Crystal Structure of Human SCO1

IMPLICATIONS FOR REDOX SIGNALING BY A MITOCHONDRIAL CYTOCHROME c OXIDASE “ASSEMBLY” PROTEIN*

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Human SCO1 and SCO2 are copper-binding proteins involved in the assembly of mitochondrial cytochrome c oxidase (COX). We have determined the crystal structure of the conserved, intermembrane space core portion of apo-hSCO1 to 2.8 Å. It is similar to redox active proteins, including thioredoxins (Trx) and peroxiredoxins (Prx), with putative copper-binding ligands located at the same positions as the conserved catalytic residues in Trx and Prx. SCO1 does not have disulfide isomerization or peroxidase activity, but both hSCO1 and a sco1 null in yeast show extreme sensitivity to hydrogen peroxide. Of the six missense mutations in SCO1 and SCO2 associated with fatal mitochondrial disorders, one lies in a highly conserved exposed surface away from the copper-binding region, suggesting that this region is involved in protein-protein interactions. These data suggest that SCO functions not as a COX copper chaperone, but rather as a mitochondrial redox signaling molecule.

Cytochrome c oxidase (COX),1 or complex IV of the mitochondrial respiratory chain, catalyzes the transfer of electrons from cytochrome c to molecular oxygen and pumps protons across the mitochondrial inner membrane to establish a proton gra-dient for ATP synthesis (1). It is composed of 3—4 polypeptides in bacteria, 10—12 in yeast, and 13 in mammals. In eukaryotes, the three largest, and most evolutionarily conserved, subunits (I, II, and III) are encoded by mitochondrial DNA and contain most of the prosthetic groups (3 coppers, 1 magnesium, 1 sodium, and 2 hemes); they perform the electron transfer and proton pumping functions. The other subunits, encoded by nuclear DNA, are presumably required for modulation of COX activity (2).

Additional nuclear gene products are also required for the assembly of COX (1), including those responsible for synthesis of heme A, transport and insertion of metal cofactors, and proper co-assembly of the mitochondrial DNA- and nuclear DNA-encoded subunits. Numerous COX assembly genes have been identified in yeast (3), with many of these having human homologues, including COX10, COX11, COX15, COX17, COX19, LRPPRC, OXA1, PET112, SCO1, SCO2, and SURF1.

The SCO family of proteins (an acronym for synthesis of cytochrome c oxidase) contains a highly conserved CXXXC motif that presumably binds copper, and therefore is hypothesized to transport copper to COX (1). Both humans and yeast have two SCO-like genes, but they are paralogs (i.e. gene duplication occurring in the two lineages independently), not orthologs (i.e. divergence from a common ancestral gene pair) (4). Human SCO1 (hSCO1) is a 301-residue polypeptide consisting of a ~40-residue mitochondrial targeting signal at its N terminus, a ~45-residue domain protruding into the mitochondrial matrix, a ~20-residue domain spanning the mitochondrial inner membrane, and a C-terminal region of ~185 residues protruding into the intermembrane space (IMS) (Fig. 1 and Supplementary Materials Fig. 1). Human SCO2 (hSCO2) is 35 residues shorter than hSCO1, due mainly to a smaller matrix domain (Supplementary Materials Fig. 1).

Mutations in hSCO1 cause fatal infantile hepatocerephalomyopathy (5), whereas mutations in hSCO2 cause fatal infantile cardioencephalomyopathy (4, 6—9). Both disorders are associated with severe COX deficiency in affected tissues, but the reason for the “tissue specificity” in the two disorders is unknown. Remarkably, the COX deficiency in cells from patients with hSCO2 mutations (6, 10) and in bacteria harboring a null mutation in YpmQ, the SCO-like homolog in Bacillus subtilis (11), could be rescued by the addition of copper to the growth medium, but the mechanism by which this rescue occurred is unknown; the addition of copper to yeast lacking Sco1p, however, could not rescue COX function (12).

To understand better the role of the SCO family members in disease, we have solved the crystal structure of hSCO1, using MAD phasing. Insights gained from the structure, biochemical characterization, and results reported in the literature imply...
that hSCO1 is a redox active protein that participates in mitochondrial redox sensing and signaling.

**EXPERIMENTAL PROCEDURES**

**Protein Expression and Purification**—The entire C-terminal cytoplasmic domain (i.e., the region protruding into the IMS) of hSCO2 (residues 79–286, ~22 kDa) (Supplementary Materials Fig. 1) was isolated by PCR, inserted into pET-24D (Novagen), and expressed in BL21-DE3 cells using standard protocols. Overexpressed hSCO2 was purified to homogeneity using standard chromatographic techniques. Briefly, cell debris was removed from sonicated cells by centrifugation (150,000 × g for 30 min). The supernatant was immediately passed over a preparative scale Sepharose-Q column (Amersham Biosciences) and the bound hSCO2 was washed and eluted with a gradient (100 to 500 mM NaCl). Fractions containing the protein (determined by SDS-PAGE) were concentrated to 200 ml and passed over a Sepharose G-75 sizing column (Amersham Biosciences) equilibrated in 50 mM Tris (pH 8.0), 200 mM NaCl, 1 mM EDTA, and 1 mM DTT. Fractions containing the protein were concentrated to ~500 μl at 30 mg/ml and the buffer was exchanged for crystallization trials (10 mM Tris, 100 mM NaCl, 5 mM EDTA, 10 mM DTT).

Purified hSCO2 protein was digested with a set of proteases and analyzed by mass spectrometry and N-terminal protein sequencing to determine domain boundaries. The N-terminal boundary starts at residue Gly682 with the same C terminus as the full-length sequence (Supplementary Materials Fig. 1). Based on this analysis, we also made a truncated hSCO1 expression construct (residues 138–301).

For bacterial expression of hSCO1 in the presence of added copper, we followed the approach detailed in Nittis et al. (13). Selenomethionyl-labeled hSCO1 protein was prepared as described elsewhere (14) and purified as described above.

**Crystallization**—Approximately 30 mg/ml purified hSCO1 and hSCO2 proteins were subjected to hanging drop crystallization trials at 4 and 20 °C, using sparse matrix factorials (Hampton Research). Based on the initial trials, each crystallization trial was optimized further based on crystal size and protein precipitation. We obtained crystals of hSCO1 using 20–38% PEG-8000, 50–200 mM NaSCN, and the level of observed protein precipitation. We obtained crys-

Data Collection and Reduction—All data were collected at NSLS-X4A at Brookhaven National Laboratory using an ASDC Quantum4R CCD detector. Despite screening a number of cryoconditions, it proved difficult to produce a uniform spot shape over the entire image. The crystal diffracted to better than 2.8 Å and a four-wavelength MAD data set was collected at 100 K using inverse beam geometry in 20° swaths. A weak anomalous signal was observed. Best data quality was obtained using the anomalous signal from the isomorphous copper-minus hSCO1 structure and a CXXXC motif as well as the other residues reported to be critical for SCO function. The crystals belong to the P212121 space group with 4 ml and passed over a Sepharose G-75 sizing column (Amersham Biosciences) equilibrated in 50 mM Tris (pH 8.0), 200 mM NaCl, 1 mM EDTA, and 1 mM DTT.

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**Overall Structure**—The overall fold of the core hSCO1 fragment (Fig. 1) is similar to that of thioredoxin (Trx) superfamily members (22), as predicted, despite a barely recognizable se-
**TABLE I**

Diffraction data

| λ (Å)                  | λ₁ (Low remote) | λ₂ (Inflection) | λ₃ (Peak) | λ₄ (High remote) |
|------------------------|-----------------|-----------------|-----------|-----------------|
| a                      | 0.9920          | 0.9793          | 0.9789    | 0.9686          |
| Bragg spacings (Å)     | 20 to 2.8       | 20 to 2.8       | 20 to 2.8 | 20 to 2.8       |
| Reflections            |                 |                 |           |                 |
| Measured               | 298,241         | 297,261         | 295,721   | 290,813         |
| Unique                 | 16,546          | 16,392          | 16,464    | 16,913          |
| Completeness (%)       | 99.3            | 99.3            | 99.3      | 99.4            |
| λ₁ (overall/last shell)* | 26.9/6.9       | 27.4/6.8        | 26.7/6.8  | 24.7/4.7        |
| R_{sym} (overall/last shell)* | 7.3/31.4    | 7.2/26.9        | 7.9/30.6  | 8.1/41.4        |

* Outer shell of the data (2.90–2.80).

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**Fig. 1.** Crystal structure of hSCO1. 

**a.** Schematic of hSCO1. Numbers denote sizes, in residues. MTS, mitochondrial targeting signal; TM, transmembrane region; JM, “juxtamembrane” region. **b.** Ribbon diagram of the overall fold of core hSCO1. Helices are in cyan, strands and loops are in purple. **c.** Sequence alignment of hSCO1 with human peroxiredoxin 2 (P2119; Residues in blue are completely conserved within each family, residues in red are active site residues. Above the sequences are lethal point mutation and the secondary structure elements as determined by definition of secondary structure of proteins (47) (cyan bars represent α-helices, purple represent β-strands). Residues that were aligned structurally are shaded with violet boxes; the SCO loop is shaded in yellow. **d.** Structural comparison of human SCO1, human Prx (48) (1PRX), and human Trx (49) (1ERU). Each polypeptide was aligned structurally, rotated from the view in b by 90° about the vertical axis and −25° about the normal to the figure, and then translated for visual clarity. Note that each structure contains the thioredoxin core (compare the α-β schematics below each structure), but that Sco and Prx contain several additional secondary elements and loops. A major deviation from Trx is insertion of the SCO loop between α4 and β5 found in Sco, Prx, and GPX. At the base of this insert are the functional residues His260 in hSCO1 and Arg160 in Prx2 (see Fig. 2). The figure was prepared with PyMol.
Asp204 is succeeded by a completely conserved pro-Trx fold, where it is in close proximity to the C site with the C motif found in all SCO homologs localized to a position analogous to that of the active site CXXC cysteines in Trx and other related oxidoreductases (Fig. 2). Superposition with other proteins that contain the Trx fold indicated that hSCO1 is most similar to family members of the peroxiredoxin (Prx) and glutathione peroxidase (GPX) families. Besides differences in length and in the conformation of several loops, the major difference between SCO and Prx/GPX is in the nature of the active site residues (Fig. 2): whereas SCO has two cysteines, Prx has only one cysteine in the active site and GPx has a selenocysteine (both corresponding to the C-terminal Cys in the CXXXC of SCO). The CXXXC motif itself is actually very specifically conserved as (T/S)XCPD(V/I)CP in authentic SCO proteins (Supplementary Materials Fig. 1).

**Active Site and SCO Loop**—The CXXXC site of hSCO1 is located at the β3–α2 turn (residues 167–171) in the heart of the Trx fold, where it is in close proximity to the β4–α3 turn (residues 204–209) and the base of an extended loop between α4 and β8 (residues 242–262); the latter is absent in Trx and Trx-like family members (e.g. protein-disulfide isomerase, phosducin) (Fig. 1). Peroxiredoxin family members do have similar features (Fig. 1d), but their loops between α4 and β8 are considerably shorter (Fig. 1e). The uniquely extended loop in hSCO1 includes two antiparallel β-strands (β6 and β7) at its base, and other authentic SCO family members have similarly extended insertions with highly conserved sequences for the base segments (Supplementary Materials Fig. 1); hence, we designate this feature as the “SCO loop.” Despite being solvent exposed in all three independent molecules of the crystal, the SCO loop segments all have continuous electron density for exposed in all three independent molecules of the crystal, the SCO loop. Despite being solvent extended insertions with highly conserved sequences for the similar features (Fig. 1). Peroxiredoxin family members do have specifically conserved as (T/S)XCPD(V/I)CP in authentic SCO proteins (Supplementary Materials Fig. 1).

Specifically, completely conserved Asp204 (hSCO1 numbering throughout unless indicated otherwise) makes a hydrogen bond to the guanidinium group of completely conserved Arg207 (3.2 Å) and to the hydroxyl group of completely conserved Tyr256 (2.7 Å), which is at the base of the extended loop. Arg207 is involved in stacking interactions with Tyr256 and with His260 (although His268 is not strongly conserved among species). In addition, Asp204 is succeeded by a completely conserved proline, Pro205, which limits the dihedral angles available to Asp204. The carboxyl group of completely conserved Asp204 makes several H-bonds: one to the hydroxyl group of conserved Ser/Thr167 (2.57 Å) preceding the CXXXC motif; another to the backbone amide of Ser/Thr167 (2.75 Å); and one to the hydroxyl group of conserved Ser/Thr202 (2.68 Å). Finally, completely conserved His260 from β3 is hydrogen bonded to Tyr163 (3.53 Å) from β3 preceding the CXXXC site (Fig. 3b).

Recently, an NMR structure of the distant SCO relative from B. subtilis, YpmQ, was published by Balatri et al. (24). Whereas the core of the molecule is similar to that of hSCO1 (with the exception of a misplaced C-terminal helix; see Supplementary Materials Fig. 3), there are major differences in structure at the active site and at the adjacent SCO loop compared with the hSCO1 structure presented here (Supplementary Materials Fig. 3). The NMR structure of YpmQ indicates that this region is highly flexible, whereas the crystal structure of hSCO1 indicates that it is well ordered. As noted above, this order is not because of lattice contacts. Rather, much of the discrepancy between the hSCO1 and YpmQ structures is likely because of sequence differences. Sequence similarity is low overall (30% identity) and several key positions do not have the canonical SCO residues. Specifically, the otherwise conserved CPD-(V/I)CP sequence is replaced by CETICP in YpmQ, and the shortened SCO loop of YpmQ is also missing conserved features at its base: Arg242 is replaced and Asp205 is deleted. Also, the completely conserved Arg207 and Tyr256 of hSCO1 (which stack together and hydrogen bond to Asp204) are replaced in YpmQ by Asn and Asp, respectively; in fact, Arg207 is the site of a lethal point mutation in hSCO2 (7). Moreover, SCO-conserved Tyr163 and Gly165 are replaced in YpmQ by Asp and Ile, respectively; these changes would necessarily alter SCO-like interactions with His260. Because much of the active site region is disordered in the YpmQ structure, it is difficult for us to make functional comparisons with hSCO1.

**SCO1 and Copper**—In the crystal structure, the position of the conserved residues Cys169, Cys173, and His260, which are implicated in Cu(I) binding at the CXXXC site by EXAFS and by mutagenesis experiments in yeast Sco1 (13), are in close proximity to each other in hSCO1 (Fig. 2). Based on small molecule studies (25), the atomic configuration of the Cu(I) ligands should be trigonal planar. However, using each allowed rotamer for the imidazole-N of His260 and for the thiol-S of Cys169 and Cys173 of the CXXXC motif, we calculated minimal distances of 2.84, 3.29, and 3.71 Å, respectively, to the oxygen atom of a water molecule (with weak density) that resides in the center of these residues. These lengths are considerably greater than the expected values of 1.98 ± 0.10 Å for Cu(I)-N and 2.25 ± 0.10 Å for Cu(I)-S. Alternatively, based on an ideal Cu(I)-S distance of 2.25 Å and a perfect trigonal planar bonding arrangement, a distance of 3.9 Å between each copper ligand (i.e. S-S or N-S) would be required. Again, no combination of side chain rotamers of these cysteine produced distances within 0.2 Å of the idealized bonding distance. Backbone conformational changes in hSCO1 would be required to form a Cu(I)-binding site.

The absence of Cu(I) allows the sulphydryl groups of the CXXXC motif to make H-bonds: Cys169 makes a 3.03-Å H-bond to the backbone amide nitrogen of Cys173 and the sulphydryl group of Cys173 makes a weak 3.54-Å H-bond to the hydroxyl group of nearly conserved Tyr163. Side chain torsional changes place the sulfur atoms of these cysteine residues in position for disulfide bond formation.

There are several other nearby residues that likely affect the ability of SCO to bind copper. These include the strongly con-
served Phe\textsuperscript{166}, which makes van der Waals contact to Cys\textsuperscript{169}, and Tyr\textsuperscript{163}, which could donate an axial hydroxyl group to the putative trigonal planar Cu(I) site (Fig. 2). Finally, the region surrounding the Cu(I) site, including Val\textsuperscript{172}, Leu\textsuperscript{177}, Phe\textsuperscript{200}, Ile\textsuperscript{257}, and Ile\textsuperscript{262}, is generally hydrophobic.

Characterization of SCO Function—The striking similarities between hSCO1 and Trx, and especially the position of the reactive cysteines, suggested that hSCO1 might have disulfide reductase activity. However, using an insulin precipitation assay (20), we found that neither hSCO1 with, or without, added copper catalyzed insulin precipitation, implying that hSCO1 lacks observable thioredoxin activity (Fig. 4a).

Likewise, similarity to the Prx and GPX families suggested that SCO might have peroxidase activity. We therefore tested
hSCO1 for peroxidase activity with H$_2$O$_2$ as the substrate and used DTT and reduced glutathione to regenerate the active site cysteines. Compared with the control experiment using glutathione peroxidase, hSCO1 shows negligible peroxidase activity toward hydrogen peroxide (Fig. 4b). However, we cannot rule out peroxidase activity toward other substrates (e.g. lipid peroxides).

As noted above, however, our construct of hSCO1 did prove to be highly sensitive to hydrogen peroxide. The effect of peroxide on the UV-visual spectral characteristics of hSCO1 that we produced in copper-supplemented medium is similar to that

**Fig. 3. Stereoview of the backbone structure of hSCO1.** a, Ca trace. The view here is rotated from the view in Fig. 1b by $-25^\circ$ around the vertical axis. Every 20th residue is marked in black; highly conserved residues are marked in blue; mutations that are associated with COX deficiency are marked in red. b, a highly conserved H-bond network near the SCO loop (see text).
specific to the copper-bound state of the SCO homolog PrrC from *Rhodobacter sphaeroides* (26), but these features were abolished by addition of peroxides (Fig. 4c).

We also tested the hydrogen peroxide sensitivity of SCO by challenging both yeast *sco1* and *sco2* null mutants with hydrogen peroxide, and assessed the resulting colony forming potential by spot tests on rich glucose (YPD) medium. The ΔSCO1 mutant (Fig. 4b), but not the ΔSCO2 mutant (data not shown), showed extreme sensitivity to 6 mM H₂O₂, whereas the isogenic parental strain W303 (Table III) displayed no sensitivity to H₂O₂ nor did another *pet* mutant deleted for COX14 (Fig. 4b). Because ΔCOX14 and ΔSCO1 have similar COX-deficient phenotypes (12, 27), the peroxide sensitivity of ΔSCO1 was not due simply to the secondary loss of COX. A plasmid expressing wild-type Sco1p was sufficient to rescue the peroxide sensitivity of ΔSCO1 (Fig. 4b), confirming the specificity of the phenotype.

**Point Mutations Causing Human Mitochondrial Disease**—A number of mutations in SCO have been mapped and characterized functionally. Besides mutations in bacteria (11) and yeast (28) that presumably disrupt the copper-binding site, there are 6 known pathogenic missense mutations in human SCO proteins: one in hSCO1 (P174L) (5), and 5 in hSCO2...
The mutation of Glu176 to Lys (corresponding to E140K in hSCO2), which is mutated to proline, lies toward the C-terminal. The mutation of Arg207 to Trp (corresponding to R171W in hSCO2) disrupts a salt bridge between Glu 176 and Lys179 (Lys143 in hSCO2) and places two positive charges next to each other. The mutation of Arg207 to Trp (corresponding to R171W in hSCO2) disrupts the conserved active site interactions discussed above, because the indole could not replace the hydrogen-bonding potential of the guanidinium group properly. Although it is possible to move the indole side chain to make a favorable stacking interaction with conserved Tyr256, the side chain of completely conserved Asp204 would also have to undergo an unfavorable conformational change to accommodate the indole ring (Fig. 2). Ile197 (corresponding to Leu151 in hSCO2), which is mutated to proline, lies toward the C-terminal end of a-helix 1, thereby disrupting an H-bond to the backbone carbonyl of Val183. Consequently, the I187P mutation likely destabilizes the protein.

The most difficult mutation to rationalize, and potentially most instructive one, is that of Thr261 to Phe (corresponding to E140K in hSCO2). This mutation, at the base of the SCO loop, directs the phenyl ring into the solvent, away from the active site but couples to it through backbone constraints to His260. The surface surrounding Thr261 is mostly hydrophobic, involving highly conserved nearby residues Tyr244, Tyr256, Ile263, and Tyr276. Replacing Thr261 with Phe in our structure and carrying out several rounds of energy minimization on the mutated model showed no significant deviations in side chain or backbone positions compared with the wild-type crystal structure, confirming that this mutation ought to be tolerated. Thus, the lethality of T261F, both in humans (4) and yeast (29), and its location in a surface-exposed hydrophobic patch near the putative copper-binding site (Fig. 5), suggests that this region may be involved in an important protein-protein interaction (discussed below).

Electrostatics—The electrostatic surface of hSCO1 is negatively charged (Fig. 5a). Similarly, energy-minimized homology models of hSCO2 and the R. spheroides homolog, PrRc, also indicate a predominately negative surface (data not shown). Because Lode et al. (30, 31) reported a stable complex between yeast Cox2p and Sco1p/Sco2p, we mapped the electrostatic potential of subunit II from bovine COX (32). Interestingly, this surface is also negatively charged (data not shown). However, without detailed electrostatic calculations and further structural data, it is difficult to speculate on the meaning of these results.

DISCUSSION

The analysis of the crystal structure of the C-terminal core region of human SCO1 implies that SCO is not a chaperone that transfers copper to COX II, but that redox sensitivity of its copper binding properties participates in signaling events. Arguments supporting these assertions are discussed in order.

The Role of Copper in SCO Function—The role of copper in SCO proteins is an important, and currently unresolved, issue. Delivery of copper to the CuA site in yeast Cox2p is thought to occur in a “bucket brigade” fashion, in which Cox17p first shuttles copper from the cytosol to the mitochondrial intermembrane space and then “hands off” copper to Sco1p (27) for eventual insertion into Cox2p (28, 29). (The function of Sco2p in this process is less clear, as a yeast sco2 null did not cause respiratory deficiency, and overexpression of yeast Sco2p could only rescue a Cox17p point mutation, but not the null mutation (27).) However, this scenario may be incorrect, for a number of reasons.

First, the current model of metal chaperones indicates that transfer between “donor” and “recipient” proteins occurs with metal-binding sites that are similar in structure (33). The copper-binding sites in COX II and SCO differ dramatically, and the similarities between the CXXX sequences in COX II and SCO (12) are merely superficial: the CuA site is bincular and each cysteine is involved in bridging both coppers (32), whereas EXAFS and EPR indicate that SCO binds only one copper atom (13, 26). Recently the NMR structure of Cox17p was published (34), and superposition of its copper-binding site on Sco1p shows significant differences. The nature of these differences is difficult to assess at present. Second, although SCO is present in all phyla, COX17, the presumed partner of SCO for copper transfer, is apparently absent from all known prokaryotic genomes. Third, a number of prokaryotes that do contain SCO do not express a COX complex containing a CuA site (they typically use a different pathway to generate a proton gradient (e.g. quinol oxidases)) (35). Finally, in those cases where copper could rescue COX activity (6, 10, 11), it is difficult to know if the rescue was because of a direct effect (e.g. via stimulation of copper uptake by residual SCO protein and/or by COX II) or an indirect one (e.g. via activation or deactivation of other genes following addition of a relatively toxic metal). We note that the reaction of Cu(I) with O2 produces oxoyradicals, and that the reaction of Cu(II) with H2O2 damages DNA (36). Thus, the addition of Cu(II) to SCO-mutant cell lines could have induced antioxidant genes that may have substituted for the redox sensitivity of SCO, over and above any putative copper-transporting function.

Whereas we have presented arguments suggesting that SCO is not a copper chaperone, the observations from EXAFS (13), NMR (24), atomic absorption (24, 26), UV-visible spectroscopy (26), and our data clearly indicate that SCO does bind copper. Thus, in view of our findings, it may be that copper is present in SCO not for eventual transfer to COX II, but rather to control the conformational state of SCO, perhaps as an allosteric regulator of its association with COX II. In particular, we note that Cu(I) can be oxidized to Cu(II) in the presence of peroxides, leading to the loss of copper from SCO.

SCO as a Potential Signaling Molecule—The potential redox-sensing capability of SCO and its structural similarity to the peroxiredoxin family support the hypothesis that SCO is a signaling molecule, as the Prx family itself has been implicated in a number of signaling pathways. For example, expression of...

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2 J. C. Williams, R. Friedman, and E. A. Schon, unpublished data.

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**TABLE III**

| Yeast strains | Source |
|---------------|--------|
| W303-1A       | a ade2-1 his3-1,15 leu2-3,112 trp1-1 ura3-1 | —a |
| W303SCO1      | a ade2-1 his3-1,15 leu2-3,112 trp1-1 ura3-1 SCO1::URA3 | Ref. 12 |
| W303SCO14     | a ade2-1 his3-1,15 leu2-3,112 trp1-1 ura3-1 SCO14::HIS3 | Ref. 12 |

**a** A kind gift of Dr. Rodney Rothstein, Department of Genetics and Development, Columbia University.

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(C133S (9), E140K (4), L151P (8), R171W (7), and S225F (4)).
peroxiredoxin II in HeLa cells reduced the ability of hydrogen peroxide to activate tumor necrosis factor-α-induced JNK and p38 signaling, and also attenuated extracellular signal-regulated kinase signaling (37). Recently, the bacterial peroxiredoxin, AhpC, was proposed to act as a component of a signaling “floodgate” (38): at low hydrogen peroxide levels AhpC reduces H₂O₂ and is regenerated by AhpD; at high levels, AhpD cannot turn over AhpC fast enough and AhpC becomes overoxidized, thus allowing the peroxide to oxidize other signaling partners.

The observation that H₂O₂ is lethal to yeast cells lacking Sco1p supports our supposition that the peroxide sensitivity of hSCO1 has physiological relevance. However, the peroxide sensitivity of Sco1p is not in and of itself definitive proof that SCO is a redox-sensitive molecule. In particular, yeast cells lacking Sco2p are not H₂O₂-sensitive (data not shown), but neither are they COX-deficient (12). On the other hand, it may be more than coincidental that COX deficiency and H₂O₂ sensitivity in yeast are correlated phenomena.

Additional insight concerning signaling in hSCO1 comes from studies of the photosynthetic regulatory response (prr) system in R. sphaeroides, a photosynthetic prokaryote. R. sphaeroides switches between photosynthesis and respiration depending on available light and O₂ levels. The expression of most of the photosynthetic genes in R. sphaeroides is under control of a histidine kinase-mediated two-component signaling system consisting of PrrA and PrrB. PrrC, which is the third member of the prrBCA operon, encodes a SCO homolog that interacts with the cbb₃ cytochrome c oxidase and inhibits the activation of PrrB (39), an interaction that is strikingly reminiscent of the interaction between Sco1p/Sco2p and Cox2p in yeast (30, 31). Moreover, the kinase activity of RegB, the PrrB homolog in R. capsulatus, is regulated by a redox-sensitive cysteine (40).

The connection of SCO to PrrC and to two-component sensing is intriguing, but there is currently no evidence for conventional two-component histidine kinases in multicellular animals. However, elements of bacterial two-component systems have been hypothesized to reside in mitochondria (41), including pyruvate dehydrogenase kinases (PDK1–PDK4) (42) and branched-chain α-keto acid-dehydrogenase kinase (BCKDK) (43). In fact, branched-chain α-keto acid-dehydrogenase kinase, a mammalian mitochondrial matrix protein that inactivates the branched-chain α-keto acid dehydrogenase complex by phosphorylation, has a three-dimensional structure similar to the prokaryotic two-component kinase domain (43). We are currently carrying out experiments to further characterize Sco1p and Sco2p peroxide sensitivities, potential enzymatic activities, and potential signaling pathways.

A Model for SCO Redox Sensing and Signaling—If SCO is indeed a copper-binding signaling molecule, how might it operate? With respect to the presumed copper-binding functionality of SCO, we deem it significant that although there is a COX assembly defect in hSCO1 mutant fibroblasts, copper insertion into COX II is apparently not affected in these cells (44), implying that the assembly problem occurs downstream of copper insertion into COX II (e.g. when COX I associates with nucleus-encoded COX IV and COX Va (44)).

Rather than transferring copper to COX, we believe that SCO acts as a copper-dependent redox switch. In particular, the oxidation state of Cu(I) is compatible with the trigonal planar configuration of the copper-binding site (25), whereas the oxidation state of Cu(II) is not. Thus, upon oxidation of Cu(I) to Cu(II), Cu(II) would be released from SCO, freeing the reactive CXXX cysteines to participate in a peroxidase reaction, as has been reported in other systems (45). Conversely, upon reduction of Cu(II) to Cu(I), Cu(I) could bind to the thiolated cysteines in the metal-free CXXX reactive site. In this way, the oxidation state of copper in SCO could act as a trigger for redox signaling, affecting COX function and perhaps other SCO binding partners. We note in this regard that hydrogen peroxide modifies tryptophan residues in bovine COX subunits IV and VIIa, thereby causing loss of assembled COX (46), and that several matrix side nucleus-encoded COX subunits have been implicated in signaling via both ATP and thyroid hormone (2). Taken together, these results also support the view that SCO has effects on COX integrity that go beyond copper transfer to COX.

With respect to redox signaling, we note that both humans and yeast express two SCO isoforms that have non-identical functions (in humans, mutations in SCO1 and SCO2 yield different clinical presentations; in yeast, a null of Sco1p, but not of Sco2p, produces respiratory deficiency). In both organisms, the amino acid sequences of the two paralogs differ in several key areas, especially in the N-terminal region, where the two isoforms have small matrix-side peptides that differ substantially in primary sequence (Supplementary Materials Fig. 1); notably, this N-terminal region is either missing or contains fewer than five residues in prokaryotes (Supplementary Materials Fig. 1). We speculate that conformational changes in SCO because of the redox state may be transduced to the matrix domain, which in turn interacts with the additional nucleus-encoded subunits of the COX complex. We note that soluble, IMS-localized fragments of both Sco1p and Sco2p make a stable complex with a soluble, IMS-localized Cox2p fragment (30, 31). In this regard, Thr²₃¹ in hSCO1 (corresponding to the lethal point mutation S225F in hSCO2) that lies in the middle of a well conserved, hydrophobic surface region, presumably interacts with the IMS-exposed region of COX II; this interaction may be sensitive to conformational changes of the COX loop. In this scenario, the two isoforms may interact differently with the COX holoprotein, resulting in isoform-specific expression of a downstream signal in response to
changes in redox potential that cause Cu(I) to be oxidized to Cu(II) (or vice versa).

Conclusions—Our finding that SCO is a member of the peroxiredoxin family has changed our view of the nature of this protein as an "assembly" factor for cytochrome c oxidase. In particular, the loss of the integrity of COX holoprotein structure and COX function in cells containing pathogenic mutations in SCO may not be simply a consequence of a failure to transfer copper from the latter to the former. Rather, SCO deficiency may disturb the ability of the mitochondrion to sense the redox state of the organelle or to react with peroxide signals, with COX deficiency being a downstream event. Of course, we cannot completely rule out copper transfer (albeit with a different mechanism than the current model), and leave open the possibility that SCO might be a molecule with multiple functions, namely, copper transfer and SCO function in cells containing pathogenic mutations in particular, the loss of the integrity of COX holoprotein structure and Cu(II) (or vice versa).

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