The P174L Mutation in Human Sco1 Severely Compromises Cox17-dependent Metallation but Does Not Impair Copper Binding*

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Paul A. Cobine†, Fabien Pierrel†, Scot C. Leary§1, Florin Sasarman§, Yih-Chern Horng§, Eric A. Shoubridge§2, and Dennis R. Winge§3

From the †Departments of Medicine and Biochemistry, University of Utah Health Sciences Center, Salt Lake City, Utah 84132 and the §Department of Human Genetics and Montreal Neurological Institute, McGill University, Montreal, Quebec H3A 2B4, Canada

Sco1 is a metallochaperone that is required for copper delivery to the Cu₄ site in the CoxII subunit of cytochrome c oxidase. The only known missense mutation in human Sco1, a P174L substitution in the copper-binding domain, is associated with a fatal neonatal hepa-topathy; however, the molecular basis for dysfunction of the protein is unknown. Immortalized fibroblasts from a SCO1 patient show a severe deficiency in cytochrome c oxidase activity that was partially rescued by overexpression of P174L Sco1. The mutant protein retained the ability to bind Cu(I) and Cu(II) normally when expressed in bacteria, but Cox17-mediated copper transfer was severely compromised both in vitro and in a yeast cytoplasmic assay. The corresponding P175L substitution in yeast Sco1 was impaired in suppressing the phenotype of cells harboring the weakly functional C57Y allele of Cox17; however, it was functional in sco1Δ yeast when the wild-type COX17 gene was present. Pulse-chase labeling of mitochondrial translation products in SCO1 patient fibroblasts showed no change in the rate of CoxII translation, but there was a specific and rapid turnover of CoxII protein in the chase. These data indicate that the P174L mutation attenuates a transient interaction with Cox17 that is necessary for copper transfer. They further suggest that defective Cox17-mediated copper metallation of Sco1, as well as the subsequent failure of Cu₄ site maturation, is the basis for the inefficient assembly of the cytochrome c oxidase complex in SCO1 patients.

Cytochrome c oxidase (CcO) is the terminal enzyme of the energy-transducing respiratory chain in mitochondria of eukaryotes and in certain prokaryotes. The enzyme catalyzes the reduction of molecular oxygen and couples this reduction with proton translocation across the inner membrane to generate the membrane potential used to synthesize ATP. The mammalian enzyme consists of 13 subunits, three of which, CoxI, CoxII and CoxIII, are mitochondrially encoded and form the catalytic core. Structurally conserved domains within subunits I and II contain the copper and heme cofactors that are essential for the catalytic competence of the holoenzyme (I). Cox1 consists of two heme A moieties, one of which interacts with a mononuclear copper site forming a heterometallic site, designated heme A₃–Cu₄. Two additional copper ions exist in a cysteine-bridged, binuclear, mixed valent center in CoxII designated Cu₃. Assembly of individual structural subunits into a functional holoenzyme complex within the mitochondrial inner membrane requires more than 30 accessory factors (2).

Isolated CcO deficiency, one of the most commonly recognized causes of respiratory chain defects in humans, is associated with a wide spectrum of clinical phenotypes (3, 4), and autosomal recessive mutations have been identified in six nuclear genes that encode CcO assembly factors in these patients (3–9). Two such factors, SCO1 and SCO2, encode metallochaperones that play a role in the copper ion metallation of the Cu₄ site in CoxII. This step in CcO assembly requires a single Sco protein in yeast (Sco1), but both Sco proteins are essential in humans. Recent studies with immortalized fibroblasts from SCO1 and SCO2 patients suggest that Sco1 and Sco2 have non-overlapping but cooperative functions in the maturation of the Cu₄ site (10), although their specific molecular roles in CcO assembly have yet to be defined.

Sco1 was first implicated in copper delivery to CcO by the observation that the respiration-deficient phenotype of a cox17Δ yeast mutant was suppressed by overexpression of SCO1 (11). Deletion of the SCO1 gene in yeast cells produces a respiratory phenotype attributable to a lack of CcO activity. Structural studies of Sco1 show that it has a globular domain with a thioredoxin fold consisting of a central four-stranded β-sheet covered with flanking helices that protrude into the mitochondrial intermembrane space (IMS) (12). Two other notable features of Sco proteins are a conserved pair of cysteiny1 residues in a CXXC motif and a conserved histidyl residue. These residues are spatially close in the apo-Sco1 structure in a solvent-exposed pocket (12).

Sco1 is capable of binding a single Cu(I) ion (18). X-ray absorption spectroscopy suggests that the Cu(I) is ligated via two sulfur donors and a nitrogen. Mutation of either the Cys or His residues abolishes Cu(I) binding and results in a nonfunctional CcO complex (18). Cox17 is the likely physiological copper donor to Sco1 (19) consistent with the postulate that Sco1 mediates Cu(I) transfer from Cox17 to CoxII. Sco proteins also bind Cu(II) (13). The Cu(II) site resembles a type II Cu(II) site with a higher coordination number than the three-coordinate Cu(I) site. A D238A substitution in yeast Sco1 abrogated Cu(II) coordination, leading to a nonfunctional protein. These data suggest that both Cu(I) and Cu(II) binding are critical for normal Sco1 function (13).

Mutations in either hSCO1 or hSCO2 result in pronounced CcO deficiency and lead to different, early onset, fatal clinical phenotypes (4, 6, 7, 14). SCO2 mutations are associated with neonatal encephalocar-
diomyopathy, whereas SCO1 patients present with neonatal hepatic failure and ketoacidotic coma. These distinct clinical phenotypes are not a result of tissue-specific expression of the two genes, as SCO1 and SCO2 are ubiquitously expressed and exhibit a similar expression pattern in different human tissues (4).

All reported SCO2 patients carry an E140K missense mutation on one allele and are either homozygous for this mutation or are compound heterozygotes. Homozygous patients have a delayed onset of the disease pathology and a more prolonged course of disease as compared with heterozygotes (14). SCO1 mutations have only been identified in a single pedigree in which the reported patients carried a nonsense mutation on one allele and a P174L missense mutation on the second allele (5). Both the E140K and P174L substitutions are adjacent to the CXXXC sequence motif.

Human Sco1 and Sco2 are nonfunctional in yeast sco1Δ cells (15). However, a chimeric protein consisting of 158 residues from the N terminus of yeast Sco1 fused to a C-terminal segment of hSco1, but not hSco2, is functional. A P174L substitution was found to attenuate its function (15, 16), although the CoxO deficiency in yeast sco1Δ cells harboring the mutant yeast/human Sco1 chimera could be rescued for growth on a nonfermentable carbon by the addition of 0.2% CuSO4 (16). This suggests that P174L Sco1 retains some residual function; however, the molecular defect in Sco1 function that results from this amino acid substitution remains unknown. The present study was therefore initiated to investigate whether copper binding is defective in the P174L mutant Sco1. We report that although copper binding is wild type in the mutant Sco1, its metallation by Cox17 is severely compromised.

**MATERIALS AND METHODS**

**Yeast Strains and Human Cell Lines**—All yeast strains used were in the W303 background (MAT a, ade2-1, his3-11,15, leu2,3,112, trp1-1, ura3-1). Cells were cultured with glucose, raffinose, or galactose as carbon sources as described (19). DNA transformations were performed using a lithium acetate protocol.

Primary cell lines from control SCO1 and SCO2 patient skin fibroblasts were immortalized and cultured as described previously (10). Preparation and supplementation of the growth media with copper histidine (Cu-His) was also as described elsewhere (10).

**Plasmids**—The construction of YEp–GAL1–hSCO1 and pHis–hSCO1 were described previously (13). The P174L mutation was introduced into these plasmids using the QuikChange™ site-directed mutagenesis kit (Stratagene, La Jolla, CA). A pRS413 vector (YCP) expressing yeast SCO1 with a C-terminal HA tag sequence under the control of the CYC1 promoter was also replaced with a MET25 terminator was used as a template to generate the P153L mutation. The entire insert was then subcloned into a pRS423 vector (YEp). The plasmid encoding Cyb2-C57Y Cox17 was generated by cloning the CYC1 promoter with a C-terminal HA tag sequence under the control of the MET25 terminator into these plasmids using the QuikChange™ site-directed mutagenesis kit (Stratagene, La Jolla, CA). A pRS413 vector (YCP) expressing yeast SCO1 containing 200 μCi/ml [35S]methionine, anisomycin (100 μg/ml) and a reversible cytotoxic translation inhibitor and chased for up to 17.5 h in regular Dulbecco’s modified Eagle’s medium. Total cellular protein (50 μg) was resuspended in loading buffer containing 93 mM Tris–HCl pH 6.7, 7.5% glycerol, 3.5% SDS, 0.25 mg of bromphenol blue/ml, and 3% mercaptoethanol, sonicated for 3–8 s, loaded, and run on 12–20% polyacrylamide gradient gels.

**Immunoblot Analysis of Yeast Proteins**—Protein (10–50 μg) from the mitochondrial fraction was electrophoresed on a 15% SDS-PAGE system and transferred to nitrocellulose (Bio-Rad). Membranes were blocked in 1× phosphate-buffered saline (50 mM Na2PO4, 100 mM NaCl (pH 7.0), 0.01% Tween 20, and 10% milk solution) prior to detection with appropriate antibodies and visualization with Pierce chemiluminescence reagents using a horseradish peroxidase-conjugated secondary antibody. Antiserum to porin (Por1) was from Molecular Probes. Rabbit anti-Sco1 antisera was generated as described previously (18).

**Immunoblot Analysis of Human Proteins**—Immortalized human fibroblasts and myoblasts were differentially permeabilized using digitonin to generate mitochondrially enriched fractions and were subsequently solubilized in phosphate-buffered saline containing 1.5% lauryl maltoside supplemented with complete protease inhibitor mixture (Roche Applied Science) (10). Equal amounts of protein were fractionated on 12% SDS-PAGE and transferred to nitrocellulose. Membranes were blocked with polyclonal antiserum raised against human Sco1 and CoxII and a monoclonal anti-Por1 antibody (Calbiochem). Following incubation with the relevant secondary antibody, immunoreactive proteins were detected by luminol-enhanced chemiluminescence (Pierce).

**Miscellaneous**—Protein concentration and CCo and citrate synthase activities in human cell lines were measured as described elsewhere (10).
Cox17-mediated Metallation of Human Sco1

RESULTS

Immortalized fibroblasts from a SCO1 patient with the P174L substitution exhibited a severe deficiency in CcO, which is reflected in both low residual enzyme activity and low steady-state levels of CoxII protein (Fig. 1, A and C). Transduction of patient fibroblasts with a retroviral virus overexpressing SCO1 completely restored wild-type CcO activity (Fig. 1A) (10), whereas overexpression of the P174L mutant SCO1 only partially rescued the CcO deficiency (Fig. 1A). These changes in CcO activity were accompanied by parallel increases in the steady-state levels of CoxII protein (Fig. 1C). Residual CcO activity in P174L-overexpressing SCO1 patient fibroblasts could be further increased by supplementing the growth media with Cu-His (Fig. 1A), an effect that was not attributable to altered levels of Sco1 protein (data not shown). These data demonstrate that the P174L mutant Sco1 retains sufficient residual function to allow for some assembly of the CcO holoenzyme.

Although the reduced CcO activity in SCO1 patient cells could be due in part to reduced Sco1 protein levels (10) (Fig. 1C), the inability of overexpressed P174L Sco1 mutant to fully restore CcO activity argues that some aspect of its function is impaired. We previously showed that overexpression of either the wild-type or the P174L mutant Sco1 proteins exerted a comparable dominant-negative effect on residual CcO activity and residual CoxII protein levels (Fig. 1, B and D). This phenotype was robust and could not be perturbed by the presence of Cu-His in the growth media. These results suggest that at least a fraction of overexpressed P174L Sco1 is metallated in SCO2 fibroblasts.

To further evaluate the molecular defect in the P174L mutant Sco1, N-terminal truncates of wild-type and P174L human Sco1 were expressed and purified (13) to characterize their copper binding properties. Both wild-type Sco1 and the P174L mutant protein eluted from gel filtration in fractions corresponding to monomeric molecules, and each contained 0.9 mol eq of copper (Table 1). Dialysis of the copper-containing proteins overnight in 1 mM EDTA/1 mM dithiothreitol resulted in only a slight depletion of bound copper; both wild-type and P174L Sco1 proteins retained 0.8 mol eq bound copper. The copper binding avidity to wild-type and P174L Sco1 was therefore similar. Titration of the proteins with varying levels of the Cu(I)-specific chelator BCS revealed a similar BCS concentration dependence in Cu(I) depletion, further suggesting that Cu(I) coordination is similar in the two proteins (Fig. 2).

The Cu(II) content, assessed by the quantity of copper that is not BCS-titratable, was 0.4 mol eq for both the wild-type and P174L Sco1 (Table 1). Absorption spectroscopy of purified wild-type Sco1 revealed the expected transitions of the Cu(II) chromophore in the visible spectral region with maxima at 360 and 480 nm (Fig. 3A). The P174L Sco1 exhibited the same transitions, confirming that the mutant Sco1 binds copper in a manner similar to wild-type Sco1. Porin served as an internal loading control.

Table 1

| Protein | Total Cu/protein | Cu(II)/protein | Post-dialysis total Cu/protein | Post-dialysis Cu(II)/protein |
|---------|-----------------|----------------|------------------------|----------------------------|
| hSco1   | 0.94 ± 0.1      | 0.40 ± 0.05    | 0.80 ± 0.1             | 0.40 ± 0.07                |
| hSco1 P174L | 0.95 ± 0.1 | 0.44 ± 0.05    | 0.78 ± 0.2             | 0.42 ± 0.05                |

Protein samples were quantified for total copper by inductively coupled plasma-optical emission spectroscopy (ICP-OES) analysis. The Cu(II) content was calculated by subtracting BCS-reactive Cu(I) (incubation with 25 mM BCS for 30 min) from the total copper amount and confirmed by quantitation of the Cu(II) EPR signal. Protein was quantified by amino acid analysis (n = 3).

FIGURE 1. Complementation analysis in SCO1 and SCO2 patient cell lines transduced with retroviral expression vectors expressing either wild-type SCO1 or P174L SCO1. A and B, CcO activity, expressed as a percentage of control (n = 3–11), in untransduced SCO1 and SCO2 patient fibroblasts (base line) and in cells overexpressing either wild-type or P174L Sco1. C and D, immunoblot analysis of expression of Sco1 and CoxII in control and patient fibroblast (C) and myoblast (D) lines in untransduced cells (−) and in cells overexpressing either wild-type Sco1 or P174L Sco1. Porin served as an internal loading control.
Cu(II) in a manner analogous to that of the wild-type protein. Comparable Cu(II) coordination between wild-type and P174L Sco1 was further confirmed by electron paramagnetic resonance spectroscopy; both proteins exhibited similar hyperfine splitting of the g_z component of the Cu(II) signal (Fig. 3B). Far UV circular dichroism revealed that the wild-type and mutant Sco1 proteins exhibited similar bulk structure, with comparable ellipticity in the two proteins, suggesting that the P174L substitution fails to grossly perturb the tertiary conformation of the protein (Fig. 4). Collectively, these data strongly suggest that the molecular defect caused by the P174L substitution does not result from aberrant copper binding.

We demonstrated previously, using both in vitro and in vivo approaches, that Cox17 is a Cu(I) donor to yeast and human Sco1 (13, 19). To investigate whether P174L Sco1 can be metallated by Cox17, purified wild-type and mutant Sco1 proteins were initially tested in an in vitro transfer assay. CuCox17 exhibits a characteristic luminescence at 580 nm that, upon addition of apo-Sco1, leads to an attenuation in emission due to Cu(I) transfer (19). The addition of apo-hSco1 to CuCox17 resulted in a rapid attenuation in the Cox17 luminescence (Fig. 5A). Subsequent purification of hSco1 by Ni-NTA chromatography and quantitation of bound copper confirmed that the quenching of Cox17 luminescence was attributable to copper transfer (Fig. 5C). The eluate contained only hSco1, and thus the observed copper transfer between Cox17 and hSco1 was likely mediated by transient interactions between the two proteins (Fig. 5D). The specificity of the Cu(I) transfer from Cox17 to hSco1 was verified by using a C57Y mutant, Cox17, that we previously demonstrated as failing to transfer Cu(I) to yeast Sco1 (19). As found earlier, incubation of hSco1 with the yeast C57Y mutant Cox17 failed to show any Cu(I) transfer as assessed by the lack of diminution in the Cox17 emission spectrum (Fig. 5A).

In contrast to wild-type hSco1, incubation of CuCox17 with P174L hSco1 failed to show either a diminution in CuCox17 emission or Cu(I) transfer after recovery of the mutant protein (Fig. 5B and C). Increasing the concentration of CuCox17 by 5-fold in the reaction resulted in limited transfer of Cu(I) to the purified P174L hSco1 sample to a final stoichiometry of 0.4 mol eq (data not shown). As expected, incubation of the yeast C57Y mutant Cox17 and the P174L hSco1 failed to show any Cu(I) transfer (Fig. 5B).

The inability of P174L hSco1 to be metallated by CuCox17 was further confirmed using the yeast cytosolic assay (19). Constructs encoding the globular domains of hSco1 and P174L hSco1 as His tag fusions were expressed from the GAL1 promoter on a high copy YEp plasmid. The proteins accumulated in the yeast cytoplasm, as they lacked their usual mitochondrial targeting sequence and transmembrane domains. Purification of hSco1 from the cytoplasm of cells co-expressing Cox17 and cultured in synthetic complete medium resulted in recovery of hSco1 with a bound copper content of 0.9 mol eq (Fig. 6). In contrast, co-expression of Cox17 and P174L hSco1 yielded only residual copper (0.1 mol eq) in the purified mutant Sco1, consistent with the above in vitro data.

The failure of Cox17 to metallate P174L Sco1 may in part explain the very low steady-state levels of CoxII protein observed in SCO1 patient fibroblasts (Fig. 1C). To evaluate the effects of the P174L substitution on CoxII expression at the protein level, pulse-chase labeling experiments of mitochondrial translation products were conducted. The rates of CoxII protein synthesis were comparable in control and SCO1 patient

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**FIGURE 2.** Competition of hSco1 and hSco1 P174L with the Cu(I)-chelator BCS. Cu(BCS)₂ complex was measured by monitoring the absorbance at 483 nm using a molar extinction coefficient of 12,250 cm⁻¹ M⁻¹. 20 μM hSco1 (bold line) and hSco1 P174L (dashed line) were incubated with varying concentrations of BCS, and the Cu(BCS)₂ complex was quantified after 10 min. Representative data are shown (n = 3).

**FIGURE 3.** Spectroscopic characterization of the Cu(II) site in hSco1 and hSco1 P174L. A, absorption spectra of purified hSco1 (bold line) and hSco1 P174L (dashed line). B, EPR spectra of purified hSco1 (top) and hSco1 P174L (bottom) with each sample present at a final copper concentration of 0.5 mM. The spectra were recorded at 77 K at a power setting of 19.8 milliwatts and a modulation amplitude of 4.0 G. Representative data are shown (n = 3).

**FIGURE 4.** Far UV circular dichroism spectra of purified hSco1 and hSco1 P174L. The concentration of purified hSco1 (bold line) and hSco1 P174L (dashed line) was 25 μM.
fibroblasts; however, there was a dramatic reduction in the stability of nascent CoxII in 
SCO1 patient fibroblasts during the chase (Fig. 7A).

The temporal loss of CoxII preceded similar reductions in the levels of CoxI and CoxIII, the other two mitochondrially encoded structural subunits (Fig. 7B). Radiolabeled CoxII was undetectable 8 h post-chase in 
SCO1 patient fibroblasts (Fig. 7B). In contrast, roughly 20% of the total radiolabeled CoxII pool from the pulse remained in control fibroblasts, an amount that persisted until the final time point at 17.5 h (Fig. 7B).

Pro-174 is a conserved residue in eukaryotic Sco proteins. To determine whether a corresponding mutation in yeast 
SCO1 attenuated function, a P153L substitution was engineered in yeast 
SCO1 and expressed on a centromere-based vector from its natural promoter. Yeast cells lacking Sco1 are respiration-deficient and lack CcO activity. Transformation of 
SCO1/H9004 cells with a mutant 
SCO1 encoding the P153L substitution suppressed the growth phenotype of 
SCO1/H9004 cells on ethanol/glycerol (Fig. 8) and restored wild-type oxygen consumption, suggesting that the P153L mutant protein was functional. As a second test of functionality, we evaluated the ability of mutant 
SCO1 to suppress the respiratory deficiency of 
cox17-1 cells. This partially functional allele of Cox17 contains a C57Y substitution, and glycerol growth of 
cox17-1 cells can be restored by overexpression of 
SCO1 (11). To test for potential suppression by mutant Sco1, we used 
cox17Δ cells harboring a plasmid expressing C57Y Cox17 as a fusion protein with the Cyb2 heme domain (designated Cox17-1*) to allow for its efficient accumulation within the IMS. Mutant 
cox17-1 cells with either wild-type or the P153L mutant 
SCO1 were plated on glycerol-containing medium. Whereas wild-type 
SCO1 reversed the growth phenotype on glycerol/ethanol when expressed from a YCp plasmid under its natural promoter (Fig. 7A), complementation of the phenotype by the P153L Sco1 required a much higher level of expression of the protein (Fig. 7A). Western anal-

FIGURE 5. Cox17-mediated copper transfer to 

hSco1 and hSco1 P174L. CuCox17 or CuCox17-C57Y present at 20 µM was incubated with 50 µM apo hSco1 or hSco1 P174L for the in vitro transfer reaction. A, the luminescence of CuCox17 before (solid line, top panel) and after the addition of apo-hSco1 (dashed line, top panel) is shown. The bottom panel shows the luminescence of CuCox17-C57Y before (solid line) and after the addition of apo-hSco1 (dashed line). Rel, relative. B, the luminescence of CuCox17 before (solid line, bottom panel) and after the addition of apo-hSco1 P174L (dashed line, bottom panel) is shown. C, the copper content of His-tagged hSco1 and hSco1 P174L after purification by Ni-NTA chromatography (eluate fractions) is shown by white bars (n = 3). The unbound fraction containing only Cox17 is shown by black bars. D, SDS-PAGE of the eluate fractions containing hSco1 and hSco1 P174L.

FIGURE 6. Copper transfer assay from Cox17 to hSco1 and hSco1 P174L in yeast cytoplasm. Yeast cells containing plasmids expressing yeast Cox17 and the His-tagged soluble domains of either hSco1 or hSco1 P174L were cultured as described under "Material and Methods." The His-tagged hSco1 proteins were purified on Ni-NTA, and the copper content of the eluted fractions is shown (n = 3). The purity of the hSco1 in the eluted fractions was determined by SDS-PAGE (inset).
ysis revealed that the wild-type and mutant protein accumulated to similar extents (Fig. 9B), ruling out the possibility that the inactivity of P153L Sco1 arose from reduced levels of the mutant protein.

The ability of Sco1 to restore glycerol growth of cox17/H9004 cells was dependent on the presence of C57Y Cox17. In the absence of C57Y Cox17, suppression of cox17/H9004 cells requires SCO1 overexpression and the addition of exogenous copper to the growth medium. Partial suppression of cox17/H9004 cells by overexpression of P153L Sco1 occurred in the presence of exogenous copper added to the growth medium (Fig. 9A).

**DISCUSSION**

The present study clearly demonstrates that the P174L substitution in hSco1 does not affect the ability of the protein to bind and retain Cu(I) or Cu(II) but, rather, impairs its ability to receive copper from the metallochaperone Cox17. Although the P174L variant has wild-type copper binding properties, its function in vivo is severely compromised as evidenced by the pronounced CcO assembly defect in SCO1 patient tissues and fibroblasts (6, 10). Overexpression of the mutant SCO1 in patient fibroblasts only partially suppressed the CcO deficiency, consistent with a loss of function mutation.

Using *in vitro* and *in vivo* copper transfer assays we previously showed that Cox17 transfers Cu(I) to both Sco1 and Cox11 in yeast (19) and that hCox17 transfers Cu(I) to hSco1 (13). The specificity of this transfer reaction is highlighted by a mutant form of Cox17 (C57Y) that fails to transfer Cu(I) to Sco1 despite normal Cu(I) binding and transfer to

**FIGURE 7.** Synthesis and turnover of mitochondrial CcO subunits in SCO1 fibroblasts. A, fibroblasts from the SCO1 patient and a control were pulse-labeled for 1 h with [35S]methionine in the presence of a reversible inhibitor of cytoplasmic translation (anisomycin) followed by chase in regular medium for various intervals of time (shown in hours at the top) to assess the rate of synthesis and the stability of mitochondrial CcO subunits. Ten of the 13 mitochondrially encoded structural subunits of the oxidative phosphorylation complexes are indicated at the left: CO, subunits of CcO; ND, subunits of complex I; cyt b, cytochrome b subunit of complex III; ATP6, subunit of complex V. The temporal decrease of the three mitochondrially encoded subunits of CcO in control patient fibroblasts is expressed as a percentage of radioactive counts incorporated following a 0.2-h chase period. The end points of the experiment (0.2 and 17.5 h chase) were assessed in five independent experiments, and the refined time course for the chase portion of the experiment was done singly.

**FIGURE 8.** Suppression of cox17-1 cells by yeast Sco1 P153L. A, transformants of cox17-1 cells were spotted onto selective plates containing 2% glucose or 2% lactate-glycerol with and without 1 mM CuSO4 as follows: empty plasmid (VEC), a plasmid encoding Cyb2-C57Y Cox17 (Cox17-1*) in combination with YCp or YEp plasmids encoding either wild-type Sco1-HA (strip A) or Sco1-HA P153L (strip B) under the control of the endogenous SCO1 (sco) or MET25 promoters (met). B, immunoblot analysis confirmed equal protein levels of Sco1-HA or Sco1-HA P153L in the cox17-1 cells. Mitochondrial protein, Por1, is shown as a loading control.

**FIGURE 9.** Suppression of cox17-1 cells by yeast Sco1 P153L. A, transformants of cox17Δ cells were spotted onto selective plates containing 2% glucose or 2% lactate-glycerol with and without 1 mM CuSO4 as follows: empty plasmid (VEC), a plasmid encoding Cyb2-C57Y Cox17 (Cox17-1*) in combination with YCp or YEp plasmids encoding either wild-type Sco1-HA (strip A) or Sco1-HA P153L (strip B) under the control of the endogenous SCO1 (sco) or MET25 promoters (met). B, immunoblot analysis confirmed equal protein levels of Sco1-HA or Sco1-HA P153L in the cox17-1 cells. Mitochondrial protein, Por1, is shown as a loading control.
Cox11. The P174L substitution in Sco1 also appears to perturb the interaction with Cox17, as its metabolism in both the in vitro assay and the in vivo yeast cytosolic assay was at residual levels.

The failure of Cox17 to metabolize P174L hSco1 in vitro could be partially reversed by substantially increasing Cox17 concentrations in the transfer reaction, suggesting that overexpression of either P174L Sco1 or hCox17 might rescue CcO activity in SCO1 patient fibroblasts. Although we demonstrated partial rescue of the CcO phenotype by overexpressing P174L Sco1, we did not observe any rescue by overexpressing Cox17, even though immunoblot analysis of whole cell extracts indicated that we were able to overexpress it 2–3-fold using retroviral expression vectors (data not shown). Cox17, however, is distributed between the cytoplasm and the mitochondrial IMS (20), and it is possible that this experiment did not result in a significant enough increase in Cox17 concentration in this compartment to promote copper loading of Sco1 in patient cells.

An attenuation of the interaction between Cox17 and the mutant hSco1 is further supported by the observation that P153L Sco1 in yeast is impaired in suppressing the phenotype of cox17-1 cells harboring the C57Y substitution in Cox17. Whereas overexpression of wild-type Sco1 suppressed the growth phenotype of cox17-1 cells on ethanol/glycerol, expression of P153L SCO1 from a YCp vector failed to restore CcO activity. Interestigly, the P153L substitution in yeast Sco1 apparently does not compromise the interaction with Cox17 as seriously as the P174L mutation in the human protein. No respiratory phenotype was observed in sco1Δ cells expressing the P153L Sco1.

The exact nature of the Cox17−Sco1 interaction is not yet clear. We have failed to observe an interaction using affinity purification of Sco1 from both in vivo and in vitro copper transfer assays (19), suggesting that Cox17 donates Cu(I) through ligand exchange reactions in a transient protein-protein interaction. We propose that this complex is attenuated by the C57Y substitution in Cox17 and by the P174L substitution in Sco1. This could arise if Pro-174 is part of the interaction interface or, alternatively, if P174L alters Sco1 conformation. It is conceivable that Sco1 exists in two subconformational states, an open conformer receptive to Cu(I) binding and a closed state poised for transfer to Cox11. The failure of Cox17 to donate Cu(I) to P174L Sco1 could arise if Sco1 exists in the closed conformer, mimicking the copper-loaded state.

We cannot rule out the possibility that the P174L substitution impairs copper transfer from Sco1 to Cox11; however, the fact that the CcO deficiency in SCO1 patient fibroblasts is partially reversed by substantially increasing Cox17 concentrations in vitro indicates that this experiment did not result in a significant enough increase in Cox17 concentration in this compartment to promote copper loading of Sco1 in patient cells.

The most important ramification of the P174L mutation in Sco1 is the failure to incorporate newly synthesized Cx21 into the holoenzyme complex. This stalls the CcO assembly process, resulting in accumulation of a subassembly and the production of a limited amount of active holoenzyme (22). Mitochondria contain an AAA protease system in the inner membrane that is responsible for the quality control of inner membrane proteins (23), rapidly turning over newly synthesized mitochondrial proteins that are not incorporated into nascent enzyme complexes. Although it is therefore not surprising to find that nascent, mitochondrial encoded Cox subunits are degraded more rapidly in cells with a CcO assembly defect than in controls, SCO1 patient cells are unique in that the turnover of newly synthesized CoxII occurs more rapidly than that of CoxI and CoxIII. We have not observed this specificity in other cell lines from patients with other isolated CcO assembly defects and similarly low residual CcO activity. This suggests that it is the inefficient maturation of the CuA site that results in the disproportionately rapid degradation of apo-CoxII molecules, further emphasizing that CoxII is the molecular target of Sco1.

Overexpression of P174L Sco1 as well as wild-type hSco1 exerts a dominant-negative effect on CcO activity in SCO2 patient fibroblasts, an effect that we previously showed depends on intact copper binding in the wild-type protein (13). Mutant Sco1 must be at least partly copper-loaded, as it retains limited function. It is possible that even a small pool of metallated Sco1 would be sufficient to cause the dominant-negative effect in SCO2 patient cells, as these cells had very low steady-state levels of Sco2 protein. Alternatively, the P174L substitution could stabilize a conformation mimicking the copper-loaded state. Future studies will address the effect of the P174L substitution on the conformation of Sco1 and its interaction with Sco2.

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