Suppression of a *sec63* Mutation Identifies a Novel Component of the Yeast Endoplasmic Reticulum Translocation Apparatus

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Mutations in the *SEC63* gene are associated with defects in protein translocation into the endoplasmic reticulum (ER) as well as in nuclear protein localization in *Saccharomyces cerevisiae*. To identify proteins that might interact and/or function with SEC63p, we cloned a high copy suppressor (*HSS1*) of the temperature-sensitive lethal phenotype of the *sec63-101* mutant. *HSS1* is an allele-specific *sec63* suppressor that encodes an integral ER membrane glycoprotein of 206 amino acids with the N-terminus in the ER lumen and C-terminal region in the cytoplasm. Haploid strains disrupted for *HSS1* are temperature-sensitive for growth and accumulate precursor forms of Kar2p and invertase. The *HSS1* null allele is synthetically lethal in combination with mutations affecting ER translocation. We propose that HSS1p is important for ER translocation and interacts with previously identified components of the yeast translocation apparatus. *HSS1* is identical to *SEC66*, which encodes a glycoprotein complexed with SEC62p and SEC63p.

INTRODUCTION

Many factors involved in either targeting of proteins to the endoplasmic reticulum (ER) or translocation across the ER membrane have been characterized (for recent reviews, see Rapoport, 1992; Sanders and Schekman, 1992). Most proteins destined for translocation across the ER membrane are targeted to the ER by a characteristic apolar stretch of amino acids termed the signal sequence. In mammalian cells, the targeting is generally mediated by a ribonucleoprotein, signal recognition protein SRP, that binds signal sequences as they emerge from ribosomes (Walter and Blobel, 1980, 1982). These SRP-polypeptide-ribosome complexes are brought to the ER membrane by the interaction of SRP with membrane-bound SRP receptors (Gilmore *et al.*, 1982; Meyer *et al.*, 1982). In a GTP-dependent reaction, the ribosome-bound polypeptide chain dissociates from SRP (Connolly and Gilmore, 1986, 1989). The interaction of such ribosome-bound nascent chains with Sec61p, a proposed protein-conducting channel, suggests that SRP-targeted proteins may be transferred to the translocation

machinery at Sec61p (Görlich *et al.*, 1992). Other membrane and peripheral membrane proteins have been proposed to transiently bind nascent chains as they are translocated across the ER membrane (for a review, see Hartmann and Rapoport, 1992).

In yeast cells, proteins destined for ER translocation may also be targeted to the ER membrane by SRP. Genes encoding homologues of mammalian SRP or SRP receptor components have been identified (Felici *et al.*, 1989; Hann *et al.*, 1989, 1992; Amaya *et al.*, 1990; Ogg *et al.*, 1992; Stirling and Hewitt, 1992), and depletion of these homologs causes a translocation defect in vivo (Amaya and Nakano, 1991; Hann and Walter, 1991; Ogg *et al.*, 1992; Stirling and Hewitt, 1992). Mutations in four essential genes (*SEC61*, *SEC62*, *SEC63*, and *KAR2*) are associated with translocation defects (Deshaies and Schekman, 1987, 1989; Toyn *et al.*, 1988; Rothblatt *et al.*, 1989; Sadler *et al.*, 1989; Vogel *et al.*, 1990; Stirling *et al.*, 1992), and genetic and biochemical evidence indicates physical interactions between their protein products (Rothblatt *et al.*, 1989; Deshaies *et al.*, 1991; Scidmore, Okamura, and Rose, unpublished data). Sec61p, Sec62p, and Kar2p can be cross-linked to translocating polypeptide chains in yeast ER membranes (Müsch *et al.*, 1992; Sanders *et al.*, 1992), suggesting that they comprise components of a common translocation apparatus.

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Table 1. Yeast strains used in this study

Strain	Genotype	Source
W303	MAT α , ura3-1, leu2-3,112, his3-11,15, trp1-1, ade2-1, cyt1::HIS3	A. Tzagoloff (Columbia University, New York, NY)
PSY30	MAT α , ura3-1, leu2-3,112, his3-11,15, trp1-1, ade2-1, cyt1::HIS3, sec63-106	Nelson <i>et al.</i> , (1993)
PSY76	MAT α , ura3-1, leu2-3,112, his3-11,15, trp1-1, cyt1::HIS3, sec63-101	Nelson <i>et al.</i> , (1993)
PSY97	MAT α , ura3, leu2-3,112, his3, cyt1::HIS3, sec63-1	Nelson <i>et al.</i> , (1993)
PSY139	MAT α , ura3-52, leu2-3,112, trp1-1, ade2, sec61-2	Nelson <i>et al.</i> , (1993)
PSY140	MAT α , ura3-52, leu2-3,112, trp1-1, ade2-1, cyt1::HIS3, sec62-1	Nelson <i>et al.</i> , (1993)
PSY198	MAT α /MAT α , ura3-52/ura3-52, leu2-3,112/leu2-3,112, trp1/+, ade2-101/+, his3 Δ 200/his3 Δ 200	J. Broach (Princeton University, Princeton, NJ)
PSY199	MAT α , ura3, leu2-3,112, trp1, cyt1::HIS3, sec63-107	Nelson <i>et al.</i> , (1993)
PSY202	MAT α , ura1, his4, trp1, ade1, ade2, lys2, sec63-108	Nelson <i>et al.</i> , (1993)
PSY393	MAT α /MAT α , ura3-52/ura3-52, leu2-3,112/leu2-3,112, trp1/+, ade2-101/+, his3 Δ 200/his3 Δ 200, hss1::LEU2/+	This study
PSY394	MAT α , ura3-52, leu2-3,112, ade2-101, his3 Δ 200	This study
PSY395	MAT α , ura3-52, leu2-3,112, ade2-101, his3 Δ 200, hss1::LEU2	This study
PSY396	MAT α , ura3-52, leu2-3,112, trp1, his3 Δ 200, hss1::LEU2	This study
PSY397	MAT α , ura3-52, leu2-3,112, trp1, his3 Δ 200	This study
CSY128	MAT α , ura3-52, leu2-3,112, ade2, trp1-1, his3, sec65-1	Stirling <i>et al.</i> , (1992)
MBY9-3B	MAT α , ura3-52, leu2-3,112, trp1-289, sec59-1	R. Schekman (University of California, Berkeley)
MS176	MAT α , ura3-52, kar2-159	Rose <i>et al.</i> , (1990)

The *SEC63* gene encodes an ER membrane protein with three hydrophobic regions (Sadler *et al.*, 1989; Feldheim *et al.*, 1992). The C-terminal two-thirds of Sec63p is located in the cytoplasm, whereas the region between the second and third hydrophobic regions is located in the ER lumen (Feldheim *et al.*, 1992; Kurihara and Silver, 1992). Several *sec63* mutations have been characterized, and they map to topologically distinct regions of Sec63p (Nelson *et al.*, 1993). Four *sec63* mutations (*sec63-101*, *-106*, *-107*, *-108*) map to the cytoplasmic region of Sec63p, whereas the *sec63-1* mutation maps to the ER luminal region. All of the mutations result in single amino acid changes.

Sec63p can form a complex with Sec61p, Sec62p, a 23-kDa protein (p23), and a 31.5-kDa glycoprotein (gp31.5), suggesting interactions between these proteins may be important for their function (Deshaies *et al.*, 1991). The effect of *sec62* and *sec63* mutations on the cross-linking of Sec61p to nascent chains supports this idea (Sanders *et al.*, 1992). Genetic interactions have been detected between *sec63* and *kar2* mutants (Scidmore, Okamura, and Rose, unpublished data), suggesting that Sec63p also interacts with Kar2p. If the effect of *sec63* mutations were to weaken such interactions, then overexpressing an interacting protein might suppress the *sec63* phenotype. Overexpression of a protein might also suppress the *sec63* phenotype if the overexpressed protein had similar cellular functions as Sec63p or the Sec63p complex. We have identified such a protein that suppresses *sec63-101* when overexpressed. We have cloned the corresponding gene and show that it is a novel gene important for ER translocation.

MATERIALS AND METHODS

Yeast Methods and Strains

Yeast growth media was prepared, and genomic DNA was prepared as described in Rose *et al.* (1990). Lithium acetate transformation of yeast cells was performed as in Ito *et al.* (1983). The genotypes of the yeast strains used in this study are listed in Table 1. To construct PSY393 (*HSS1*/ Δ *hss1*), PSY198 (*HSS1*/*HSS1*) was transformed with the products of a complete *Bam*HI/*Hind*III digestion of plasmid pTK108. PSY393 is a stable Leu⁺ transformant of PSY198. For double mutant analyses, auxotrophic selections were used to identify diploids generated by crosses. For crosses involving Δ *hss1* and W303, *sec61-2*, *sec62-1*, *sec63-1*, or *sec63-101*, Leu⁺His⁺ diploids were selected. *sec65-1*, *kar2-159*, and *sec59-1* were first transformed with the Ura⁺ yeast vector YCp50 (Rose *et al.*, 1987), then crossed to Δ *hss1* and Leu⁺Ura⁺ diploids selected.

Plasmids

Plasmids were constructed using standard techniques as in Sambrook *et al.* (1989). The vectors YEp213 (Broach *et al.*, 1979), YCp50 (Rose *et al.*, 1987), and YEp352 (Hill *et al.*, 1986) were used for cloning and as controls. Plasmids pCS15 (CEN, LEU2, SEC61) and pRD15 (CEN, LEU2, SEC62) were the generous gift of Randy Schekman (University of California, Berkeley). The plasmids pTK1 (2 μ , LEU2, SEC63) and pMR397 (CEN, URA3, KAR2) have been described previously (Rose *et al.*, 1989; Sadler *et al.*, 1989). pHSS20 corresponds to the *sec63-101* suppressing plasmid from the multicopy LEU2-based yeast genomic library of Nasmyth and Tatchell (1980). pHSS20 contains an $\approx$ 8-kilobase (kb) genomic fragment inserted into the *Bam*HI site of YEp13. Plasmids pTK100–pTK103 were constructed by cloning fragments containing the pHSS20 genomic insert into YEp213. pTK100 represents a 4-kb *Hind*III fragment inserted into the *Hind*III site of YEp213. pTK101 and pTK103 were constructed by cloning 2.8-kb and 2.5-kb *Bam*HI fragments into the *Bam*HI site of YEp213. pTK102 represents a 3-kb *Hind*III/Sph I fragment inserted into the *Hind*III/Sph I sites of YEp213. pTK104, pTK107, and pTK112 were constructed by cloning a 1.3-kb *Bam*HI/*Hind*III fragment of pHSS20 into the *Bam*HI/*Hind*III sites of YEp213, pBluescript KS+ (Stratagene, La Jolla, CA), and

YEp352, respectively. pTK106 and pTK109 contain a 1-kb *Sph* I (10-mer at *EcoRV* site)/*Hind*III fragment of pHSS20 into the *Sph* I/*Hind*III sites of YEp213 and YCp50, respectively. pTK108 was constructed by inserting the *LEU2* gene as a 1.5-kb *Ssp* I fragment from pJJ250 (Jones and Prakash, 1990) into the filled-in *EcoRI*/*Mun* I sites of pTK107.

Site-directed Mutagenesis and myc-tag Construction

Site-directed mutagenesis was performed according to the Altered Sites system (Promega, Madison, WI). The 1.3-kb *Bam*HI/*Hind*III fragment of pTK107 was inserted into the *Bam*HI/*Hind*III sites of pAlter (Promega) to create pTK111. The primer 5'-GATGGAAGAC-TAGTCAATTAG-3' was used to create a *Spe* I site near the C-terminus of the *HSS1* ORF (pTK114). pTK115 was constructed by *Spe* I digestion of pTK114 followed by insertion of the *myc*-encoding fragment

5'-CTAGCCGAGCAGAAGCTGATT-

AGCGAGGAAGATCTGAAT-3'

3'-GGCTCGTCTTCGACTAATCGCT-

CCTTCTAGACTTAGATC-5'.

pTK119 and pTK121 represent the *HSS1*-*myc*-containing *Bam*HI/*Hind*III fragment of pTK115 cloned into the *Bam*HI/*Hind*III sites of YCp50 and YEp352, respectively. To construct the glycosylation site mutant pTK122, the primer 5'-CTATATAAAAGGGCTCTATTGGG-3' was used to change an asparagine to a lysine at amino acid 123.

DNA Sequencing and Polymerase Chain Reaction (PCR)

DNA sequence was determined by using the dideoxy-chain termination method and Sequenase (United States Biochemical, Cleveland, OH). The 1.3-kb *Bam*HI/*Hind*III insert of pTK107 or fragments of the 1.3-kb inserts cloned into pBluescript KS+ were sequenced using either the reverse or -20 pBluescript primers. Genomic DNA was amplified by PCR according to standard procedures (Saiki, 1990) using the pair of primers TK23 (5'-GACGAATTCCTTGCTGTCAATAC-3') and TK24 (5'-TGGGAATCCAAGTAGTGAGCAAG-3') flanking the *Bam*HI and *Hind*III sites, respectively, of pTK107. TK23 corresponds to sequences (-28 to -5) relative to the *Bam*HI site (+1), and TK24 corresponds to sequences (+1393 to +1370) relative to the *Hind*III site (+1342).

Western Blotting

For analysis of Kar2p, whole cell extracts were prepared by glass bead lysis in sodium dodecyl sulphate (SDS) sample buffer (10% glycerol, 1% β -mercaptoethanol, 2% SDS, 65 mM Tris(hydroxymethyl)-aminomethane [Tris]-HCl, pH 6.8, 5 μ g/mL bromophenol blue). After boiling for 2 min, cell debris and glass beads were removed by centrifugation and extracts from 0.5 OD equivalents of cells were subjected to SDS-polyacrylamide gel electrophoresis (PAGE). Electrophoresed proteins were transferred to nitrocellulose paper (Schleicher and Schuell, Keene, NH) in transfer buffer (25 mM Tris base, 192 mM glycine). Blots were preincubated in phosphate-buffered saline (PBS)/0.1% Tween-20 containing 5% nonfat dried milk (Carnation, Los Angeles, CA) and then incubated for 30 min at room temperature with a 1:10 000 dilution of anti-Kar2 (Mark Rose, Princeton University, Princeton, NJ). After washing with PBS, blots were incubated for 1 h at room temperature with a 1:5000 dilution of horseradish peroxidase (HRP)-conjugated anti-rabbit IgG (Promega) and developed by enhanced chemiluminescence (Amersham International, Arlington Heights, IL).

For analysis of *Hss1*-*myc* protein, extracts were prepared from cells at OD600 = 0.5 by glass bead lysis in solubilization buffer (10 mM Tris-HCl, pH 6.6, 1% SDS, 2% β -mercaptoethanol). Lysates were boiled for 5 min, and soluble extract was collected after centrifugation.

For tunicamycin-treated cells, 10 μ g/mL tunicamycin was added directly to growth medium for 2 h before harvesting cells. For endoH-treated samples (Sigma, St. Louis, MO), extracts from 0.5 OD equivalent of cells were incubated overnight at 37°C with an equivalent volume of endoH buffer (50 mM sodium citrate, pH 5.5) containing 0.004 units endoH. Electrophoresed proteins were transferred to nitrocellulose in transfer buffer (25 mM Tris, 192 mM glycine, 0.01% SDS, 20% methanol). Blots were developed as for Kar2p except primary incubation was performed at 4°C overnight with anti-*myc* monoclonal antibody 9E10 (1:50 dilution) followed by HRP-conjugated anti-mouse IgG (Promega).

Immunoprecipitations

Immunoprecipitation of invertase was performed essentially as described in Rothblatt and Schekman (1989), using the identical lysis, immunoprecipitation, and wash buffers. Cells were grown overnight at 23°C in SD-met + 5% glucose to OD600 = 0.5 then harvested and resuspended in SD-met + 0.1% glucose for 20 min to induce secretory invertase. Two OD equivalents of cells were labeled with 60 μ Ci Tran[³⁵S]Label (ICN Radiochemicals, Irvine, CA) either for 20 min at 23°C or for 20 min at 37°C after a 30-min preshift from 23 to 37°C. For tunicamycin-treated samples, tunicamycin was added (10 μ g/mL) to cultures 15 min before labeling. Labeled extracts were incubated overnight at 4°C with 3 μ L anti-invertase antibody (Mark Rose, Princeton University, Princeton, NJ) and then incubated with Protein A-Sepharose (Pharmacia LKB, Uppsala, Sweden) for 2 h at room temperature. The precipitated proteins were electrophoresed on a 7.5% SDS gel, and the gel was dried and exposed to XAR5 film (Eastman Kodak, Rochester, NY) to visualize labeled proteins.

Immunofluorescence

Five milliliters of cells were grown to OD600 = 0.5 at 30°C. 0.6 mL of 37% formaldehyde was directly added to the cultures and the cells fixed for 2 h at 30°C. The fixed cells were harvested, washed twice in solution P (1.1 M sorbitol, 0.1 M potassium phosphate, pH 7.5), then resuspended in 1 mL solution P with 5 μ L of glucylase (Dupont, Wilmington, DE) and 5 μ L of 1 mg/mL zymolyase-100 (ICN, Costa Mesa, CA). After a 1-h incubation at 30°C, cells were harvested, washed once in solution P, and resuspended in solution P. Cells were attached to multiwell slides treated with 0.1% poly-L-lysine (Sigma) then permeabilized by 6 min in ice-cold methanol and 30 s in ice-cold acetone. The permeabilized cells were incubated overnight at room temperature with anti-*myc* monoclonal antibody 9E10 diluted 1:4 dilution in incubation buffer (Solution P with 2 mg/mL bovine albumin). After three washes with Solution P, cells were incubated for 2 h at room temperature with a 1:500 dilution of fluorescein isothiocyanate (FITC)-conjugated anti-mouse IgG (Jackson ImmunoResearch Laboratories, West Grove, PA) in incubation buffer. After three washes with Solution P, cells were briefly treated with 1 μ g/mL 4',6-diamidino-2-phenyl-indole (DAPI, Sigma) then washed twice more with Solution P. Coverslips were mounted with PBS/90% glycerol containing 1 mg/mL p-phenylenediamine, and the slides visualized with a Zeiss Axioskop fluorescence microscope (Thornwood, NY).

RESULTS

To identify proteins that may interact with or function in the same cellular processes as SEC63p, we isolated a high-copy suppressor of the *sec63-101* (originally *np11-1*, see Nelson *et al.*, 1993) mutant. The *sec63-101* allele was chosen from among five *sec63* mutants (*sec63-1*, -101, -106, -107, -108) for two reasons. First, *sec63-101* has a tight temperature-sensitive lethal growth phe-

Figure 1. Identification of a high-copy suppressor (*HSS1*) of *sec63-101*. (A) Plasmids containing *HSS1* region of yeast genome were tested for suppression of the *sec63-101* ts⁻ defect. pHSS20 contains an 8-kb genomic fragment in the YEp13 *Bam*HI site. pTK plasmids contain fragments of the 8-kb pHSS20 genomic insert in YEp213. pTK104 contains a 1.3-kb *Bam*HI-*Hind*III fragment. Restriction enzyme sites: B, *Bam*HI; H, *Hind*III. + indicates growth at 36°C. (B) *HSS1*-containing plasmids were tested for suppression of the ts⁻ defects of various translocation mutants. Vector is YEp213 and *HSS1* plasmid is pTK106 for all mutants except *kar2-159*. Vector and *HSS1* plasmid are YCp50 and pTK112, respectively, for *kar2-159*. Wild-type plasmid is pTK1 for *sec63* alleles, pRD15 for *sec61-2*, pCS15 for *sec62-1*, and pMR397 for *kar2-159*. Yeast transformants were selected at 23 or 30°C and then streaked at 36°C. Plates depicting growth at 36°C are shown.

notype at 36°C (Sadler *et al.*, 1989). Second, the mutation in *sec63-101* has been mapped and sequenced (Nelson *et al.*, 1993).

Specific Suppression of *sec63-101* by *HSS1*

A high-copy suppressor of the 36°C temperature-sensitive growth phenotype of *sec63-101* (*HSS1*) was cloned

BamHI
 1 GGATCCGATTTCACATTGACACCATCTTTCTCATTAGCGGGCTTGTCCGAATAGTTAAGGATCAGCATGTCCCGTGCTTTAGTCTAGATCCATCACTGTTTCGATCAGCTAGTTTCAGA
 121 AACAGCATGAATACCTTGACCGGGCTTCTCACAACAGTAAATGTGTCGACATCGGCATTGGGGTCCGAGATTACCCACCAACTTTTCAATGACCGTTCCGAAAAGTCGTTTCTTGACA
 241 AGAAACCTGTGTGTACCGTTTTTGTATCTAAATCTGATAAGCATACTTCACTTAAATGTATATCGATATCAGTAGTATAGGGAAATTTTCTTCAGAGTACTGTCTATTATTGGCCAC
 361 TCTTCGTTCTGTATGTTACGAGGGCGTTCCTTAAATGGGTAGACGCATCTTATTACCCGCCAAAAACGTCAAAAGTTTATAGAACACGCTCTAAAAGTTGAAATAATATGTGAAAAAT
 481 TGATGAAATATTAATGAAATGGCTTATTAAACGAATTCAGTACAGGAAAGAGGTACGCACAACACTTGTAGTTTGCCAATATGTCCGAATTTAATGAAACAAAATTCCTCAACAACGG
 1 MetSerGluPheAsnGluThrLysPheSerAsnAsnGly
 601 GACGTTTTTTGAAACGGAAGAGCCAATTGTGGAGACGAAATCAATCTCGTTTATACCCCACTCATATATGTCTTTATTCTGGTGGTTCCTTGTGATGTTGCTTCAAGCTACAGAAA
 14 ThrPhePheGluThrGluGluProIleValGluThrLysSerIleSerValTyrThrProIleTyrValPheIleLeuValValSerLeuValMetPheIleSerSerTyrArgLys
 721 GAAGCAGGCCAAAAAATTAGTGAGCAACCATCCATATTGACGAAAAAGATGCCATGATCTGTATTTCAAAATAAGGAAATGAGTGAAAAATGAAAAAATTCACGAGAAGGTGTGAA
 54 LysGlnAlaLysLysIleSerGluGlnProSerIlePheAspGluAsnAspAlaHisAspLeuTyrPheGlnIleLysGluMetSerGluAsnGluLysIleHisGluLysValLeuLys
 841 GGCGCTTTATTGAACAGAGGAGCAGAATCTGTAGACGATCATTAAAGTTAAAGAGTTGGCTCCTCAGATAAACCTTCTATATAAAAAATGGCTCTATTGGGGAGGATTACTGGAAGAG
 94 AlaAlaLeuLeuAsnArgGlyAlaGluSerValArgArgSerLeuLysLeuLysGluLeuAlaProGlnIleAsnLeuLeuTyrLysAsnGlySerIleGlyGluAspTyrTrpLysArg
 961 ATTTGAACTGAAGTTAAATTAATGAATTTGAAATTTAAAGATCTTTACAAGAAGCTGAAAGATTGCAACCGGGCTGGGTTCAATTGTTGCTTATGGTTTGAAGAAATTTGCTTTAA
 134 PheGluThrGluValLysLeuIleGluLeuGluPheLysAspThrLeuGlnGluAlaGluArgLeuGlnProGlyTrpValGlnLeuPheValMetValCysLysGluIleCysPheAsn
 1081 TCAAGCTCTCTTAGACGTTATCAATCAATCTTGAACGGAAAGAAGTGTGTTAAAGAGTGGGAGCTGAAAAATAAATATGATGGAAGATTAGTCAATTAGTGCTACTGTGTGCAAA
 174 GlnAlaLeuSerArgArgTyrGlnSerIleLeuLysArgLysGluValCysIleLysGluTrpGlnLeuLysIleAsnAsnAspGlyArgLeuValAsnEnd
 1201 GATATGTATTCGCTCGTTCAGTGTTTTTTTAAAAATATGTATAGAATTGTCTATTCTCGCTTAAAAATAGTTATAAGTATATACAATAACAATAAATGATAAAGAAATATGCAGTG
 HindIII
 1321 AAAAGAAAAAATTATGAAGCTT 1342
 XbaI

Figure 2. The sequence of *HSS1* corresponding to the 1342-bp insert in pTK104 that suppresses the *ts⁻* defect of *sec63-101*. The restriction enzyme sites indicated over the nucleotide sequence were used for plasmid constructions. The bold-type nucleotides correspond to those changed by site-directed mutagenesis. The deduced amino acid sequence of *HSS1p* is shown in bold type below the nucleotide sequence. The single and double lines under the amino acid sequence denote potential N-linked glycosylation sites and a hydrophobic region, respectively. The 252-bp region between the *Xba* I and *Hind*III sites is identical to the 3' untranslated region of the yeast *SMY2* gene (Genbank release 72.0). The *HSS1* sequence has been submitted to the EMBL database under the accession number X72224.

from the *LEU2*-based multicopy yeast genomic library of Nasmyth and Tatchell (1980). Six thousand *Leu⁺* *sec63-101* transformants representing approximately three genome equivalents were selected at 30°C, and the resulting transformants were tested for growth at 36°C by replica printing. Three colonies grew at 36°C, and plasmids isolated from all three colonies conferred 36°C temperature-resistance when reintroduced into *sec63-101*. Restriction enzyme mapping indicated that two of the three plasmids contained *SEC63* within their inserts. Thus, one plasmid (pHSS20, Figure 1A) that contains *HSS1*, a high copy suppressor of *sec63-101* was cloned. Several overlapping fragments of the 8-kb genomic insert of pHSS20 were cloned into the 2 μ -based YEp213 vector, and the suppressing ability of these subclones was analyzed (Figure 1A). A 1.3-kb *Bam*HI-*Hind*III fragment containing *HSS1* (pTK104) was sufficient to confer temperature-resistance to *sec63-101*. *HSS1* does not suppress the temperature-sensitivity of all *sec63* mutations but is specific for the *sec63-101* allele (Figure 1B). *HSS1* also does not suppress the temperature-sensitive phenotype of other ER translocation mutants (Figure 1B), such as *sec61-2*, *sec62-1*, and *kar2-159*, or of *np14-1* (Bossie *et al.*, 1992), another nuclear localization mutant.

Primary Structure of *HSS1p*

The primary structure of *HSS1p* was deduced from sequencing the 1.3-kb *Bam*HI-*Hind*III fragment of pTK104 (Figure 1A). The 1342-base pair (bp) DNA sequence contains one complete open reading frame (563–1180 bp, Figure 2) that would encode a 206 amino acid protein. A 1-kb *Eco*RV-*Hind*III subclone containing this open reading frame fully suppressed the temperature-sensitivity of *sec63-101* (Figure 1B), confirming that this open reading frame is encoded by *HSS1*. Comparison of the 1342-bp sequence with Genbank (Release 72.0) revealed that the 252-bp region between the *Xba* I and *Hind*III sites is identical to the 3' untranslated region of the yeast *SMY2* gene. An open reading frame starting at bp 283 and extending beyond the *Bam*HI site corresponds to the amino-terminus of NPL4p (DeHoratius and Silver, personal communication). Because *np14* has been mapped to Chromosome II (Bossie *et al.*, 1992), we conclude that *HSS1* also lies on Chromosome II. The deduced amino acid sequence of *HSS1p* reveals a single hydrophobic region between amino acids 34–48 as well as three potential N-linked glycosylation sites (Figure 2). The amino acid sequence shares no homology with any database sequences.

Figure 3. $\Delta hss1$ strains are temperature-sensitive for growth at 37°C. (A) Construction of $hss1$ null allele. pTK107 contains the 1.3-kb BamHI-HindIII insert of pTK104 cloned in pBlueScript KS+. The Hss1p coding region is indicated by a black box. pTK108 was constructed from pTK107 by inserting the *LEU2* gene (▨) between the EcoRI and MunI sites. *LEU2* replaces the HSS1 promoter region and 161 N-terminal amino acids of Hss1p. TK23 and TK24 represent PCR primers flanking the BamHI and HindIII sites. (B) The 2.3-kb BamHI-HindIII fragment from pTK108 was used to transform diploid strain PSY198 to generate the *Leu*⁺ diploid strain PSY393. Five PSY393 tetrads were dissected, and four viable haploid progeny were obtained for each tetrad at 30°C. Primers TK23 and TK24 were used to amplify genomic DNA from one such tetrad (PSY394-PSY397) to confirm proper integration and 2:2 segregation of $hss1$ disruption. As indicated, 1.3-kb and 2.3-kb PCR products are amplified from *HSS1* and $hss1::LEU2$ strains, respectively. (C) $\Delta hss1$ strains are temperature-sensitive for growth at 37°C. Numbers depicted on plates correspond to strain numbers in B.

HSS1p Is Required for Growth at Elevated Temperatures

Targeted gene disruption of *HSS1* was used to determine if HSS1p is required for cell viability. An $hss1::LEU2$ null allele was created by replacing the HSS1 N-terminal coding region (161 amino acids) with a *LEU2* gene fragment in a bacterial vector (pTK108, Figure 3A). Targeted integration of the $hss1::LEU2$ allele was performed in the diploid strain PSY198 (*leu2/leu2*), and stable *Leu*⁺ integrants sporulated at 30°C. Four viable spores were

recovered from each of nine complete tetrads, and proper integration of *LEU2* at *HSS1* was confirmed by PCR for one such tetrad (Figure 3B). When replica plated at 14 and 23°C, all spores grew, but when replica plated at 37°C, only two *Leu*⁺ spores from each tetrad grew, indicating that $hss1::LEU2$ strains are temperature-sensitive for growth (Figure 3C). This temperature-sensitivity is because of disruption of *HSS1* and not due to disruption of a neighboring gene, because *HSS1* on a centromeric plasmid (pTK109) rescued the 37°C growth defect of $hss1::LEU2$ (Figure 5). Thus, HSS1p is not required for cell viability but is essential for growth at elevated temperatures.

Secretory Precursors Accumulate in $\Delta hss1$ Cells

Because *HSS1* was cloned as a high-copy suppressor of *sec63-101*, we considered if cells lacking *HSS1* ($\Delta hss1$ SEC63) show similar defects to *sec63* mutants. *Sec63* mutants are temperature-sensitive for growth like $\Delta hss1$ strains and are defective to varying degrees both in ER protein translocation and in nuclear protein localization (Toyn *et al.*, 1988; Rothblatt *et al.*, 1989; Sadler *et al.*, 1989).

$\Delta hss1$ strains were assayed for precursor accumulation of two proteins, Kar2p and invertase (Figure 4). Kar2p

Figure 4. Secretory precursor accumulation in $\Delta hss1$ cells. (A) Kar2 was detected in whole cell extracts of indicated strains grown at 30°C by Western blotting as described in MATERIALS AND METHODS. The positions of pre-Kar2 and mature Kar2 are indicated. (B) Invertase was immunoprecipitated from PSY396 ($\Delta hss1$) and PSY397 (*HSS1*) cells pulse-labeled with [³⁵S]methionine as described in MATERIALS AND METHODS. Cells were grown and labeled at 23°C or shifted from 23 to 37°C for 30 min before labeling at 37°C. Where indicated, cells were treated with 10 μg/mL tunicamycin (TM) for 15 min before labeling. Proteins precipitated by anti-invertase antibodies are displayed by SDS-PAGE. The positions of cytoplasmic (cytolnv), precursor (prelnv), ER forms (glnv[ER]), and Golgi forms (glnv[Sec]) of invertase are indicated. Cytoplasmic invertase was not efficiently precipitated.

Table 2. Synthetic lethal combinations of $\Delta hss1$ with translocation mutations

Cross	(Live:Dead) Tetrads Picked ^a	Genotype of spores ^b
$\Delta hss1 \times W303$	(4:0) 18	2 $\Delta hss1$, 2 HSS1
$\Delta hss1 \times sec61-2$	(4:0) 4	2 $\Delta hss1$ SEC61, 2 HSS1 <i>sec61-2</i>
	(3:1) 11	1 HSS1 SEC61, 1 $\Delta hss1$ SEC61, 1 HSS1 <i>sec61-2</i> , 1 $\Delta hss1$ <i>sec61-2</i> ^c
	(2:2) 5	2 HSS1 SEC61, 2 $\Delta hss1$ <i>sec61-2</i> ^c
$\Delta hss1 \times sec62-1$	(4:0) 3	2 $\Delta hss1$ SEC62, 2 HSS1 <i>sec62-1</i>
	(3:1) 11	1 HSS1 SEC62, 1 $\Delta hss1$ SEC62, 1 HSS1 <i>sec62-1</i> , 1 $\Delta hss1$ <i>sec62-1</i> ^c
	(2:2) 1	2 HSS1 SEC62, 2 $\Delta hss1$ <i>sec62-1</i> ^c
$\Delta hss1 \times sec63-1$	(4:0) 3	2 $\Delta hss1$ SEC63, 2 HSS1 <i>sec63-1</i>
	(3:1) 13	1 HSS1 SEC63, 1 $\Delta hss1$ SEC63, 1 HSS1 <i>sec63-1</i> , 1 $\Delta hss1$ <i>sec63-1</i> ^c
	(2:2) 2	2 HSS1 SEC63, 2 $\Delta hss1$ <i>sec63-1</i> ^c
$\Delta hss1 \times sec63-101$	(4:0) 4	2 $\Delta hss1$ SEC63, 2 HSS1 <i>sec63-101</i>
	(3:1) 16	1 HSS1 SEC63, 1 $\Delta hss1$ SEC63, 1 HSS1 <i>sec63-101</i> , 1 $\Delta hss1$ <i>sec63-101</i> ^c
	(2:2) 5	2 HSS1 SEC63, 2 $\Delta hss1$ <i>sec63-101</i> ^c
$\Delta hss1 \times sec65-1$	(4:0) 16	2 $\Delta hss1$ SEC65, 2 HSS1 <i>sec65-1</i>
	(3:1) 12	1 HSS1 SEC65, 1 $\Delta hss1$ SEC65, 1 HSS1 <i>sec65-1</i> , 1 $\Delta hss1$ <i>sec65-1</i> ^d
	(2:2) 5	2 HSS1 SEC65, 2 $\Delta hss1$ <i>sec65-1</i> ^d
$\Delta hss1 \times kar2-159$	(4:0) 2	2 $\Delta hss1$ KAR2, 2 HSS1 <i>kar2-159</i>
	(4:0) 16	1 HSS1 KAR2, 1 $\Delta hss1$ KAR2, 1 HSS1 <i>kar2-159</i> , 1 $\Delta hss1$ <i>kar2-159</i> ^d
	(4:0) 2	2 HSS1 KAR2, 2 $\Delta hss1$ <i>kar2-159</i> ^d
$\Delta hss1 \times sec59-1$	(4:0) 2	2 $\Delta hss1$ SEC59, 2 HSS1 <i>sec59-1</i>
	(4:0) 11	1 HSS1 SEC59, 1 $\Delta hss1$ SEC59, 1 HSS1 <i>sec59-1</i> , 1 $\Delta hss1$ <i>sec59-1</i>
	(4:0) 4	2 HSS1 SEC59, 2 $\Delta hss1$ <i>sec59-1</i>

Crosses were performed and diploids selected as in MATERIALS AND METHODS.

^a Number of tetrads picked with corresponding (Live:Dead) spore segregation at 23°C.

^b Genotypes were determined by segregation of LEU2 and ts markers. For each double mutant cross, three rows of genotype combinations are shown. Top to bottom, they represent parental ditype, tetratype, and nonparental ditype tetrads.

^c Spores inviable at 23°C.

^d Spores inviable at 30°C, viable at 23°C.

is a soluble ER luminal protein synthesized in a precursor form (pre-Kar2) with a cleavable N-terminal signal sequence (Rose *et al.*, 1989). The mature form of Kar2p migrates as a 76-kDa species, whereas pre-Kar2 migrates as a 78-kDa species. In *sec63-1* cells, pre-Kar2 accumulation is seen at 23, 30°C, and after a 2-h shift from 23 to 37°C (Figure 4A, lane 1). In $\Delta hss1$ strains, pre-Kar2 was detected in cells grown at 23°C, after a 2-h shift from 23 to 37°C and at 30°C (Figure 4A, lanes 3 and 4). Only mature Kar2p was detected in HSS1⁺ cells grown at 30°C (Figure 4A, lanes 2 and 5).

Invertase is synthesized in two forms, a cytoplasmic form (61 kDa) and a secretory form (63 kDa), which differs from the cytoplasmic form by the inclusion of an N-terminal signal sequence (Carlson and Botstein, 1982). After signal sequence cleavage, the secretory invertase is glycosylated at multiple sites in the ER and undergoes further modification in the Golgi before being secreted (Esmon *et al.*, 1981). In HSS1⁺ cells pulse-labelled with ³⁵S-methionine either at 23°C or after a 30-min shift to 37°C, the ER and Golgi forms of invertase were detected (Figure 4B, lanes 1 and 2). Similarly, at 23°C both the ER and Golgi forms of invertase were detected in $\Delta hss1$ cells (Figure 4B, lane 3). In cells grown at 23°C and treated with the glycosylation inhibitor tunicamycin (Figure 4B, lane 4) (Olden *et al.*, 1979), the

unmodified ER form of invertase was detected that co-migrates with the cytoplasmic form of invertase. After a 30-min shift from 23 to 37°C, the ER form and precursor form of invertase that migrated with slightly lower mobility than the cytoplasmic form were detected in $\Delta hss1$ cells (Figure 4B, lane 5).

Because *sec63* mutants accumulate normally nuclear proteins in the cytoplasm (Sadler *et al.*, 1989), we determined if $\Delta hss1$ cells also are defective in nuclear protein localization. Judging by indirect immunofluorescence using antibodies directed against Np13p, a normally nuclear protein (Bossie *et al.*, 1992), no cytoplasmic accumulation of Np13p was seen in $\Delta hss1$ cells either grown at 30°C or preshifted for 4 h from 23 to 36°C. Thus, at least for one endogenous nuclear protein, $\Delta hss1$ cells are not defective in nuclear localization.

Genetic Interactions Between $\Delta hss1$ and Previously Identified Translocation Mutants

Genetic interactions between HSS1 and other genes known to be involved in ER protein translocation (SEC61, SEC62, SEC63, SEC65, KAR2) were analyzed by constructing double mutant combinations ($\Delta hss1$ *sec* or $\Delta hss1$ *kar2*). The inviability or reduced viability of double mutants (synthetic lethality) can indicate proteins

HSS1 C-terminus
(pTK109, pTK112)

aa200 - DGRLVN - aa206

HSS1-myc C-terminus
(pTK119, pTK121, pTK122)

aa200 - DGRLAEQKLISEEDLNLVN - aa219
9E10 epitope

Figure 5. A *myc*-tag was introduced at the C-terminus of Hss1p as described in MATERIALS AND METHODS. The C-terminal amino acids of HSS1p and HSS1-*myc* are shown. The *myc* sequence recognized by anti-*myc* monoclonal antibody (mAb) 9E10 is underlined. Plasmids expressing HSS1p (pTK109, pTK112) or HSS1-*myc* (pTK119, pTK121, pTK122) were transformed into PSY396 ($\Delta hss1$) and *sec63-101*, and transformants were analyzed for growth at indicated temperatures. pTK122 is a mutant derivative of pTK121 as described in Figure 7.

that interact and/or perform similar functions (Huffaker *et al.*, 1987) and has previously been shown for combinations of translocation mutants (Rothblatt *et al.*, 1989; Scidmore, Okarmura, and Rose, unpublished data). Haploid strains with *sec61-2*, *sec62-1*, *sec63-1*, *sec63-101*, *sec65-1*, or *kar2-159* mutations, all of which are viable at 23°C, were mated to $\Delta hss1$ strains of the opposite mating type. The resulting diploids were sporulated at 23°C, and the genotype of the resulting spores was determined by scoring *Leu*⁺ phenotype at 23°C (because of *hss1::LEU2*) and/or by scoring temperature-sensitivity at 37°C (because of $\Delta hss1$, *sec*, or *kar2* mutations). The tetatype:parental ditype:nonparental ditype distribution of progeny for each cross was also determined, on the basis of the spore genotypes within each tetrad.

Synthetic lethal combinations at 23°C were apparent between $\Delta hss1$ and *sec61-2*, *sec62-1*, *sec63-1*, or *sec63-101*, judging by spore genotype and approximate 4:1:1 distribution of tetatype:parental ditype:nonparental ditype tetrads within each cross (Table 2). Synthetic lethality was also observed between $\Delta hss1$ *kar2-159* and $\Delta hss1$ *sec65-1* combinations at 30°C ($\Delta hss1$ *KAR2*, *HSS1* *kar2-159*, $\Delta hss1$ *SEC65*, and *HSS1* *sec65-1* cells can all grow at 30°C).

Because $\Delta hss1$ was synthetically lethal in combination with all tested ER mutations affecting translocation (*sec61-2*, *sec62-1*, *sec63-1*, *sec63-101*, *kar2-159*), we determined if $\Delta hss1$ is synthetically lethal with any early secretory mutation affecting the ER. We found that $\Delta hss1$ is not synthetically lethal in combination with

sec59-1, an early secretory mutation affecting glycosylation (Berstein *et al.*, 1989), as $\Delta hss1$ *sec59-1* cells grew at both 23 and 30°C (Table 2). Thus, *HSS1* genetically interacts with genes involved in ER translocation but not generally with genes involved in early stages of secretion.

HSS1p Localizes to the ER

To further characterize HSS1p, a *myc*-tagged version of HSS1p was constructed. The *c-myc* epitope EQKLI-SEEDLN, which is recognized by the monoclonal antibody 9E10 (Evan *et al.*, 1985), was inserted at the C-terminus of the HSS1p open reading frame such that the predicted size of HSS1-*myc* would be 219 amino acids (Figure 5). HSS1-*myc* was judged to be fully functional by two criteria. First, when expressed from its own promoter on a single copy plasmid (pTK119), it rescued the temperature-sensitive growth phenotype of PSY396 ($\Delta hss1$) (Figure 5). Second, when expressed from its own promoter on a high copy number plasmid (pTK121), it fully suppressed the temperature-sensitive growth phenotype of *sec63-101* (Figure 5).

The intracellular localization of HSS1-*myc* was determined by indirect immunofluorescence using 9E10 anti-*myc* antibody. When HSS1-*myc* was expressed from its own promoter in a $\Delta hss1$ strain (PSY396), a mostly perinuclear *myc* staining pattern was seen (Figure 6) similar to that seen for ER proteins such as Kar2p and Sec63p (Rose *et al.*, 1989; Feldheim *et al.*, 1992; Kurihara

Figure 6. Immunofluorescence localization of HSS1-myc. PSY396 ($\Delta hss1$) was transformed with pTK119 (YCp50-HSS1-myc), and the transformants were prepared for immunofluorescence as described in MATERIALS AND METHODS. HSS1-myc was detected by sequential binding of anti-myc mAb 9E10 followed by FITC-conjugated anti-mouse IgG (FITC anti-myc). Nuclear DNA staining (DAPI) and Nomarski images of cells are also shown.

and Silver, 1992). No *myc*-staining was seen in PSY396 transformed with the YCP50 vector. A functional HSS1- β -galactosidase fusion also was localized to the ER using antibodies directed against β -galactosidase. The localization of HSS1p to the ER is consistent with a role for HSS1p in protein translocation and suggests that HSS1p

might physically interact with previously identified ER components of the translocation machinery. Because the primary sequence of HSS1p contains a predicted membrane-spanning region, HSS1p is likely to be an integral ER membrane protein.

HSS1 Is Identical to SEC66

The combined genetic and biochemical data above suggest that HSS1p is an ER protein that is important for translocation and may interact with previously identified components of the translocation machinery. The predicted size and features of HSS1p are similar to those of two genetically unidentified proteins, p23 and gp31.5, that are part of a membrane-bound complex along with Sec61p, Sec62p, and Sec63p (Deshaies *et al.*, 1991). SEC66, the gene encoding gp31.5, has been cloned (Feldheim *et al.*, 1993), and comparison of HSS1 and SEC66 sequences indicates that they are identical.

Membrane Topology of HSS1p

Because HSS1 is identical to SEC66, we verified that HSS1p encodes a glycoprotein (gp31.5). HSS1-myc has three potential N-linked glycosylation sites in its primary sequence (pTK121, Figure 7). HSS1-myc was detected as a ≈ 30 -kDa species in Western blots of cell extracts using the 9E10 anti-myc monoclonal antibody (Figure 7, lanes 4 and 7). The 9E10 antibody cross-reacted with a yeast protein of ≈ 32 kDa in Western blots as seen in

Figure 7. Modification of the N-terminal region of HSS1p by N-linked glycosylation. The hydrophobic region (■), potential N-linked glycosylation sites, and location of *myc* tag are depicted for pTK121 encoding HSS1-myc. Site-directed mutagenesis was used to destroy the potential N-linked glycosylation site at amino acid 123 (NGS $\rightarrow$ KGS) as described in MATERIALS AND METHODS. pTK122 encoding this mutant HSS1-myc is depicted. Cell extracts were prepared from PSY396 ($\Delta hss1$) transformants, and Western blotting was performed with anti-myc 9E10 as in MATERIALS AND METHODS. Where indicated, cells were grown in the presence of 10 μ g/mL tunicamycin for 2 h (lanes 3, 6, and 9) or extracts treated with Endo H (lanes 2, 5, and 8). The positions of glycosylated (G) and unglycosylated (U) forms of HSS1p are indicated. * is a yeast protein that cross-reacts with the 9E10 antibody.

extracts of cells transformed with the YEp352 vector (Figure 7, lanes 1–3) or with HSS1-*myc* plasmids (Figure 7, lanes 4–9).

Two strategies were employed to determine that HSS1p is a glycoprotein. In the first strategy, solubilized cell extracts were denatured in SDS and treated with the enzyme endoglycosidase H (endoH), which cleaves sugar moieties off of N-linked glycoproteins. If HSS1-*myc* was an N-linked glycoprotein, then endoH would cleave off sugar moieties and HSS1-*myc* would migrate with higher mobility in denaturing gels. In Western blots of cell extracts treated with endoH, HSS1-*myc* no longer was detected as a ≈ 30 -kDa species but rather was detected as a faster migrating ≈ 26 -kDa species (Figure 7, compare lanes 4 and 7 to lanes 5 and 8). In the second strategy, extracts were prepared from cells grown for 2 h in the presence of the N-linked glycosylation inhibitor tunicamycin (10 μ g/mL). If HSS1-*myc* were normally a glycoprotein, a faster migrating form would be detected in cells treated with tunicamycin. As in endoH-treated cell extracts, a ≈ 26 -kDa species of HSS1-*myc* was detected in extracts treated with tunicamycin (Figure 7, lanes 6 and 9). The small fraction of ≈ 30 -kDa species represents HSS1-*myc* synthesized before tunicamycin treatment. The ≈ 26 -kDa HSS1-*myc* species in extracts prepared from tunicamycin-treated cells migrates with slightly higher mobility than that in endoH-treated extracts, because endoH leaves a GlcNAc attached to sugar-modified asparagine residues.

Because HSS1p is an N-linked glycoprotein, its ER luminal regions may be predicted by determining which sites are modified by glycosylation. Site-directed mutagenesis was performed to mutate the potential N-linked glycosylation site carboxyl-terminal to the hydrophobic region (pTK122, Figure 7). Elimination of this site leaves only the two potential sites amino-terminal to the predicted membrane-spanning region. This mutant HSS1-*myc* still migrated as a ≈ 30 -kDa species in untreated cell extracts (Figure 7, lane 7) and as an ≈ 26 -kDa species in endoH- or tunicamycin-treated cell extracts (Figure 7, lanes 8 and 9), indicating that only the amino-terminal sites are normally glycosylated. Because N-linked glycosylation only occurs in the ER lumen, the amino-terminus of HSS1p is ER luminal, whereas the carboxyl-terminal region is predicted to be cytoplasmic. This type II integral membrane prediction is consistent with topology predictions based on the composition of amino acids flanking the membrane-spanning regions of proteins (Hartmann *et al.*, 1989; von Heijne, 1992). The predicted cytoplasmic region of Hss1p, as for many other membrane proteins, contains many basic residues flanking its membrane-spanning region.

DISCUSSION

We have identified a high copy suppressor of *sec63-101* and cloned the corresponding gene (*HSS1*). The primary

sequence of HSS1p is 206 amino acids long with a single hydrophobic region and three potential N-linked glycosylation sites. HSS1p, like Sec63p, is an ER membrane protein with a predicted C-terminal cytoplasmic region. HSS1p is required for growth at elevated temperatures, because haploid cells disrupted for the *HSS1* promoter and N-terminal coding region grow at 17, 23, and 30°C, but not at 37°C.

Both pre-Kar2 and preinvertase accumulated in Δ *hss1* cells. If the Δ *hss1* cells are defective for ER translocation, then untranslocated precursor proteins would accumulate in the cytoplasm. Alternatively, if the Δ *hss1* cells are defective for signal peptide processing, then translocated precursor forms would accumulate within the ER lumen. The experiments performed do not distinguish between the two alternative possibilities. Using an independently isolated null allele of *HSS1*, Feldheim *et al.* (1993) have demonstrated that Δ *hss1* cells have a translocation defect. Although HSS1p is important for ER translocation, it is not essential, as Δ *hss1* cells are temperature-sensitive for growth. In this respect, *HSS1* is similar to yeast genes involved in the SRP-targeting pathway. Like Δ *hss1* cells, haploid cells disrupted for *SEC65*, *SRP54*, *SRP101*, or *SCR1* are slow-growing but viable and are defective for ER translocation (Amaya and Nakano, 1991; Hann and Walter, 1991; Ogg *et al.*, 1992; Stirling and Hewitt, 1992). It has been suggested that although yeast cells normally utilize SRP, they can adapt to the loss of SRP because an SRP-independent translocation pathway is induced (Ogg *et al.*, 1992). Similarly, cells may normally utilize Hss1p in translocation but can survive in the absence of Hss1p because mechanisms exist for replacing Hss1p function.

Double mutant combinations between Δ *hss1* and other translocation mutations were inviable or had reduced viability relative to the respective single mutations. This phenomenon, termed "synthetic lethality" has been observed among genes whose products physically interact and/or perform similar functions (Hufaker *et al.*, 1987). Synthetic lethal combinations were detected between Δ *hss1* and four essential genes (*sec61*, *sec62*, *sec63*, and *kar2*) whose products participate in a common translocation pathway (Rothblatt *et al.*, 1989; Deshaies *et al.*, 1991; Sanders *et al.*, 1992). Thus, HSS1p may perform an important but nonessential function in the essential translocation pathway mediated by Sec61p, Sec62p, Sec63p, and Kar2p. The high copy suppression of *sec63-101* by *HSS1* is also consistent with a functional interaction between HSS1p and Sec63p. The genetic predictions for HSS1p-Sec63p interaction were confirmed by the identity of HSS1p with gp31.5 (Sec66p), a protein that is found in an ER membrane-bound complex (Sec complex) with Sec61p, Sec62p, Sec63p, and a 23-kDa protein (Deshaies *et al.*, 1991; Feldheim *et al.*, 1993).

HSS1 specifically suppressed *sec63-101* among all *sec63* alleles tested. This may indicate a specific defect

associated with the *sec63-101* allele or that *sec63-101* has the weakest phenotype among the *sec63* mutants. Allele-specific high-copy suppression has previously been detected among members of the Sec protein complex, as high copy *SEC62* can suppress *sec63-1* but not *sec63-101* (Deshaies and Schekman, 1990). The membrane topology prediction for Hss1p indicates that it may have a large cytoplasmic domain, like Sec62p and Sec63p (Deshaies and Schekman, 1990; Feldheim *et al.*, 1992; Kurihara and Silver, 1992). The C-terminus of Hss1p may be important for its translocation function, since a Hss1-beta-galactosidase fusion that removes 30 amino acids from the Hss1p C-terminus rescues the temperature-sensitivity of Δ *hss1* but does not suppress *sec63-101* when overexpressed.

The synthetic lethality between Δ *hss1* and *sec65* genetically indicates that the Sec complex and SRP-targeting machinery may functionally interact during translocation, as *SEC65* encodes a subunit of SRP. Biochemical evidence for an interaction between ER targeting pathways and the Sec complex has previously been shown by cross-linking of translocating polypeptides to Sec61p and Sec62p (Musch *et al.*, 1992; Sanders *et al.*, 1992). Mutations in *SEC62* or *SEC63* decreased the amount of polypeptide cross-linked to Sec61p, indicating that Sec62p and Sec63p function are required for interactions between Sec61p and translocating polypeptides (Sanders *et al.*, 1992). Sec62p and Sec63p were proposed to affect Sec61p-polypeptide interactions by increasing the affinity of targeting pathways for Sec61p and/or by modifying Sec61p to allow more efficient polypeptide interaction (Sanders *et al.*, 1992). Perhaps gp31.5 (Hss1p) also performs a similar function, because it is tightly complexed with Sec62p and Sec63p. Together with their physical interactions, a functional overlap between gp31.5, Sec62p, and Sec63p in affecting Sec61p function would be consistent with the synthetic lethality between all tested combinations of *sec61*, *sec62*, *sec63*, and Δ *hss1* mutations.

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