The *Saccharomyces cerevisiae* SCS2 Gene Product, a Homolog of a Synaptobrevin-Associated Protein, Is an Integral Membrane Protein of the Endoplasmic Reticulum and Is Required for Inositol Metabolism

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The *Saccharomyces cerevisiae* SCS2 gene has been cloned as a suppressor of inositol auxotrophy of *CSE1* and *hac1/ire15* mutants (J. Nikawa, A. Murakami, E. Esumi, and K. Hosaka, J. Biochem. 118:39–45, 1995) and has homology with a synaptobrevin/VAMP-associated protein, VAP-33, cloned from *Aplysia californica* (P. A. Skehel, K. C. Martin, E. R. Kandel, and D. Bartsch, Science 269:1580–1583, 1995). In this study we have characterized an SCS2 gene product (Scs2p). The product has a molecular mass of 35 kDa and is C-terminally anchored to the endoplasmic reticulum, with the bulk of the protein located in the cytosol. The disruption of the SCS2 gene causes yeast cells to exhibit inositol auxotrophy at temperatures of above 34°C. Genetic studies reveal that the overexpression of the INO1 gene rescues the inositol auxotrophy of the SCS2 disruption strain. The significant primary structural feature of Scs2p is that the protein contains the 16-amino-acid sequence conserved in yeast and mammalian cells. The sequence is required for normal Scs2p function, because a mutant Scs2p that lacks the sequence does not complement the inositol auxotrophy of the SCS2 disruption strain. Therefore, the Scs2p function might be conserved among eukaryotic cells.

The *Saccharomyces cerevisiae* SCS2 gene was identified as a multicopy suppressor of inositol auxotrophy of *CSE1* and *ire15* mutants (25). *CSE1* mutants show dominant inositol auxotrophy in the presence of choline in the growth medium (14). *CSE1* mutants cannot activate the expression of *INO1*, which encodes inositol-1-phosphate synthase, an essential protein for inositol biosynthesis in yeast cells (8). In yeast, the expression of *INO1* and other phospholipid biosynthetic genes is regulated in response to the amount of the soluble lipid precursors inositol and choline (3, 27). Genetic analysis of *CSE1* mutants has revealed that *CSE1* is a factor involved in the regulation of *INO1* expression, although the gene has not been cloned yet (13).

*ire15* mutants have defects in the expression of the inositol transporter gene (*ITR1*) in addition to that of *INO1*. Three human genes which can suppress the growth defect of *ire15* mutants have been isolated. They encode transforming growth factor β receptor type II, protein phosphatase type 2A subunit A, and the 14-3-3 protein (23). These results suggest that yeast cells contain a signal transduction mechanism similar to those found in mammalian cells. Although it is likely that the ER/NRC membrane is required for the exocytosis of neurotransmitters (37). As synaptobrevin homologs have been isolated from yeast and are involved in protein secretion pathways (10, 32), it is likely that VAP-33 homologs exist in yeast and participate in the regulation of yeast exocytic pathways.

The ability of SCS2 to suppress the inositol auxotrophy of *CSE1* and *hac1/ire15* mutants and its structural relationship to

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VAP-33 lead to the assumption that SCs2 is involved both in the regulation of membrane biogenesis through the activation of phospholipid biosynthetic gene expression and in intracellular membrane transport through the activation of fusion of transport vesicles. To investigate this assumption, we have characterized the SCs2 gene product. In this paper we show that the gene is involved in the activation of the INO1 expression and that the gene product (Scs2p) is a 35-kDa type II integral membrane protein. On the other hand, we failed to obtain any line of evidence that the gene is required for protein secretion, although Scs2p is localized to the ER, where protein and lipid biosynthesis and transport vesicle formation take place.

**MATERIALS AND METHODS**

**Media and strains.** Yeast extract-peptide-dextrose (YPD) and yeast minimal media were described by Kaiser et al. (16) and Klüg et al. (17). When added, inositol (myo-inositol; Sigma) was at a final concentration of 100 μM/pLP.

The preparation of INO1 and INO2 genes was described by Kagawa et al. (15). Strains used were CTY182 (MATa ura3-52 lys2-801 ade2-101 his3-200 leu2-3,112 trp1-901 his3-112 trp1-63 pdr1-201 his3-200 trp1-63 pdr1-201 his3-200 trp1-63 pdr1-201 trp1-63 pdr1-201 his3-200 ura3-52 leu2-3,112 leu2-3,112 [36]), YPH500 (MATa his3-13 ura3-52 lys2-801 his3-200 trp1-901 ade2-101 leu2-3,112 [36]), and YK36 (CTY182 scs2::TRP1).

**Construction of SCS2 disruption.** The SCS2 gene was amplified by PCR from pSC2, which contains the HindIII-ClaI fragment of the SCS2 genome (25). The forward and reverse primers used were 5′-CCAAAGTTTGGCATGCAGCAGC C′-3′ and 5′-CCGAATTCTAGTATTGTAAAGGC-3′, respectively. An EcoRI site engineered at the 5′-end of the reverse primer. The PCR fragment was cut with HindIII and EcoRI, and the 1.3-kb fragment was inserted into the same sites of YEplac13 (26) to generate YEp (Sac2p) and pKY151 (YEp[SCS2]), respectively.

**SCS2 disruption.** To disrupt the SCS2 gene, the coding region corresponding to amino acids 4 to 219 was replaced with the URA3 or TRP1 gene. To this end, an additional PRL site, other than the endogenous one, was generated in the SCs2 gene by mutagenesis of the nucleotide at position +9 from T to A (position +1 refers to the A residue of the ATG start codon). The PCR fragment was cut with PstI and self-ligated to generate pKY144, which lacks 643 nucleotides from position +11 to +658. The URA3 or TRP1 marker gene was incorporated into the PRL site of pKY144 to generate pKY145 and pKY159, respectively. The HindIII/EcoRI fragments of pKY145 and pKY159 were used for one-step gene replacement (16). YPH500, YPH501, and CTY182 were used for SCS2 disruption.

The identities of disruption strains were verified by PCR analysis of genomic DNA prepared from transformed cells and Western blotting.

**Polyclonal antibody against the GST-tagged Scs2p protein.** Construction of a partial antigen-Scs2p polyclonal antibody. PCR was performed with pKY134 as a template. The forward primers, 5′-GATCCCTCGAGTTGTTGGTG-3′ and 5′-GAATTCCTAGTATTGTAAAGGC-3′, respectively, were designed to construct a BamHI site at the 5′-end of the reverse primer. The PCR fragment was cut with BamHI and EcoRI, and ligated into BamHI/EcoRI-digested pPIEX-1 (Pharmacia Biotech) to yield pPIEX-Scs2. Nucleotides 54-243 were amplified with the primers 5′-GATGTTCCAGATTACGCTGAAATTTCCCCTGACGTG-3′ and 5′-GGATATCACGAGGTGGGCTCCACGGCCCGGAG-3′ from T to A to introduce an EcoRI site. This modification does not change the coding sequence of the SCS2 amino acid sequence. The Accl site used for insertion of the PCR product amplified by using the forward primer 5′-GTATACCCATAC GATGTCCTCAGAATTCTGGGAGG-3′ and the reverse primer 5′-CTTGGTTTGGCATGGGCTTGG-3′. The forward primer was constructed to place the HA-coding sequence 5′ to nucleotide +13. The resulting fragment was subcloned into YCPV-1 and YCPV195 to generate pKY106 [YCPV(Scs2p)] and pKY107 [YCPV(Scs2p)]. By this construction, the N-terminal 13 amino acids (pKY107) of Scs2p were expressed in the yeast cytoplasm. Following induction with 0.01 mg of trypsin inhibitor (Sigma) was added to a final concentration of 0.4 mg/ml.

**Immunoblotting.** Protein samples were separated by sodium dodecyl sulfate–10% polyacrylamide gel electrophoresis (SDS–10% PAGE) and transferred to a nitrocellulose membrane (0.45-μm pore size; Toyo Roshi). Immunoblotting was performed by using the anti-Scs2p polyclonal antibody (a gift from V. A. Bankaitis, University of Alabama at Birmingham) (2). Secondary antibodies were alkaline phosphatase-conjugated anti-mouse immunoglobulin G (IgG) and anti-rabbit IgG (Promega).

**Immunofluorescence.** Appropriate yeast strains were grown to the early log-arithmetic stage in complete or uracil-deficient medium for plasma membrane. Cells were fixed by direct addition of formaldehyde (final concentration, 4%) and incubation with gentle agitation at 30°C for 30 min. Cells were centrifuged at 700 × g for 5 min, resuspended in 1 ml of spheroplasting buffer containing 4% formaldehyde, and incubated overnight at 4°C with gentle agitation. Fixed cells were resuspended and resuspended in 0.1 ml of spheroplasting buffer containing 20 μg of Yersinia enterocolitica T18 (Sigma) per ml and 5 μg of β-mercaptoethanol per ml and incubated for 1 h at 30°C. Spheroplasts were centrifuged, washed once, and applied to poly-L-lysine-coated coverslips. Cells were treated with ice-cold methanol for 5 min and blocked with 1% bovine serum albumin per ml in PBS–0.1% Tween 20. Primary antibody was applied, followed by anti-Scs2p polyclonal antibody, a rabbit-anti-HA polyclonal antibody (a gift from R. Hirata, The Institute of Physical and Chemical Research [RIKEN], Wako, Japan) (12), and the anti-Dnp1p monoclonal antibody. Secondary antibodies were fluorescein isothiocyanate-conjugated anti-mouse IgG and tetramethylrhodamine isothiocyanate-conjugated anti-rabbit IgG (Biomedical Technologies). Antibody incubations were for 1 h at room temperature, with four washes with PBS–0.1% Tween 20. Prior to a final rinse, cells were incubated with 5 μg of DAPI (4′,6-diamidino-2-phenylindole dihydrochloride) (Sigma) per ml. Cells were mounted with 90% glycerol–10% PBS containing 1 mg of p-phenylenediamine (Sigma) per ml. Fluorescence images were recorded with a fluorescence microscope equipped with a cooled charge-coupled device camera (PROVIS AX-70; Olympus).

**Secretion assay.** Secretion of invertebrate was analyzed by invertebrate activity staining (21). Cells were grown in YPD medium at 25°C and shifted to YP with 0.1% glucose. After incubation at 30°C for 2 h, the cells were washed with ice-cold 10 mM NaCl, and were converted to spheroplasts as described above. After centrifugation of the spheroplasts at 800 × g for 3 min, intracellular (pellet) and extracellular (supernatant) fractions were obtained. The intracellular fraction was resuspended in 0.5 ml of water containing 2% Triton X-100. Samples were resolved on 6.75% nondenaturing polyacrylamide gels. After electrophoresis, gels were incubated in 0.2 M sodium acetate (pH 4.8) containing 0.2 M sucrose for 1 h at 30°C and were stained with 0.1% 2,3,5-triphenyltetrazolium chloride in 0.1 M NaOH. A halo assay was carried out according to the method of Sprague (39) with RC687 (MATa am2) as a test strain.
RESULTS

SCS2 disruption mutants show inositol auxotrophy. In order to study the physiological role of the SCS2 gene, we have constructed SCS2 disruption (scs2Δ) strains. A diploid strain (YPH501) was transformed with the scs2::URA3 gene, in which the SCS2 coding region for residues 7 through 221 was replaced with URA3, and Ura− transformants were selected and purified. Transformants which had both intact SCS2 and scs2::URA3 genes were selected and subjected to sporulation and tetrad analysis. All four viable spores from 20 tetrads grew on inositol-free medium. As expected, an SCS2 null allele as the sole copy of this gene demonstrated that the SCS2 gene was not essential for yeast viability. This finding made it possible to construct SCS2 disruption strains by transforming haploid cells directly. To this end, two independent yeast strains, CTY182 and YPH500, were transformed with scs2::URA3 and scs2::TRP1, respectively, and transformants were purified. Since isogenic strains were available, we studied the nature of scs2Δ by using the disruption strains (KY356 and KY360) derived from CTY182 and YPH500 for further studies.

As the SCS2 gene was originally isolated as a suppressor of the inositol auxotrophy of CSE1 and hac1/ire15 mutants (25), we examined the viability of scs2Δ mutants on inositol-free medium. As expected, an scs2Δ strain (KY360) could not grow well on inositol-free medium compared to the parental strain (YPH500). The growth defect was marked when cells were incubated at temperatures of above 34°C (Fig. 1). Another scs2Δ strain (KY356) derived from CTY182 also showed a similar growth deficiency. Therefore, the inositol auxotrophy was independent of genetic background and marker genes. To prove that the inositol auxotrophy was caused by the SCS2 gene disruption, we examined whether incorporation of the SCS2 gene into the scs2Δ strain rescues the auxotrophy. As shown in Fig. 1, scs2Δ strains harboring SCS2 on a centromere-based (CEN) plasmid [YCp(SCS2)] could grow on inositol-free medium at 37°C. Interestingly overproduction of SCS2 from a multicopy 2μ plasmid [YEp(SCS2)] could not rescue the defect efficiently (see Fig. 5B). Since even wild-type cells (CTY182) did not grow well at 37°C when the SCS2 gene was overexpressed by the 2μ plasmid (data not shown), overproduction of the SCS2 gene would be toxic to yeast at 37°C.

In yeast an essential step of inositol biosynthesis is the conversion of glucose-6-phosphate to inositol-1-phosphate, which is catalyzed by the INO1 gene product. The expression of the INO1 gene is controlled by the positive regulators INO2 and INO4, which encode basic helix-loop-helix proteins. The INO2 and INO4 gene products form a heterodimer that interacts with the upstream activating sequence of the INO1 gene and activates INO1 expression (1). To investigate the cause for the scs2Δ inositol auxotrophy, the INO1 or INO2 gene was incorporated into an scs2Δ strain (KY360). As shown in Fig. 2, overproduction of INO1 from the CEN plasmid [YCp(INO1)] and of INO2 from the 2μ plasmid [YEp(INO2)] could rescue the growth defect. The results suggest that an increase in INO1 expression levels can rescue the scs2Δ inositol auxotrophy.

Scs2p is a 35-kDa type II integral membrane protein. To gain insight into the physiological role of SCS2, we investigated the nature of the SCS2 gene product (Scs2p). An affinity-purified polyclonal antibody raised against a GST-SCS2 fusion protein recognized a 35-kDa band in the LSS of wild-type cells (YPH500) (Fig. 3A, lane 1), and the signal intensity of the band was increased in the lysate of cells carrying YEp(SCS2) (data not shown). The 35-kDa band was not observed in the

FIG. 1. Inositol auxotrophy of scs2Δ strains. KY360 (scs2Δ::TRP1) cells transformed with YCplac33 (control), YCp(SCS2), or YCp(HA-SCS2) were streaked for isolation on either inositol-containing (INO+) or inositol-free (INO−) minimal medium and incubated at 34°C for 72 h or at 37°C for 96 h.
fraction prepared from an \textit{scs2}Δ strain (KY360) (Fig. 3A, lane 2), suggesting that structurally homologous proteins with similar molecular masses were not expressed to the extent that they could be visualized by Western blotting. Other than the 35-kDa band, a 66-kDa band, which was not reacted with a preimmune serum, was detected in lysates of \textit{scs2}Δ cells (Fig. 3A, lane 2).

The nucleotide sequence of \textit{SCS2} predicts a protein of 26.9 kDa. The discrepancy between the estimated and the observed molecular masses indicates the existence of posttranslational modifications. In fact, the \textit{SCS2}-encoded sequence contains three potential N-linked glycosylation sites (25). However, since Scs2p does not appear to be sensitive to digestion with endoglycosidase H\textsubscript{f} (data not shown), it is unlikely that these sites are utilized. In addition, phosphorylation of Scs2p was not detected by immunoprecipitation of cell lysates labeled with \textsuperscript{32}Porthophosphate (data not shown).

A hydrophobic stretch of 16 amino acids at the carboxy terminus of Scs2p (25) suggests that it is bound to membranes by insertion of this region into the membranes. Cell lysates of a wild-type strain (CTY182) were subjected to a series of centrifugation steps at 800, 12,000, and 100,000 \textit{g}. The supernatant fractions at 800, 12,000, and 100,000 \textit{g} are referred to as LSS, 12S, and 100S, respectively, and the pellet fractions at 12,000 and 100,000 \textit{g} are called 12P and 100P, respectively. As shown in Fig. 3B, Scs2p detected by Western blotting was found exclusively in the LSS and 12P fractions, in which the ER and the nuclear membranes are enriched (4). In fact, the ER membrane protein marker, dolichol phosphate mannosynthase (Dpm1p) (31), was found exclusively in the LSS and 12P fractions. The ER luminal protein marker, Kar2p (34), was also enriched in those fractions, indicating that the integrity of the membrane fractions remained intact during the centrifugation. As expected, the cytosolic/Golgi protein marker, a phosphatidylinositol/phosphatidylcholine transfer protein (Sec14p) (2, 4), was associated mainly with the 12S and 100S fractions. These results indicate that Scs2p is associated with membranes. This was also confirmed by differential solubilization of the 12P fraction. Scs2p was not released into the supernatant by treatment with a high salt concentration (1 M KCl), sodium carbonate (pH 11), or 2 M urea, all of which extract peripheral membrane proteins (Fig. 3C, lanes 1 to 8). On the other hand, about 60% of Scs2p was solubilized in the presence of 1% Triton X-100 (Fig. 3C, lanes 9 and 10). These results suggest that Scs2p is an integral membrane protein.

The topology of Scs2p with respect to the cytosol was examined by a protease protection assay. For this assay, the N-terminal region of the protein should be recognized specifically by a monoclonal antibody. To this end, we constructed an HA-\textit{SCS2} gene, which encodes an \textit{SCS2} gene product tagged with nine amino acids from the influenza virus HA protein (the HA tag) at its amino terminus. The fusion protein (HA-Scs2p) encoded by the HA-\textit{SCS2} gene retained the normal Scs2p activity because it suppressed the inositol auxotrophy of the \textit{scs2}Δ strain at 34°C, although the suppression was not sufficient at 37°C (Fig. 1). On Western blots, an anti-HA monoclonal antibody (clone 12CA5) recognized a 40-kDa band (Fig. 3A, lane 5). This band was not present in extracts made from

**FIG. 2.** Inositol auxotrophy of \textit{scs2}Δ strains transformed with \textit{INO1} or \textit{INO2}. KY360 (\textit{scs2}Δ::\textit{TRP1}) cells transformed with YCp\textsubscript{lac33} [vector control for YCp(\textit{INO1})], YEplac195 [vector control for YEp(\textit{INO2})], YCp(\textit{INO1}), or YEplac195 were streaked for isolation on either inositol-containing (\textit{INO+}), or inositol-free (\textit{INO−}) minimal medium and incubated at 34°C for 72 h.
strains lacking the HA-SCS2 gene (Fig. 3A, lanes 1, 2, and 4) and was more abundant in strains containing HA-SCS2 on the 2μm plasmid (data not shown). Treatment of the 12P fraction from yeast cells harboring HA-SCS2 with trypsin (0.1 mg/ml) for 30 min at 4°C digested the HA-Scs2p protein into several peptides, irrespective of whether the treatment was carried out in the absence or presence of Triton X-100 (Fig. 3D, top panel). The anti-HA polyclonal antibody showed HA-Scs2p localization much more clearly, probably because of the high specificity of the antibody (Fig. 4g). These results exclude the possibility that the staining pattern in Fig. 4a shows the localization of the 66-kDa protein observed in Fig. 3A. Thus, cumulatively, these results indicate that Scs2p is an integral ER membrane protein.

Scs2p antibody stained cells in a pattern that includes the nuclear membrane, projections of membrane from the nucleus, and membranes just beneath the plasma membrane (Fig. 4a). The staining pattern is very similar to that with the anti-Dpm1p antibody (Fig. 4b), indicating that Scs2p is colocalized with Dpm1p, the ER membrane protein. On the other hand, only faint staining of the cytoplasm was observed in scs2Δ cells with the anti-Scs2p antibody (Fig. 4d), while localization of Dpm1p was similar to that in wild-type cells (Fig. 4b and e). The anti-HA polyclonal antibody showed HA-Scs2p localization much more clearly, probably because of the high specificity of the antibody (Fig. 4g). These results indicate that Scs2p is an integral ER membrane protein.

Scs2p has a 16-amino-acid conserved sequence. Protein and nucleotide database searches by using FASTA and BLAST protocols have revealed that a 16-amino-acid sequence which corresponds to residues 37 through 53 of Scs2p is well con-
served between yeast and mammalian gene products (Fig. 5A).

In the sequences shown in Fig. 5A, mouse and human homologs were deduced from cDNA sequences of expressed sequence tags. The *Schizosaccharomyces pombe* homolog function is unknown. The *Aplysia* homolog (VAP-33) was studied at the protein level (see below). Two other ER membrane proteins of yeast and rat liver, Cdc48p (an ER membrane protein required for fusion of ER membranes [9, 18]) and TER ATPase (a protein associated with transition vesicles between the ER and the Golgi complex [44]), have the consensus sequence, although similarities are not significant. Nematode MSP1A, which also has a similar sequence, is a member of the major sperm protein family expressed specifically in crawling sperm (33, 40).

The *Aplysia* homolog is a synaptobrevin/VAMP binding protein, VAP-33, required for neurotransmitter release. Since Scs2p has 26.8% identity and 66.3% similarity in the N-terminal 190 residues with VAP-33, we examined whether Scs2p is involved in protein secretion pathways. However, scs2Δ cells secreted invertase, a major yeast secretory protein, as well as did wild-type cells in YPD medium at 30°C. Moreover, there was no difference between wild-type and scs2Δ cells in the electrophoretic mobility of the secreted invertase (Fig. 6A), suggesting that the sugar modification of invertase in scs2Δ cells was normal under these conditions. The secretion of another marker, α-factor, was also examined by the halo assay (39). As shown in Fig. 6B, scs2Δ cells could secrete the protein as efficiently as wild-type cells.

To investigate whether the conserved region of Scs2p is crucial for its function, we constructed a mutant Scs2p protein which lacks the region by removing residues 36 through 53. As shown in Fig. 5B, overproduction of the protein (Scs2pΔ36-53) from the 2μm plasmid failed to suppress inositol auxotrophy of the scs2Δ strain, although Scs2pΔ36-53 expression was not lessened significantly (data not shown). Since Scs2p overproduction was toxic, as described above, there is a possibility that the failure to rescue the inositol auxotrophy is due to the toxic effect. However, overproduction of Scs2pΔ36-53 from the CEN plasmid also failed to suppress the auxotrophy (Fig. 6B). The results suggest that the region is required for normal Scs2p function.

**DISCUSSION**

In this study we found that scs2Δ strains showed inositol auxotrophy. They could form isolated colonies in the absence of inositol at 30°C, even though the growth rate was not as high as that of wild-type cells. Significant growth defects in inositol-
free medium were observed when cells were incubated at temperatures of above 34°C (Fig. 1). The observed inositol auxotrophy was relatively weak compared to those of other inositol-auxotrophic mutants (ino1 and ino2 mutants) (7, 14, 19, 28). The finding that overproduction of INO1 or INO2 rescued the inositol auxotrophy (Fig. 2) suggests that Scs2p is a transcriptional factor like Ino2p or Ino4p (1). In fact, both SCS2 and INO2 are multicopy suppressors of CSE1 inositol auxotrophy, and an increase in INO1 mRNA levels is observed when SCS2 is overproduced in CSE1 mutants (25). However, since we have found that Scs2p is an integral membrane protein of the ER (Fig. 4), it is unlikely that Scs2p is a conventional transcriptional factor which directly binds to the upstream activation site of the INO1 gene.

Studies on sterol-regulatory element binding protein 1 (SREBP-1), found in mammalian cells, provide a possibility for the Scs2p function. SREBP-1 is a transcriptional factor which is associated with ER membranes and has a molecular mass of 125 kDa. Surprisingly, in cells deprived of cholesterol, the protein is cleaved to release a 68-kDa N-terminal fragment. The 68-kDa fragment is then targeted to the nucleus, where it binds to the sterol-regulatory element of the low-density lipoprotein receptor (LDLR) gene.
VAP-33, which is required for neurotransmitter release, is the only characterized protein which has an overall similarity with Scs2p. Since we have failed to obtain any line of evidence that Scs2p is involved in protein secretion, function may not be conserved between Scs2p and VAP-33, although there remains a possibility that a functionally redundant protein(s) may substitute for Scs2p function. The localization of Scs2p on the ER membrane (Fig. 4) does not favor the assumption that Scs2p is directly associated with yeast synaptobrevin homologs (Snclp and Snclp [10, 32]), because synaptobrevin is a membrane protein of secretory vesicles which are derived from the Golgi complex. However, it might be possible that Scs2p is associated with the other yeast synaptobrevin homologs found on vesicular carriers responsible for protein transport from the ER to the Golgi complex (22). Identification of a protein(s) which binds to Scs2p would give insight into this question.

Although the biochemical activity of Scs2p is still unknown, the existence of the conserved 16-amino-acid sequence (Fig. 5A) suggests that the Scs2p function is conserved between yeast and mammalian cells. Interestingly, Scs2p, VAP-33, Cdc48p, and TER ATPase, all of which contain the conserved sequence, are associated with the cytoplasmic face of biomembranes (Fig. 3) (9, 18, 37, 44). This fact implies that the sequence serves as a targeting or anchoring signal for those proteins to associate with this specific membrane region. Therefore, more detailed studies of the sequence are expected to reveal a novel protein motif which is required for the association with membranes in various eukaryotic cells.

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FIG. 6. Disruption of SCS2 has no effect on protein secretion. (A) Sugar modification of invertase. Cells were grown in YPD medium at 25°C and shifted to YP medium with 0.1% glucose. After 2 h of incubation at 30°C, cells were converted to spheroplasts and separated into the intracellular (I) and extracellular (E) fractions. These fractions were subjected to native gel activity staining for invertase. The position of nonglycosylated (cytosolic) invertase is indicated by an arrow. Strains used were CTY182 (wild type [WT]) (lanes 1 and 2) and KY356 (scs2Δ::URA3) (lanes 3 and 4). (B) Halo assay for α-factor production. YPH500 (WT) and KY360 (scs2Δ::TRP1) cells were spotted on a lawn of a-mating-type cells (MATa αt2) on a plate and incubated at 30°C for 48 h to allow halos to develop.

poprotein receptor (42, 43). By analogy, it seems likely that Scs2p is a novel membrane-bound transcriptional factor which moves to the nucleus from the ER in response to inositol starvation and induces the expression of INO1. Unfortunately, we have failed to find migration of Scs2p to the nucleus in response to inositol starvation by immunofluorescence analysis (unpublished data). More detailed studies using cell-free systems might reveal the localization change.

Ire1p is one of the ER membrane proteins whose disruption causes inositol auxotrophy (5, 20, 26). Ire1p is an ER transmembrane kinase and transmits a signal of unfolded-protein accumulation in the ER to the nucleus. A basic leucine zipper transcription factor, Hac1p/Ire1p, is required for the unfolded-protein response (UPR) and binds to the UPR element in the promoters of UPR-regulated genes (6). As inositol-containing lipids are major phospholipid components of yeast membranes, Ire1p is postulated to regulate the coordinated biogenesis of both the protein and lipid components of the ER (30). Since overproduction of Scs2p suppresses inositol auxotrophy of ire15Δ/hac1Δ mutants and scs2Δ strains are sensitive to tunicamycin treatment that induces the UPR (26a), it seems likely that Scs2p is involved in a signal transduction pathway similar to the Ire1p pathway. While Ire1p is activated by the UPR, Scs2p may be activated by heat shock, because the scs2 inositol auxotrophy become significant at high temperatures (Fig. 1 and 5B).


