R. W., Tiranti, V., Arnold, S., Fernandez-Vizarra, E., and Zeviani, M. (2017) MR-1S interacts with PET100 and PET117 in module-based assembly of human cytochrome c oxidase. *Cell Rep.* **18,** 1727–1738 CrossRef Medline
9. McEwen, J. E., Ko, C., Kloeckner-Gruissem, B., and Poyton, R. O. (1986) Nuclear functions required for cytochrome c oxidase biogenesis in *Saccharomyces cerevisiae*. Characterization of mutants in 34 complementation groups. *J. Biol. Chem.* **261,** 11872–11879 Medline
10. Tzagoloff, A., and Dieckmann, C. L. (1990) PET genes of *Saccharomyces cerevisiae*. *Microbiol. Rev.* **54,** 211–225 Medline
11. Shoubridge, E. A. (2001) Cytochrome c oxidase deficiency. *Am. J. Med. Genet.* **106,** 46–52 CrossRef Medline
12. Khalimonchuk, O., Rigby, K., Bestwick, M., Pierrel, F., Cobine, P. A., and Winge, D. R. (2008) Pet191 is a cytochrome c oxidase assembly factor in *Saccharomyces cerevisiae*. *Eukaryot. Cell* **7,** 1427–1431 CrossRef Medline
13. Huigsloot, M., Nijtmans, L. G., Szklarczyk, R., Baars, M. J., van den Brand, M. A., Hendriksfranssen, M. G., van den Heuvel, L. P., Smeitink, J. A., Huynen, M. A., and Rodenburg, R. J. (2011) A mutation in C2orf64 causes impaired cytochrome c oxidase assembly and mitochondrial cardiomyopathy. *Am. J. Hum. Genet.* **88,** 488–493 CrossRef Medline
14. Longen, S., Bien, M., Bihlmaier, K., Kloeppel, C., Kauff, F., Hammermeister, M., Westermann, B., Herrmann, J. M., and Riemer, J. (2009) Systematic analysis of the twin cx(9)c protein family. *J. Mol. Biol.* **393,** 356–368 CrossRef Medline
15. Horn, D., Zhou, W., Trevisson, E., Al-Ali, H., Harris, T. K., Salviati, L., and Barrientos, A. (2010) The conserved mitochondrial twin Cx9C protein Cmc2 is a Cmc1 homologue essential for cytochrome c oxidase biogenesis. *J. Biol. Chem.* **285,** 15088–15099 CrossRef Medline
16. Rahman, S., Taanman, J. W., Cooper, J. M., Nelson, I., Hargreaves, I., Meunier, B., Hanna, M. G., García, J. J., Capaldi, R. A., Lake, B. D., Leonard, J. V., and Schapira, A. H. (1999) A missense mutation of cytochrome oxidase subunit II causes defective assembly and myopathy. *Am. J. Hum. Genet.* **65,** 1030–1039 CrossRef Medline
17. Clark, K. M., Taylor, R. W., Johnson, M. A., Chinnery, P. F., Chrzanowska-Lightowlers, Z. M., Andrews, R. M., Nelson, I. P., Wood, N. W., Lamont, P. J., Hanna, M. G., Lightowlers, R. N., and Turnbull, D. M. (1999) An mtDNA mutation in the initiation codon of the cytochrome *c* oxidase subunit II gene results in lower levels of the protein and a mitochondrial encephalomyopathy. *Am. J. Hum. Genet.* **64,** 1330–1339 CrossRef Medline
18. Horváth, R., Schoser, B. G., Müller-Höcker, J., Völpel, M., Jaksch, M., and Lochmüller, H. (2005) Mutations in mtDNA-encoded cytochrome c oxi-

dase subunit genes causing isolated myopathy or severe encephalomyopathy. *Neuromuscul. Disord.* **15,** 851–857 CrossRef Medline
19. Szklarczyk, R., Wanschers, B. F., Nijtmans, L. G., Rodenburg, R. J., Zschocke, J., Dikow, N., van den Brand, M. A., Hendriks-Franssen, M. G., Gilissen, C., Veltman, J. A., Nooteboom, M., Koopman, W. J., Willems, P. H., Smeitink, J. A., Huynen, M. A., and van den Heuvel, L. P. (2013) A mutation in the FAM36A gene, the human ortholog of COX20, impairs cytochrome c oxidase assembly and is associated with ataxia and muscle hypotonia. *Hum. Mol. Genet.* **22,** 656–667 CrossRef Medline
20. Doss, S., Lohmann, K., Seibler, P., Arns, B., Klopstock, T., Zühlke, C., Freimann, K., Winkler, S., Lohnau, T., Drungowski, M., Nürnberg, P., Wiegers, K., Lohmann, E., Naz, S., Kasten, M., *et al.* (2014) Recessive dystonia-ataxia syndrome in a Turkish family caused by a COX20 (FAM36A) mutation. *J. Neurol.* **261,** 207–212 CrossRef Medline
21. Calvo, S. E., Compton, A. G., Hershman, S. G., Lim, S. C., Lieber, D. S., Tucker, E. J., Laskowski, A., Garone, C., Liu, S., Jaffe, D. B., Christodoulou, J., Fletcher, J. M., Bruno, D. L., Goldblatt, J., Dimauro, S., *et al.* (2012) Molecular diagnosis of infantile mitochondrial disease with targeted next-generation sequencing. *Sci. Transl. Med.* **4,** 118ra110 CrossRef Medline
22. Baertling, F., A. M. van den Brand, M., Hertecant, J. L., Al-Shamsi, A., P. van den Heuvel, L., Distelmaier, F., Mayatepek, E., Smeitink, J. A., Nijtmans, L. G., and Rodenburg, R. J. (2015) Mutations in COA6 cause cytochrome c oxidase deficiency and neonatal hypertrophic cardiomyopathy. *Hum. Mutat.* **36,** 34–38 CrossRef Medline
23. Stroud, D. A., Maher, M. J., Lindau, C., Vögtle, F. N., Frazier, A. E., Surgenor, E., Mountford, H., Singh, A. P., Bonas, M., Oeljeklaus, S., Warscheid, B., Meisinger, C., Thorburn, D. R., and Ryan, M. T. (2015) COA6 is a mitochondrial complex IV assembly factor critical for biogenesis of mtDNA-encoded COX2. *Hum. Mol. Genet.* **24,** 5404–5415 CrossRef Medline
24. Stiburek, L., Vesela, K., Hansikova, H., Hulkova, H., and Zeman, J. (2009) Loss of function of Sco1 and its interaction with cytochrome c oxidase. *Am. J. Physiol. Cell Physiol* **296,** C1218–C1226 CrossRef Medline
25. Valnot, I., Osmond, S., Gigarel, N., Mehaye, B., Amiel, J., Cormier-Daire, V., Munnich, A., Bonnefont, J. P., Rustin, P., and Rötig, A. (2000) Mutations of the SCO1 gene in mitochondrial cytochrome c oxidase deficiency with neonatal-onset hepatic failure and encephalopathy. *Am. J. Hum. Genet.* **67,** 1104–1109 CrossRef Medline
26. Leary, S. C., Antonicka, H., Sasarman, F., Weraarpachai, W., Cobine, P. A., Pan, M., Brown, G. K., Brown, R., Majewski, J., Ha, K. C., Rahman, S., and Shoubridge, E. A. (2013) Novel mutations in SCO1 as a cause of fatal infantile encephalopathy and lactic acidosis. *Hum. Mutat* **34,** 1366–1370 CrossRef Medline
27. Jaksch, M., Ogilvie, I., Yao, J., Kortenhaus, G., Bresser, H. G., Gerbitz, K. D., and Shoubridge, E. A. (2000) Mutations in SCO2 are associated with a distinct form of hypertrophic cardiomyopathy and cytochrome c oxidase deficiency. *Hum. Mol. Genet.* **9,** 795–801 CrossRef Medline
28. Papadopoulou, L. C., Sue, C. M., Davidson, M. M., Tanji, K., Nishino, I., Sadlock, J. E., Krishna, S., Walker, W., Selby, J., Glerum, D. M., Coster, R. V., Lyon, G., Scalais, E., Lebel, R., Kaplan, P., *et al.* (1999) Fatal infantile cardioencephalomyopathy with COX deficiency and mutations in SCO2, a COX assembly gene. *Nat. Genet.* **23,** 333–337 CrossRef Medline
29. Bourens, M., Boulet, A., Leary, S. C., and Barrientos, A. (2014) Human COX20 cooperates with SCO1 and SCO2 to mature COX2 and promote the assembly of cytochrome c oxidase. *Hum. Mol. Genet.* **23,** 2901–2913 CrossRef Medline
30. Leary, S. C., Kaufman, B. A., Pellecchia, G., Guercin, G. H., Mattman, A., Jaksch, M., and Shoubridge, E. A. (2004) Human SCO1 and SCO2 have independent, cooperative functions in copper delivery to cytochrome c oxidase. *Hum. Mol. Genet.* **13,** 1839–1848 CrossRef Medline
31. Leary, S. C., Sasarman, F., Nishimura, T., and Shoubridge, E. A. (2009) Human SCO2 is required for the synthesis of CO II and as a thiol-disulphide oxidoreductase for SCO1. *Hum. Mol. Genet.* **18,** 2230–2240 CrossRef Medline
32. Stiburek, L., Vesela, K., Hansikova, H., Pecina, P., Tesarova, M., Cerna, L., Houstek, J., and Zeman, J. (2005) Tissue-specific cytochrome c oxidase assembly defects due to mutations in SCO2 and SURF1. *Biochem. J.* **392,** 625–632 CrossRef Medline
33. Coenen, M. J., Smeitink, J. A., Smeets, R., Trijbels, F. J., and van den Heuvel, L. P. (2005) Mutation detection in four candidate genes (OXA1L, MRS2L, YME1L and MIPEP) for combined deficiencies in the oxidative phosphorylation system. *J. Inherit. Metab. Dis.* **28,** 1091–1097 CrossRef Medline
34. Sacconi, S., Salviati, L., and Trevisson, E. (2009) Mutation analysis of COX18 in 29 patients with isolated cytochrome c oxidase deficiency. *J. Hum. Genet.* **54,** 419–421 CrossRef Medline
35. Takahashi, Y., Kako, K., Kashiwabara, S., Takehara, A., Inada, Y., Arai, H., Nakada, K., Kodama, H., Hayashi, J., Baba, T., and Munekata, E. (2002) Mammalian copper chaperone Cox17p has an essential role in activation of cytochrome c oxidase and embryonic development. *Mol. Cell. Biol.* **22,** 7614–7621 CrossRef Medline
36. Weraarpachai, W., Antonicka, H., Sasarman, F., Seeger, J., Schrank, B., Kolesar, J. E., Lochmüller, H., Chevrette, M., Kaufman, B. A., Horvath, R., and Shoubridge, E. A. (2009) Mutation in TACO1, encoding a translational activator of COX I, results in cytochrome c oxidase deficiency and late-onset Leigh syndrome. *Nat. Genet.* **41,** 833–837 CrossRef Medline
37. Bonnefoy, N., Fiumera, H. L., Dujardin, G., and Fox, T. D. (2009) Roles of Oxa1-related inner-membrane translocases in assembly of respiratory chain complexes. *Biochim. Biophys. Acta* **1793,** 60–70 CrossRef Medline
38. Jia, L., Dienhart, M., Schramp, M., McCauley, M., Hell, K., and Stuart, R. A. (2003) Yeast Oxa1 interacts with mitochondrial ribosomes: the importance of the C-terminal region of Oxa1. *EMBO J.* **22,** 6438–6447 CrossRef Medline
39. Szyrach, G., Ott, M., Bonnefoy, N., Neupert, W., and Herrmann, J. M. (2003) Ribosome binding to the Oxa1 complex facilitates co-translational protein insertion in mitochondria. *EMBO J.* **22,** 6448–6457 CrossRef Medline
40. Ott, M., Prestele, M., Bauerschmitt, H., Funes, S., Bonnefoy, N., and Herrmann, J. M. (2006) Mba1, a membrane-associated ribosome receptor in mitochondria. *EMBO J.* **25,** 1603–1610 CrossRef Medline
41. Haque, M. E., Elmore, K. B., Tripathy, A., Koc, H., Koc, E. C., and Spremulli, L. L. (2010) Properties of the C-terminal tail of human mitochondrial inner membrane protein Oxa1L and its interactions with mammalian mitochondrial ribosomes. *J. Biol. Chem.* **285,** 28353–28362 CrossRef Medline
42. Haque, M. E., Spremulli, L. L., and Fecko, C. J. (2010) Identification of protein-protein and protein-ribosome interacting regions of the C-terminal tail of human mitochondrial inner membrane protein Oxa1L. *J. Biol. Chem.* **285,** 34991–34998 CrossRef Medline
43. Bonnefoy, N., Kermorgant, M., Groudinsky, O., Minet, M., Slonimski, P. P., and Dujardin, G. (1994) Cloning of a human gene involved in cytochrome oxidase assembly by functional complementation of an oxa1− mutation in *Saccharomyces cerevisiae*. *Proc. Natl. Acad. Sci. U.S.A.* **91,** 11978–11982 CrossRef Medline
44. Stiburek, L., Fornuskova, D., Wenchich, L., Pejznochova, M., Hansikova, H., and Zeman, J. (2007) Knockdown of human Oxa1l impairs the biogenesis of F1Fo-ATP synthase and NADH:ubiquinone oxidoreductase. *J. Mol. Biol.* **374,** 506–516 CrossRef Medline
45. Bourens, M., and Barrientos, A. (2017) Human mitochondrial cytochrome c oxidase assembly factor COX18 acts transiently as a membrane insertase within the subunit 2 maturation module. *J. Biol. Chem.* **292,** 7774–7783 CrossRef Medline
46. Elliott, L. E., Saracco, S. A., and Fox, T. D. (2012) Multiple roles of the Cox20 chaperone in assembly of *Saccharomyces cerevisiae* cytochrome c oxidase. *Genetics* **190,** 559–567 CrossRef Medline
47. Lorenzi, I., Oeljeklaus, S., Ronsor, C., Bareth, B., Warscheid, B., Rehling, P., and Dennerlein, S. (2016) The ribosome-associated Mba1 escorts Cox2 from insertion machinery to maturing assembly intermediates. *Mol. Cell. Biol.* MCB.00361–16
48. Hell, K., Tzagoloff, A., Neupert, W., and Stuart, R. A. (2000) Identification of Cox20p, a novel protein involved in the maturation and assembly of cytochrome oxidase subunit 2. *J. Biol. Chem.* **275,** 4571–4578 CrossRef Medline
49. Fiumera, H. L., Broadley, S. A., and Fox, T. D. (2007) Translocation of mitochondrially synthesized Cox2 domains from the matrix to the intermembrane space. *Mol. Cell. Biol.* **27,** 4664–4673 CrossRef Medline

50. Saracco, S. A., and Fox, T. D. (2002) Cox18p is required for export of the mitochondrially encoded *Saccharomyces cerevisiae* Cox2p C-tail and interacts with Pnt1p and Mss2p in the inner membrane. *Mol. Biol. Cell* **13,** 1122–1131 CrossRef Medline
51. Fiumera, H. L., Dunham, M. J., Saracco, S. A., Butler, C. A., Kelly, J. A., and Fox, T. D. (2009) Translocation and assembly of mitochondrially coded *Saccharomyces cerevisiae* cytochrome c oxidase subunit Cox2 by Oxa1 and Yme1 in the absence of Cox18. *Genetics* **182,** 519–528 CrossRef Medline
52. Broadley, S. A., Demlow, C. M., and Fox, T. D. (2001) Peripheral mitochondrial inner membrane protein, Mss2p, required for export of the mitochondrially coded Cox2p C tail in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* **21,** 7663–7672 CrossRef Medline
53. Speno, H., Taheri, M. R., Sieburth, D., and Martin, C. T. (1995) Identification of essential amino acids within the proposed Cu_A binding site in subunit II of cytochrome *c* oxidase. *J. Biol. Chem.* **270,** 25363–25369 CrossRef Medline
54. Glerum, D. M., Shtanko, A., and Tzagoloff, A. (1996) Characterization of COX17, a yeast gene involved in copper metabolism and assembly of cytochrome oxidase. *J. Biol. Chem.* **271,** 14504–14509 CrossRef Medline
55. Chacinska, A., Pfannschmidt, S., Wiedemann, N., Kozjak, V., Sanjuán Szklarz, L. K., Schulze-Specking, A., Truscott, K. N., Guiard, B., Meisinger, C., and Pfanner, N. (2004) Essential role of Mia40 in import and assembly of mitochondrial intermembrane space proteins. *EMBO J.* **23,** 3735–3746 CrossRef Medline
56. Mesecke, N., Terziyska, N., Kozany, C., Baumann, F., Neupert, W., Hell, K., and Herrmann, J. M. (2005) A disulfide relay system in the intermembrane space of mitochondria that mediates protein import. *Cell* **121,** 1059–1069 CrossRef Medline
57. Banci, L., Bertini, I., Cefaro, C., Cenacchi, L., Ciofi-Baffoni, S., Felli, I. C., Gallo, A., Gonnelli, L., Luchinat, E., Sideris, D., and Tokatlidis, K. (2010) Molecular chaperone function of Mia40 triggers consecutive induced folding steps of the substrate in mitochondrial protein import. *Proc. Natl. Acad. Sci. U.S.A.* **107,** 20190–20195 CrossRef Medline
58. Sideris, D. P., Petrakis, N., Katrakili, N., Mikropoulou, D., Gallo, A., Ciofi-Baffoni, S., Banci, L., Bertini, I., and Tokatlidis, K. (2009) A novel intermembrane space-targeting signal docks cysteines onto Mia40 during mitochondrial oxidative folding. *J. Cell Biol.* **187,** 1007–1022 CrossRef Medline
59. Heaton, D., Nittis, T., Srinivasan, C., and Winge, D. R. (2000) Mutational analysis of the mitochondrial copper metallochaperone Cox17. *J. Biol. Chem.* **275,** 37582–37587 CrossRef Medline
60. Punter, F. A., and Glerum, D. M. (2003) Mutagenesis reveals a specific role for Cox17p in copper transport to cytochrome oxidase. *J. Biol. Chem.* **278,** 30875–30880 CrossRef Medline
61. Cobine, P. A., Ojeda, L. D., Rigby, K. M., and Winge, D. R. (2004) Yeast contain a non-proteinaceous pool of copper in the mitochondrial matrix. *J. Biol. Chem.* **279,** 14447–14455 CrossRef Medline
62. Vest, K. E., Leary, S. C., Winge, D. R., and Cobine, P. A. (2013) Copper import into the mitochondrial matrix in *Saccharomyces cerevisiae* is mediated by Pic2, a mitochondrial carrier family protein. *J. Biol. Chem.* **288,** 23884–23892 CrossRef Medline
63. Vest, K. E., Wang, J., Gammon, M. G., Maynard, M. K., White, O. L., Cobine, J. A., Mahone, W. K., and Cobine, P. A. (2016) Overlap of copper and iron uptake systems in mitochondria in *Saccharomyces cerevisiae*. *Open Biol.* **6,** 150223 CrossRef Medline
64. Horng, Y. C., Cobine, P. A., Maxfield, A. B., Carr, H. S., and Winge, D. R. (2004) Specific copper transfer from the Cox17 metallochaperone to both Sco1 and Cox11 in the assembly of yeast cytochrome *c* oxidase. *J. Biol. Chem.* **279,** 35334–35340 CrossRef Medline
65. Banci, L., Bertini, I., Ciofi-Baffoni, S., Hadjiloi, T., Martinelli, M., and Palumaa, P. (2008) Mitochondrial copper(I) transfer from Cox17 to Sco1 is coupled to electron transfer. *Proc. Natl. Acad. Sci. U.S.A.* **105,** 6803–6808 CrossRef Medline
66. Morgada, M. N., Abriata, L. A., Cefaro, C., Gajda, K., Banci, L., and Vila, A. J. (2015) Loop recognition and copper-mediated disulfide reduction underpin metal site assembly of CuA in human cytochrome oxidase. *Proc. Natl. Acad. Sci. U.S.A.* **112,** 11771–11776 CrossRef Medline
67. Schulze, M., and Rödel, G. (1988) SCO1, a yeast nuclear gene essential for accumulation of mitochondrial cytochrome c oxidase subunit II. *Mol. Gen. Genet.* **211,** 492–498 CrossRef Medline
68. Glerum, D. M., Shtanko, A., and Tzagoloff, A. (1996) SCO1 and SCO2 act as high copy suppressors of a mitochondrial copper recruitment defect in *Saccharomyces cerevisiae*. *J. Biol. Chem.* **271,** 20531–20535 CrossRef Medline
69. Ghosh, A., Pratt, A. T., Soma, S., Theriault, S. G., Griffin, A. T., Trivedi, P. P., and Gohil, V. M. (2016) Mitochondrial disease genes COA6, COX6B and SCO2 have overlapping roles in COX2 biogenesis. *Hum. Mol. Genet.* **25,** 660–671 CrossRef Medline
70. Nittis, T., George, G. N., and Winge, D. R. (2001) Yeast Sco1, a protein essential for cytochrome *c* oxidase function is a Cu(I)-binding protein. *J. Biol. Chem.* **276,** 42520–42526 CrossRef Medline
71. Horng, Y. C., Leary, S. C., Cobine, P. A., Young, F. B., George, G. N., Shoubridge, E. A., and Winge, D. R. (2005) Human Sco1 and Sco2 function as copper-binding proteins. *J. Biol. Chem.* **280,** 34113–34122 CrossRef Medline
72. Banci, L., Bertini, I., Ciofi-Baffoni, S., Leontari, I., Martinelli, M., Palumaa, P., Sillard, R., and Wang, S. (2007) Human Sco1 functional studies and pathological implications of the P174L mutant. *Proc. Natl. Acad. Sci. U.S.A.* **104,** 15–20 CrossRef Medline
73. Cobine, P. A., Pierrel, F., Leary, S. C., Sasarman, F., Horng, Y. C., Shoubridge, E. A., and Winge, D. R. (2006) The P174L mutation in human Sco1 severely compromises Cox17-dependent metallation but does not impair copper binding. *J. Biol. Chem.* **281,** 12270–12276 CrossRef Medline
74. Pacheu-Grau, D., Bareth, B., Dudek, J., Juris, L., Vögtle, F. N., Wissel, M., Leary, S. C., Dennerlein, S., Rehling, P., and Deckers, M. (2015) Cooperation between COA6 and SCO2 in COX2 maturation during cytochrome c oxidase assembly links two mitochondrial cardiomyopathies. *Cell Metab.* **21,** 823–833 CrossRef Medline
75. Lode, A., Kuschel, M., Paret, C., and Rödel, G. (2000) Mitochondrial copper metabolism in yeast: interaction between Sco1p and Cox2p. *FEBS Lett.* **485,** 19–24 CrossRef Medline
76. Rigby, K., Cobine, P. A., Khalimonchuk, O., and Winge, D. R. (2008) Mapping the functional interaction of Sco1 and Cox2 in cytochrome oxidase biogenesis. *J. Biol. Chem.* **283,** 15015–15022 CrossRef Medline
77. Banci, L., Bertini, I., Calderone, V., Ciofi-Baffoni, S., Mangani, S., Martinelli, M., Palumaa, P., and Wang, S. (2006) A hint for the function of human Sco1 from different structures. *Proc. Natl. Acad. Sci. U.S.A.* **103,** 8595–8600 CrossRef Medline
78. Ghosh, A., Trivedi, P. P., Timbalia, S. A., Griffin, A. T., Rahn, J. J., Chan, S. S., and Gohil, V. M. (2014) Copper supplementation restores cytochrome *c* oxidase assembly defect in a mitochondrial disease model of COA6 deficiency. *Hum. Mol. Genet.* **23,** 3596–3606 CrossRef Medline
79. Horn, D., Al-Ali, H., and Barrientos, A. (2008) Cmc1p is a conserved mitochondrial twin CX9C protein involved in cytochrome c oxidase biogenesis. *Mol. Cell. Biol.* **28,** 4354–4364 CrossRef Medline
80. Nobrega, M. P., Bandeira, S. C., Beers, J., and Tzagoloff, A. (2002) Characterization of COX19, a widely distributed gene required for expression of mitochondrial cytochrome oxidase. *J. Biol. Chem.* **277,** 40206–40211 CrossRef Medline
81. Barros, M. H., Johnson, A., and Tzagoloff, A. (2004) COX23, a homologue of COX17, is required for cytochrome oxidase assembly. *J. Biol. Chem.* **279,** 31943–31947 CrossRef Medline
82. Bourens, M., and Barrientos, A. (2017) A CMC1-knockout reveals translation-independent control of human mitochondrial complex IV biogenesis. *EMBO Rep.* **18,** 477–494 CrossRef Medline
83. Bode, M., Woellhaf, M. W., Bohnert, M., van der Laan, M., Sommer, F., Jung, M., Zimmermann, R., Schroda, M., and Herrmann, J. M. (2015) Redox-regulated dynamic interplay between Cox19 and the copper-binding protein Cox11 in the intermembrane space of mitochondria facilitates biogenesis of cytochrome c oxidase. *Mol. Biol. Cell* **26,** 2385–2401 CrossRef Medline
84. Dela Cruz, R., Jeong, M. Y., and Winge, D. R. (2016) Cox1 mutation abrogates need for Cox23 in cytochrome c oxidase biogenesis. *Microb. Cell* **3,** 275–284 CrossRef Medline

85. Mootha, V. K., Bunkenborg, J., Olsen, J. V., Hjerrild, M., Wisniewski, J. R., Stahl, E., Bolouri, M. S., Ray, H. N., Sihag, S., Kamal, M., Patterson, N., Lander, E. S., and Mann, M. (2003) Integrated analysis of protein composition, tissue diversity, and gene regulation in mouse mitochondria. *Cell* **115,** 629–640 CrossRef Medline
86. Forner, F., Foster, L. J., Campanaro, S., Valle, G., and Mann, M. (2006) Quantitative proteomic comparison of rat mitochondria from muscle, heart, and liver. *Mol. Cell. Proteomics* **5,** 608–619 CrossRef Medline
87. Williams, J. C., Sue, C., Banting, G. S., Yang, H., Glerum, D. M., Hendrickson, W. A., and Schon, E. A. (2005) Crystal structure of human SCO1: implications for redox signaling by a mitochondrial cytochrome c oxidase "assembly" protein. *J. Biol. Chem.* **280,** 15202–15211 CrossRef Medline
88. Kojer, K., Bien, M., Gangel, H., Morgan, B., Dick, T. P., and Riemer, J. (2012) Glutathione redox potential in the mitochondrial intermembrane space is linked to the cytosol and impacts the Mia40 redox state. *EMBO J.* 15;31(14):3160–82 CrossRef Medline
89. Kojer, K., Peleh, V., Calabrese, G., Herrmann, J. M., and Riemer, J. (2015) Kinetic control by limiting glutaredoxin amounts enables thiol oxidation in the reducing mitochondrial intermembrane space. *Mol. Biol. Cell* **26,** 195–204 CrossRef Medline