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Molecular Pathogenesis of Genetic and Inherited Diseases

Unexpected Vascular Enrichment of SCO1 over SCO2 in Mammalian Tissues

Implications for Human Mitochondrial Disease

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Mammalian SCO1 and SCO2 are evolutionarily-related copper-binding proteins that are required for the assembly of cytochrome *c* oxidase (COX), a mitochondrial respiratory chain complex, but the exact roles that they play in the assembly process are unclear. Mutations in both *SCO1* and *SCO2* are associated with distinct clinical phenotypes as well as tissue-specific COX deficiency, but the reason for such tissue specificity is unknown. We show in this study that although both genes are expressed ubiquitously in all mouse and human tissues examined, surprisingly, SCO1 localizes predominantly to blood vessels, whereas SCO2 is barely detectable in this tissue. To our knowledge, SCO1 is the first known example of a mitochondrial protein that is strongly expressed in the vasculature. We also show that the expression of SCO1, but not of SCO2, is very high in liver (the tissue most affected in *SCO1*-mutant patients), whereas the reverse holds true in muscle (the tissue most affected in *SCO2*-mutant patients). Our findings may help explain the differences in clinical presentations due to mutations in *SCO1* and *SCO2* and provide clues regarding the partially nonoverlapping functions of these two proteins. ***(Am J Pathol 2010, 177:2541–2548; DOI: 10.2353/ajpath.2010.100229)***

Cytochrome *c* oxidase (COX), also called complex IV of the respiratory chain, is located within the mitochondrial inner membrane. It accepts electrons from cytochrome *c* and passes them to oxygen, while pumping protons across the inner membrane to help establish a proton gradient for ATP synthesis. COX functions as a dimer: each monomer contains 13 subunits, two heme groups (heme *a* and *a3*, both located in subunit I), three copper ions (two in the Cu_A site in subunit II and one in the Cu_B site in subunit I), a zinc ion, and a magnesium ion.[1] The three largest subunits (I, II, and III), which are encoded by mitochondrial DNA, form the catalytic core of the enzyme and contain the prosthetic groups; they perform the electron transfer and proton pumping functions. The 10 smaller subunits are encoded by nuclear DNA and have regulatory and structural functions. Additional nuclear-encoded factors are required for the maturation and assembly of the structural subunits into the COX complex, including those responsible for synthesis of heme A and the transport and insertion of metal cofactors.[2–4] A number of COX assembly factors have been identified in yeast, many of which have human homologs, including CMC1, COX10, COX11, COX15, COX17, COX18, COX19, COX20, LRPPRC, OXA1, PET191, SCO1, SCO2, and SURF1.[4]

Both humans and yeast have two SCO genes (SCO stands for synthesis of cytochrome *c* oxidase), *SCO1* and *SCO2*, which were derived from a gene duplication event that occurred in the two lineages independently.[5] Both SCO proteins in humans are essential, whereas in yeast only SCO1 is required for cell viability on nonfermentable carbon sources.[6] The SCO family of proteins has been implicated in the transport of copper to COX and is thought to bind copper at a highly conserved CxxxC

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This article is dedicated to Eduardo Bonilla, an outstanding scientist/morphologist and a beloved friend, who passed away on April 7, 2010. He will be missed.

Supplemental material for this article can be found on *http://ajp.amjpathol.org*.

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domain.[7] However, structural analysis of human SCO1 has shown a similarity to redox-active proteins, including thioredoxins and peroxiredoxins,[8–10] and the protein is peroxide-sensitive,[8] "raising the possibility that SCO1 - and perhaps human SCO2 (whose structure has not yet been deteremined) as well, as the two proteins are similar in the region containing the thioredoxin-like fold[8] - might also function as a thiol:disulfide oxidoreductase.[8,10–12]" Based on the extreme sensitivity to H_2O_2 of a SCO1-null mutant in yeast,[8,13] the SCO proteins have been proposed to function as copper-dependent redox switches.[8,10] More recent findings suggest that the SCO proteins play a regulatory role in the maintenance of cellular copper homeostasis.[14] On the other hand, SCO2 is regulated positively by p53 in both humans and mice, thereby modulating the cellular balance between glycolysis and respiration.[15] Interestingly, the effect of p53 seems to be tissue-specific, in that SCO2 expression is responsive to p53 in liver, but not in muscle.[16] Despite the progress in characterizing the function of the SCO proteins, which have been shown to have nonoverlapping, cooperative functions,[12,14] it still remains unclear why two SCO proteins are required for survival in humans.

Mutations in both human *SCO1* and *SCO2* cause severe COX deficiencies, because of a failure in the assembly of the COX holoenzyme, but different tissues are affected. Mutations in *SCO1* cause fatal infantile hepato-encephalomyopathy,[17] whereas mutations in *SCO2* cause fatal infantile cardio-encephalomyopathy[5]; at present it is unclear why this "tissue specificity" exists.

To try to understand this tissue specificity, we analyzed *SCO1* and *SCO2* transcription and protein expression in different human and mouse tissues. Our results suggest that although there is no strict tissue specificity for either protein, SCO1 exhibits an unexpected and differential pattern of expression in the vasculature, which, to our knowledge, is a novel feature for a mitochondrial protein.

Materials and Methods

Northern Blot Analysis

A human multiple-tissue blot with mRNA from eight tissues (Clontech, Mountain View, CA) and a mouse multiple-tissue blot with mRNA from 10 tissues (Ambion, Austin, TX) was used for Northern blot analysis. For human *SCO1*, a single-stranded radiolabeled probe spanning exons 4 to 6 (nucleotides [nt] 627 to 1072, GenBank NM_004589.2) was generated by *in vitro* transcription using the MAXIScript SP6/T7 kit (Ambion) in the presence of [α-^{32}P]dUTP and was hybridized to the blot. For human *SCO2* (nt 128–872, GenBank NM_005138.2), as well as mouse *Sco1* and *Sco2*, double-stranded DNA fragments were generated by PCR and labeled with [α-^{32}P]dCTP using the Random Primed DNA labeling kit (Roche Diagnostics, Indianapolis, IN). The mouse *Sco1* probe spanned exons 2 to 6 (nt 280 to 857, GenBank NM_001040026.1); the mouse *Sco2* probe included 600 bp of the coding region (nt 329 to 928, GenBank NM_001111288.1). The probes were hybridized overnight at 68°C. The membranes were stripped in boiling 0.5% SDS. The human blot was probed sequentially, as follows: *SCO1* exposed 4 days; *SCO2* exposed 11 days; and β-actin (cDNA control probe provided by Clontech) exposed 12 hours. The mouse blot was probed with *Sco1* for 7 days, with *Sco2* for 7 days, and with β-actin (nt 32 to 468, GenBank NM_007393.3) for 4 hours.

In Situ *Hybridization*

Sections (10 μm) of frozen human muscle were cut, air-dried, and fixed in 4% paraformaldehyde. Sections were incubated in prehybridization solution (50% formamide, 5× standard saline citrate [SSC], 10× Denhardt's solution, 0.3 mg/ml *Escherichia coli* tRNA, and 2.5 mg/ml herring sperm DNA) for 2 hours at 70°C. Probes were generated by *in vitro* transcription (DIG RNA labeling mix from Roche Diagnostics) according to the manufacturer's protocol using a linearized plasmid containing 447 bp of *Sco1* (nt 627 to 1072, GenBank NM_004589.2) or 352 bp (nt 181 to 533, GenBank NM_005138.2) of the coding region of *Sco2* as a template, in both the sense (control) and antisense orientations. Probes were hybridized overnight at 70°C. After removal of unbound probe (by washing in 5× SSC for 5 minutes at 70°C and 3 times in 0.2× SSC for 30 minutes at 70°C), the slides were blocked and incubated with anti-DIG antibody overnight at 4°C. The sections were developed with Fast Red solution (Sigma-Aldrich, St. Louis, MO).

Immunohistochemical Analyses

For immunohistochemical analysis, aorta, brain, heart, kidney, liver, and muscle tissues were frozen in isopentane cooled in liquid nitrogen. The tissues were cryosectioned (8 μm thick) and fixed for 10 minutes at room temperature (RT) in 4% paraformaldehyde in PBS. Tissues were rinsed in PBS followed by an antigen retrieval step in citrate buffer (10 mmol/L citric acid, pH 6.0, at 100°C for 45 minutes). Slides were then incubated in 0.4% Triton X-100 in PBS for 20 minutes at RT. For fluorescence staining, the sections were blocked for 1 hour at RT in SuperBlock blocking buffer (Pierce Chemical, Rockford, IL) containing 160 μl/ml avidin block (Vector Laboratories, Burlingame, CA). An antibody was raised in chicken against an 18-amino acid (aa) peptide from aa 113 to 130 (KHVKKEKAEKLEKERQRH) of human SCO1 (Lampire Biological Laboratories, Pipersville, PA) and a rabbit polyclonal antibody was raised against a 14-aa peptide from aa 236 to 249 (LFTDYYGRSRSAEQ) of human SCO2 (Zymed Laboratories, Invitrogen, Carlsbad, CA). Antibodies were diluted 1:100 in SuperBlock blocking buffer containing 160 μl/ml biotin block (Vector Laboratories) and were added to the tissue sections overnight at 4°C. Secondary biotinylated chicken and rabbit antibodies (Amersham Biosciences, Piscataway, NJ)

were added for 1 hour at RT, followed by incubation with streptavidin fluorescein (Amersham Biosciences) for 1 hour at RT. For peroxidase staining, the sections were blocked with sheep and donkey serum. Primary and secondary antibodies were as above but were diluted in 1% bovine serum albumin in 1× PBS, followed by addition of avidin and biotinylated horseradish peroxidase H (Vectastain ABC Kit, Vector Laboratories) for 1 hour at RT. The staining was visualized with diaminobenzidine, followed by dehydration and mounting with Permount.

Background stainings were performed as above but in the absence of the relevant primary antibody. The specificity of the staining was confirmed by antigen competition experiments. The antibodies were incubated with the relevant peptides before immunolabeling; a dramatic reduction in signal intensity to background levels was observed.

Western Blotting

For Western blotting of proteins separated by SDS-polyacrylamide gel electrophoresis, 15 μg of crude mouse mitochondrial or cytosolic proteins were electrophoresed through a 12% polyacrylamide gel and electroblotted onto polyvinylidene difluoride filters (Bio-Rad Laboratories, Hercules, CA). For protein detection, we used a rabbit polyclonal antibody raised against aa 272 to 285 (EFLDYFGQNKRKGE) of human SCO1 (Zymed Laboratories) and the above-mentioned rabbit polyclonal antibody to SCO2. Both SCO antibodies were affinity-purified using the SulfoLink Kit (Pierce Chemical) according to the manufacturer's protocol. The loading controls were antibodies against the Cox1 subunit of complex IV (459600, Invitrogen) at a dilution of 1:400 and against the Ndufa9 subunit of complex I (459100, Invitrogen) at a dilution of 1:1000. The membranes were stripped between probing with the different antibodies. We used ImageJ (NIH, Bethesda, MD) to quantitate the intensities of the immunoblot signals.

Results

Transcriptional Analysis of Sco1 *and* Sco2

Using Northern blot analysis of multiple tissues, we showed that transcripts of mouse *Sco1* (~1.4 kb) and *Sco2* (~1.1 kb) were present in all tissues examined (Supplemental Figure 1, see *http://ajp.amjpathol.org*). We also confirmed that transcripts of human *SCO1* (~1.7 kb) and *SCO2* (~0.9 kb), were present in all tissues analyzed (not shown), as reported previously.[5,18]

Western Blot Analysis to Detect SCO1 and SCO2 Protein Expression

We performed immunoblot analysis to detect Sco1 and Sco2 in the cytosol and in crude mitochondria derived from five mouse tissues (brain, heart, kidney, muscle, and liver) processed immediately after sacrifice (Figure 1). As anticipated and consistent with the Northern blotting results, we detected Sco1 in the mitochondrial fraction but not in the cytosolic fraction in all tissues analyzed (Figure 1A). Relative to the signal for Cox1, a mitochondrial DNA-encoded subunit of complex IV of the mitochondrial respiratory chain (Figure 1), and for Ndufa9, a nucleus-encoded subunit of complex I of the respiratory chain (not shown), the highest amounts of Sco1 were detected in liver and the lowest amounts in skeletal muscle (Figure 1B). A similar analysis to detect Sco2 showed that it too was expressed in all tissues analyzed (Figure 1A), with the highest levels in muscle and the lowest levels in liver (Figure 1B). Interestingly, although the amounts of Sco1 and Sco2 in brain, heart, and kidney, were comparable, in liver there was about a 15-fold greater amount of Sco1 than of Sco2, whereas conversely, in skeletal muscle there was approximately a sevenfold greater amount of Sco2 than Sco1. These results were replicated in a separate experiment (not shown).

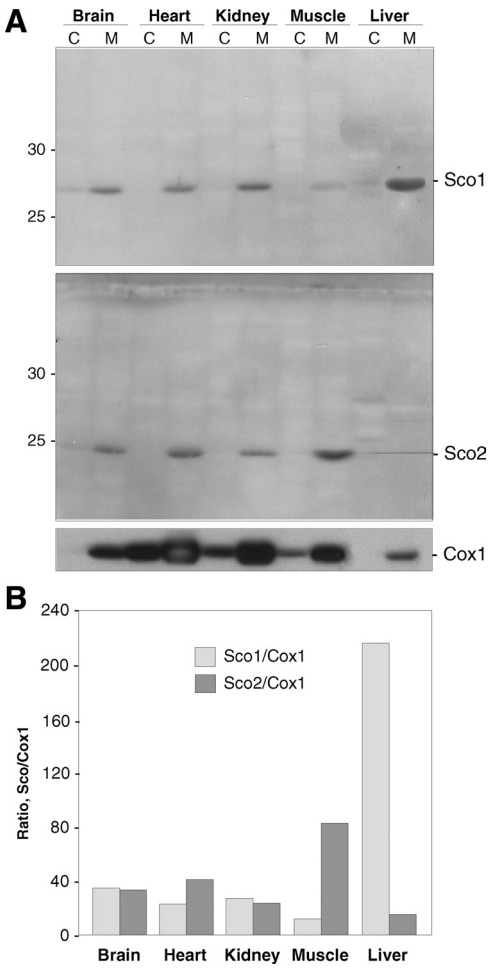

Figure 1. Expression of Sco1 and Sco2 in various mouse tissues. **A:** Western blot. C, cytoplasmic fraction; M, crude mitochondrial fraction. Molecular mass markers, in kDa, at left. Cox1 was used as a loading control. **B:** Quantitation of the amount of Sco1 and Sco2 in the tissues analyzed in **A**, normalized to that of Cox1. Note the differentially higher levels of Sco1 in liver and of Sco2 in muscle.

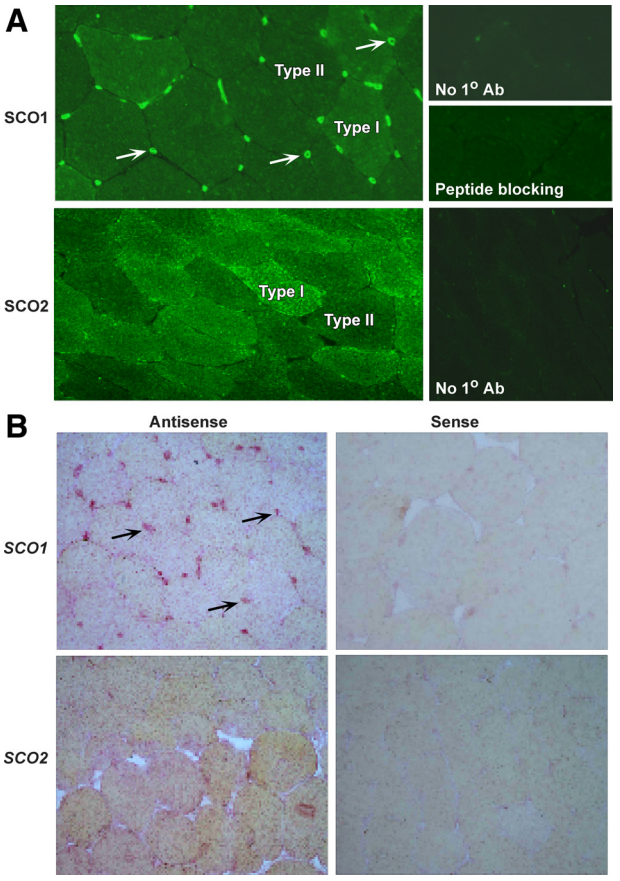

Figure 2. SCO expression in human muscle. **A:** Immunofluorescence to detect SCO1 (**top panels**) and SCO2 (**bottom panels**); control immunostaining was performed in the absence of the respective primary antibodies or with a blocking peptide, as indicated. Note greater expression of both proteins in type I versus type II fibers and the expression of SCO1 but not SCO2 in endomysial blood vessels (**arrows**) ($n = 7$ experiments). **B:** *In situ* hybridization in human muscle with *SCO1* and *SCO2* antisense and sense (control) probes. Note the preferential expression in blood vessels of *SCO1* mRNA (**arrows**), and the apparent absence of *SCO2* mRNA ($n = 2$).

Immunolocalization of SCO1 and SCO2 in Tissues

Cryostat sections of frozen human skeletal muscle were analyzed by immunofluorescence microscopy to detect SCO1 and SCO2, using antibodies generated against amino acid sequences specific to the central region of SCO1 and the C terminus of SCO2. Both proteins were expressed throughout the muscle fibers, with a stronger signal in type I versus type II fibers, consistent with a mitochondrially targeted protein involved in oxidative energy metabolism (Figure 2A). However, we also observed an unexpectedly strong signal for SCO1 in the vasculature, with no obvious evidence of any signal for SCO2; SCO1 was present both in endomysial and perimysial vessels.

Because of the unexpected and surprising localization of SCO1 in the muscle vasculature, we performed *in situ* hybridization analysis to detect both *SCO1* and *SCO2* transcripts in human skeletal muscle. Consistent with the immunohistochemical results, we found both transcripts in the muscle fibers, but in the vasculature we observed intense signals only for the *SCO1* transcripts, whereas *SCO2* transcripts were essentially absent (Figure 2B).

To determine the generality of these results, we performed immunohistochemical analyses of multiple tissues. Cryostat sections of frozen human (Figure 3)[19] and mouse (Supplemental Figure 2, see *http://ajp.amjpathol.org*) heart, kidney, liver, and muscle were analyzed by immunofluorescence microscopy and of brain by immunoperoxidase staining. As with the initial analysis in skeletal muscle, both SCO1 and SCO2 were expressed in all tissues examined, with a relatively uniform distribution for SCO2. For SCO1, however, besides a generally uniform level of immunostain in the "bulk" tissue, we consistently observed intense signals in the vasculature, which appeared to be negative for SCO2 signals (Figure 3). Besides strong signals in the blood vessels of the heart, liver, kidney, and brain, and the portal veins and the sinusoids of the liver, we also observed extremely intense staining of glomeruli in the kidney. The glomerular staining was presumably also localized to blood vessels, because these structures contain numerous capillaries.[20] The immunohistochemical results were confirmed with additional antibodies raised against other epitopes in SCO1 and SCO2 (not shown).

Localization of SCO1 and SCO2 within the Vasculature

To investigate more precisely the location of SCO1 and SCO2 within the vasculature, we examined the pattern of immunofluorescence in heart, liver, and kidney at higher magnification (Figure 4A). In all three tissues, we observed immunostain for SCO1 in the vascular smooth muscle but much more intense immunostain in the endothelia (Figure 4A). In an artery from skeletal muscle, SCO1 was again expressed in both smooth muscle and endothelial cells, but at high magnification we observed more immunostain in the luminal portion of the intima (Figure 4B). Analysis of frozen sections of aorta from human (Figure 4C) and mouse (data not shown) showed SCO1 immunostain in both the smooth muscle and the endothelial cells, with a somewhat greater staining intensity in the latter (Figure 4C, top center panel), whereas the signal for SCO2 was elevated only slightly above background (Figure 4C, compare bottom center and right panels). Consistent with the immunohistochemical results, Western blotting of crude mitochondria from human aorta showed a much stronger signal for SCO1 compared with that for SCO2, which was virtually undetectable (Figure 4D). Taken together, these results indicate that, compared with SCO2, SCO1 is expressed preferentially in both the smooth muscle and endothelial cells of blood vessels, but the degree of expression in these two vascular compartments can vary among tissues.

Immunolocalization of SCO1 and SCO2 in Early Development

In an analysis of Sco2-mutated mice, we recently showed that Sco2 is required for early mouse development, as

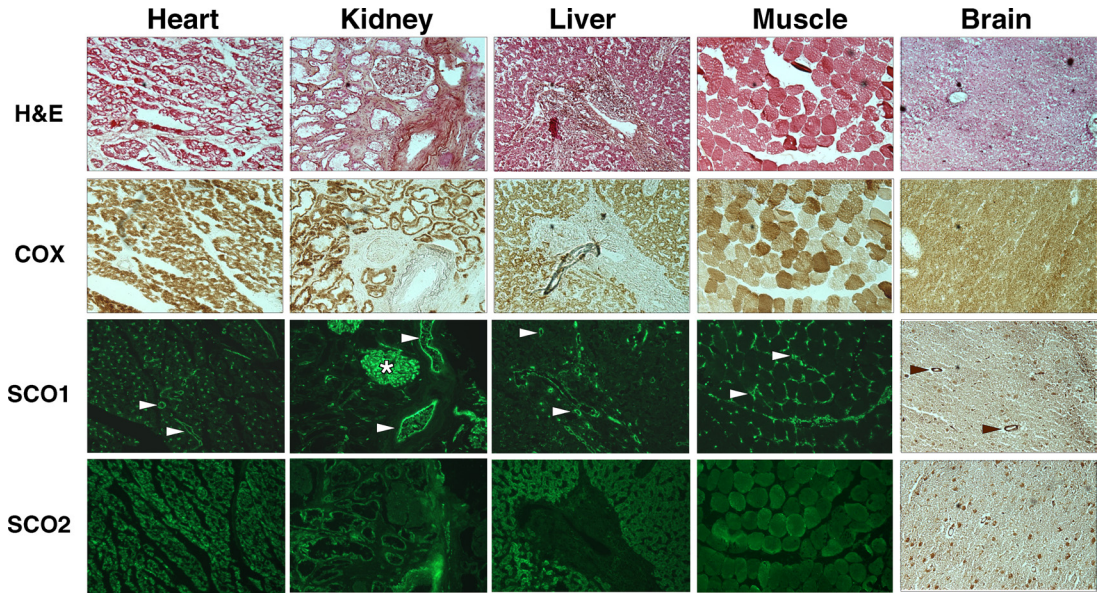

Figure 3. Immunostaining of the indicated human tissues to detect SCO1 and SCO2 (alkaline phosphatase method for brain; immunofluorescence for the other tissues); control immunostaining was performed in the absence of the respective primary antibodies (Figure 2A). Note the preferential expression of SCO1 in blood vessels in all tissues examined (**arrowheads**), as well as in the kidney glomerulus (**asterisk**), which is highly vascularized. Note that it seems as if COX activity is absent in the glomerulus; in fact, it is present, but at reduced levels compared with those in the surrounding tubules, because of the comparatively fewer mitochondria in the glomerulus. This phenomenon has been observed by others.[19] Also shown are H&E staining and histochemistry to detect COX activity ($n = 6$).

ablation of *Sco2* resulted in early embryonic lethality, before day 8.5 post coitum.[21] To investigate further whether Sco1 is also expressed during early gestation, we used immunohistochemistry to detect both Sco1 and Sco2 in early embryos at the blastocyst stage (3.5 days post coitum) (Figure 5). We detected both Sco1 and Sco2 proteins at this stage of development. These results highlight the importance of both proteins for early development and also imply that Sco1 and Sco2 have nonoverlapping functions at this developmental stage, because Sco1 could not rescue the lethality attributable to loss of Sco2.[21]

Discussion

We have shown here that although both SCO1 and SCO2 are expressed essentially ubiquitously in all tissues we examined, there is a differential expression pattern for the two proteins, both at the transcriptional and translational level, in humans and in mice. Most strikingly, we found that SCO1 has an unexpected differential pattern of expression for a mitochondrial protein, namely in the vasculature. For example, in muscle, although SCO1 protein was present at low levels in all muscle fibers, it was located predominantly in the blood vessels, whereas SCO2 was expressed almost exclusively in the muscle fibers, with little evidence of any vascular expression (a result that was also confirmed at the transcriptional level by *in situ* hybridization). The vascular-specific pattern of SCO1 expression compared with that of SCO2 was observed in every adult tissue examined, including heart, skeletal muscle, aorta, kidney, liver, and brain.

Many mitochondrial proteins have tissue-specific isoforms, including 5 of the 10 nucleus-encoded subunits of COX (IV, VIa, VIb, VIIa, and VIII[22,23]), but in these cases the tissue specificity is typically between a ubiquitously expressed isoform and a tissue-specific isoform (ie, expression only in heart/muscle [eg, COX7A1[24]], testis [eg, COX6B2[25]], or lung [eg, COX4I2[26]]). In the case of SCO, the "specificity" resides not among different tissues, but within each tissue in the vasculature. Given the ostensible role of the SCO proteins in COX assembly and copper transport, one might have expected any potential tissue-specific expression of SCO to fall along the muscle/nonmuscle divide (as an example). However, this was not the case, because both genes are expressed ubiquitously in all tissues, but with different degrees of expression *within* each tissue (ie, SCO1 versus SCO2 in the vasculature). We note that a standard Northern blot analysis of RNA derived from whole tissues would not be able to distinguish vascular from nonvascular expression of *SCO1* versus *SCO2*, because the RNAs prepared from these tissues contain messages derived from both the cell bodies and the interstitial blood vessels.

As noted above, aside from the predominance of Sco1 in the vasculature, Sco1 and Sco2 proteins were expressed at similar levels in most mouse tissues, with two notable exceptions: Sco1 was present at much higher levels than Sco2 in liver, whereas the reverse was true in skeletal muscle. This skewing of the expression of the two proteins might explain, at least in part, why patients with *SCO1* mutations have significant liver involvement,[17,27] whereas those with *SCO2* mutations have cardiac/muscle involvement.[5] Moreover, the observed levels of SCO2 protein in brain and muscle also correlate with the phenotype in patients with *SCO2* mutations.[5] Perhaps in human liver, low levels of SCO2 cannot compensate for the loss of SCO1, whereas in human heart and muscle low

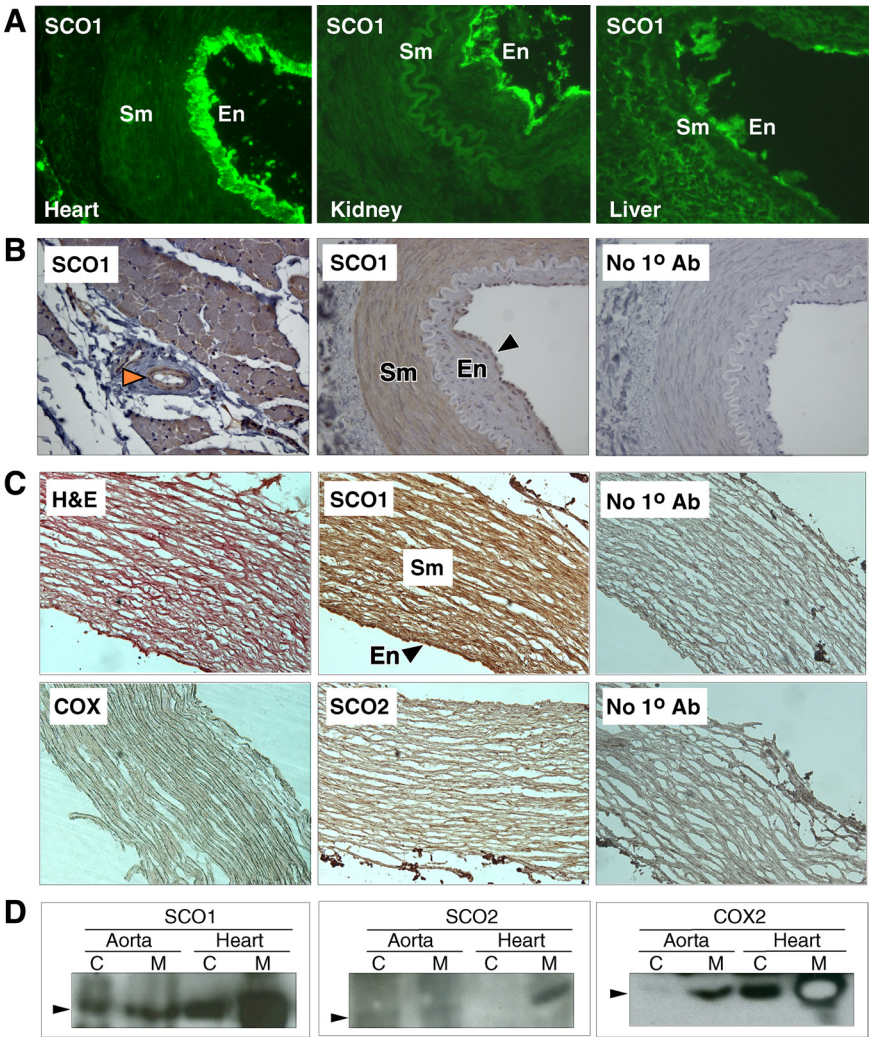

Figure 4. SCO expression in blood vessels. **A:** Immunofluorescence to detect SCO1 in the tissues indicated. Note staining of both smooth muscle (Sm) and endothelium (En), with more intense staining of the latter ($n = 6$). **B:** Immunohistochemical staining of human muscle for SCO1 (immunoperoxidase method). Note preferential staining of blood vessels (**arrowhead** indicates a perimysial vessel; **left panel**). At higher magnification (**center panels**), a vessel shows staining of both the smooth muscle and the endothelium, especially on the luminal side (**arrowhead**). Control staining without first antibody is at right. **C:** Immunostaining of human aorta, showing H&E stain, histochemistry to detect COX activity, and immunostaining to detect SCO1 and SCO2, along with the respective controls in the absence of first antibody, as indicated. Note the more intense staining of SCO1 above background compared with that for SCO2 ($n = 4$). **D:** Western blotting to detect SCO1 and SCO2 in cytoplasmic (C) and crude mitochondrial (M) fractions from human aorta compared with heart (50 μg loaded in each lane); COX2 was used as the loading control. Note the virtual absence of SCO2 in aorta, consistent with the immunohistochemical results.

levels of SCO1 cannot compensate for the loss of SCO2. In support of this idea, it has been shown that there is a tissue-specific decrease in COX activity in patients with SCO2 mutations, especially in muscle and brain.[28] However, liver retained high residual levels of COX activity in these patients,[28] even though SCO2 is expressed at very low levels in this tissue.

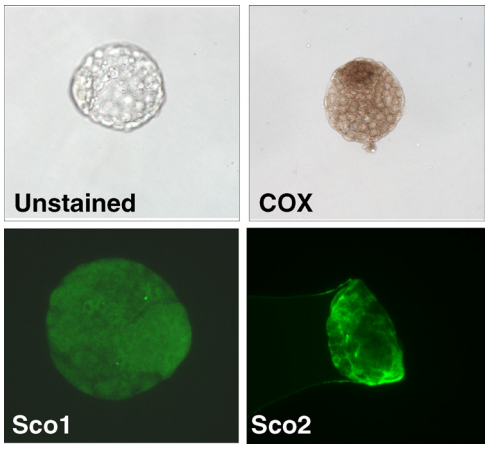

Figure 5. Analysis of mouse embryos (3.5 days post coitum). Histochemistry to detect COX activity and immunohistochemistry to detect Sco1 and Sco2. Note that the Sco2-stained embryo was folded over onto itself.

If this line of reasoning is correct, it would imply that the two SCO proteins must have at least some degree of functional overlap. On the other hand, our data on SCO protein expression in embryos (Figure 5) and studies by others[14] indicate that the two proteins can have separate, nonoverlapping functions. Moreover, a lack of complementation between the two proteins has also been reported in cell culture, in which reduced copper levels and COX activity due to mutations in either *SCO1* or *SCO2* could be suppressed by overexpression of SCO2 but not by SCO1.[29] Most likely the two proteins have only partially nonoverlapping functions, with some degree of cooperative behavior and functional overlap between them,[14] especially in cells in which both proteins are expressed.

What might those nonoverlapping functions be? The primary proposed function for SCO is as a copper chaperone for COX,[30] and, in fact, addition of Cu to SCO2 patient cells rescued COX activity.[31–33] However, a second function for SCO might be as a redox sensor,[8,12,29,34] as is the case for PrrC, the SCO homolog in the aquatic organism *Rhodobacter sphaeroides*,[35] which senses

changes in diurnal oxygen concentration to signal the shift from photosynthesis to respiration. Although both SCO1 and SCO2 may indeed transport copper, perhaps SCO1 has an additional function in redox sensing, especially in the vasculature. The vascular localization and structural similarity of SCO1 to the thioredoxin and peroxiredoxin protein families[8,11,34] support the idea that SCO1 might function not only as a copper chaperone but also as a copper-binding redox-sensing protein. This could explain why SCO1 is so abundant in the vasculature, where oxygen levels are highly variable.[34] On the other hand, SCO1 could sense oxygen levels and act as a signaling molecule to downstream targets that might, for example, regulate the transcription or translation of COX subunits.

In summary, we have shown that the two SCO genes are expressed ubiquitously in human and mouse tissues, but protein levels vary among tissues, possibly explaining some of the differences in the pathological conditions of patients harboring *SCO1* and *SCO2* mutations. Furthermore, we have shown that high levels of SCO1 protein localize to the vasculature in all tissues examined, a novel and unexpected location for a mitochondrial protein.

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References

1. Tsukihara T, Aoyama H, Yamashita E, Tomizaki T, Yamaguchi H, Shinzawa-Itoh K, Nakashima R, Yaono R, Yoshikawa S: Structures of metal sites of oxidized bovine heart cytochrome c oxidase at 2.8 Å. Science 1995, 269:1069–1074
2. Herrmann JM, Funes S: Biogenesis of cytochrome oxidase-sophisticated assembly lines in the mitochondrial inner membrane. Gene 2005, 354:43–52
3. Fontanesi F, Soto IC, Horn D, Barrientos A: Assembly of mitochondrial cytochrome c-oxidase, a complicated and highly regulated cellular process. Am J Physiol Cell Physiol 2006, 291:C1129–C1147
4. Barrientos A, Gouget K, Horn D, Soto IC, Fontanesi F: Suppression mechanisms of COX assembly defects in yeast and human: insights into the COX assembly process. Biochim Biophys Acta 2009, 1793:97–107
5. Papadopoulou LC, Sue CM, Davidson MM, Tanji K, Nishino I, Sadlock JE, Krishna S, Walker W, Selby J, Glerum DM, Van Coster R, Lyon G, Scalais E, Lebel R, Kaplan P, Shanske S, De Vivo DC, Bonilla E, Hirano M, DiMauro S, Schon EA: Fatal infantile cardioencephalomyopathy with COX deficiency and mutations in SCO2, a COX assembly gene. Nat Genet 1999, 23:333–337
6. Glerum DM, Shtanko A, Tzagoloff A: SCO1 and SCO2 act as high copy suppressors of a mitochondrial copper recruitment defect in Saccharomyces cerevisiae. J Biol Chem 1996, 271:20531–20535
7. Lode A, Kuschel M, Paret C, Rodel G: Mitochondrial copper metabolism in yeast: interaction between Sco1p and Cox2p. FEBS Lett 2000, 485:19–24
8. Williams JC, Sue C, Banting GS, Yang H, Glerum DM, Hendrickson WA, Schon EA: Crystal structure of human SCO1: implications for redox signaling by a mitochondrial cytochrome c oxidase "assembly" protein. J Biol Chem 2005, 280:15202–15211
9. Abajian C, Rosenzweig AC: Crystal structure of yeast Sco1. J Biol Inorg Chem 2006, 11:459–466
10. Abriata LA, Banci L, Bertini I, Ciofi-Baffoni S, Gkazonis P, Spyroulias GA, Vila AJ, Wang S: Mechanism of Cu_A assembly. Nat Chem Biol 2008, 4:599–601
11. Chinenov YV: Cytochrome c oxidase assembly factors with a thioredoxin fold are conserved among prokaryotes and eukaryotes. J Mol Med 2000, 78:239–242
12. Leary SC, Sasarman F, Nishimura T, Shoubridge EA: Human SCO2 is required for the synthesis of CO II and as a thiol-disulphide oxidoreductase for SCO1. Hum Mol Genet 2009, 18:2230–2240
13. Banting GS, Glerum DM: Mutational analysis of the Saccharomyces cerevisiae cytochrome c oxidase assembly protein Cox11p. Eukaryot Cell 2006, 5:568–578
14. Leary SC, Kaufman BA, Pellecchia G, Guercin GH, Mattman A, Jaksch M, Shoubridge EA: Human SCO1 and SCO2 have independent, cooperative functions in copper delivery to cytochrome c oxidase. Hum Mol Genet 2004, 13:1839–1848
15. Matoba S, Kang JG, Patino WD, Wragg A, Boehm M, Gavrilova O, Hurley PJ, Bunz F, Hwang PM: p53 regulates mitochondrial respiration. Science 2006, 312:1650–1653
16. Park JY, Wang PY, Matsumoto T, Sung HJ, Ma W, Choi JW, Anderson SA, Leary SC, Balaban RS, Kang JG, Hwang PM: p53 improves aerobic exercise capacity and augments skeletal muscle mitochondrial DNA content. Circ Res 2009, 105:705–712
17. Valnot I, Osmond S, Gigarel N, Mehaye B, Amiel J, Cormier-Daire V, Munnich A, Bonnefont JP, Rustin P, Rötig A: Mutations of the SCO1 gene in mitochondrial cytochrome c oxidase deficiency with neonatal-onset hepatic failure and encephalopathy. Am J Hum Genet 2000, 67:1104–1109
18. Petruzzella V, Tiranti V, Fernandez P, Ianna P, Carrozzo R, Zeviani M: Identification and characterization of human cDNAs specific to BCS1. PET112, SCO1, COX15, and COX11, five genes involved in the formation and function of the mitochondrial respiratory chain Genomics 1998, 54:494–504
19. Nakada K, Inoue K, Ono T, Isobe K, Ogura A, Goto YI, Nonaka I, Hayashi JI: Inter-mitochondrial complementation: mitochondria-specific system preventing mice from expression of disease phenotypes by mutant mtDNA. Nat Med 2001, 7:934–940
20. Ballermann BJ: Contribution of the endothelium to the glomerular permselectivity barrier in health and disease. Nephron Physiol 2007, 106:19–25
21. Yang H, Brosel S, Acin-Perez R, Slavkovich V, Nishino I, Khan R, Goldberg IJ, Graziano J, Manfredi G, Schon EA: Analysis of mouse models of cytochrome c oxidase deficiency owing to mutations in Sco2. Hum Mol Genet 2010, 19:170–180
22. Kadenbach B, Stroh A, Huther FJ, Reimann A, Steverding D: Evolutionary aspects of cytochrome c oxidase. J Bioenerg Biomembr 1991, 23:321–334
23. Hüttemann M, Lee I, Liu J, Grossman LI: Transcription of mammalian cytochrome c oxidase subunit IV-2 is controlled by a novel conserved oxygen responsive element. FEBS J 2007, 274:5737–5748
24. Arnaudo E, Hirano M, Seelan RS, Milatovich A, Hsieh CL, Fabrizi GM, Grossman LI, Francke U, Schon EA: Tissue-specific expression and chromosome assignment of genes specifying two isoforms of subunit VIIa of human cytochrome c oxidase. Gene 1992, 119:299–305
25. Hüttemann M, Jaradat S, Grossman LI: Cytochrome c oxidase of mammals contains a testes-specific isoform of subunit VIb—the counterpart to testes-specific cytochrome c? Mol Reprod Dev 2003, 66:8–16
26. Hüttemann M, Kadenbach B, Grossman LI: Mammalian subunit IV isoforms of cytochrome c oxidase. Gene 2001, 267:111–123
27. Stiburek L, Vesela K, Hansikova H, Hulkova H, Zeman J: Loss of function of Sco1 and its interaction with cytochrome c oxidase. Am J Physiol Cell Physiol 2009, 296:C1218–C1226
28. Stiburek L, Vesela K, Hansikova H, Pecina P, Tesarova M, Cerna L, Houstek J, Zeman J: Tissue-specific cytochrome c oxidase assembly defects due to mutations in SCO2 and SURF1. Biochem J 2005, 392:625–632
29. Leary SC, Cobine PA, Kaufman BA, Guercin GH, Mattman A, Palaty J, Lockitch G, Winge DR, Rustin P, Horvath R, Shoubridge EA: The human cytochrome c oxidase assembly factors SCO1 and SCO2 have regulatory roles in the maintenance of cellular copper homeostasis. Cell Metab 2007, 5:9–20
30. Cobine PA, Pierrel F, Winge DR: Copper trafficking to the mitochondrion and assembly of copper metalloenzymes. Biochim Biophys Acta 2006, 1763:759–772

31. Jaksch M, Paret C, Stucka R, Horn N, Muller-Hocker J, Horvath R, Trepesch N, Stecker G, Freisinger P, Thirion C, Muller J, Lunkwitz R, Rodel G, Shoubridge EA, Lochmuller H: Cytochrome c oxidase deficiency due to mutations in SCO2, encoding a mitochondrial copper-binding protein, is rescued by copper in human myoblasts. Hum Mol Genet 2001, 10:3025–3035
32. Salviati L, Hernandez-Rosa E, Walker WF, Sacconi S, DiMauro S, Schon EA, Davidson MM: Copper supplementation restores cytochrome c oxidase activity in cultured cells from patients with SCO2 mutations. Biochem J 2002, 363:321–327
33. Freisinger P, Horvath R, Macmillan C, Peters J, Jaksch M: Reversion of hypertrophic cardiomyopathy in a patient with deficiency of the mitochondrial copper binding protein Sco2: is there a potential effect of copper? J Inher Metab Dis 2004, 27:67–79
34. Banci L, Bertini I, Calderone V, Ciofi-Baffoni S, Mangani S, Martinelli M, Palumaa P, Wang S: A hint for the function of human Sco1 from different structures. Proc Natl Acad Sci USA 2006, 103:8595–8600
35. Eraso JM, Kaplan S: From redox flow to gene regulation: role of the PrrC protein of Rhodobacter sphaeroides 2.4.1. Biochemistry 2000, 39:2052–2062