SEC11 is Required for Signal Peptide Processing and Yeast Cell Growth

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Abstract. Among the collection of temperature-sensitive secretion mutants of *Saccharomyces cerevisiae*, *sec11* mutant cells are uniquely defective in signal peptide processing of at least two different secretory proteins. At 37°C, the restrictive growth temperature, *sec11* cells accumulate core-glycosylated forms of invertase and acid phosphatase, each retaining an intact signal peptide. In contrast, other *sec* mutant strains in which transport of core-glycosylated molecules from the endoplasmic reticulum is blocked show no defect in signal peptide cleavage. A DNA fragment that complements the *sec11-7* mutation has been cloned. Genetic analysis indicates that the complementing clone contains the authentic *SEC11* gene, and that a null mutation at the *SEC11* locus is lethal.

The DNA sequence of *SEC11* predicts a basic protein (estimated pI of 9.5) of 167 amino acids including an NH_2-terminal hydrophobic region that may function as a signal and/or membrane anchor domain. One potential N-glycosylation site is found in the 18.8-kD (Sec 11p) predicted protein. The mass of the *SEC11* protein is very close to that found for two of the subunits of the canine and hen oviduct signal peptidases. Furthermore, the chromatographic behavior of the hen oviduct enzyme indicates an overall basic charge comparable to the predicted pI of the Sec11p.

THE initial event in protein localization is achieved by interaction of a signal (leader) or transit peptide on a transported molecule with a cytoplasmic or membrane bound receptor. Properly targeted precursors are then translocated across the membrane, followed by proteolytic removal of the NH_2-terminal signal or transit peptide by highly specialized enzymes located within the target organelle.

A variety of the specialized processing proteases have been purified. *Escherichia coli* contains two such enzymes: leader peptidase and lipoprotein signal peptidase (40, 44). Both enzymes are integral membrane proteins of the cytoplasmic membrane and possess single subunits with relative molecular masses of 36,000 and 18,000, respectively. Both enzymes are essential for bacterial cell growth, but not for translocation of secretory proteins across the membrane. Repression of synthesis of leader peptidase causes improperly folded forms of secretory proteins to accumulate in the periplasm (11).

The eukaryotic counterparts of these processing proteases are more diverse and complex. Signal peptidase from dog pancreas and hen oviduct microsomes has been purified and found to consist of a complex of six polypeptides with molecular masses of 25, 23, 21, 18, and 12 kD (2, 15). Processing of mitochondrial precursor proteins requires a matrix localized, metalloendoprotease (4, 25) which consists of a 57-kD catalytic subunit and a nonassociated stimulatory protein of 52 kD (Neupert, W., personal communication).

In spite of the structural differences between prokaryotic and eukaryotic secretory protein peptidases, the substrate specificities are remarkably similar. Both enzymes cleave eukaryotic and prokaryotic secretory proteins in vitro at the correct peptide bond (29, 42). This conservation of activity has led to the suggestion that other subunits associated with the eukaryotic peptidase may perform other functions associated with polypeptide translocation (15).

Identification of a gene product required for signal peptide processing in yeast would allow a genetic approach to defining peptidase-associated proteins and their function in polypeptide translocation into the endoplasmic reticulum (ER).[1] We have shown previously that amino acid substitutions at the signal peptide cleavage site of yeast invertase result in accumulation of core glycosylated precursor forms of the enzyme within the lumen of the ER (5, 36). The block in secretion of mutant invertase is not complete even with substitutions that eliminate signal cleavage (5). For this reason we expect that mutations in the signal peptidase would show a pleiotropic but incompletely restrictive defect in secretion of molecules that have cleavable signal peptides. Inasmuch as this delay in secretion of diverse molecules is likely to compromise cell growth, we expect such a mutation will be found only in a conditionally lethal strain.

In this report we describe the characteristics of a secretion defective mutant, *sec11*, whose properties fit the predictions of a signal peptidase lesion.

Materials and Methods

Strains, Plasmids, and Materials

Table I lists the sources of bacterial and yeast strains used in this study. YPD liquid broth contained 1% Bacto-Yeast extract, 2% Bacto-Peptone (Difco Laboratories, Detroit, MI), and 2–5% glucose. Wickerham's minimal

1. *Abbreviations used in this paper*: Endo H, endoglycosidase H; ER, endoplasmic reticulum.

Table I. Bacterial and Yeast Strains

Strain	Genotype	Source of reference
X2180-1B	*gal2, MATα*	YGSC*
SEY2102	*gal2, his4-519, ura3-52 leu2-3,112, suc2-Δ9, MATα*	S Emr (13)
SEY2102-s1I	*gal2, his4-519, leu2-3,112, suc2-s1, MATα*	I. Schauer (36)
SF273-1A	*sec11-7, gal2, MATa*	This laboratory
SF274-2A	*sec12-4, gal2, MATa*	This laboratory
SF276-1A	*sec13-1, gal2, MATa*	This laboratory
SF280-3B	*sec16-2, gal2, MATa*	This laboratory
SF281-1B	*sec17-1, gal2, MATa*	This laboratory
SF282-1A	*sec18-1, gal2, MATa*	This laboratory
SF298-1B	*sec19-1, gal2, MATa*	This laboratory
SF284-1B	*sec20-1, gal2, MATa*	This laboratory
SF286-1B	*sec21-1, gal2, MATa*	This laboratory
SF303-2C	*sec22-3, gal2, MATa*	This laboratory
SF309-2D	*sec23-1, gal2, MATa*	This laboratory
PBY401A	*ura3-52, his4-519, leu2-3,112 gal2, MATα*	This study
PBY408A	*sec11-7, ura3-52, his4-519, leu2-3,112 gal2, MATα*	This study
PBY6	*SEC11/sec-11-7, SUC2/suc2-Δ9, his4-519/his4-519, leu2-3,112/leu2-3,112, ura3-52/ura3-52, gal2/gal2, MATa/MATα*	This study
Escherichia coli		
SE10	*F⁻ Δ(lac-pro)ara rpsL thi pyrF74::Tn5 (Ø80dlacZΔM15)*	S. Emr (14)
MC1061	*F⁻ araD139 Δ(araABOIC-leu)7679 Δlac$_X$74 galU galK rpsL hsdR*	M.J. Casadaban (10)
TG-1	*Δ(lacpro) supE, thi, F'traD36, proAB lac^q ZΔM15 hsdD5*	T. Gibson (16)

* Yeast Genetics Stock Center, University of California, Berkeley, California.

medium (43) was used with 2–5% glucose. Solid media were supplemented with 2% Bacto-Agar (Difco Laboratories). *E. coli* plasmids pUC9 (28), pUC12 (27), pUC18 (46), and pGEM1 (26), and *E. coli*–yeast shuttle plasmids YEp13 (7), pSEY8 (14), YIp5 (6), and pRB58 (9) have been described previously. pSEYc68 is identical to pSEYc58 (14) except for the multiple-cloning site which was derived from pUC18 (46). The yeast genomic DNA library originally constructed by Nasmyth (31) contains DNA pieces resulting from a *Sau*3A partial digest inserted into the Bam HI site of YEp13. For low sulfate or low phosphate medium, chloride salts replaced sulfate or phosphate salts, respectively. Ammonium sulfate or potassium phosphate was added to the desired concentration. The absorbance of dilute cell suspensions was measured in a 1-cm cuvette at 600 nm in a Gilford 2451A spectrophotometer (Oberlin, OH). Liquid cultures were grown in flasks or tubes with agitation, and experiments were initiated with exponentially growing cells at an OD_{600} of 0.7–1.5.

All reagents used were of analytical grade, except non-fat dry milk used for Western blotting was purchased at the local grocery store. [α-^{32}P]-dCTP, $H_2{}^{35}SO_4$, [α-^{35}S]dCTP and Na[^{125}I] were obtained from Amershan (Arlington Heights, IL). Lyticase (Fraction II, 30,000–80,000 U/ml) was prepared as described by Scott and Schekman (37). Invertase antiserum was prepared as described earlier (36). Anti-acid phosphatase serum was generously provided by G. Schatz (Biocenter, University of Basel, Switzerland). Endoglycosidase H (endo H) was obtained from P. Robbins (Biology Dept., Massachusetts Institute of Technology, Cambridge, MA).

Cloning and DNA Sequencing

Yeast strain PBY 408A was grown in YPD medium to an OD_{600} of 1 and cells were converted to spheroplasts which were transformed with the genomic DNA library of Nasmyth (31). Transformants were selected on minimal medium lacking leucine at 24°C. After 24 h at 24°C, at which time transformants began to appear, plates were transferred to the *sec* mutant restrictive temperature of 37°C. Colonies that continued growth were selected, replated, and plasmid DNA was isolated from each (3), and used to transform *E. coli* strain SE10. Plasmid DNA was recovered from individual *E. coli* transformants, and complementation of both temperature-sensitive growth and leucine auxotrophy was reconfirmed by transformation of the original strain PBY 408A.

Plasmid pCB1132, isolated from the genomic DNA bank, contains a 6.5-kb insert in YEp13 that includes the *SEC11* gene (Fig. 1 *A*). Plasmid pCB1133 consists of pSEY8 carrying the 1.4-kb Bam HI/Sac I fragment (Fig. 1 *C*) cloned into the pUC12-derived multiple-cloning site. pCB1134 contains the 1.4-kb Bam HI/Sac I fragment (Fig. 1 *C*) on the centromere plasmid pSEYc68. pCB*sec11::LEU2* contains the 0.4 kb Bam HI/Pvu II fragment and the 0.5 kb Eco RV/Sac I fragment separated by a 3.1-kb Bgl II insert that carries the *LEU2* gene (Fig. 1 *E*) on a pUC18-derived vector. The yeast integration vector pCBY511 contains the *SEC11* gene and was derived from YIp5 by insertion of the Bam HI/Sph I fragment (Fig. 1 *B*) into the Bam HI and Sph I enzyme-treated vector.

Restriction endonuclease digestion and T^4 DNA ligase reactions were carried out according to the supplier's instructions. Standard techniques of plasmid isolation, agarose gel electrophoresis, and DNA transformation of *E. coli* and *S. cerevisiae* have been described elsewhere (21, 24, 39). DNA sequencing was performed using the dideoxy chain termination method of Sanger et al. (35). The 1.4-kb Bam HI/Sac I fragment (Fig. 1 *C*) that contains the *SEC11* gene was isolated from pCB1132 and self-ligated to form concatameric DNA. Sonication was used to obtain random DNA fragments of 0.3–0.7 kb which were subcloned into pUC9 that was digested with Sma I. Clones were propagated in TG-1 cells and prepared for DNA sequencing. M13 primer was a gift of W. Rottman and E. Penhoet (this Department).

For Northern blot analysis fragments D1 and D2 (Fig. 1) were subcloned into pGEM1. The recombinant plasmids were converted to linear forms and strand specific [α-^{32}P]CTP labeled RNA was prepared (supplier's protocol) and used as hybridization probes. Poly(A)$^+$RNA purified from yeast strain X2180-1B was electrophoresed in an agarose gel containing 2.2 M formaldehyde. Transfer of RNA to nitrocellulose and RNA–RNA hybridization was performed as described elsewhere (24).

Accumulation of Invertase in sec Mutant Strains

sec mutant strains were grown in minimal medium containing 5% glucose to 1 OD_{600}/ml. Cells (8 OD_{600} U) were centrifuged at 24°C, washed with

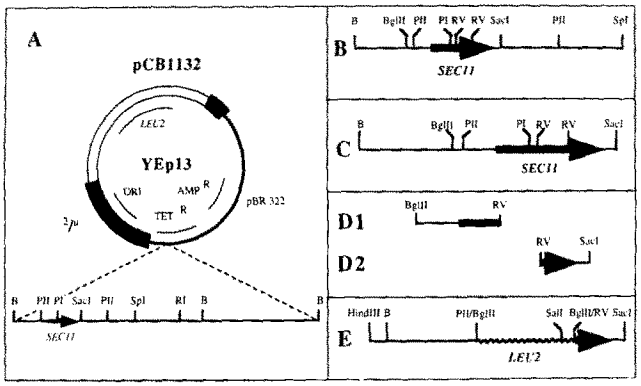

Figure 1. (*A*) Restriction map of the 6.5-kb insert in plasmid pCB1132 which contains the authentic *SEC11* gene. The heavy arrow indicates the coding region of *SEC11* and its direction of transcription. Fragments B-E are different subclones and constructs which were ligated into vectors as described in the text. *B*, Bam HI; *PI*, Pvu I; *PII*, Pvu II; *RV*, Eco RV; *Sp I*, Sph I.

8 ml distilled water and resuspended in 4 ml fresh minimal medium +0.1% glucose. The cell suspension was divided into 4 aliquots of 2 OD_{600} U each. Two aliquots received tunicamycin (10 μg/ml) followed by a 15-min incubation of all samples at 24°C. One treated and one untreated sample were left at 24°C, while the other two were incubated at 37°C for 3 h. Each incubation was terminated with 1 ml ice cold 20 mM NaN_3 and cooled on ice for 10 min, followed by centrifugation in an IEC table top centrifuge for 3 min at room temperature. Cells were washed with 2 ml ice cold 10 mM NaN_3, centrifuged and placed on ice. Cell pellets were mixed with 0.3 g glass beads (0.3–0.5 mm), 0.3 ml of Laemmli dissociation buffer (22) was added, and cells were agitated on a Vortex mixer at top speed for 30 s, 4 times with 30-s intervals on ice. Cell homogenates were heated for 5 min in a boiling water bath. The extracts were centrifuged at top speed for 1 min in an IEC table top centrifuge and supernatant fractions removed and stored at −20°C. Aliquots (24 μl) were resolved by 7.5% SDS-PAGE, which was then followed by immunoblot analysis (8). Antiserum directed against invertase and [^{125}I]Protein A were used to detect the different forms of invertase.

Radiolabeling, Immunoprecipitation, and Treatment with Endo H

Cultures of PBY401A and PBY408A were grown up to 1 OD_{600} Unit cells/ml in minimal medium +5% glucose and 0.1 mM ammonium sulfate. 12 OD_{600} U of PBY401A and 14 OD_{600} U of PBY408A cells were harvested by centrifugation for 5 min at 6,000 *g*. Cells were washed in water and resuspended to 1 OD_{600} U/ml minimal medium +0.1% glucose. After 30 min at 37°C, during which time the synthesis of invertase was derepressed and the *sec11* mutant block was imposed, 0.15 mCi $H_2{}^{35}SO_4/OD_{600}$ was added and cells were radiolabeled for 5 min at 37°C. Cultures were adjusted to 0.5% glucose, 1 mM ammonium sulfate, and 100 μg/ml cycloheximide, to initiate the chase period. Aliquots of 2 OD_{600} U of cells were taken at various times and added to chilled tubes that contained 20 μl 1 M NaN_3. Cells were washed once with 2 ml ice cold 10 mM sodium azide and held on ice until the conclusion of the experiment. Cells from each time point of chase were centrifuged and resuspended in 400 μl spheroplasting buffer (1.4 M sorbitol, 50 mM potassium phosphate, pH 7.4, 10 mM sodium azide, 80 mM β-mercaptoethanol, 50 U lyticase per OD_{600} unit of cells), incubated for 1 h at 30°C, and spheroplasts collected by centrifugation for 10 min at 3,000 *g*. Supernatant fractions were adjusted to 0.5% SDS and incubated for 5 min in a boiling water bath. Spheroplasts were resuspended in 100 μl 2% SDS and heated for 5 min in boiling water. SDS-treated supernatant and pellet fractions were diluted to 1 ml with PBS containing 1% Triton X-100. 50 μl (~5 mg protein) of nonradioactive extract from strain SEY2102 was added to each sample to compete for precipitation of unrelated proteins (36). 40 μl of IgG Sorb, prepared as described by the manufacturer, was added for 30 min at 4°C. IgG Sorb was removed by centrifugation at 12,000 *g* for 20 min and supernatant fractions were transferred to new microfuge tubes. Invertase specific antiserum was added (4 μl/OD_{600} unit cell equivalent) and samples were incubated overnight at 4°C. For each microliter of antiserum used, 5 μl of 20% Protein A-Sepharose 4B was added to precipitate the immune complex. The precipitates were washed twice with 1 ml PBS, 1% Triton X-100, 0.1% SDS and twice with 1 ml 10 mM Tris-HCl, pH 8, 50 mM sodium chloride. The washed complexes were solubilized in 30 μl dissociation buffer (50 mM Tris-HCl, pH 6.8, 2% SDS, 2 mM EDTA, 0.01% Bromphenol Blue, 10% glycerol, 1% 2-mercaptoethanol) heated in a boiling water bath for 5 min and stored at −20°C. After centrifugation in a microfuge for 2 min, the supernatant fractions were subjected to gel electrophoresis on 6% SDS polyacrylamide slab gels according to the procedure of Laemmli (22). Gels were fixed, prepared for fluorography by incubation for 25 min in 1 M sodium salicylate, dried, and allowed to expose Kodak X-Omat AR film at −70°C. Fluorograms were quantified by scanning with a Kratos model SD3000 spectrodensitometer coupled to a Kratos SDS300 density computer (Kratos Analytical Instruments, Ramsey, NJ) and an integrator (model No. 3380A, Hewlett-Packard Co., Palo Alto, CA).

For treatment with endo H, 10-μl aliquots of immunoprecipitated invertase resuspended in dissociation buffer were diluted 4-fold and adjusted to 250 mM sodium citrate, pH 5.5, 5 mM sodium azide. Samples were heated in boiling water for 5 min, cooled to 37°C, and treated with 0.5 mU of endo H overnight at 37°C. Incubations (40 μl) were mixed with 10 μl 5× dissociation buffer (250 mM Tris-HCl, pH 6.8, 10% SDS, 10 mM EDTA, 0.05% Bromphenol Blue, 50% glycerol, 5% β-mercaptoethanol), heated in boiling water, and subjected to SDS-gel electrophoresis.

NH_2-Terminal Analysis of Invertase and Acid Phosphatase Accumulated in sec11 Cells at 37°C

PBY408A harboring pRB58 was grown in minimal medium containing 5% glucose and 100 μM sulfate to 1 OD_{600} unit/ml. Eight OD_{600} cell equivalents were resuspended in 4 ml minimal medium (no sulfate) + 0.1% glucose + 4 μM leucine and incubated for 15 min at 37°C. Four mCi $H_2{}^{35}SO_4$ and 0.5 mCi of [^{3}H]leucine were added and cells incubated for 3 h at 37°C. An equal volume of ice-cold 20 mM sodium azide was added and centrifuged cells were lysed by agitation with glass beads as described above. Immune precipitation and PAGE were performed as described above and the labeled protein identified after exposure of the dried gel to Kodak X-Omat AR film for 3 d at room temperature. Bands corresponding to the ER forms of invertase were excised, electroeluted, and prepared for sequential Edman degradation as described previously (36). Preparation of radiolabeled acid phosphatase was performed identically except strain SF273-1A and growth media depleted of $PO_4{}^{3-}$ were used. Material released in each of the first 18 cycles (for invertase) or 27 cycles (for acid phosphatase) was measured for (^{3}H) counts per minute in a Searle Delta 300 liquid scintillation spectrometer.

Results

sec11 Mutant Cells Accumulate Core-glycosylated Glycoprotein Precursors

In a search for pleiotropic mutations that affect signal peptide processing, a collection of yeast secretory mutants was examined for accumulation of core-glycosylated invertase in a signal peptide-unprocessed form. Yeast cells produce two forms of invertase, both encoded by the *SUC2* gene (9). A cytoplasmic, unglycosylated species is produced constitutively and a glycosylated, secreted form is synthesized in response to glucose deprivation. The secreted enzyme is made as a precursor with a 19 amino acid signal peptide that is cleaved upon arrival in the ER lumen. *sec* mutant strains that are known to accumulate secretory proteins in the ER (33) were derepressed for synthesis of secreted invertase at the restrictive growth temperature of 37°C for 3 h, in the presence or absence of tunicamycin, an inhibitor of N-linked oligosaccharide synthesis. An invertase mutant form (*suc2-s1*) that shows reduced signal peptide processing because of an ala → val substitution at position −1 of the signal peptide cleavage site (36) was examined in parallel. Whole cell extracts

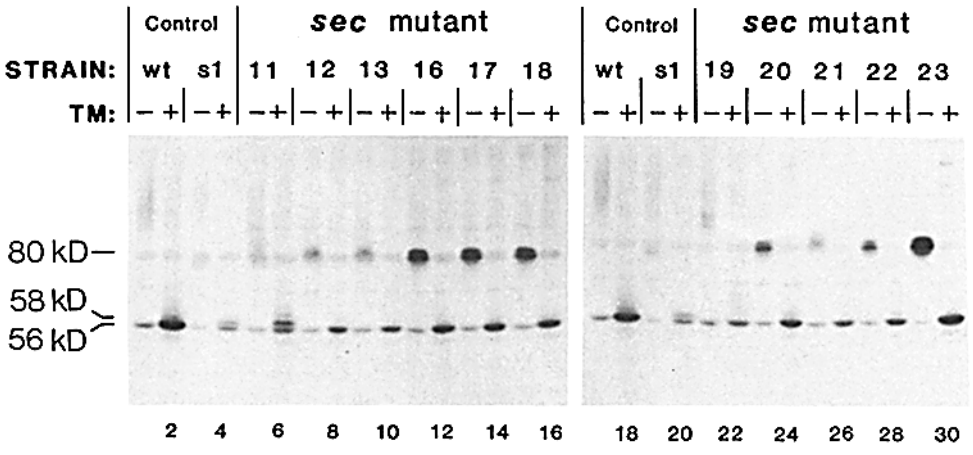

Figure 2. Accumulation of invertase in different *sec* mutant cells at 37°C. Wild-type strains PBY-401A (*SUC2*) or SEY2102-sII (*suc2-sl*) and *sec* mutant strains (all *SUC2*) were grown to mid-logarithmic phase in minimal medium + 5% glucose, washed, and resuspended in the same medium + 0.1% glucose. One half of each culture was treated with 10 μg/ml tunicamycin and all cultures incubated at 24°C for 15 min, followed by a 3-h incubation at 37°C. Total cell protein was extracted, separated on 7.5% polyacrylamide slab gels and analyzed by immunoblotting with anti-invertase antiserum and [^{125}I]protein A. *wt*, Wild-type strain; *sl*, wild-type strain carrying the *suc2-sl* invertase mutation integrated into the genome; *TM*, tunicamycin; 80, 58, and 56 kD denote the positions of migration on the polyacrylamide gel of core glycosylated, signal peptide-unprocessed, and -processed forms of invertase, respectively.

were prepared and accumulated invertase species detected by SDS-PAGE and immunoblotting (Fig. 2). Wild-type and mutant cells incubated in the absence of tunicamycin (Fig. 2, odd-numbered lanes) revealed core-glycosylated (M_r ~ 80,000) and cytoplasmic (M_r ~ 56,000) forms of invertase. In the presence of tunicamycin, secretory invertase produced by wild-type cells migrated as a 56-kD species, whereas sl-invertase had an apparent molecular weight of 58,000 due to retention of the signal peptide (Fig. 2, lane *4*). Among the *sec* mutants examined, only *secII* accumulated a form of invertase that comigrated with sl invertase (Fig. 2, lanes *4* and *6*).

A relation between the secretory defect and accumulation of the invertase precursor was established by incubating *secII* mutant cells at permissive, semi-permissive and restrictive temperatures (24°, 30°, 37°C). Wild-type and *secII* mutant strains were derepressed for synthesis of invertase and one half of each culture treated with tunicamycin. Cell cultures were shifted to the respective temperatures for 3 h, followed by extraction of cell proteins, separation by SDS–PAGE and immunoblot analysis. A temperature-dependent accumulation of core glycosylated invertase in untreated samples, and of the 58-kD precursor species in tunicamycin-treated samples, is documented by the data shown in Fig. 3. This characteristic was found in 10 independent *secII* isolates. Furthermore, the processing defect co-segregated with temperature-sensitive growth among the haploid meiotic progeny of *secII/SECII* heterozygotes.

A more direct demonstration of deficient signal peptide processing came from NH_2-terminal sequence analysis of radiolabeled glycoprotein precursors accumulated in *secII* at 37°C. Mutant cells were labeled with [^{3}H]leucine and $^{35}SO_4^{2-}$ for 3 h at 37°C, and core-glycosylated invertase, isolated by immune precipitation from whole cell extracts, was purified by SDS–PAGE. Sequential Edman degradation showed [^{3}H] label released at positions expected for leucine in the signal peptide of invertase (Fig. 4 *A*). Previous direct sequencing of invertase accumulated in the ER of another pleiotropic mutant (*secI8*) showed cycles of [^{3}H]leucine release expected for the signal peptide-processed species (36). Another secreted protein, acid phosphatase, whose efficient export depends both on faithful signal peptide cleavage (18) and on *SECII* (33), was also examined by NH_2-terminal sequencing of antigen immuno-purified from [^{3}H]leucine and [^{35}S]SO_4^{2-} double-labeled *secII* cells. Fig. 4 *B* shows that this protein also accumulated as an unprocessed precursor. Unlike this phenotype of *secII*, signal cleavage defective sl-invertase has no effect on secretion of acid phosphatase (36).

Secretion of Invertase Is Delayed in secII Mutant Cells

We showed before that uncleaved preinvertase was delayed but not blocked in transport through the secretory pathway (5). Single amino acid substitutions at the signal peptide cleavage site increased the half time of secretion from 2–70 min. The secretion delay phenotype of these signal peptide substrate mutations was consistent with the incompletely re-

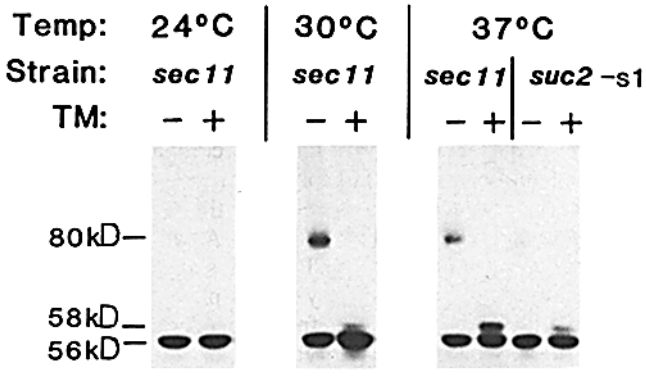

Figure 3. Temperature-dependent accumulation of different intracellular forms of invertase in *secII* mutant cells. Wild-type (SEY2102-sII) and *secII* mutant (PBY408A) strains were treated the same as described in Fig. 2, except that the 3-h incubation was performed either at 24°C, 30°C or 37°C. *suc2-sl*, wild-type strain carrying the *suc2-sl* mutant gene integrated into the genome; *TM*, tunicamycin. 80, 58, and 56 kD, denote the positions on the SDS-polyacrylamide gel of core-glycosylated, signal peptide-unprocessed and -processed forms of invertase, respectively. Cytoplasmic invertase migrates as a 56-kD species.

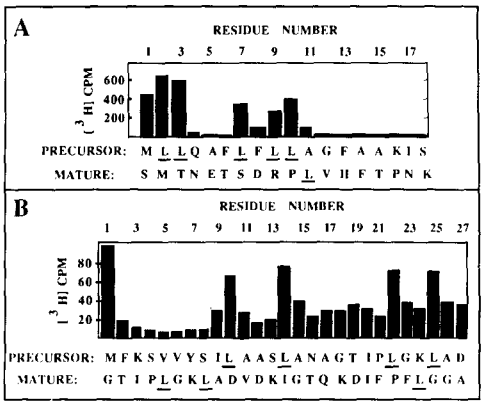

Figure 4. Amino terminal analysis of invertase and acid phosphatase accumulated in *secII* mutant cells at 37°C. Depicted are the ^{3}H-leucine counts per minute of the first 18 and 27 cycles of Edman degradation of invertase (*A*) or acid phosphatase (*B*), respectively. The predicted amino termini of precursor and mature forms of invertase and acid phosphatase are presented at the bottom of each panel. The ^{3}H signal observed for the initiating methionine of invertase and acid phosphatase precursor polypeptides was due to the presence of $[^{35}S]SO_4^{2-}$ in the labeling reaction.

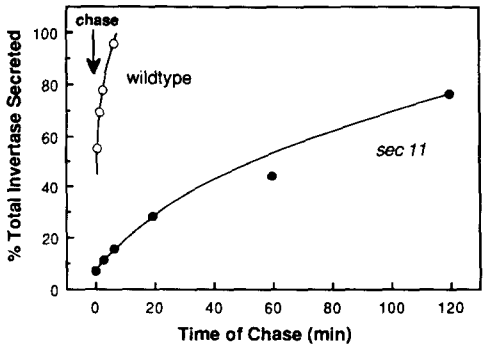

Figure 5. Kinetics of invertase secretion in wild type and *secII* mutant cells at 37°C. *secII* Mutant (PBY408A) and wild-type cells (PBY401A) were grown under selective conditions in minimal medium + 5% glucose to mid-logarithmic phase. Cells were washed and incubated in minimal medium + 0.1% glucose for 30 min at 37°C, conditions that derepress synthesis of invertase and impose the *secII* mutant block. Cells were labeled with $[^{35}S]SO_4^{2-}$ for 5 min and then exposed to conditions of chase for the indicated times. Spheroplasts were prepared and invertase was immunoprecipitated from spheroplast supernatant and pellet fractions. This material was treated with endoglycosidase H and deglycosylated invertase forms were separated on 6% SDS polyacrylamide slab gels. After fluorography, cross-reacting material was quantified by densitometric scanning.

strictive secretion defective phenotype of all *secII* isolates (33). A test of the delay in invertase secretion was performed with wild type and *secII* cells pulse-labeled for 5 min with $[^{35}S]SO_4^{2-}$ followed by a chase period at 37°C. Half of the labeled invertase was secreted by wild-type cells during the 5-min pulse radiolabeling while ~60 min was required for export of an equivalent amount from *secII* cells (Fig. 5). Thus, the secretion block was severe though clearly incomplete.

Cloning and Genetic Analysis of the SEC11 Gene

Yeast strain PBY408A was transformed with a yeast genomic DNA library contained on the multicopy vector YEp13. Transformants were selected simultaneously for complementation of leucine auxotrophy and growth at 37°C, the restrictive temperature for *secII* cells. Among 8,000 Leu$^+$ transformants, 6 showed a Ts$^+$ phenotype. Plasmids were isolated from these yeast transformants, propagated in *E. coli*, and *secII* complementing activity was re-evaluated by transformation of *secII* mutant cells. Two different plasmids (pCB1132 and pCB1112) were found to complement the Ts$^-$ and leucine auxotrophic phenotypes in the second screen. Restriction enzyme analysis revealed the presence of the same genomic region in both plasmids. A restriction map of the smaller genomic clone pCB1132 is shown in Fig. 1 *A*. Subcloning into a single-copy CEN plasmid (pSEYc68) defined a 1.4-kb Bam HI/Sac I fragment (Fig. 1 *C*) that contained the complementing activity.

Since yeast integrates linear yeast DNA fragments by homologous recombination with the corresponding chromosomal sequences (34), it was possible to demonstrate that the authentic *SEC11* gene was obtained. The large Bam HI/Sph I fragment (Fig. 1 *B*) was subcloned into the *URA3*-containing integration vector YIp5, producing plasmid pCBY511. A Sac I restriction site adjacent to the putative *SEC11* gene was cleaved to produce linear molecules which were introduced by transformation into a heterozygous diploid strain PBY6 (*SEC11/secII*). Five stable Ura$^+$ transformants (PBY61 to 65) were sporulated and subjected to tetrad analysis. Integration of pCBY511 adjacent to the *SEC11* locus would result in cosegregation of *secII* with Ura$^-$ and *SEC11* with Ura$^+$. All spores of PBY64 and 65 showed 2:2 segregation of Ts$^+$:Ts$^-$ and coincident 2:2 segregation of Ura$^+$: Ura$^-$ phenotypes confirming integration at the *SEC11* locus (Table II). All spores of PBY61 to 63 showed a Ts$^+$ phenotype, consistent with integration at the *secII* locus in these transformants.

DNA/Protein Sequence and Transcription of SEC11

The nucleotide sequence of a 1.2-kb DNA fragment containing the *SEC11* gene was determined. As shown in Fig. 6, *SEC11* contained a single uninterrupted open reading frame

Table II. The Cloned DNA Contains the Authentic SEC11 Gene

Transformant	ASCI dissected	Ts$^+$:Ts$^-$	Ura$^+$:Ura$^-$
PBY61*	15	4:0 (15)§	2:2 (15)
PBY62	16	4:0 (16)	2:2 (16)
PBY63	20	4:0 (19)	2:2 (20)
PBY64‡	15	2:2 (14)	2:2 (15)
PBY65	16	2:2 (15)	2:2 (16)

The diploid strain PBY6 (*sec11-7/SEC11*) was transformed with the SacI-linearized plasmid pCBY511, 5 independent Ura$^+$ transformants were picked and subjected to tetrad analysis.

* The three strains PBY61, 62 and 63 represent an integration of the linearized plasmid adjacent to the *sec11-7* mutant locus, giving rise to 4 wild-type spores.

‡ The two strains PBY64 and 65 resulted from integration of pCBY511 at the wild type locus of *SEC11*. 2 of the 4 spores have a duplicated *SEC11* locus and two are *sec11*, thus Ts$^-$ for growth. None of the Ts$^-$ spores were Ura$^+$ suggesting a tight linkage of the cloned fragment B (Fig. 1) to the *SEC11* locus.

§ The numbers in parenthesis describe the number of asci that segregated accordingly.

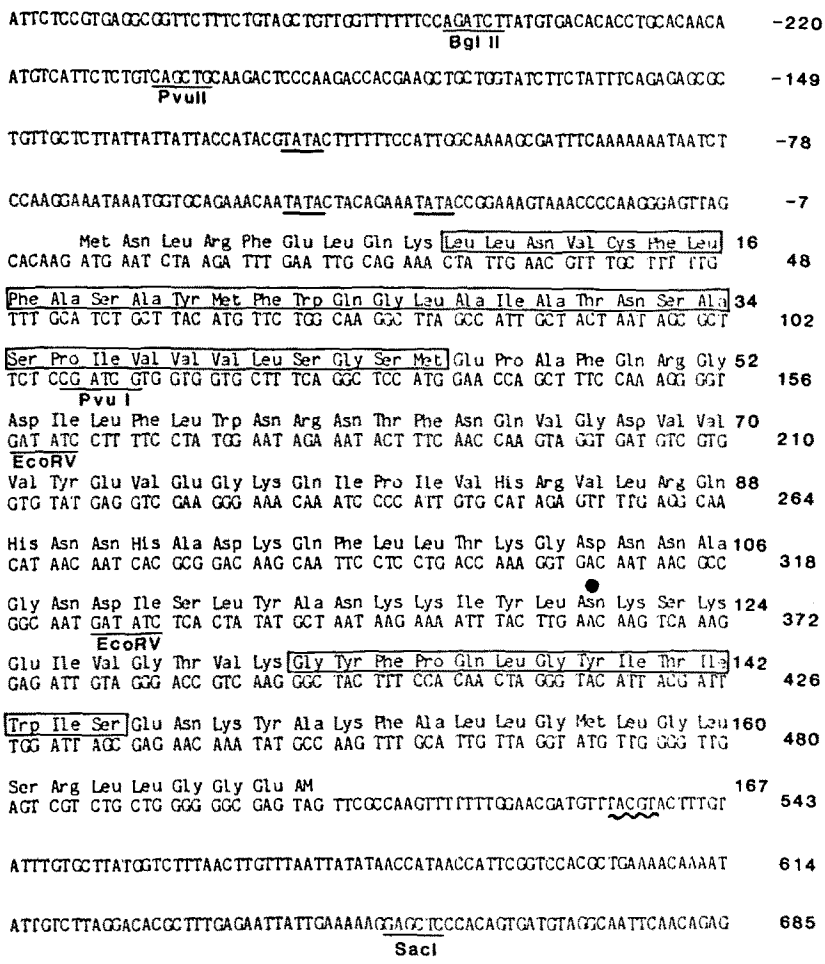

Figure 6. Nucleotide sequence of the *SEC11* gene and the predicted amino acid sequence of Sec11p. Both strands of the *SEC11* gene and flanking regions were sequenced entirely. Underlined are restriction endonuclease sites that were used in this study and three TATA elements that may be involved in initiation of transcription. The squiggled line denotes a 3′ terminal signal for polyadenylation. Numbers refer to the nucleotides or amino acids relative to the initiating A or methionine of the coding sequence, respectively. Encased are hydrophobic stretches of the presumptive *SEC11* gene product. •, Denotes a possible glycosylation site for NH_2-linked oligosaccharides.

starting at the nucleotide designated +1 and terminating after nucleotide 501. This open reading frame (Sec11p) predicts a basic polypeptide (estimated pI ~9.5) of 167 amino acids (18% charged) with a molecular weight of 18,825. Hydropathic analysis (Fig. 7) performed by the method of Hopp and Woods (20) detected an amino terminal hydrophobic stretch of 36 amino acids (Fig. 6, encased) preceded by three charged amino acids. This configuration is reminiscent of a eukaryotic signal peptide. A second hydrophobic stretch of 14 amino acids was identified between amino acids 132 and 145. The asparagine at position 121 of Sec 11p is a possible site for N-linked glycosylation. No significant sequence homology has been found to proteins in the Dayhoff protein sequence bank at the University of California (San Francisco) or in the data bank from the National Biomedical Research Foundation (23).

Examination of the DNA sequence upstream from *SEC11* revealed several stretches of A residues and three TATA sequences which are thought to be involved in positioning the start of transcription (17). Also highlighted in Fig. 6 (squiggled line) are sequences that may be involved in transcription termination and polyadenylation. The sequence TAG . . . 27 nucleotides . . . TACGT . . . , located between nucleotides 502–536, is homologous to a conserved sequence that may function in termination and polyadenylation of yeast mRNAs (47).

Transcription of *SEC11* was examined by Northern hybridization. Two different DNA fragments (Fig. 1, fragments D1 and D2) were subcloned into pGEM1 and strand specific ^{32}P-labeled RNA was transcribed using either SP6 or T7 RNA polymerase. RNA probes complementary to both DNA fragments detected the same *SEC11* mRNA transcript of 0.85 kb (data not shown) and confirmed the direction of transcription as shown in Fig. 1. No other transcript originating from DNA contained within the BglII/SacI fragment was identified.

The Essential Nature of SEC11

The conditional lethality of *sec11* mutations was consistent with either a thermosensitive *sec11* gene product, or with a null mutation that exposed an independent thermosensitive process. Disruption of the chromosomal *SEC11* locus should differentiate between these two possibilities. The 0.53 kb PvuII/EcoRV fragment of *SEC11* was replaced by a 3.1-kb insert carrying the *LEU2* gene (Fig. 1 *E*). A 3.8-kb Bam HI/Sac I fragment containing the disrupted *SEC11* was introduced into PBY6 (*SEC11/sec11-7*) and diploid Leu$^+$ transformants were sporulated and dissected into tetrads (Fig. 8). Among 44 asci analyzed from two transformants, both of which had a disruption of the *sec11* allele, 42 gave rise to two viable spores and 2 tetrads produced only one progeny. All viable spores were Leu$^-$/Ts$^+$, while other loci (MAT*a*/MATα and *SUC2*/*suc2*-Δ9) were unaffected and segregated independently of the lethal phenotype. The results confirmed the essential role of the *SEC11* locus in cell growth.

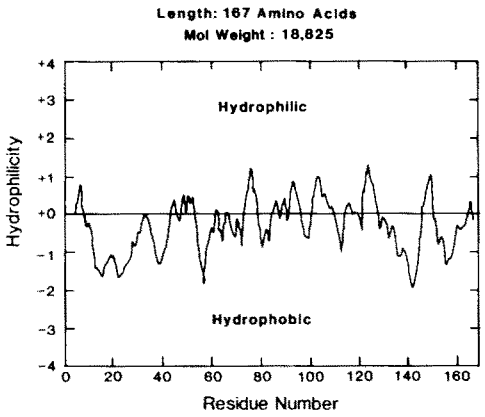

Figure 7. Hydrophilicity plot of the *SEC11* gene product. The analysis was performed with a minimum length peptide of 6 amino acids (20).

Discussion

The pleiotropic, secretion-defective yeast mutant *sec11* accumulates signal peptide unprocessed forms of glycoproteins in the lumen of the ER. Incubation of mutant cells at a restrictive temperature causes a dramatic delay but not an absolute block in secretion of precursor proteins. A similar, though enzyme specific defect is seen with signal peptide cleavage mutations in the invertase and acid phosphatase structural genes (5, 18, 36). The delay in secretion may result from an association of the hydrophobic signal peptide with the lumenal surface of the ER membrane. Schauer et al. (36) showed that an unprocessed mutant form of invertase resists saponin solubilization from the ER while signal peptide processed forms are readily released.

Among the *sec* mutant strains that accumulate enzymatically active invertase in an intracellular, non-cytoplasmic pool, *sec11* appears unique in displaying no proliferation of membrane-bounded organelles (33). Intracellular membrane proliferation may occur only when integral membrane components fail to be transported to a normal destination. If membrane proteins lack cleaved signal peptides, then a lesion in the peptidase might not create exaggerated membrane profiles. In support of this, genes for several yeast plasma membrane proteins have been sequenced and reveal no typical NH_2-terminal signal peptide (30, 38). Similarly, *sec11* cells are selectively blocked in the export of a subset of major periplasmic proteins (32). It will be interesting to determine whether *sec11* interferes with the biogenesis of known plasma membrane proteins.

The *sec11* mutation most likely interferes with signal peptidase function directly rather than indirectly such as in the generation of a peptidase inhibitor. All 10 mutant isolates of *sec11*, though differing in the severity of the lesion, are genetically recessive and display the same processing defect. Identification of Sec11p as a subunit of yeast signal peptidase awaits purification of the yeast enzyme and generation of Sec11p-specific antiserum.

Sec11p has a predicted size of 18.8 kD and a pI of 9.5. In size it resembles two of the subunits of eukaryotic signal peptidase. Hen oviduct and canine peptidase have in common one non-glycosylated subunit and two to three glycoprotein subunits that vary only in content of oligosaccharide chains

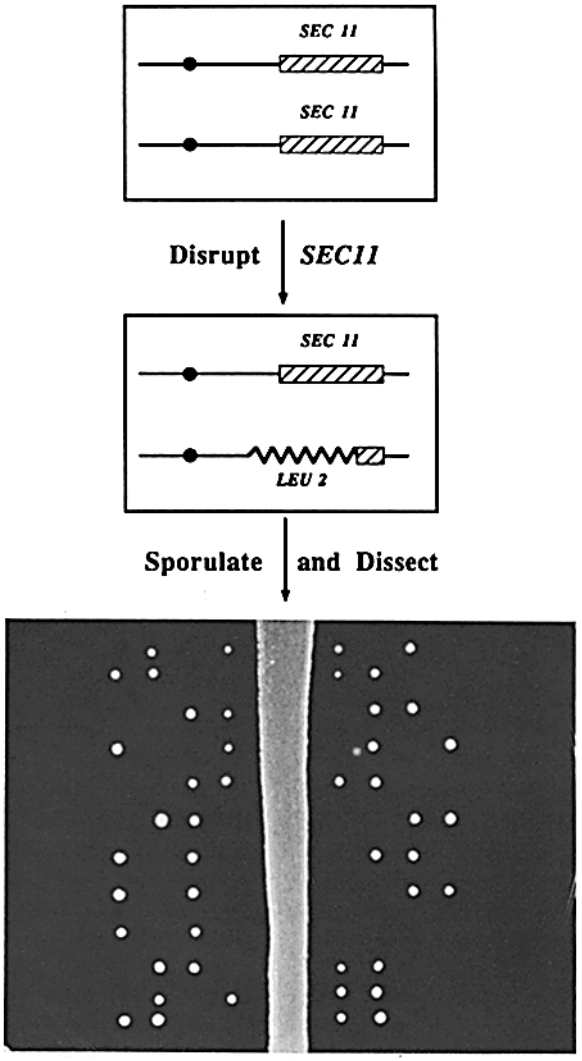

Figure 8. The *SEC11* gene is essential for cell growth. One *SEC11* locus in the diploid PBY6 was disrupted by one step gene displacement with fragment E (see Fig. 1). Diploid Leu^+ integrants were sporulated for 5 d on acetate containing sporulation plates. Tetrads were dissected and the spores subsequently scored for leucine auxotrophy. The figure displays 23 tetrads. Each tetrad resulted in two viable Leu^- spores.

(1, 2, 15). Furthermore, the hen oviduct complex has a very basic charge as suggested by the purification characteristics on DEAE- and CM-cellulose (2).

If Sec11p is related to one of the two higher eukaryotic peptidase subunits it may serve either in a catalytic or facilitating role. Inasmuch as the eukaryotic and prokaryotic signal (leader) peptidases possess remarkably similar peptide bond specificities, a sequence homology between the catalytic subunit of the eukaryotic enzyme and the single subunit of the prokaryotic enzyme is anticipated. No such homology was seen in a direct comparison of *SEC11* and the *E. coli LEP* gene. Hence, Sec11p may provide some non-catalytic function in signal peptide processing. A similar possibility has recently been described for the mitochondrial matrix protease responsible for removing transit peptides from mitochondrial precursors. One protein, defined both biochemically and genetically (45), lacks catalytic activity, but dramatically stimulates the action of a non-associated matrix

protease (W. Neupert, personal communication). The facilitating subunit may bind the translocated precursor and expose the transit peptide cleavage site in a conformation favorable for protease action. Similarly Secl1p could position the signal peptidase adjacent to a translocating secretory polypeptide. This possibility may be explored by testing genetic interactions between *SEC11* and the genes that are required for secretory protein translocation (12).

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