The present studies were undertaken to further characterize the properties of Sco1p, a constituent of the mitochondrial inner membrane implicated in copper transfer to cytochrome oxidase. We report a procedure capable of yielding Sco1p of >95% purity. Sco1p has been purified from strains of Saccharomyces cerevisiae that overexpress the protein. The amino-terminal sequence of purified Sco1p indicates that the first 40 amino acids of the primary translation product constitute a mitochondrial targeting sequence that is proteolytically cleaved during import. We estimate that Sco1p constitutes 0.08% total mitochondrial proteins in wild type yeast and 5% in the transformant used for the purification. Sco1p contains ~1 mol of copper/mol protein. The copper is not removed by the treatment of Sco1p with EDTA, indicating that it is bound with high affinity. Purified Sco1p sediments identical to Sco1p in crude extracts of mitochondria from wild type yeast or from a strain transformed with SCO1 on a high copy plasmid. Native Sco1p has an estimated mass of 88 kDa, suggesting that it is a homotrimer. Sco1p expressed as a soluble protein lacking the internal 17 amino acids of the membrane-anchoring domain has been localized in the matrix. The protein has also been targeted to the intermembrane space. Neither soluble matrix nor intermembrane-localized Sco1p is able to complement a sco1 mutant, suggesting that only the membrane form with the carboxyl-terminal domain facing the intermembrane space is able to exert its normal function.

The proposed function of Sco1p as a copper transferase was based on the ability of SCO1 to act as a high copy suppressor of cox17 mutants (6), the presence in Sco1p of a domain with sequence similarity to the copper binding site of Cox2p (6), and the physical interaction of Sco1p with Cox2p (9). The involvement of Sco1p in mitochondrial copper metabolism is more directly supported by recent studies showing that a soluble fragment of Sco1p, expressed in Escherichia coli, binds 1 copper/molecule of protein (10).

To learn more about the properties of Sco1p, we have purified the native protein from yeast and characterized its copper-binding property. We have also determined the site at which Sco1p is processed by the matrix protease and the size of the native protein. The evidence obtained with constructs expressing Sco1p lacking the membrane-spanning domain or having its normal import signal substituted with the leader of cytochrome c1 shows that the localization and orientation of the protein are essential for its function.

MATERIALS AND METHODS

Yeast Strains and Media—Sco1p was purified from two different strains of S. cerevisiae. E428/U1/ST5 is a sco1 mutant (6) transformed with pG41/ST5, a high copy plasmid containing the wild type SCO1 gene on a 1.9-kilobase pair EcoRI fragment (Fig. 1). E428/U1/ST28 was obtained by transformation of the same mutants with pG41/ST28, which contains both SCO1 and COX17 on a 1.2-kilobase pair HindIII fragment (Fig. 1). For large-scale purifications, cells maintained on minimal galactose (yeast nitrogen base plus 2% galactose) were inoculated and grown to stationary phase in liquid galactose medium (YPgal) containing 4% galactose, 1% yeast extract, and 1% peptone with or without 50 μM copper sulfate.

Construction of Genes Expressing Modified Sco1p—A gene lacking the sequence for the internal membrane-spanning domain of Sco1p was constructed by PCR amplification of the gene in pG41/ST5 (6) with the bidirectional primers described by Buchwald et al. (11). The resultant plasmid, pG41/ST23, was identical to pG41/ST5 with the exception that it lacked the internal 51 nucleotides coding for amino acid residues 75–90 of Sco1p (Fig. 1).

The sequence of CYT1 (12) coding for the 5'-untranslated region and amino-terminal import and intermembrane targeting signal was obtained by PCR amplification of yeast nuclear DNA with the forward PCR primer (Primer 1) 5’-AGACTATCTGAGCTTCAATGAGGCGC-3’ and the reverse primer (Primer 2) 5’-TGCAATCGGAGTCTCGTGAAGGCC-3’.

The fragments were cloned in YEp351 (13) linearized with SacI and BamHI yielding pGI01/ST10. SCO1 was amplified with the forward primer (Primer 1) 5’-AGACTATCTGAGCTTCAATGAGGCGC-3’ and the reverse primer (Primer 2) 5’-TGCAATCGGAGTCTCGTGAAGGCC-3’.

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primer (Primer 3) 5'-GCCGTGACTGCTCAATGGGACGAGAAC-CATTA-3' and the reverse primer (Primer 4) 5'-CGATACACCGTC-GAAGGGTGTAG-3'. The PCR product lacking the sequence coding for the amino-terminal 40 residues of the import signal was digested with BclI and SacI and cloned into pG101/ST10. The gene in the resultant plasmid (pG41/ST24) codes for the following sequence at the junction of cytochrome c₁ and Sco1p: glu-alα ↓ met-thr-alα-alα-Asp-gln-ser-asn-gly where the arrow demarcates the processing site in cytochrome c₁, the lower-case residues are part of the cytochrome c₁ leader, the capitalized residues are created by the new restriction site at the junction, and the italicized residues correspond to the amino-terminal end of mature Sco1p. This gene was further modified by removing the sequence coding for the transmembrane segment by PCR amplification of pG41/ST24 with the bidirectional primers described by Buchwald et al. (11). The resultant plasmids were designated as pG41/ST44 (Fig. 1). All the high copy plasmids were used to transform the null strain W303 sco1/H9004 in a mating protocol (10). The concentration of Sco1p was determined by quantitative Western blot analysis. Purified Sco1p was used to obtain a standard curve relating the concentration of Sco1p to the absorbance of the Coomassie blue stained protein. The materials obtained after steps 3 and 5–10 can be stored frozen at −80 °C.

In step 2, the spheroplasts were centrifuged at 2,600 × g for 20 min, washed twice with 3 liters of 1.2 M sorbitol, and lysed in 1.2 liters of STE buffer (0.5 M sorbitol, 50 mM Tris-HCl, pH 7.5, and 1 mM phenylmethylsulfonyl fluoride). The lysed spheroplasts were homogenized in a Waring blender for 40 s and centrifuged at 640 × g for 10 min. The supernatant was collected, and the pellet was washed with 600 ml of STE buffer (0.5 M sorbitol, 50 mM Tris-HCl, pH 7.5, and 1 mM phenylmethylsulfonyl fluoride). The first supernatant and wash were combined and centrifuged at 640 × g to remove remaining cell debris.

In step 3, the mitochondria obtained by centrifugation of the supernatant from step 2 at 14,700 × g for 30 min were washed three times in 0.5 M sorbitol and 50 mM Tris-HCl, pH 7.5, suspended in the same buffer at a protein concentration of 20–30 mg/ml, and sonicated irradiated for 45 s in a 100-ml beaker with a Branson sonifier using a microtip probe at a power output of 60 watts. The submitochondrial particles (SMP) were sedimented in a Beckman ultracentrifuge at 79,000 × g for 45 min and suspended in Tris-HCl, pH 7.5 at a final protein concentration of 20 mg/ml.

In step 4, to the SMP suspension were added solid KCl to a final concentration of 1 M, 0.01 volumes of 20 mg/ml phenylmethylsulfonyl fluoride, and 0.1 volumes of 10% potassium deoxycholate. After centrifugation at 79,000 × g for 10 min, the supernatant containing Sco1p was collected. The materials obtained after steps 3 and 5–10 can be stored frozen at −80 °C.

In step 5, to the deoxycholate extract from step 4 was added an equal volume of cold water and 20% potassium deoxycholate to a final concentration of 0.5%. Saturated ammonium sulfate (4°C) was added to a final concentration of 26% saturation, and the precipitate was removed by centrifugation at 79,000 × g for 10 min. The clear reddish supernatant was adjusted to 42% ammonium sulfate saturation with cold saturated ammonium sulfate. The greenish pellet obtained after centrifugation at 79,000 × g for 10 min was dissolved in 15 ml of TT buffer (20 mM Tris, pH 7.5, and 0.05% Triton X-100) and was desalted on a 120-ml column of Sephadex G-50 equilibrated in TT buffer. The protein eluates were eluted as a green-colored band in ~30 ml.

In step 6, the fraction from step 5 was diluted to 200 ml with TT buffer and applied to a 5 × 17-cm column of Cibacron Blue 3GA cross-linked to agarose (Sigma). The column was washed sequentially with 1) 400 ml of TT buffer, 2) 200 ml of TT buffer containing 1.0 M KCl, 3) 200 ml of TT buffer, 4) 500 ml of a 0–0.5 M linear gradient of potassium deoxycholate in TT buffer, and 5) 700 ml of 0.5% potassium deoxycholate in TT buffer. Fractions (15 ml) were collected, separated on a 12% polyacrylamide gel, and stained with silver. Most of Sco1p elutes in the potassium deoxycholate gradient and subsequent 0.5% deoxycholate wash. Fractions containing Sco1p were pooled (~1 liter). When frozen, this material may develop a white precipitate upon thawing. The precipitate can be removed on a 0.45-μm filter without loss of Sco1p.

In step 7, the pool from the Cibacron Blue column was applied to a 50-ml column of hydroxyapatite (Bio-Gel HTP, Bio-Rad) equilibrated with TT buffer. Following loading of the sample, the column was washed twice with 3 liters of 1.2 M sorbitol and suspended in 1.2 liters of buffer containing 1.2 M sorbitol, 30 mM potassium phosphate, pH 7.5, 1 mM EDTA, 0.15 M β-mercaptoethanol, and 0.5 mg/ml zymolyase 20,000 (ICN Biochemicals). After incubation at 37 °C for 3 h, 80–90% of the cells were converted to spheroplasts.

The PCR product lacking the sequence coding for the cytochrome c₁ presequence is shown by the broken line and bar in pG41/ST24 and ST44.

**Fig. 1. Physical map of pG41/ST5, ST23, pG24, ST28, and ST44.** The open arrows indicate the direction of transcription of SCO1 and COX17. The locations of the restriction sites for EcoRI (E), BglII (G), SacI, and HindIII (H) are marked on the inserts. The sequences coding for the SCO1 import signal and transmembrane domain are represented by the stippled and solid bars, respectively. The 5'-untranslated region and the sequence coding for the cytochrome c₁ presequence are shown by the broken line and bar in pG41/ST24 and ST44.

| Fraction                  | Total protein*       | Sco1p*     | Total Sco1p | Recovery | Purification factor |
|---------------------------|----------------------|------------|-------------|----------|---------------------|
|                           | mg                   | mg/mg      | mg          | %        |                     |
| SMP                       | 1840                 | 0.069      | 126         | 100      | —                   |
| Deoxycholate extract      | 969                  | 0.09       | 87.2        | 69       | 1.3                 |
| 28–42% sat. AS precipitate | 280                  | 0.17       | 47.6        | 37       | 2.5                 |
| Pool from Cibacron Blue   | 71                   | 0.39       | 26.9        | 21       | 5.5                 |
| Hydroxylapatite           | 44                   | 0.27       | 11.8        | 9.4      | 3.9                 |
| Mono S                    | 4.3                  | 0.89       | 3.8         | 3        | 12.8                |
| Mono Q                    | 3                    | 1          | 3           | 2.3      | 14.4                |

* The purification of Sco1p was carried out with mitochondria prepared from E428/U1/ST5 grown in 32 liters of YPGal medium.

* The concentration of Sco1p was determined by quantitative Western blot analysis. Purified Sco1p was used to obtain a standard curve relating amounts of protein to the density of the signals obtained with the antisera against the carboxyl-terminal peptide. The density of the signals was quantitated with a Visage 110 Bioimager (Millipore).

* The protein fraction precipitated between 28 and 40% saturation in ammonium sulfate.
The antibody-antigen complex was visualized by treatment of the blot with 125I-protein A and exposure to x-ray film. The density of the signals was measured with a densitometer. The indicated amounts of E428/U1/ST5 (WT) and E428/U1/ST28 (ST5) mitochondria and purified Sco1p were separated on a 12% polyacrylamide gel. The proteins were transferred to nitrocellulose paper, and the Western blot was treated with antibody against Sco1p. Most of the extra stain above the 66-kDa marker in lanes 5, 6, 10, and 11 is because of a staining artifact. The antibody-antigen complex was visualized by treatment of the blot with 125I-protein A and exposure to x-ray film. The density of the signals was measured with a densitometer. The indicated amounts of E428/U1/ST5 (WT) and E428/U1/ST28 (ST5) mitochondria and purified Sco1p were separated on a 12% polyacrylamide gel. The proteins were transferred to nitrocellulose paper, and the Western blot was treated with antibody against Sco1p. Most of the extra stain above the 66-kDa marker in lanes 5, 6, 10, and 11 is because of a staining artifact. B, preparation of Sco1p purified from E428/U1/ST5 used for copper analysis. C, preparation of Sco1p purified from E428/U1/ST28 used for copper analysis.

**FIG. 2. Purification of Sco1p.** A, the fractions are numbered as in Table I. Proteins were separated on a 12% polyacrylamide gel and silver-stained. Mature Sco1p migrates slightly faster than the carbonic anhydrase (31 kDa) standard. The arrowhead in the margin identifies Sco1p. Most of the extra stain above the 66-kDa marker in lanes 5, 6, and 7 is because of a staining artifact. B, preparation of Sco1p purified from E428/U1/ST5 used for copper analysis. C, preparation of Sco1p purified from E428/U1/ST28 used for copper analysis.

**TABLE I.** Proteins were separated on a 12% polyacrylamide gel and silver-stained. Mature Sco1p migrates slightly faster than the carbonic anhydrase (31 kDa) standard. The arrowhead in the margin identifies Sco1p. Most of the extra stain above the 66-kDa marker in lanes 5, 6, and 7 is because of a staining artifact. B, preparation of Sco1p purified from E428/U1/ST5 used for copper analysis. C, preparation of Sco1p purified from E428/U1/ST28 used for copper analysis.

| Preparation | Strain | Growth Conditions | Treatment | Copper in buffer | Mole Sco1p |
|-------------|--------|-------------------|-----------|------------------|------------|
| 1           | E428/U1/ST5 | 3% YPGal        | 5× concentrated by lyophilization | 30 | 0.7 |
| 2           | E428/U1/ST5 | 4% YPGal + 10 μM CuSO₄ | None | <10 | 1.0 |
| 3           | E428/U1/ST5 | 4% YPGal + 10 μM CuSO₄ | Plus copper⁺ | 10 | 1.0 |
| 4           | E428/U1/ST28 | 4% YPGal + 10 μM CuSO₄ | Plus copper in the presence of Cox17p⁺ | 10 | 0.85 |
| 5           | E428/U1/ST28 | 4% YPGal + 10 μM CuSO₄ | EDTA | <10 | 1.0 |
| 6           | E428/U1/ST28 | 4% YPGal + 10 μM CuSO₄ | EDTA | 24 | 0.7 |

* Sco1p was reduced in the presence of 0.1 mM DTT for 10 min on ice. The DTT was removed on a Sephadex G-25 column that had been treated with DTT and then equilibrated with a degassed/nitrogen-saturated buffer containing 100 mM NaCl in TT buffer (TTNaCl). The reduced Sco1p eluted from the Sephadex column was incubated at 23 °C with an equal volume of saturated CuCl (approximately 0.2 mM). EDTA was added to a concentration of 0.5 mM and the mixture further incubated for 45 min at 23 °C. Copper and EDTA were removed on a Sephadex G-100 column equilibrated with nitrogen-saturated TTNaCl buffer. Fractions containing protein were pooled, concentrated on a Centricon filter (Amicon), and analyzed for copper.

⁺ Sco1p was reduced in the same way as above except that the 

**TABLE II.** Copper content of purified Sco1p

| Preparation | Strain | Growth Conditions | Treatment | Copper in buffer | Mole Sco1p |
|-------------|--------|-------------------|-----------|------------------|------------|
| 1           | E428/U1/ST5 | 3% YPGal        | 5× concentrated by lyophilization | 30 | 0.7 |
| 2           | E428/U1/ST5 | 4% YPGal + 10 μM CuSO₄ | None | <10 | 1.0 |
| 3           | E428/U1/ST5 | 4% YPGal + 10 μM CuSO₄ | Plus copper⁺ | 10 | 1.0 |
| 4           | E428/U1/ST28 | 4% YPGal + 10 μM CuSO₄ | Plus copper in the presence of Cox17p⁺ | 10 | 0.85 |
| 5           | E428/U1/ST28 | 4% YPGal + 10 μM CuSO₄ | EDTA | <10 | 1.0 |
| 6           | E428/U1/ST28 | 4% YPGal + 10 μM CuSO₄ | EDTA | 24 | 0.7 |

* Sco1p was reduced in the presence of 0.1 mM DTT for 10 min on ice. The DTT was removed on a Sephadex G-25 column that had been treated with DTT and then equilibrated with a degassed/nitrogen-saturated buffer containing 100 mM NaCl in TT buffer (TTNaCl). The reduced Sco1p eluted from the Sephadex column was incubated at 23 °C with an equal volume of saturated CuCl (approximately 0.2 mM). EDTA was added to a concentration of 0.5 mM and the mixture further incubated for 45 min at 23 °C. Copper and EDTA were removed on a Sephadex G-100 column equilibrated with nitrogen-saturated TTNaCl buffer. Fractions containing protein were pooled, concentrated on a Centricon filter (Amicon), and analyzed for copper.

⁺ Sco1p was reduced in the same way as above except that the 

**FIG. 3. Concentration of Sco1p in wild type and E428/U1/ST5 mitochondria.** The indicated amounts of E428/U1/ST5 (ST5) and E303-1A (WT) mitochondria and purified Sco1p were separated on a 12% polyacrylamide gel. The proteins were transferred to nitrocellulose paper, and the Western blot was treated with antibody against Sco1p. The antibody-antigen complex was visualized by treatment of the blot with 125I-protein A and exposure to x-ray film. The density of the signals obtained with purified Sco1p was determined with a Visage 110 Biosis magnifier (Miller) and was used to estimate the concentration of Sco1p in the two different samples of mitochondria.

**FIG. 4. Sedimentation analysis of Sco1p in sucrose gradients.** A, a suspension of wild type mitochondria from W303-1A was adjusted to a protein concentration of 20 mg/ml in 20 mM Tris-Cl, pH 7.5, and 1 mM KCl. A 10% solution of potassium deoxycholate was added to a final concentration of 1%, and the mixture was centrifuged at 100,000 × g for 15 min. The clear supernatants (300 μl) were collected and mixed with 75 μl of a solution containing 2.5 mg of hemoglobin in 20 mM Tris-Cl, pH 7.5, and 0.05% Triton X-100. B, mitochondria from the transformant E428/U1/ST5 were extracted as in A. The extract (100 μl) was mixed with 200 μl of 0.4% deoxycholate, 0.5 mM KCl, 20 mM Tris-Cl, pH 7.5, and 75 μl of the hemoglobin solution. C, purified Sco1p (20 μg in 20 μl) was diluted with 300 μl of 0.4% deoxycholate, 0.5 mM KCl, 20 mM Tris-Cl, pH 7.5, and 74 μl of the hemoglobin solution. Each sample was loaded on 4.8 ml of a 12–30% linear sucrose gradient containing 20 mM Tris-Cl, pH 7.5, 0.05% Triton X-100, and 1 mM phenylmethylsulfonyl fluoride. The gradients were centrifuged at 4 °C for 44 h at 60,000 rpm in a Beckman SW 65Ti rotor. Fractions were collected and assayed for hemoglobin by absorption at 410 nm and for Sco1p by Western blot analysis. The distribution of hemoglobin is plotted, and the distribution of Sco1p is shown by the photographs in the inserts. The size of Sco1p in each gradient was calculated from its sedimentation relative to hemoglobin (18).
**Purification and Properties of Yeast Sco1p**

**RESULTS AND DISCUSSION**

**Purification of Sco1p**—The concentration of Sco1p in E428/U1/ST5 is ~50 µg/mg mitochondrial protein. This value is raised by another factor of 1.7 in SMP, the starting material used for the purification. The results of a typical fractionation are summarized in Table I and Fig. 2. The extraction of the SMPs with deoxycholate solubilizes 70% of the protein almost half of which is lost during the ammonium sulfate fractionation.

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**Miscellaneous Procedures**—Standard procedures were used for the preparation and ligation of DNA fragments and for the transformation and recovery of plasmid DNA from *E. coli* (14). Proteins were analyzed on 12% polyacrylamide gel by SDS-PAGE (15). Western blots were treated with antibodies against the Sco1p (6). Antibody-antigen complexes were visualized by a secondary reaction with 125I-protein A (16).

FIG. 5. Transport of Sco1p expressed from wild type and mutant genes. A, transport of the precursor across the inner membrane is arrested by the transmembrane domain (filled bar) that acts as a stop-transfer signal. The amino-terminal presequence (open bar) is cleaved by the matrix protease. B, the absence of the stop-transfer sequence allows the Sco1p to be completely transferred to the matrix. C, the amino-terminal of the cytochrome _c_ and recovery of plasmid DNA from preparation and ligation of DNA fragments and for the transformation superfine column equilibrated in TT buffer.

FIG. 6. Localization of wild type and mutant forms of Sco1p. Mitochondria were prepared by the method of Glick and Pon (19) from the wild type strain W303-1A (W303) and from the transformants E428/U1/ST23 (ST23) and E428/U1/ST44 (ST44) (see Fig. 1 for details of the pG41/ST23 and pG41/ST44 plasmids). One-half of the mitochondria (Mt) at a protein concentration of 8 mg/ml were diluted with 4 volumes of 0.6 M sorbitol, 20 mM Hepes, pH 7.5, and the other half was centrifuged with mitoplasts (M) by dilution with 4 volumes of 20 mM Hepes, pH 7.5. Both mitochondria and mitoplasts were incubated on ice for 1 h in the absence (−) or presence (+) of 0.1 mg/ml proteinase K. After the addition of phenylmethylsulfonyl fluoride to a final concentration of 1 mM, the samples were centrifuged at 14,000 rpm for 15 min, and the supernatants from the mitoplasts (S) were saved. The mitochondrial and mitoplast pellets were suspended in 100 µl of 0.6 M sorbitol, and 20 mM Hepes, pH 7.5, precipitated with trichloroacetic acid. The precipitates were washed with 75 ml of TT buffer and applied to a 1 ml fast protein liquid chromatography mono-Q column (Amersham Biosciences). The column was washed with 5 ml of TT buffer followed by 5 ml of 0.35 M NaCl in TT buffer. Sco1p elutes as a single 280-nm absorbing peak in ~50 ml. Because of the weak adsorption of Sco1p to mono-S, in the next step it is important to remove all the salt on the Sephadex column.

In step 8, a preparative high pressure liquid chromatography mono-S column (8 ml) (Amersham Biosciences) was washed with 25 ml of TT buffer and 1.0 M NaCl followed by 50 ml of TT buffer. After application of the desalted fraction from step 7, the column was washed with 1) 20 mM NaCl in TT buffer, 2) 80 ml of a 0.1 M linear gradient of NaCl in TT buffer, and 3) 80 ml of 0.1 M NaCl in TT buffer. Fractions of 8 ml were collected and analyzed for Sco1p on a 12% polyacrylamide gel. Sco1p elutes in ~70 ml of TT buffer peeking at 0.1 M NaCl.

In step 9, the pool from step 8 was diluted to 150 ml with TT buffer and applied to a 1 ml fast protein liquid chromatography mono-Q column (Amersham Biosciences). The column was washed with 5 ml of TT buffer followed by 5 ml of 0.35 M NaCl in TT buffer. Sco1p elutes as a single 280-nm absorbing band and is recovered in ~2 ml.

In step 10, purified Sco1p was desalted on a 10-ml Sephadex G-25 superfine column equilibrated in TT buffer.
suggested that the maturation of subunit 2 might require that Sco1p be present in the inner membrane.\(^2\) However, in that study, the location of the soluble Sco1p was not determined. Hence, if Sco1p lacking the transmembrane domain is transported to the matrix, the mislocalization could also account for failure to complement the mutant. To address this question, we first examined the compartment in which soluble Sco1p is located. Resistance against protease K (Fig. 6) indicates that the soluble protein is transported to the matrix compartment. The matrix localization implies that the hydrophobic transmembrane domain acts as a stop-transfer sequence.

The dependence of Sco1p function on its localization and/or membrane association was further examined by directing the soluble protein to the intermembrane space. The sequence of \(SCO1\) starting from codon 41 was fused to the sequence encoding the cytochrome \(c_1\) presequence (12). The gene was further modified by removing the sequence coding for the transmembrane domain. The cytochrome \(c_1\) presequence consists of an amino-terminal mitochondrial targeting signal followed by a hydrophobic sorting sequence (20). The targeting signal directs the amino terminus to the matrix where it is cleaved by the matrix-processing protease. The hydrophobic part of the presequence anchors the precursor to the inner membrane (21) after which cleavage by the Imp protease (22) causes the release of the mature amino terminus in the intermembrane space (21).

The predication was that substitution of the cytochrome \(c_1\) bipartite signal for its normal import signal would cause Sco1p to be localized in the intermembrane space (Fig. 5C). The intermembrane localization of Sco1p expressed from this construct (pG41/ST44) was confirmed by its proteinase K sensitivity in mitoplasts but not mitochondria (Fig. 6). Most of the intermembrane Sco1p was solubilized by alkaline extraction of mitochondria with carbonate (Fig. 7). However, the protein probably has some residual hydrophobic character, because it is only partially released when mitochondria are converted to mitoplasts (Fig. 6). The retargeted soluble Sco1p failed to rescue the mutant, indicating that its postulated function in copper transfer probably requires that it be anchored to the membrane.

It is of interest that \(sco1\) mutants also fail to be complemented by the \(SCO1\) gene in pG41/ST24. The gene in this plasmid consists of the cytochrome \(c_1\) presequence fused to the entire \(SCO1\) sequence coding for the mature protein including the transmembrane domain. The product of this gene is processed to the mature-size Sco1p that is located in the intermembrane space, but unlike the native Sco1p, it is not located as an intrinsic membrane protein (data not shown). This indicates that the transmembrane domain of Sco1p is a stop-transfer rather than a membrane-targeting/insertion sequence (21). The inability of this gene to complement the \(sco1\) mutant emphasizes the importance of both compartmentation and membrane topology for Sco1p activity.

REFERENCES
1. Glerum, D. M., Shtanko, A., and Tragoull, A. (1996) J. Biol. Chem. 271, 14504–14509
2. Schulze, M., and Rodel, G. (1989) Mol. Gen. Genet. 216, 37–43
3. Beers, J., Glerum, D. M., and Tragoull, A. (1997) J. Biol. Chem. 272, 33191–33196
4. Valentine, J. S., and Gralla, E. B. (1997) Science 278, 817–818
5. Heaton, D., N., George, G. N., Garrison, G., and Winge, D. R. (2000) Biochem-

\(^2\) The finding that soluble Sco1p expressed in \(E. coli\) has bound copper (10) makes the alternate explanation that membrane localization is required for copper addition less probable.
Purification and Properties of Yeast Sco1p

6. Glerum, D. M., Shtanko, A., and Tzagoloff, A. (1996) J. Biol. Chem. 271, 20531–20536
7. Hiser, L., Di Valentin, M., Hamer, A. G., and Hosler, J. P. (2000) J. Biol. Chem. 275, 619–623
8. Tzagoloff, A., Capitanio, N., Nobrega, M. P., and Gatti, D. (1990) EMBO J. 9, 2759–2764
9. Lode, A., Kuschel, M., Paret, C., and Rodel, G. (2000) FEBS Lett. 485, 19–24
10. Nittis, T., George, G. N., and Winge, D. R. (2001) J. Biol. Chem. 276, 42520–42526
11. Buchwald, P., Krummeck, G., and Rodel, G. (1991) Mol. Gen. Genet. 229, 413–442
12. Sadler, J., Suda, K., Schatz, G., Kaudewitz, F., and Haid, A. (1984) EMBO J. 3, 2137–2143
13. Hill, J. E., Myers, A. M., Koerner, T. J., and Tzagoloff, A. (1986) Yeast 2, 163–167
14. Maniatis, T., Fritsch, E. F., and Sambrook, J. (1982) Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
15. Laemmli, U. K. (1970) Nature 227, 680–685
16. Schmidt, R. J., Myers, A. M., Gillham, N. W., and Boynton, J. E. (1984) Mol. Biol. Ecol. 1, 317–334
17. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951) J. Biol. Chem. 193, 265–275
18. Martin, R. G., and Ames, B. N. (1961) J. Biol. Chem. 236, 1372–1379
19. Glick, B., and Pon, L. A. (1995) Methods Enzymol. 260, 213–223
20. Gasser, S. M., Ohashi, A., Daum, G., Bohni, P. C., Gibson, J., Reid, G. A., Yonetani, T., and Schatz, G. (1982) Proc. Natl. Acad. Sci. U. S. A. 79, 267–271
21. Arnold, I., Folsch, H., Neupert, W., and Stuart, R. A. (1998) J. Biol. Chem. 2731, 469–476
22. Nunnari, J., Fox, T. D., and Walter, P. (1993) Science 262, 1997–2004
Purification and Characterization of Yeast Sco1p, a Mitochondrial Copper Protein
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