JOURNAL OF BACTERIOLOGY, Apr. 1998, p. 1700–1708 Vol. 180, No. 7
0021-9193/98/$04.00+0

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

SATOSHI KAGIWADA,[1]* KOHEI HOSAKA,[2] MASAYUKI MURATA,[3] JUN-ICHI NIKAWA,[4] AND AKIRA TAKATSUKI[1]

Animal and Cellular Systems Laboratory, The Institute of Physical and Chemical Research (RIKEN), Wako, Saitama 351-01,[1] Department of Basic Allied Medicine, Gunma University School of Health Sciences, Maebashi 371,[2] Ultrastructural Research Laboratory, National Institute for Physiological Sciences, Okazaki, Aichi 444,[3] and Department of Biochemical Engineering and Science, Faculty of Computer Science and Systems Engineering, Kyushu Institute of Technology, Iizuka, Fukuoka 820,[4] Japan

Received 8 October 1997/Accepted 20 January 1998

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 IIB, protein phosphatase type 2A subunit A, and the 14-3-3 protein (23). These results suggest that yeast cells contain a signal transduction mechanism resembling the human transforming growth factor β receptor-mediated pathway to induce the expression of inositol biosynthetic genes (23). The gene responsible for the *ire15* mutation is identical to *HAC1*, which encodes a transcriptional factor with a basic leucine zipper motif (24, 29).

A relationship between inositol metabolism and a signal transduction mechanism is also suggested by recent work on the *IRE1* gene (5, 20). Although *IRE1* was originally identified as a gene required for inositol prototrophy (26), it is also required for the transcription of *KAR2*, which encodes a protein chaperon, BiP, in response to the accumulation of unfolded proteins in the endoplasmic reticulum (ER). The *IRE1* gene product is a member of transmembrane serine/threonine kinases and lies in the ER/nuclear membrane. It is thought that Ire1p transmits a signal of unfolded-protein accumulation in the ER to the nucleus by a mechanism similar to those found in transmembrane kinases in the plasma membranes of higher eukaryotic cells (35).

Recently, a gene which has partial homology to *SCS2* has been cloned from *Aplysia californica* (37). The gene encodes the synaptobrevin/VAMP-associated protein VAP-33, which was identified by using the yeast two-hybrid system (37). Synaptobrevin is localized to the surface of synaptic vesicles and associates with syntaxin and SNAP-25, which are localized to the presynaptic membranes (38). Through the interaction of these proteins, the synaptic vesicles fuse with the presynaptic membranes and neurotransmitters are released from the vesicles. Since presynaptic injection of antibodies to VAP-33 inhibited the synaptic transmission, VAP-33 is considered to be 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

* Corresponding author. Present address: Department of Biology, Nara Women's University, Nara 630, Japan. Phone and fax: 81-742-20-3416.

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-peptone-dextrose (YPD) and yeast minimal media were described by Kaiser et al. (16) and Klig et al. (17), respectively. When added, inositol (*myo*-inositol; Sigma) was at a final concentration of 100 μM. The preparation of *INO1* and *INO2* genes was described by Kagiwada et al. (15). Strains used were CTY182 (*MAT***a** *ura3-52 his3-Δ200 lys2-801* [2]), YPH500 (*MAT*α *ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1* [36]), YPH501 (*MAT***a**/α *ura3-52*/*ura3-52 lys2-801*/*lys2-801 ade2-101*/*ade2-101 trp1-Δ63*/*trp1-Δ63 his3-Δ200*/*his3-Δ200 leu2-Δ1*/*leu2-Δ1* [36]), KY356 (CTY182 *scs2*Δ::*URA3*), and KY360 (YPH500 *scs2*Δ::*TRP1*).

Construction of *SCS2* vectors. The *SCS2* gene was amplified by PCR from pSC2, which contains the *Hin*dIII-*Cla*I fragment of the *SCS2* genome (25). The forward and reverse primers used were 5′-CCAAGCTTTGCATAGCGCACG C-3′ and 5′-CCGAATTCTAGTATTGTAAAGGC-3′, respectively. An *Eco*RI site was engineered at the 5′ region of the reverse primer. The PCR fragment was cut with *Hin*dIII and *Eco*RI, and the 1.3-kb fragment was inserted into the same sites of YEp*lac*195 and YCp*lac*33 (11) to generate pKY134 [YEp(*SCS2*)] and pKY151 [YCp(*SCS2*)], respectively.

***SCS2* disruption.** To disrupt the *SCS2* gene, the coding region corresponding to amino acids 4 through 219 was replaced with the *URA3* or *TRP1* gene. To this end, an additional *Pst*I 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 resultant plasmid was cut with *Pst*I and self-ligated to generate pKY144, which lacks 648 nucleotides from position +11 to +658. The *URA3* or *TRP1* marker gene was incorporated into the *Pst*I site of pKY144 to generate pKY145 or pKY159, respectively. The *Hin*dIII-*Eco*RI 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. A glutathione *S*-transferase (GST)–Scs2 fusion protein was constructed for preparation of an anti-Scs2p polyclonal antibody. PCR was performed with pKY134 as a template. The forward and reverse primers, 5′-G GATCCCCTGACGTGTTGGTG-3′ and 5′-GAATTCATTTTCTGCAGGTAC G-3′, respectively, were constructed to place a *Bam*HI site at the 5′ end and an *Eco*RI site at the 3′ end of the 645-bp segment of *SCS2* which corresponds to residues 7 through 221. The PCR product was cut with *Bam*HI and *Eco*RI and ligated into *Bam*HI-*Eco*RI-digested pGEX4T-1 (Pharmacia Biotech) to yield pKY149. Proteins which were expressed in response to induction with isopropyl-β-D-thiogalactopyranoside were purified from DH5α cells harboring pKY149 by using Bulk GST Purification Modules (Pharmacia Biotech). Rabbit antiserum against the purified proteins was passed through GST-Sepharose 4B beads to remove antibodies against GST, and then the serum was affinity purified with antigen conjugated to Sepharose 4B beads.

HA epitope tagging. A 9-amino-acid epitope recognized by antihemagglutinin (anti-HA) antibody was introduced into the *SCS2* coding sequence by the following method. The *SCS2* gene was modified by mutagenesis of the nucleotide at position +12 from T to A to introduce an *Acc*I site. This modification does not change the *SCS2* amino acid sequence. The *Acc*I site was used for insertion of the PCR product amplified by using the forward primer 5′-GTATACCCATAC GATGTTCCAGATTACGCTGAAATTTCCCCTGACGTG-3′ and the reverse primer 5′-CCGAATTCTAGTATTGTAAAGGC-3′. The forward primer was constructed to place the HA-coding sequence 5′ to nucleotide +13. The resulting fragment was subcloned into YCp*lac*33 and YEp*lac*195 to generate pKY166 [YCp(HA-*SCS2*)] and pKY167 [YEp(HA-*SCS2*)]. By this construction, the N terminus of Scs2p was changed from MSAVEI to MSAVYPYDVPDYAEI (the HA epitope residues are underlined).

***SCS2*(Δ36-53) construction.** To construct *SCS2*(Δ36-53), a DNA fragment (AA1-35) which contains 989 bp from nucleotide −442 to +547 was ligated with a DNA fragment (AA54-243) which contains 726 bp from nucleotide +602 to +1327. AA1-35 was amplified by PCR with the primer 5′-CCAAGCTTTGCA TAGCGCACGC-3′ and 5′-CTGCAGTGGTTTGGTCTGAATTGTTGG-3′. AA54-243 was amplified with the primers 5′-CTGCAGTTGTTGCTCCAGGT G-3′ and 5′-CCGAATTCTAGTATTGTAAAGGC-3′. AA1-35 was digested with *Hin*dIII and *Pst*I and subcloned into *Hin*dIII-*Pst*I-digested pKY144 to create pKY168. AA54-243 was cut with *Pst*I and subcloned into *Pst*I-digested pKY168 to create YEp(*SCS2*Δ36-53). YEp(*SCS2*Δ36-53) was cut with *Hin*dIII and *Eco*RI and was inserted into the same site of YCp*lac*33 to generate YCp-(*SCS2*Δ36-53).

Cell fractionation. Preparation of subcellular fractions was performed as described by Cleves et al. (4) with minor modifications. Appropriate yeast strains were grown to an optical density at 600 nm of 0.4 in 100 ml of YPD with shaking at 26°C. The cells were washed with 10 mM NaN_3, converted to spheroplasts by resuspension in 10 ml of spheroplasting buffer (1.1 M sorbitol, 50 mM potassium phosphate [pH 7.4], 10 mM NaN_3) containing 20 μg of Zymolyase 20T (Seikagaku Kogyo) per ml and 5 μg of β-mercaptoethanol per ml, and incubated at 30°C for 60 min. Spheroplasts were washed with the spheroplasting buffer and resuspended in 6 ml of ice-cold lysis buffer (0.3 M sorbitol, 10 mM potassium phosphate, pH 7.4). The cells were incubated on ice for 20 min with occasional gentle agitation. Lysates were adjusted to 1.1 M sorbitol and centrifuged at 800 × *g* for 5 min to remove unlysed cells. The low-speed supernatant (LSS) was centrifuged at 12,000 × *g* for 15 min to yield pellet (12P) and supernatant (12S) fractions. The 12S fraction was centrifuged at 100,000 × *g* for 1 h in a TLA45 rotor (Beckman Instruments) to yield pellet (100P) and supernatant (100S) fractions.

Treatment of membranes. In order to extract peripheral proteins associated with the 12P fraction, 40 μl of the 12P fraction was incubated with 10 μl of either H_2O, 5 M KCl, 10 M urea, 0.5 M Na_2CO_3, or 5% Triton X-100. The mixtures were incubated for 30 min on ice and centrifuged at 100,000 × *g* for 30 min. Pellets were resuspended in 50 μl of lysis buffer. For the protease protection assay, the 12P fraction of KY360 harboring YCp(HA-*SCS2*) was incubated with 0.1 mg of trypsin (type III; Sigma) per ml in the presence or absence of 0.1% Triton X-100. At the beginning or end of the incubation (4°C, 30 min), soybean 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 mouse anti-HA monoclonal antibody (clone 12CA5; Boehringer Mannheim), a mouse anti-Dpm1p monoclonal antibody (clone 5C5-A7; Molecular Probes), a rabbit anti-Kar2p polyclonal antibody (a gift from K. Kohno, Nara Institute of Science and Technology, Nara, Japan) (41), or a rabbit anti-Sec14p 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 logarithmic stage in complete or uracil-deficient medium for plasmid maintenance. 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 centrifuged and resuspended in 0.5 ml of spheroplasting buffer containing 20 μg of Zymolyase 20T 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 mg of bovine serum albumin per ml dissolved in phosphate-buffered saline (PBS). Primary antibodies used were the rabbit 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-Dpm1p 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 invertase was analyzed by invertase 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 NaN_3 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 10% glycerol 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 (*MAT***a** *sst2*) as a tester strain.

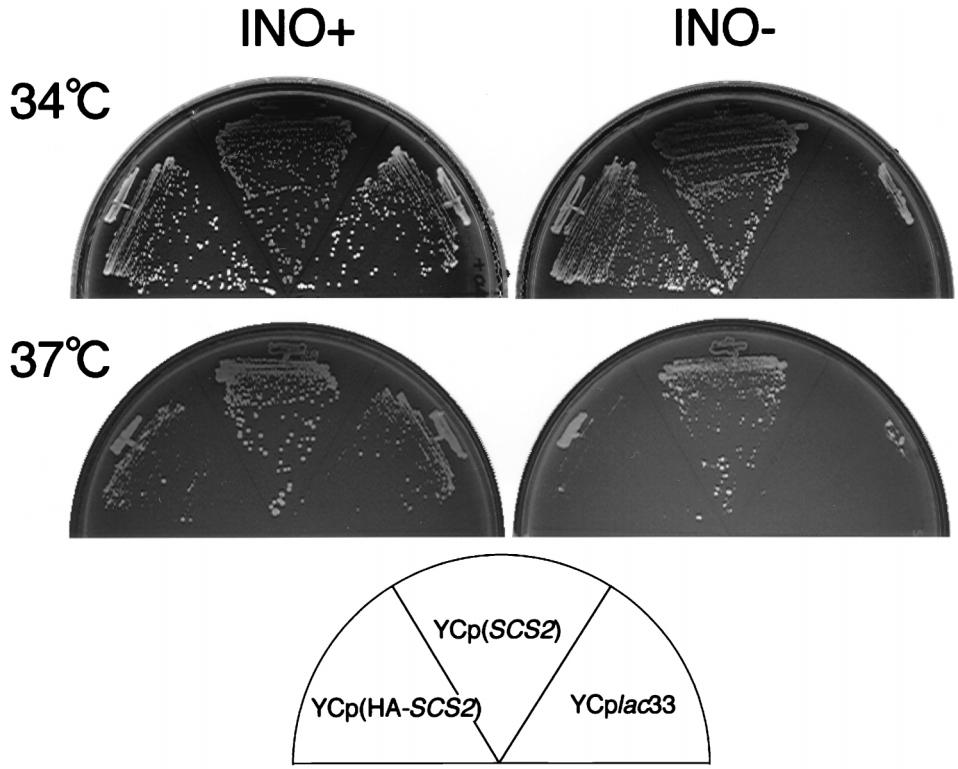

FIG. 1. Inositol auxotrophy of *scs2*Δ strains. KY360 (*scs2*Δ::*TRP1*) cells transformed with YCp*lac*33 (control), YCp(*SCS2*), or YCp(HA-*SCS2*) were streaked for isolation on either inositol-containing (INO+) or inositol-free (INO−) minimal medium and incubated at 34°C for 72 h or at 37°C for 96 h.

RESULTS

***SCS2* disruption mutants show inositol auxotrophy.** In order to study the physiological role of the *SCS2* gene, we have constructed *SCS2* disruption (*scs2*Δ) 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 a YPD plate. The ability to generate haploid yeast strains with the *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 *scs2*Δ 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 *scs2*Δ mutants on inositol-free medium. As expected, an *scs2*Δ 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 *scs2*Δ 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 *scs2*Δ strain rescues the auxotrophy. As shown in Fig. 1, *scs2*Δ 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 2μm 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 2μm 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 *scs2*Δ inositol auxotrophy, the *INO1* or *INO2* gene was incorporated into an *scs2*Δ strain (KY360). As shown in Fig. 2, overproduction of *INO1* from the CEN plasmid [YCp(*INO1*)] and of *INO2* from the 2μm plasmid [YEp(*INO2*)] could rescue the growth defect. The results suggest that an increase in *INO1* expression levels can rescue the *scs2*Δ 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

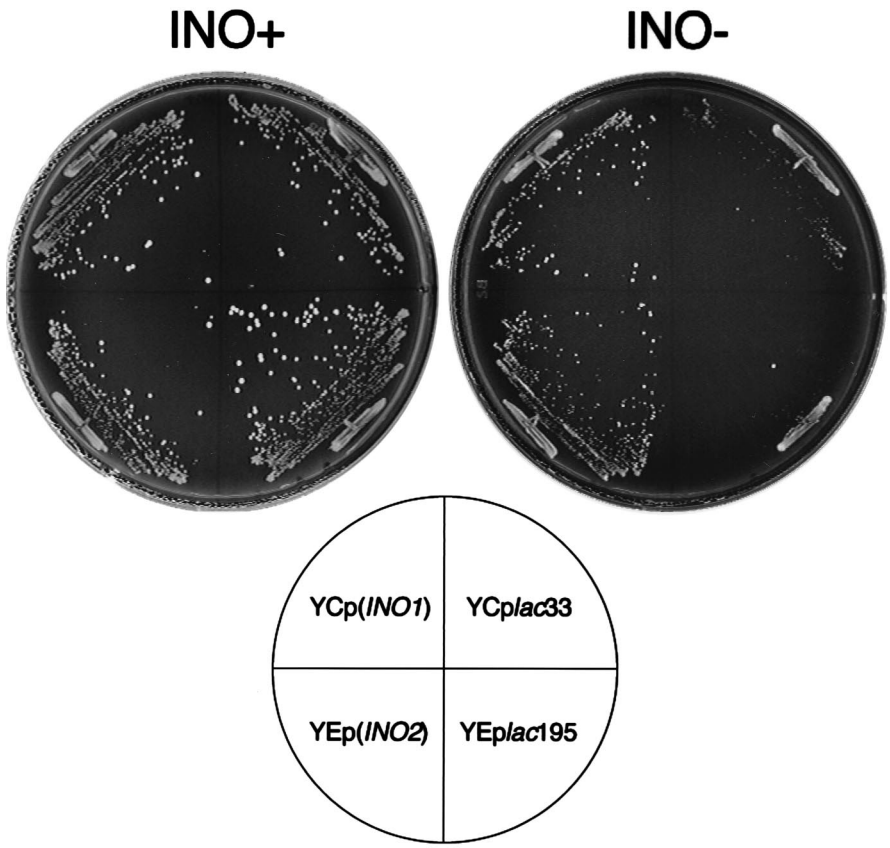

FIG. 2. Inositol auxotrophy of *scs2*Δ strains transformed with *INO1* or *INO2*. KY360 (*scs2*Δ::*TRP1*) cells transformed with YCp*lac*33 [vector control for YCp(*INO1*)], YEplac195 [vector control for YEp(*INO2*)], YCp(*INO1*), or YEp(*INO2*) were streaked for isolation on either inositol-containing (INO+), or inositol-free (INO−) minimal medium and incubated at 34°C for 72 h.

fraction prepared from an *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 *scs2*Δ cells (Fig. 3A, lane 2).

The nucleotide sequence of *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 *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_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 [^{32}P]orthophosphate (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 × *g*. The supernatant fractions at 800, 12,000, and 100,000 × *g* are referred to as LSS, 12S, and 100S, respectively, and the pellet fractions at 12,000 and 100,000 × *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 mannose synthase (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-*SCS2* gene, which encodes an *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-*SCS2* gene retained the normal Scs2p activity because it suppressed the inositol auxotrophy of the *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

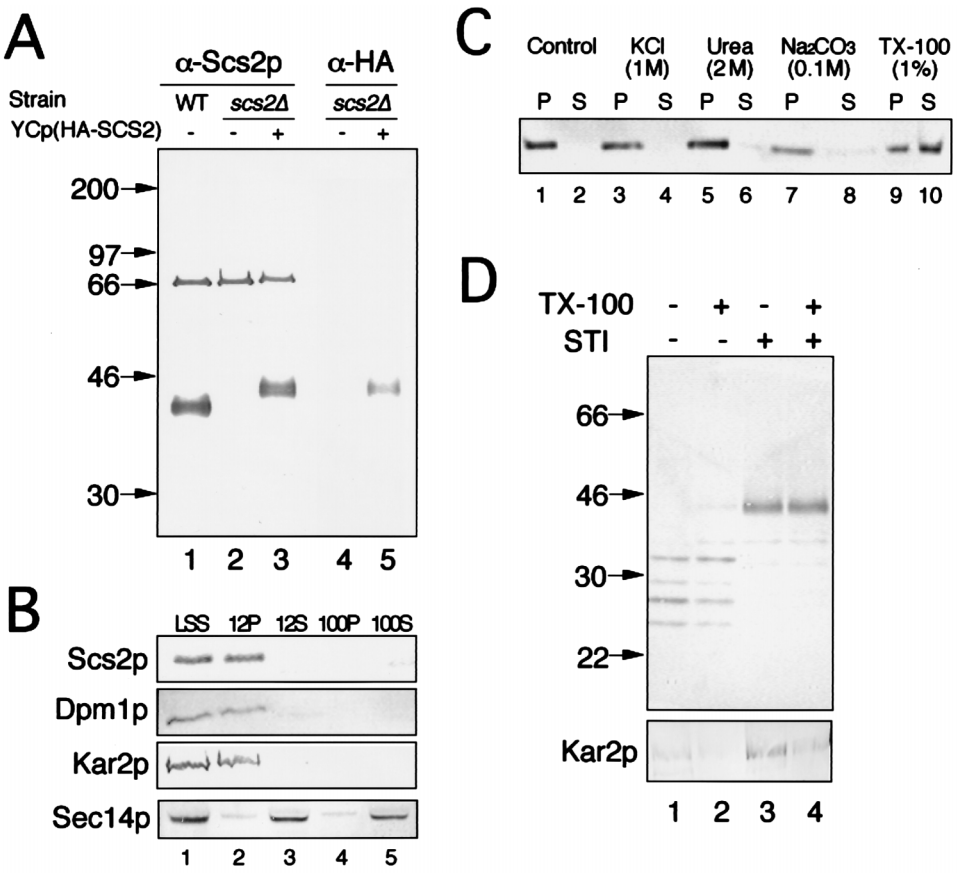

FIG. 3. Characterization of Scs2p by Western blotting. (A) Strains were grown in minimal medium, converted to spheroplasts, and lysed osmotically. The resultant lysate was centrifuged at 800 × *g* to yield LSS. The LSS was separated by SDS–10% PAGE and immunoblotted with the anti-Scs2p polyclonal antibody (α-Scs2p) (lanes 1 to 3) or the anti-HA monoclonal antibody (α-HA) (lanes 4 and 5). The strains employed were YPH500 (wild type [WT]) (lane 1) and KY360 (*scs2*Δ::*TRP1*) harboring either YCp*lac*33 (lanes 2 and 4) or YCp(HA-*SCS2*) (lanes 3 and 5). (B) The LSS fraction of wild-type cells (CTY182) was subjected to two rounds of differential centrifugation to yield 12,000 × *g* pellet and supernatant fractions (12P and 12S, respectively) and 100,000 × *g* pellet and supernatant fractions (100P and 100S, respectively). These fractions were resolved by SDS–10% PAGE and immunoblotted for the presence of Scs2p and for markers of the ER membrane (Dpm1p), ER lumen (Kar2p), and cytoplasm/Golgi membrane (Sec14p). (C) The 12P fraction of wild-type cells (CTY182) was incubated with 0.2 volume of one of the following solutions: H_2O (control), 5 M KCl, 10 M urea, 0.5 M Na_2CO_3 (pH 11), or 5% Triton X-100 (TX-100). After incubation at 4°C for 30 min, samples were centrifuged at 100,000 × *g* for 30 min to separate supernatant (S) and pellet (P) fractions. These fractions were resolved by SDS–10% PAGE and immunoblotted with the anti-Scs2p polyclonal antibody. (D) The 12P fraction of KY360 (*scs2*Δ::*TRP1*) harboring YCp(HA-*SCS2*) was incubated with 0.1 mg of trypsin per ml in the presence (lanes 2 and 4) or absence (lanes 1 and 3) of 0.1% Triton X-100 (TX-100). Soybean trypsin inhibitor (STI) was added at the beginning (lanes 3 and 4) or end (lanes 1 and 2) of the incubation. Samples were resolved by SDS–10% PAGE and immunoblotted with the anti-HA monoclonal antibody (top panel) or the anti-Kar2p polyclonal antibody (bottom panel). In panels A and C, the numbers on the left are molecular masses in kilodaltons.

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). On the other hand, Kar2p was not digested in the absence of Triton X-100 (Fig. 3D, bottom panel), indicating that membranes in the 12P fraction were not destroyed during the treatment. Taken together, all of these data are consistent with the idea that Scs2p has the topology of a type II integral membrane protein, with the N-terminal domain located in the cytoplasm and the C-terminal hydrophobic domain located inside membranes.

Scs2p is localized to the ER membrane. In order to study the localization of Scs2p, we used indirect immunofluorescence. Fixed and permeabilized cells (YPH500) were double labeled with the affinity-purified anti-Scs2p polyclonal antibody and the anti-Dpm1p monoclonal antibody (Fig. 4a to c). The anti-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 *scs2*Δ 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 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 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-

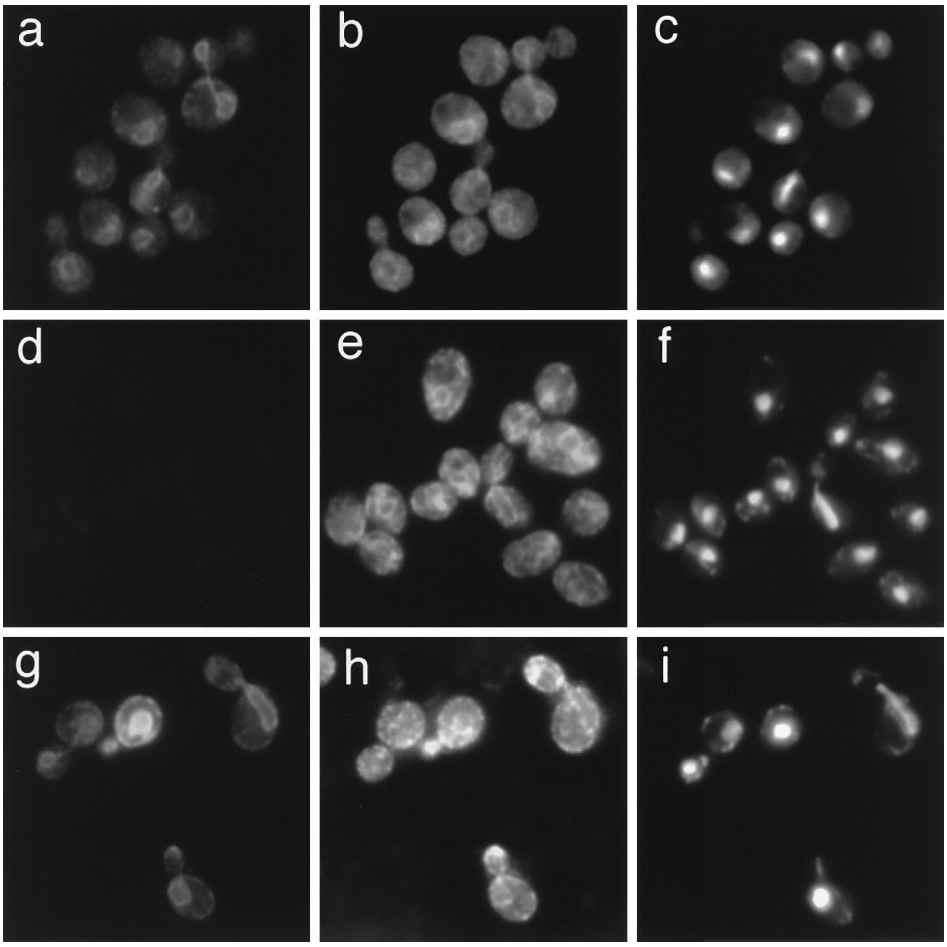

FIG. 4. Localization of Scs2p. Indirect immunofluorescence was carried out with YPH500 cells (wild type) (a, b, and c), KY360 cells (*scs2*Δ::*TRP1*) (d, e, and f), and KY360 cells harboring YCp(HA-*SCS2*) (g, h, and i). The cells in panels a to f were incubated with the rabbit anti-Scs2p polyclonal antibody and the mouse anti-Dpm1p monoclonal antibody, and cells in panels g to i were incubated with the rabbit anti-HA polyclonal antibody and the mouse anti-Dpm1p monoclonal antibody. Samples were stained with tetramethylrhodamine isothiocyanate-conjugated anti-rabbit IgG to detect Scs2p (a, d, and g), fluorescein isothiocyanate-conjugated anti-mouse IgG to detect Dpm1p (b, e, and h), and DAPI to indicate the position of the nuclei (c, f, and i).

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, *scs2*Δ 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 *scs2*Δ cells in the electrophoretic mobility of the secreted invertase (Fig. 6A), suggesting that the sugar modification of invertase in *scs2*Δ 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, *scs2*Δ 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 ($Scs2p^{\Delta 36\text{-}53}$) from the 2μm plasmid failed to suppress inositol auxotrophy of the *scs2*Δ strain, although $Scs2p^{\Delta 36\text{-}53}$ 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 $Scs2p^{\Delta 36\text{-}53}$ 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 *scs2*Δ 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-

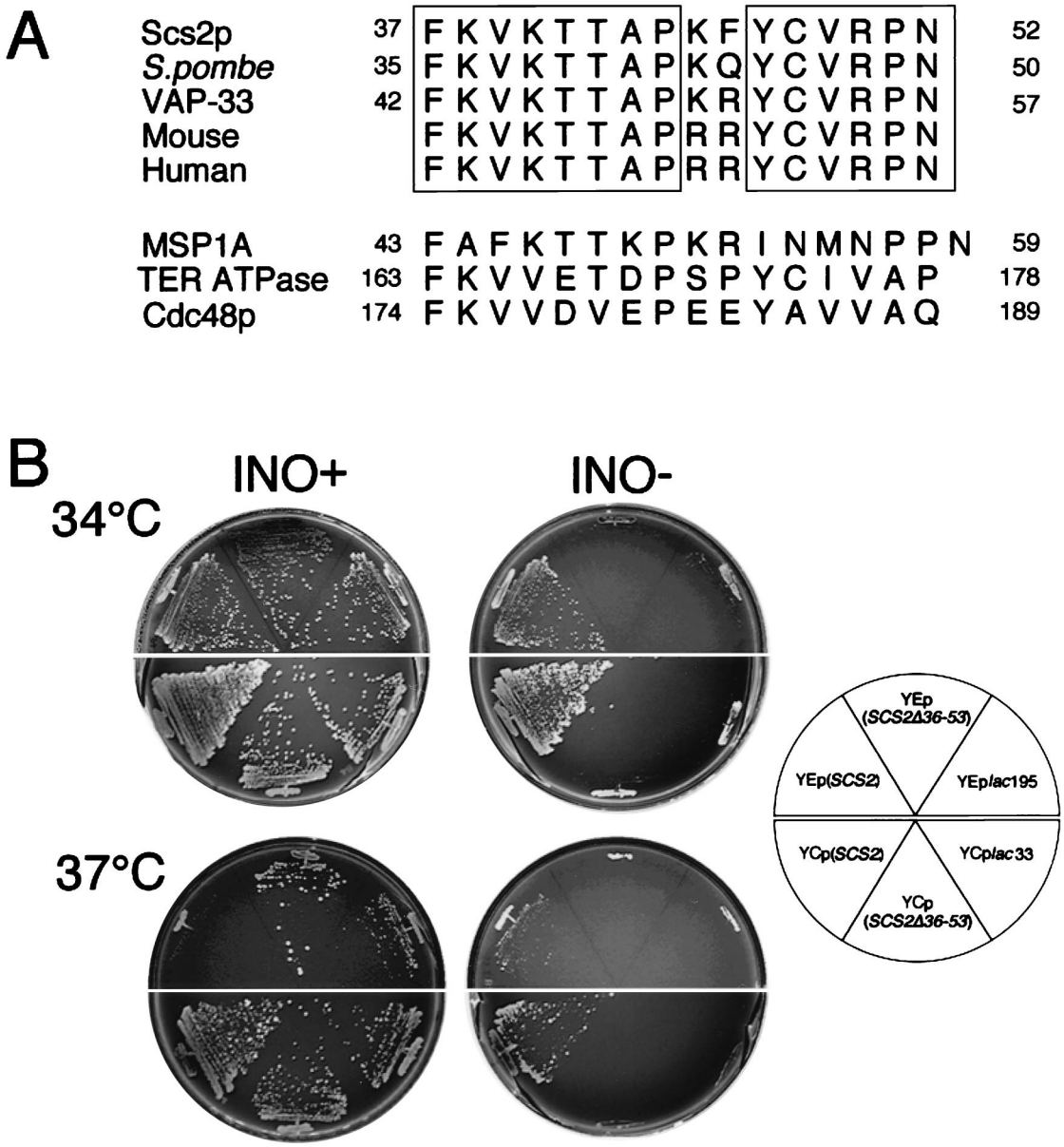

FIG. 5. The 16-amino-acid conserved region is required for Scs2p activity. (A) Alignment of a 16-amino-acid sequence of Scs2p with those of *S. pombe*, *A. californica* (VAP-33), and mouse and human homologs. The sequences of the mouse and human homologs are deduced from nucleotide sequences of expressed sequence tags. Residues identical in all five sequences are boxed. Homologous sequences of MSP1A, TER ATPase, and Cdc48p are also shown. For Scs2p, the *S. pombe* homolog, VAP-33, MSP1A, TER ATPase, and Cdc48p, the numbers refer to amino acid positions. GenBank accession numbers are D44493 (Scs2p), Z73099 (*S. pombe*), U36779 (VAP-33), W54842 (mouse), N34715 (human), P53021 (MSP1A), U11760 (TER ATPase), and X56956 (Cdc48p). (B) Overproduction of Scs2p$^{\Delta 36\text{-}53}$ failed to suppress the inositol auxotrophy of the *scs2*Δ strain. KY360 (*scs2*Δ::*TRP1*) cells transformed with YEp*lac*195, YEp(*SCS2*), YEp(*SCS2*Δ36-53), YCp*lac*33, YCp(*SCS2*), or YCp(*SCS2*Δ36-53) 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.

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 li-

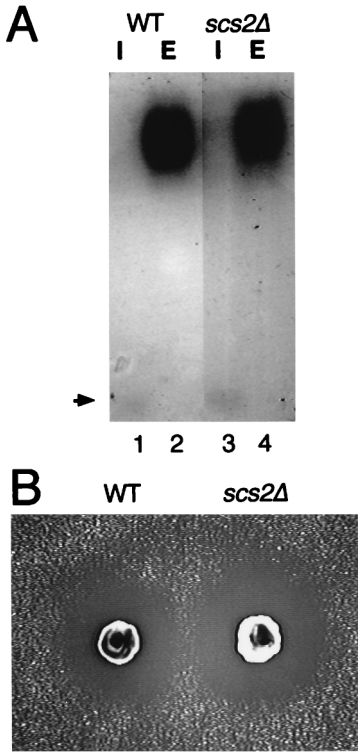

FIG. 6. Disruption of *SCS2* has no effect on protein secretion. (A) Sugar modification of invertase. Cells were grown in YPD medium at 25°C and shifted to YP medium with 0.1% glucose. After 2 h of incubation at 30°C, cells were converted to spheroplasts and separated into the intracellular (I) and extracellular (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 (*scs2*Δ::*URA3*) (lanes 3 and 4). (B) Halo assay for α-factor production. YPH500 (WT) and KY360 (*scs2*Δ::*TRP1*) cells were spotted on a lawn of **a**-mating-type cells (*MAT***a** *sst2*) 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/Ire15p, 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 *ire15*/*hac1* mutants and *scs2*Δ 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).

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 (Snc1p and Snc2p [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.

ACKNOWLEDGMENTS

We thank Vytas Bankaitis and Ryogo Hirata for providing strains and antibodies, Kenji Kohno for the anti-Kar2p antibody, and Makoto Muroi and members of Animal and Cellular Systems Laboratory of RIKEN for helpful discussions. We also thank the Division of Laboratory Animal Research of RIKEN for production of the anti-Scs2p polyclonal antibody.

S.K. was supported by the special postdoctoral scientist program of RIKEN.

REFERENCES

1. **Ambroziak, J., and S. A. Henry.** 1994. *INO2* and *INO4* gene products, positive regulators of phospholipid biosynthesis in *Saccharomyces cerevisiae*, form a complex that binds to the *INO1* promoter. J. Biol. Chem. **269:**15344–15349.
2. **Bankaitis, V. A., D. E. Malehorn, S. D. Emr, and R. Greene.** 1989. The *Saccharomyces cerevisiae SEC14* gene encodes a cytosolic factor that is required for transport of secretory proteins from the yeast Golgi complex. J. Cell Biol. **108:**1271–1281.
3. **Carman, G. M., and S. A. Henry.** 1989. Phospholipid biosynthesis in yeast. Annu. Rev. Biochem. **58:**635–669.
4. **Cleves, A. E., T. P. McGee, E. A. Whitters, K. M. Champion, J. R. Aitken, W. Dowhan, M. Goebl, and V. A. Bankaitis.** 1991. Mutations in the CDP-choline pathway for phospholipid biosynthesis bypass the requirement for an essential phospholipid transfer protein. Cell **64:**789–800.
5. **Cox, J. S., C. E. Shamu, and P. Walter.** 1993. Transcriptional induction of genes encoding endoplasmic reticulum resident proteins requires a transmembrane protein kinase. Cell **73:**1197–1206.
6. **Cox, J. S., and P. Walter.** 1996. A novel mechanism for regulating activity of a transcription factor that controls the unfolded protein response. Cell **87:** 391–404.
7. **Culbertson, M. R., and S. A. Henry.** 1975. Inositol requiring mutants of *Saccharomyces cerevisiae*. Genetics **80:**23–40.
8. **Dean, J. M., and S. A. Henry.** 1989. Biosynthesis of inositol in yeast. Primary structure of *myo*-inositol-1-phosphate synthase (EC 5.5.1.4) and functional analysis of its structural gene, the *INO1* locus. J. Biol. Chem. **264:**1274–1283.
9. **Fröhlich, K. U., H. W. Fries, M. Rudiger, R. Erdmann, D. Botstein, and D. Mecke.** 1991. Yeast cell cycle protein CDC48p shows full-length homology to the mammalian protein VCP and is a member of a protein family involved in secretion, peroxisome formation, and gene expression. J. Cell Biol. **114:** 443–453.
10. **Gerst, J. E., L. Rodgers, M. Riggs, and M. Wigler.** 1992. *SNC1*, a yeast homolog of the synaptic vesicle-associated membrane protein/synaptobrevin gene family: genetic interactions with the RAS and CAP genes. Proc. Natl. Acad. Sci. USA **89:**4338–4342.

11. **Gietz, R. D., and A. Sugino.** 1988. New yeast-*Escherichia coli* shuttle vectors constructed with *in vitro* mutagenized yeast genes lacking six-base pair restriction sites. Gene **74:**527–534.
12. **Hirata, R., L. A. Graham, A. Takatsuki, T. H. Stevens, and Y. Anraku.** 1997. VMA11 and VMA16 encode second and third proteolipid subunits of the *Saccharomyces cerevisiae* vacuolar membrane H+-ATPase. J. Biol. Chem. **272:**4795–4803.
13. **Hosaka, K., J. Nikawa, T. Kodaki, and S. Yamashita.** 1992. A dominant mutation that alters the regulation of *INO1* expression in *Saccharomyces cerevisiae*. J. Biochem. **111:**352–358.
14. **Hosaka, K., J. Nikawa, T. Kodaki, and S. Yamashita.** 1994. Cloning and characterization of the *SCS1* gene required for the expression of genes in yeast phospholipid synthesis. J. Biochem. **115:**131–136.
15. **Kagiwada, S., B. G. Kearns, T. P. McGee, M. Fang, K. Hosaka, and V. A. Bankaitis.** 1996. The yeast *BSD2-1* mutation influences both the requirement for phosphatidylinositol transfer protein function and derepression of phospholipid biosynthetic gene expression in yeast. Genetics **143:**685–697.
16. **Kaiser, C., S. Michaelis, and A. Mitchell.** 1994. Methods in yeast genetics. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
17. **Klig, L. S., M. J. Homann, G. M. Carman, and S. A. Henry.** 1985. Coordinate regulation of phospholipid biosynthesis in *Saccharomyces cerevisiae*: pleiotropically constitutive *opi1* mutant. J. Bacteriol. **162:**1135–1141.
18. **Latterich, M., K. U. Fröhlich, and R. Schekman.** 1995. Membrane fusion and the cell cycle: Cdc48p participates in the fusion of ER membranes. Cell **82:**885–893.
19. **Loewy, B. S., and S. A. Henry.** 1984. The *INO2* and *INO4* loci of *Saccharomyces cerevisiae* are pleiotropic regulatory genes. Mol. Cell. Biol. **4:**2479–2485.
20. **Mori, K., W. Ma, M. J. Gething, and J. Sambrook.** 1993. A transmembrane protein with a cdc2+/CDC28-related kinase activity is required for signaling from the ER to the nucleus. Cell **74:**743–756.
21. **Muroi, M., N. Shiragami, K. Nagao, M. Yamasaki, and A. Takatsuki.** 1994. Folimycin (concanamycin A) and bafilomycin A(1), inhibitors specific for V-ATPase, exert similar but distinct effects on intracellular translocation and processing of glycoproteins. Biosci. Biotech. Biochem. **58:**425–427.
22. **Newman, A. P., J. Shim, and S. Ferro-Novick.** 1990. *BET1*, *BOS1*, and *SEC22* are members of a group of interacting yeast genes required for transport from the endoplasmic reticulum to the Golgi complex. Mol. Cell Biol. **10:**3405–3414.
23. **Nikawa, J.** 1994. A cDNA encoding the human transforming growth factor β receptor suppresses the growth defect of a yeast mutant. Gene **149:**367–372.
24. **Nikawa, J., M. Sugiyama, K. Hayashi, and A. Nakashima.** 1997. Suppression of the *Saccharomyces cerevisiae hac1/ire15* mutation by yeast genes and human cDNAs. Gene **201:**5–10.
25. **Nikawa, J., A. Murakami, E. Esumi, and K. Hosaka.** 1995. Cloning and sequence of the *SCS2* gene, which can suppress the defect of *INO1* expression in an inositol auxotrophic mutant of *Saccharomyces cerevisiae*. J. Biochem. **118:**39–45.
26. **Nikawa, J., and S. Yamashita.** 1992. *IRE1* encodes a putative protein kinase containing a membrane-spanning domain and is required for inositol prototrophy in *Saccharomyces cerevisiae*. Mol. Microbiol. **6:**1441–1446.

26a. **Nikawa, J.** Unpublished data.

27. **Nikoloff, D. M., and S. A. Henry.** 1991. Genetic analysis of yeast phospholipid biosynthesis. Annu. Rev. Genet. **25:**559–583.
28. **Nikoloff, D. M., and S. A. Henry.** 1994. Functional characterization of the *INO2* gene of *Saccharomyces cerevisiae*: a positive regulator of phospholipid biosynthesis. J. Biol. Chem. **269:**7402–7411.
29. **Nojima, H., S.-H. Leem, H. Araki, A. Sakai, N. Nakashima, Y. Kanaoka, and Y. Ono.** 1994. Hac1: a novel yeast bZIP protein binding to the CRE motif is a multicopy suppressor for *cdc10* mutant of *Schizosaccharomyces pombe*. Nucleic Acids Res. **22:**5279–5288.
30. **Nunnari, J., and P. Walter.** 1996. Regulation of organelle biogenesis. Cell **84:**389–394.
31. **Piper, R. C., E. A. Whitters, and T. H. Stevens.** 1994. Yeast Vps45p is a Sec1p-like protein required for the consumption of vacuole-targeted, post-Golgi transport vesicles. Eur. J. Cell Biol. **65:**308–318.
32. **Protopopov, V., B. Govindan, P. Novick, and J. E. Gerst.** 1993. Homologs of the synaptobrevin/VAMP family of synaptic vesicle proteins function on the late secretory pathway in *S. cerevisiae*. Cell **74:**855–861.
33. **Roberts, T. M., and M. Stewart.** 1995. Nematode sperm locomotion. Curr. Opin. Cell Biol. **7:**13–17.
34. **Rose, M. D., L. M. Mistra, and J. P. Vogel.** 1989. *KAR2*, a karyogamy gene, is the yeast homolog of the mammalian Bip/GRP78 gene. Cell **57:**1211–1221.
35. **Shamu, C. E., and P. Walter.** 1996. Oligomerization and phosphorylation of the Ire1p kinase during intracellular signaling from the endoplasmic reticulum to the nucleus. EMBO J. **15:**3028–3039.
36. **Sikorski, R. S., and P. Hieter.** 1989. A system of shuttle vectors and yeast host strains designed for efficient manipulation of DNA in *Saccharomyces cerevisiae*. Genetics **122:**19–27.
37. **Skehel, P. A., K. C. Martin, E. R. Kandel, and D. Bartsch.** 1995. A VAMP-binding protein from *Aplysia* required for neurotransmitter release. Science **269:**1580–1583.
38. **Söllner, T., S. W. Whiteheart, M. Brunner, H. Erdjument-Bromage, S. Geromanos, P. Tempst, and J. E. Rothman.** 1993. SNAP receptors implicated in vesicle targeting and fusion. Nature **362:**318–324.
39. **Sprague, G. F., Jr.** 1991. Assay of yeast mating reaction. Methods Enzymol. **194:**77–93.
40. **Theriot, J. A.** 1996. Worm sperm and advances in cell locomotion. Cell **84:**1–4.
41. **Tokunaga, M., A. Kawamura, and K. Kohno.** 1992. Purification and characterization of Bip/Kar2 protein from *Saccharomyces cerevisiae*. J. Biol. Chem. **267:**17553–17559.
42. **Wang, X., R. Sato, M. S. Brown, X. Hua, and J. L. Goldstein.** 1994. SREBP-1, a membrane-bound transcriptional factor released by sterol-regulated proteolysis. Cell **77:**53–62.
43. **Yokoyama, C., X. Wang, M. R. Briggs, A. Admon, J. Wu, X. Hua, J. L. Goldstein, and M. S. Brown.** 1993. SREBP-1, a basic-helix-loop-helix-leucine zipper protein that controls transcription of the low density lipoprotein receptor gene. Cell **75:**187–197.
44. **Zhang, L., C. L. Ashendel, G. W. Becker, and D. J. Moore.** 1994. Isolation and characterization of the principal ATPase associated with transitional endoplasmic reticulum of rat liver. J. Cell Biol. **127:**1871–1883.