SEC7 Encodes an Unusual, High Molecular Weight Protein Required for Membrane Traffic from the Yeast Golgi Apparatus*

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Saccharomyces cerevisiae with mutations at the sec7 locus are pleiotropically deficient in protein transport within the Golgi apparatus and proliferate a large array of Golgi cisternae at a restrictive growth temperature (37°C). The SEC7 gene and its product (Sec7p) have been evaluated by molecular cloning and sequence analysis. Two genes that allow sec7 mutant cells to grow at 37°C are represented in wild-type yeast DNA libraries. A single copy of the authentic SEC7 gene permits growth of mutant cells, whereas the other gene suppresses growth deficiency only when expressed from a multicopy plasmid. The SEC7 gene is contained on a 8.4-kilobase pair SpHl restriction fragment, portions of which hybridize to a single 6-kilobase pair mRNA. The gene is essential for yeast vegetative growth. DNA sequence analysis of this region detects a single open reading frame with the potential to encode a 2008-amino acid-long hydrophilic protein of 230 kDa. Putative Sec7p contains an unusual, highly charged acidic domain of 125 amino acids with 29% glutamate, 18% aspartate, and 21% serine. Within this region, stretches of 14 consecutive glutamate residues and 13 consecutive glutamates/aspartates are predicted. This domain in Sec7p may serve a structural role to interact with lipids or proteins on the cytoplasmic surface of the Golgi apparatus.

The pathways of intracellular protein traffic have been studied by a variety of approaches. Biochemical assays that detect the participation of soluble proteins have yielded important clues concerning the mechanism of protein translocation across membranes (1, 2). Similar reactions that measure intercompartmental transport of proteins show promise (3–5), but have not yet resulted in the identification of individual proteins that facilitate this process.

An alternative that may complement the biochemical approach is the identification of gene products that are required at unique points in the secretory process. We have defined a pathway of secretion in Saccharomyces cerevisiae by the isolation and characterization of a series of conditionally lethal mutations (6). At least 23 (SEC) genes are implicated in the process of intercompartmental protein transport (7, 8). Most of the sec mutations block transport of proteins from the endoplasmic reticulum to the Golgi apparatus or from mature secretory vesicles to the plasma membrane. Two mutations (sec7 and sec14) block protein traffic within the Golgi apparatus (9, 10).

Mutations that define the sec7 locus exert a unique and dramatic effect on traffic of secretory, plasma membrane, vacuolar, and endocytic marker molecules (9–11). At a restrictive growth temperature (37°C), sec7 mutants cells accumulate multiple exaggerated Golgi-like membranes (5). Golgi cisternae collect in unusually large stacks when the incubation at 37°C is conducted in media containing low concentrations of glucose. Secretory glycoproteins accumulate within these cisternae (8, 9) and are exported when cells are returned to a permissive temperature (24°C).

Examination of the secreted pheromone a-factor accumulated in sec7 cells at the nonpermissive temperature shows that maturation of oligosaccharide chains has occurred, but that the block precedes endoproteolytic digestion of the precursor polypeptide (12, 13). However, the vacuolar protein carboxypeptidase Y exhibits both a defect in the maturation of its oligosaccharide chains and endoproteolytic cleavage of the precursor polypeptide as a result of the sec7 block (10). Hence, the SEC7 gene product (Sec7p) may regulate protein traffic from various compartments of the Golgi apparatus.

In this report, we describe the molecular cloning and analysis of the SEC7 gene.

EXPERIMENTAL PROCEDURES

Strains, Plasmids, Growth Conditions, and Materials—The yeast and bacterial strains used in this study are listed in Table I. Escherichia coli plasmid pUC18 and the M13-derived versions of M13mp18 and M13mp19 were used as described (14). Yeast centromeric plasmids YCp50 (15), pSEYc58 (16), and the high copy number (2μ-based) plasmids YEpl3 (17) and pCF35 were used as E. coli-yeast shuttle vectors. Integrating plasmid YIp6 was used as described (18). A yeast genomic DNA library, constructed with a partial Sau3A restriction enzyme digest inserted into the BamHI site of YCP50, was provided by P. Novick (Yale University, New Haven, CT) and M. Rose (Princeton University, Princeton, NJ) (19). The cDNA expression library of McKnight and McConaughy (20) was used as before.

Yeast cells were grown in YPD medium (1% yeast extract, 2% peptone, 5% glucose). Their growth was monitored by optical density at 600 nm as described (20).

Restriction endonucleases, DNA-modifying enzymes, and DNA polymerase were from Bethesda Research Laboratories; ATP and deoxynucleoside triphosphates were from Sigma; dideoxynucleoside triphosphates were from Pharmacia LKB Biotechnology Inc.; and a[β-32P]dCTP and α[32P]dCTP were from Amersham Corp. Lyticase was prepared and described as described (21).

Molecular Cloning of SEC7—Restriction endonuclease digestions and T4 DNA ligase reactions were carried out according to the...
manufacturer's instructions. Reactions with T4 DNA polymerase and E. coli DNA polymerase I (Klenow fragment) were performed as described by Maniatis et al. (22). Techniques of plasmid preparation, agarose gel electrophoresis and DNA transformations of E. coli and S. cerevisiae were as described (22-24).

 sec7 yeast (strain SF821-8A) was grown to O.D.650 nm = 1-2, converted to spheroplasts, and transformed with a genomic DNA library (25) or a cDNA expression library (26). Transformants were selected on minimal medium lacking leucine (for the genomic library) or tryptophan (for the cDNA library). After 2 days, at which time transformants began to appear, plates were transferred to the sec restrictive temperature (38°C). Colonies that continued to grow were selected and replated, and plasmid DNA was isolated from each. Yeast plasmid DNA was used to transform E. coli MC1061. Plasmid DNA was isolated from individual transformants, and confirmation of both the growth defect and the auxotrophic requirement was confirmed by transformation of the original yeast host.

Plasmid pCF36, obtained from the genomic centromere library (19), contained a 12-kb insert carrying the authentic SEC7 gene (Fig. 1A). pTA33 contained an 8.4-kb SphI fragment (Fig. 1A, fragment A) that included the entire SEC7 gene in pUC18. Subsequent subcloning was done from pTA33. Yeast pTA67 was digested with partial Sau3A and partial Yba37, and fragments of 3' end of SEC7, in pCF35 and pSEYc58, respectively. Yeast pTA34 and pTA35 contained a 7.2-kb Xhol-BamHI fragment (Fig. 1A, fragment B), missing only the 3' end of SEC7, in pCF35 and pSEYc58, respectively. Yeast pTA34 and pTA35 carried a 7.2-kb Xhol-BamHI fragment (Fig. 1A, fragment C). Equilibrium sedimentation analyses performed by the T. Gibson Research Group (27) are shown in Table 1 and in the text. Splicing of SEC7 was performed as described by Bernstein et al. (22). Random fragments (700-1000 base pairs) were generated by sonication. Samples chilled on ice were sonicated four times for 15 s using a Branson sonifier at 50-watt power. Fragments were converted to complete duplexes with the Klenow fragment of DNA polymerase as described (22). The resulting blunt end fragments were ligated into M13mplO that had been restricted by Smal and dephosphorylated by alkaline phosphatase (Pharmacia LKB Biotechnology Inc.). Recombinants were propagated in E. coli strain X2180-1B. Template preparation and DNA se-

1 The abbreviation used is: kb, kilobase pair.
probes for Northern hybridization tails, see "Experimental Procedures.

gene replacement and disruption of the SEC7 locus on the chromosome (for de-

D were used to make nick-translated DNA restriction fragments

with a yeast genomic and a yeast cDNA expression library. Transformants were selected for growth on medium lacking

tRNA, -40,000 Trp' colonies were obtained, of which

clone; Fig. 1B). The cDNA clone complemented only the sec7-1 mutation, but not another allele, sec7-4. Furthermore, the SplI-HindIII fragment of p334, which carried the complementing gene, failed to restore Ts' growth to sec7-1 cells when inserted into the single copy YCP50 centromere plasmid. From this, we suspected that p334 expressed an allele-specific suppressor function, but only when overproduced. Independent genetic evidence showed that this gene integrated at a chromosomal locus separate from the sec7 locus. For these reasons, further investigation of the nature of the p334 gene was postponed.

The genomic library yielded ~50,000 Ura' colonies, of which five were also Ts'. These five clones were identical by restriction enzyme analysis (pCF36 genomic clone; Fig. 1A), but completely distinct from the cDNA clones described above. The genomic clone complemented both the sec7-1 and sec7-4 mutations. To identify the region of the 12-kb insert that carried complementing activity, plasmid pCF36 was mutagenized by random insertion of the transposon Tn5. Five independent isolates of pCF36 with a Tn5 insertion in the yeast genomic sequence were obtained; four of these insertion events inactivated pCF36 complementation of sec7-1 (Fig. 1, Tn5-13, -14, -22, and -26). Restriction enzyme analysis of the position of transposon insertion indicated that the SEC7 complementing activity extended close to the rightmost end point of the yeast insert as depicted in Fig. 1. Additional Tn5 insertions confirmed the size of the gene and marked the 5' boundary of the SEC7 gene (data not shown).

A subclone of pCF36 that exhibited SEC7 complementing activity was a 5.5-kb SplI-SplI fragment (Fig. 1A, fragment A). This fragment introduced either on a centromere plasmid (pTAYc66) or on a multicopy plasmid (YEpTA65) restored normal growth to sec7 mutant cells at 38 °C. Similar results were seen with fragment C in single copy or multicopy plasmids (pTAYc35 and YEpTA65, respectively). Partial complementation was obtained with fragment B, whereas fragment D was unable to restore Ts' growth to sec7 cells.

Analysis of SEC7 mRNA by Northern hybridization confirmed the large size of the gene. Fragment B (Fig. 2) hybridized to a unique 6-kb species represented in poly(A)+ mRNA from a wild-type strain (Fig. 3). Fragments A and C (Fig. 2) detected the same species. By comparison with an internal standard, the SEC7 mRNA appeared to be about 20% as abundant as URA3 mRNA, or perhaps about five copies/cell.

SEC7 Encodes an Essential Gene—Homologous integration was used to demonstrate that pCF36 contained the authentic SEC7 gene. The yeast LEU2 gene was introduced in place of a 2.2-kb ClaI-ClaI fragment and then excised as a 5.5-kb EcoRI-EcoRI fragment (Fig. 2, fragment E) so that SEC7 sequences flanked the LEU2 gene. This linear molecule was introduced into the sec7/SEC7 heterozygous diploid (strain TAYD68) by spheroplast transformation. The SEC7 locus is closely linked to the aro1C and hom2 loci on the right arm of

Fig. 1. Restriction map of plasmids pCF36 (A) and p334 (B) and fragments of pCF36 subcloned into different vectors. A, pCF36 contains a 12-kb insert in YCp50; B, p334 contains a 1.2-kb insert. The black arrows describe the approximate ends of the coding region of SEC7 (for details, see "Results and Discussion"). Pa, PstI; X, XhoI; Ps, PsI; RI, EcoRI; Sp, SplI; B, BglI; C, ClaI; RV, EcoRV; Ba, BamHI; H, HindIII. ▽, sites where Tn5 transpositions have occurred. The complementation table refers to the ability of transformed multi-copy plasmids carrying the designated inserts to restore growth to sec7-1 cells at the nonpermissive temperature.

Cloning of the SEC7 Gene—The SEC7 gene was cloned by complementation of the temperature-sensitive sec7-1 defect. Mutant cells (strain SF821-8A) were transformed separately with a yeast genomic and a yeast cDNA expression library. Transformants were selected for growth on medium lacking uracil or tryptophan and for growth at 38 °C, the restrictive temperature for the sec7-1 mutation. From the cDNA library, ~40,000 Trp' colonies were obtained, of which two were also thermoresistant Ts'. These two cDNA clones were identical by restriction enzyme analysis (p334 cDNA clone; Fig. 1B). The cDNA clone complemented only the sec7-1 mutation, but not another allele, sec7-4. Furthermore, the SplI-HindIII fragment of p334, which carried the complementing gene, failed to restore Ts' growth to sec7-1 cells when inserted into the single copy YCP50 centromere plasmid. From this, we suspected that p334 expressed an allele-specific suppressor function, but only when overproduced. Independent genetic evidence showed that this gene integrated at a chromosomal locus separate from the sec7 locus. For these reasons, further investigation of the nature of the p334 gene was postponed.

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SEC7 and Golgi Traffic

The 0.7-kb EcoRI fragment (Fig. 2, fragment A) from SEC7 DNA was used to probe the blot. A URA3 DNA probe was used as an internal standard for size and quantitation.

chromosome IV (33). Integration of the linear construct into the chromosome is directed by homologous recombination and thermosensitive analysis and Southern hybridization with probes derived from the undeleted segment of cloned SEC7 demonstrated integration of LEU2 at identical loci in the thermoresistant (Ts+) and thermosensitive (ts−) diploid transformants (data not shown). Further support that integration occurred at the SEC7 (or sec) locus was obtained from the characterization of spores derived from stable Ts+ and ts− diploid transformants. The Ts+ diploid generated tetrad (17 analyzed) which segregated two viable Ts+ and two dead spores. Likewise, the ts− diploid generated tetrad (22 analyzed) consisting of two viable ts− and two dead spores. All viable spores were Leu+, demonstrating that integration had disrupted the SEC (or sec) locus and generated a lethal phenotype in the null mutant. Segregation of the other markers (aro1 and hom2) confirmed tight linkage to the SEC locus, whereas other loci (MAT, TRP1, ADE2, HIS4) segregated independently of the lethal phenotype. We conclude that integration of fragment E (Fig. 2) had occurred at the SEC7 locus and that disruption of this gene prevented spore germination. Hence, SEC7 is an essential gene for growth in yeast.

DNA Sequence Analysis of SEC7—Random cloning was used to sequence most the the Sphi-Sphi fragment (8.5 kb) of pCP36. Two short stretches of DNA that were not found among the (377) sequenced random clones were subcloned into M13 by direct procedures. The information in Fig. 4 was determined by sequencing both strands entirely.

Computer analysis of the SEC7 DNA sequence revealed a single open reading frame encoding a protein of 2008 amino acid residues (Fig. 4), with a predicted molecular weight of 227,885. The position of this open reading frame within the 8.5-kb fragment is shown by the black arrows in Fig. 1. Translation is predicted to start at an ATG 11 bp upstream from the second of two closely spaced EcoRI sites.

Potential transcriptional start sites, the TATA sequences (34), were noted at nucleotides −301, −152, and −8 relative to the putative translation start codon. A number of yeast genes that encode abundant mRNAs have transcription start sites at or very near the sequence CAAG, and a CT-rich block is often found upstream of this sequence (35). This pattern of nucleotides was noted upstream of the distal and proximal (but not the medial) TATA boxes of the 5′-SEC7 noncoding region. The relevance of these sequences to SEC7 transcription is not clear inasmuch as Northern hybridization showed SEC7 mRNA to be less abundant than URA3 mRNA (Fig. 3). This low level may relate to stability of an unusually large mRNA or to a low rate of transcription. By comparison with well-characterized yeast genes, the TATA sequence at position −152 approximates the normal position relative to the start codon.

The consensus sequence ANNAUGNNNU has been proposed as a site that marks efficient translation initiation in yeast (36). The ATG at position +1 (CUGAUGUCU) in Sec7p does not fulfill the predicted consensus requirements.

SEC7 appears not to contain introns. Consensus sequences for splice junctions were not detected. Furthermore, the continuity of the open reading frame and correspondence in size to the mRNA size suggest the absence of noncoding intervening sequences.

A termination codon (TAA) was observed 333 nucleotides downstream from the distal PstI restriction site, and an additional 286 nucleotides were sequenced. This latter region does not contain typical yeast transcription termination signals (37, 38). Whereas the first 100 nucleotides of this putative 3′-noncoding region have the usual AT-rich character (69%), the remaining 186 nucleotides are GC-rich (64%). The latter may correspond to vector sequences since the distal Sphi restriction site is contained in the plasmid (pCF35) used to express the genomic library.

Sec7p Sequence Analysis—The large open reading frame of the SEC7 sequence was used to scan the NBRF Protein Data Bank for homologies. Analysis was performed by the FASTP program (32) using the entire or smaller portions of the predicted sequence. No significant homologies to any registered protein sequences were noted. Domains characteristic of nucleotide-binding, calcium-binding, or protein kinase activity also were not detected.

Eight potential N-linked glycosylation sites (Asn-X-Thr) were noted in the Sec7p sequence. These sites, if used, would be diagnostic of a glycoprotein within the secretory pathway. Since Sec7p is required for membrane traffic from the Golgi apparatus, the protein may function from within the organelles of the secretory pathway. The N-terminal region of Sec7p was not predicted to be hydrophobic, as would be expected if Sec7p contained a signal sequence for translocation across the endoplasmic reticulum. Hydropathy analysis (39) of Sec7p predicted a strongly hydrophilic character for the polypeptide. Only one region of the sequence approximates the hydrophobic structure of a membrane anchor domain. This region, starting at amino acid 1917, extends for 19 residues and comprises 2 charged, 7 polar, and 10 hydrophobic amino acids. It seems unlikely, therefore, that SEC7 encodes either a membrane-spanning protein or a soluble luminal protein. We suspect that Sec7p resides in cytosol or is associated with the cytoplasmic surface of a membrane and therefore does not utilize any of the eight potential N-linked glycosylation sites.

One striking feature of the predicted Sec7p was the highly acidic domain near the amino terminus (Fig. 5). Beginning at residue 89, 25 of the next 29 amino acids (84%) are acidic. The segment from positions 89 to 213 has a predicted pI of 3.0 and consists of 47% acidic residues, including stretches of
Fig. 4. Sequence of the SEC7 gene. The complete sequence of the SEC7 gene as well as 724 nucleotides of flanking DNA are presented. The sequence is shown only for the coding strand of the gene. The TATA consensus sequences in the 5′ flanking region are underlined. The predicted Sec7p open reading frame, starting at nucleotide 440, is shown above the corresponding nucleotide sequence. Residue number is displayed in the open reading frame. Nucleotide number is shown for flanking DNA sequence.
FIG. 5. Highly charged region of Sec7p. The predicted amino acid sequence of Sec7p from residues 89 to 215 is highlighted. The single-letter code for amino acids is used. Content of aspartate, glutamate, and serine in this highly charged region is summarized below.

14 consecutive glutamates and 13 consecutive acidic residues. Only 2 basic residues were noted in this region, and none were found in the segment from positions 87 to 204. In contrast, the remaining Sec7p contained 14% acidic and 11% basic amino acids, giving a predicted pl of 6.0. Also somewhat unusually, the acidic domain was comprised of 21% serine, compared to 9% in the remainder of the protein.

Several other proteins with highly acidic domains have been described. Nucleoplasmin, nucleolin, high mobility group 1 (HMG-1), and CENP-B (centromere protein-B) (Refs. 40-43; also see Ref. 44 for a review) are examples of nuclear proteins that share an acidic domain. Structural roles have been proposed for these domains. Indeed, polyglutamic acid can act as a template for core histone organization and regulate nucleosome assembly in vitro (45). Although Sec7p is apparently not a nuclear localized protein, highly acidic domains may be structural motifs for cytosolic proteins as well. This domain, located near the amino terminus of the 230-kDa polypeptide, may be anchored to or function in the recognition of proteins or lipids on the cytoplasmic surface of the Golgi apparatus.

Given the predicted size of Sec7p, the polypeptide could serve as a template for the assembly of proteins and lipids into a "transport complex" required for traffic between compartments of the secretory pathway (3). Sec7p could alternatively serve as a cytoskeletal protein to enclose budding transport vesicles in a lattice structure reminiscent of clathrin coats. The polyacidic domain of Sec7p may play some essential role in the interaction of Sec7p with other components of intracellular protein traffic.

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