The Sec15 Protein Responds to the Function of the GTP Binding Protein, Sec4, to Control Vesicular Traffic in Yeast

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Abstract. SEC15 function is required at a late stage of the yeast secretory pathway. Duplication of the gene encoding the ras-like, GTP-binding protein, Sec4, can suppress the partial loss of function resulting from the sec15-1 mutation, but cannot suppress disruption of sec15. Analysis of the SEC15 gene predicts a hydrophilic protein product of 105 kD. Anti--Sec15 antibody recognizes a protein of 116-kD apparent molecular mass which is associated with a microsomal fraction of yeast in a strongly pH dependent fashion. Overproduction of Sec15 protein interferes with the secretory pathway, resulting in the formation of a cluster of secretory vesicles, and a patch of Sec15 protein revealed by immunofluorescence. The sec4-8 and sec2-41 mutations, but not mutations in other SEC genes, prevent formation of the Sec15 protein patch. We propose that Sec15 protein responds to the function of the Sec4 protein to control vesicular traffic.

The analysis of protein secretion in eukaryotic cells has established a well-defined intracellular transport pathway for secreted proteins. In this pathway, newly synthesized proteins pass from the ER through the Golgi complex to the plasma membrane. These transport events are mediated by interorganelle vesicular carriers that bud from the donor compartment and fuse with the acceptor compartment, releasing the transported proteins into the target organelle (Palade, 1975). Biochemical analysis has led to the identification of several factors required for vesicular transport through the Golgi apparatus (Wattenberg and Rothman, 1986; Block et al., 1988). Genetic analysis of the yeast secretory pathway has revealed 26 genes whose products are needed for protein transport from the ER to the plasma membrane (Novick and Schekman, 1979; Novick et al., 1980; Newman and Ferro-Novick, 1987; Segev et al., 1988). Studies of temperature-sensitive alleles have shown that 10 of these genes govern vesicular traffic from the ER to the plasma membrane.

In our earlier reports we established that one of these 10 late acting genes, SEC4, encodes a GTP-binding protein that is associated with the cytoplasmic face of secretory vesicles and the plasma membrane (Salminen and Novick, 1987; Goud et al., 1988). The evidence provided by these studies suggested that the Sec4 protein (Sec4p) cycles between the plasma membrane and the secretory vesicles. This cycle of Sec4p localization may be obligatorily coupled to a cycle of GTP binding and hydrolysis (Walworth et al., 1989). A related GTP-binding protein the YPT1 gene product, has been shown to participate in yeast secretion at an earlier stage of the pathway (Segev et al., 1988). In addition, an as yet unidentified GTP-binding protein has been implicated in controlling traffic within the Golgi apparatus in a mammalian system (Melancon et al., 1987). These findings suggest that this type of mechanism may be generally applicable to all vesicular transport events, but each class of vesicles may require a different, structurally related, GTP-binding protein.

GTP binding proteins, in general, fulfill their cellular function by regulating an effector protein. In its GTP-bound state, the GTP binding protein interacts with the effector to modulate its activity. Hydrolysis of the nucleotide curtails the interaction. One of the key questions raised by our findings concerns the nature of the Sec4 effector. Possible candidates for the effector have been identified through studies on the genetic interactions between SEC4 and other SEC genes required at the late stage of the secretory pathway (Salminen and Novick, 1987). Duplication of SEC4 either on an episome or on the chromosome was found to suppress, to varying extents, the defects imposed by mutations in a subset of the other late-acting sec genes. This same set of mutations, when combined with the sec4-8 mutation in a haploid cell, caused lethality at the permissive temperature. The strongest interaction was seen between the SEC4 and the SEC15 genes, where the duplication of SEC4 suppressed the growth defect, the secretion defect, and the accumulation of vesicles in a sec15-1 strain. In this article we report the analysis of the SEC15 gene. The aim of the work is to extend our understanding of the interaction between these two gene products. We present evidence suggesting that the Sec15 protein may be the target or a component of the target of Sec4 control.
Materials and Methods

Yeast Genetic Techniques

Yeast strains used in this study are listed in Table I. Plasmids are listed in Table II. Yeast cultures were grown in rich medium (YPD), containing 1% Bacto yeast extract, 2% Bacto peptone (Difco Laboratories Inc., Detroit, MI) and 2% glucose, or in minimal medium (SD), containing 0.7% yeast nitrogen base without amino acids (Difco Laboratories Inc.), 2% glucose, and supplemented for auxotrophic requirements as described by Sherman et al. (1974) when necessary. To induce the overproduction of the Sec15p nitorgen base without amino acids (Difco Laboratories Inc.), 2% glucose, Table II. Yeast strains used in this study are listed in Table I. Plasmids are listed in

 complementation and suppression assays have been described earlier (Salminen and Novick, 1987). The crosses, sporulation of diploids, and dissection of tetrads were done as described by Sherman et al. (1974).

Nucleic Acid Techniques

Bacteria and plasmid constructions were done as described earlier (Salminen and Novick, 1987; Goud et al., 1988). Plasmid pNB90 was isolated from a plasmid library of wild-type yeast inserts in YCp50, a centromere based shuttle vector containing yeast CEN4, URA3*; amep*, tet*. This yeast genomic library was previously described (Rose et al., 1987). Plasmid pNB140 was constructed by inserting the 6.1-kb Bam HI fragment from pNB13 into the Bam HI site of YCp50. Plasmid pNB143 was generated by deletion of the 2.4-kb Hind III fragment of pNB140. To construct the muticopy plasmid, pNB148, the 3.7-kb Hind III-Bam HI fragment from pNB143 was inserted into the Hind III-Bam HI sites of the 2-μm circle based vector, pRB307 (from collection of D. Botstein, Genentech, South San Francisco, CA).

Construction of pNB186, which contains the 3.35-kb complementing fragment, involved deletion of the 0.4-kb Bam HI-Xba I fragment of pNB143. The recessed 3' ends of the plasmids were filled using Escherichia coli pol I Klenow fragment (Boeringer Mannheim Biochemicals, Indianapolis, IN) and blunt-end ligated. This ligation recreates the Bam HI site but removes the Xba I site. Plasmids pNB187 (Cen) and pNB191(Ylp) have been described earlier (Goud et al., 1988). Integrating plasmid pNB192 was constructed by cloning the Hind III-Bam HI fragment from pNB186 in the Hind III-Bam HI sites of pNB191. Integrating plasmid pNB193, which contains the internal 1.25-kb Eco RI-Cla I fragment, was constructed by inserting this fragment in the Eco RI-Cla I sites of pNB191. Plasmid pNB291, in which the SEC15 gene is disrupted by the LEU2 gene, was constructed by cloning the ~3-kb Bgl2-Bgl2 fragment carrying LEU2 from YEp3 into the Bgl2 sites of the SEC15 gene in pNB192. To generate the linear fragment carrying the disrupted copy of SEC15, pNB291 was digested with Hind III and Bam HI.

Cloning SEC15 behind the inducible GALI promoter was as follows: pNB192 was cut with Hind III and blunt ended as above. The linear vector was further digested with Kpn I and the 440-bp Hind III-Kpn I fragment and the vector fragment were purified. The isolated Hind III-Kpn I fragment was digested with Alu I (Alu I cuts 9 bp upstream from the ATC), and the resulting two fragments 200 and 240 bp were separated in 10% acrylamide gel. The 200-bp Alu I-Kpn I fragment was isolated and ligated with the Hind III (blunt)–Kpn I digested vector in the presence of kinased Bam HI linker (dGGGATCCC; Boeringer Mannheim Biochemicals). This ligation generated plasmid pNB299, carrying the SEC15 gene on a Bam HI-Bam HI fragment. Plasmid pNB300 which contains the SEC15 gene under GALI control was constructed by cloning the Bam HI-Bam HI fragment from pNB299 into the Bam HI site of pNB187. Integrating plasmid pNB304, containing the SEC15 gene under GALI control, was constructed by cloning the 4.5-kb Pvu I–Sal I fragment from pNB300 in Pvu I–Sal I sites of pNB191.

The use of the pATH protein fusion system has been described earlier (Goud et al., 1988). Plasmid pNB164, which contains TrpF fused to an internal fragment of the SEC15 gene (amino acids 257–676), was constructed by inserting the 1.25-kb Eco RI-Cla I fragment from pNB186, in frame into the Eco RI-Cla I sites in the polylinker region of pATH11. Plasmid pNB301 which contains the TrpF fused to the amino terminal third of the Sec15 protein (amino acids 1–241) was constructed as follows: pNB299 and pATH2 were digested with Eco RI and Hind III, respectively, and the recessed ends were blunted as described above. Both vectors were then cut with Bam HI and electrophoresed into an agarose gel. The 0.7-kb Bam HI–Eco RI (blunt) fragment from pNB299, and the pATH2 vector were purified from the agarose gel. The purified fragments were religated and used to transform E. coli as described before (Goud et al., 1988).

Table I. Yeast Strains Used

<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotype</th>
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<tbody>
<tr>
<td>NY13</td>
<td>MATa, ura3-52</td>
</tr>
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<td>NY15</td>
<td>MATa, ura3-52, his4-619</td>
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<td>NY64</td>
<td>MATa, ura3-52, sec15-1</td>
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<td>MATa, LEU2-3, 112, ura3-52</td>
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<td>NY180</td>
<td>MATa, LEU2-3, 112, ura3-52</td>
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<td>NY363</td>
<td>MATa/α, LEU2-3, 112/+, ura3-52/ura3-52, his4-619/+</td>
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<tr>
<td>NY376</td>
<td>MATa, ura3-52, sec15-1, SEC4::pNB141 (SEC4, URA3)</td>
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<td>NY440</td>
<td>MATa, ura3-52, his4-619, pNB148 (2 μm, SEC15, URA3)</td>
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<td>NY451</td>
<td>MATa, ura3-52, Gal*</td>
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<td>NY456</td>
<td>MATa, ura3-52, sec4-8, Gal*</td>
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<td>NY467, 468</td>
<td>MATa, ura3-52, his4-619, SEC15::pNB192 (SEC15, URA3)</td>
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<td>NY483</td>
<td>MATa/α, LEU2-3, 112/+ , ura3-52/ura3-52, his4-619/+ , sec15::pNB193 (sec15, internal fragment, URA3)</td>
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<td>NY503</td>
<td>MATa, ura3-52, his4-619, sec6-4, Gal*</td>
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<td>NY662</td>
<td>MATa, LEU2-3, 112/LEU2-3, 112, ura3-52/ura3-52, SEC15::SEC15::LEU2</td>
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<td>NY724</td>
<td>MATa, ura3-52, Gal*, SEC15::pNB304 (GAL1-SEC15, URA3)</td>
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<td>MATa, ura3-52, Gal*, his4-619, sec5-24, SEC15::pNB304 (GAL1-SEC15, URA3)</td>
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<td>NY753</td>
<td>MATa, ura3-52, Gal*, his4-619, SEC15::pNB304 (GAL1-SEC15-1, URA3)</td>
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<td>NY754</td>
<td>MATa, ura3-52, Gal*, his4-619, sec2-41, SEC15::pNB304 (GAL1-SEC15, URA3)</td>
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Table II. Plasmids Used (see Materials and Methods for constructions)

<table>
<thead>
<tr>
<th>Plasmid</th>
<th>Derivation</th>
</tr>
</thead>
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<tr>
<td>pNB140</td>
<td>YcP50, SEC15; 16.5 kb genomic insert in the Bam HI site</td>
</tr>
<tr>
<td>pNB140</td>
<td>YcP50, SEC15; 6.1 kb Bam HI-Bam HI fragment from pNB140, in Bam HI site</td>
</tr>
<tr>
<td>pNB143</td>
<td>YcP50, SEC15; 3.7 kb Hind III-Bam HI fragment, Hind III Δ of pNB140</td>
</tr>
<tr>
<td>pNB148</td>
<td>2 μm, SEC15; 3.7 kb Hind III-Bam HI fragment from pNB143, in Hind III-Bam HI sites of vector, pRB307 (from collection of D. Botstein)</td>
</tr>
<tr>
<td>pNB164</td>
<td>pATH11, TrpE-SEC15(^{5-241}) fusion; 1.25 kb Eco RI-Cla I fragment from pNB186 into Eco RI-Cla I sites of pATH11</td>
</tr>
<tr>
<td>pNB186</td>
<td>YcP50, SEC15; 3.35 kb Hind III-Bam HI fragment, Bam HI-Xba I Δ of pNB143</td>
</tr>
<tr>
<td>pNB187</td>
<td>YcP50 with GALI promoter; 0.28 kb Eco RI-Bam HI fragment in Eco RI-Bam HI sites, expression under GALI control by cloning into the Bam HI site</td>
</tr>
<tr>
<td>pNB191</td>
<td>Ylp5 with unique Pvu II site removed by Bal31 digestion</td>
</tr>
<tr>
<td>pNB192</td>
<td>Ylp5, SEC15; 3.35 kb Hind III-Bam HI fragment from pNB186, in Hind III-Bam HI sites of pNB191</td>
</tr>
<tr>
<td>pNB193</td>
<td>Ylp5, SEC15; internal 1.25 kb Eco RI-Cla I fragment, into Eco RI-Cla I sites of pNB191</td>
</tr>
<tr>
<td>pNB291</td>
<td>Ylp5, sec15::Leu2 gene disruption; 3 kb Bgl2-Bgl2 fragment from Ylp13 (Leu2) replacing the Bgl2-Bgl2 internal fragment of SEC15 in pNB192</td>
</tr>
<tr>
<td>pNB299</td>
<td>Ylp5, SEC15; 3.1 kb Bam HI-Bam HI fragment, Hind III-Alu 1 Δ of pNB192, SEC15 leader sequence removed</td>
</tr>
<tr>
<td>pNB300</td>
<td>YcP50, GALI-SEC15; 3.1 kb Bam HI-Bam HI fragment from pNB299 into Bam HI site of pNB187</td>
</tr>
<tr>
<td>pNB301</td>
<td>pATH2, TrpE-SEC15(^{5-241}) fusion; 0.7 kb Bam HI-Eco RI (blunt) fragment from pNB299 into Bam HI and Hind III (blunt) sites of pATH2</td>
</tr>
<tr>
<td>pNB304</td>
<td>Ylp5, GALI-SEC15; 4.5 kb Pvu I-Sal I fragment from pNB300 into Pvu I and Sal I sites of pNB191</td>
</tr>
</tbody>
</table>

DNA Sequencing and Protein Homology Analyses

Nucleotide sequencing was carried out by the dideoxy chain termination method (Sanger et al., 1977) in the presence of d-35SdATP (650 Ci/mmol; Amersham Corp., Arlington Heights, IL) (Williams et al., 1986). The template DNA was obtained by subcloning restriction fragments from pNB143 into M13 phage derivatives mpl8 or mpl9. The predicted protein sequence was compared with the National Biomedical Research Foundation Library by the FASTP program in the kitup-2 mode (Lipman and Pearson, 1985).

Southern Blot Hybridization

Total yeast DNA was extracted from cells by the method of Holm et al. (1986). DNA (1 μg) was digested with Bam HI, fractionated by electrophoresis in a 0.5 % agarose gel, and transferred to nitrocellulose filters (Southern et al., 1975). The prehybridization, hybridization, and washing of the filters and the preparation of the probe were essentially as described elsewhere (Emanuel et al., 1986). In each hybridization experiment the isolated Eco RI-Cla I fragment from pNB186 was used as a probe.

Preparation of the TrpE-SEC15 Fusion Proteins and Rabbit Immunization

The fusion proteins were produced in E. coli strains, NRBI64 and NRBI301 (DH1 transformants containing the plasmid pNB164 or pNB301, respectively) essentially as described earlier (Goud et al., 1988). Cells from 200 ml culture were washed with 25 mM Tris-HCl, pH 7.0. The pellet was resuspended in 5 ml of cracking buffer (10 mM NaPi, pH 7.2, 1% β-mercaptoethanol, 1% SDS, 6 M urea [95°C, 75°C) by vortexing in a 50 ml centrifuge tube and incubated at 37°C for 30 min. To remove DNA the lysate was spun 30 min at 25,000 rpm in a 5013 rotor (Beckman Instruments, Inc., Palo Alto, CA) and the pellet was discarded. Bromo protein was electroeluted at 100 V from gel cubes in dialysis buffer and collected.

were given subcutaneously at 4wk intervals, mixing the fusion protein/PBS solution (20 μg) with Freund's incomplete adjuvant. After a titre was detected boosts were given by injecting the fusion protein/PBS solution (10 μg) into hind leg muscles. Immunization with the fusion protein isolated from NRBI301 (TrpE-SEC15\(^{5-241}\)) was done essentially as described earlier (Goud et al., 1988; Louvard et al., 1982).

The affinity purification of the antibodies was carried out as described previously (Goud et al., 1988). The crude serum was first circulated through a column containing the TrpE-protein, for 3 hrs. The flow-through was circulated 3 times through column containing the TrpE-SEC15 fusion-protein. The antibodies were eluted from the immunoabsorbent according to Guesdon and Avrameas (1976).

Electrophoresis and Immunoblotting

For SDS-PAGE, samples were heated for 5 min at 100°C in sample buffer containing 2% SDS and run on 8 or 10% slab gels according to Laemmli (1970). After transfer onto nitrocellulose (BA 83, 0.22 μm; Schleicher & Schuell, Inc., Keene, NH) overnight at 4°C, SEC15 was probed with affinity-purified antisera (αSec15\(^{5-241}\); 250 μg/ml, 1/2,000 dilution; or αSec15\(^{5-241}\); 130 μg/ml, 1/500 dilution) and radiiodinated staphylococcal protein A (0.5 μCi/ml; 30 mCi/mg; Amersham Corp.) as described elsewhere (Burnette, 1981; Goud et al., 1988) with following modifications. Filters were washed for 30 min at room temperature. After washing, the filters were dried and autoradiographed 12-24 h.

Cell Fractionation and Extraction Experiments

Cells were grown at 25°C to an A600 of 2.0 as described above. Usually 200-400 A600 units were pelleted and washed once with buffer, 10 mM Tris, pH 7, 10 mM NaCl. Cells were resuspended in spheroplast medium (50 mM Tris, pH 7.5, 10 mM NaCl, 1 M sorbitol, 40 mM β-mercaptoethanol, and 0.125 mg/ml of Zymolyase-100T; ICN Radiochemicals, Irvine, CA) at a density of 10 A600 units/ml, and converted to spheroplasts during a 45-min incubation at 7°C. The spheroplasts were pelleted and resuspended in 2 ml of ice-cold lysis buffer (0.8 M sorbitol, 20 mM triethanolamine, 1 mM EDTA, at pH 7.2 or 6.0, containing 1 mM PMSF, and 10 μl/10 ml of protease inhibitor cocktail: leupeptin, chymostatin, pepstatin, antipain, apronitin, 1 mg/ml). The lysis was homogenized with 20 strokes in 2 ml tissue grinder (Ten Broeck; Fisher Scientific Co., Pittsburgh, PA) and centrifuged at 450 g for 3 min. The homogenization protocol was repeated to the pellet (P1), and the supernatants were pooled. The total pro-

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tein concentration of the lysate was adjusted to 2.8 mg/ml with lysis buffer (SI). The pH of the lysate was adjusted to 6.5, but necessary, by adding 1 M MES-buffer pH 6.5, to 50 mM into SI. In differential centrifugation experiments the SI supernatant was spun at 12,000 rpm (100,000 gsup) in a rotor (model 50Ti; Beckman Instruments Inc., Palo Alto, CA) in 1.5 ml volume for 10 min at 4°C. The pellet (P2) was resuspended in 1.5 ml lysis buffer. The S2 supernatant was further centrifuged at 40,000 rpm (100,000 gsub) in the same rotor in 1 ml volume for 1 h at 4°C and the pellet was resuspended in 1 ml lysis buffer. In crude membrane separation centrifugation the SI supernatant was directly spun at 40,000 rpm (100,000 gsub) for the extraction experiments the low speed (450 g) supernatant was adjusted to 5 mg/ml. 0.5 ml of this concentrated lysate was mixed with an individual extracting agent at 2 × concentration (i.e., 10 M urea, 2% Triton X-100, or 2 M NaCl). The lysate was in-

Results

tation (i.e., 10 M urea, 2% Triton X-100, or 2 M NaCl). The lysate was in-

ferred six plasmids from a genomic plasmid bank (Rose et al., 1987), and all viable spores were Ura-. The nongrowing colonies 

were dissected. Two spores from each tetrad were inviable, and all viable spores were Ura+. These data establish that the cloned sequence contains the SEC15 gene.

**SEC15 Is an Essential Locus**

Although the isolation of a recessive conditional lethal mutation strongly suggests that the SEC15 gene product is essential, this was verified by constructing a gene disruption mutation by the method described by Shortle et al. (1982). Plasmid pNB193, which carries the internal Eco RI-Cla I fragment from pNB186 (Fig. 1) was cleaved at the unique Pvu II site. This linearized plasmid was used to transform NY363, a diploid strain homozygous for the ura3-52 mutation, thereby replacing one chromosomal copy of SEC15 with a duplication in which both copies are truncated (Fig. 2 B), effectively disrupting the SEC15 gene. Southern blot analysis of NY483, a diploid Ura+ transformant of NY363, verified the integration-disruption event (Fig. 2 C). The diploid transformant was shifted to sporulation media, and 12 tetrads were dissected. Two spores from each tetrad were inviable, and all viable spores were Ura+. The nongrowing colonies were arrested at the two-cell stage. This construction allows the synthesis of a truncated gene product, but since the expected, lethal result was obtained, this truncated product appears to be nonfunctional. This result establishes this putative null allele as a recessive lethal mutation, and the SEC15 gene as an essential locus.

**Figure 1.** Restriction map of the 3.35-kb complementing region containing the SEC15 gene in plasmid pNB186. The open bar shows the coding region. The start of the gene is depicted by the solid triangle. H, Hind III; K, Kpn I; Bc, Be II; E, Eco RI; G, Bgl I; P, Pvu II; V, Eco RV; C, Cla I; Hc, Hind II; X, Xba I; B, Bam HI. Note that the genomic Xba I site has been converted to Bam HI site on the plasmid.

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SEC4 Duplication Cannot Suppress a Null Allele of sec15

In our earlier study we had established that duplication of the SEC4 gene could partially (~70%) suppress the growth defect, the secretion defect, and the accumulation of vesicles in a strain carrying a temperature-sensitive sec15 defect. We have tested the ability of SEC4 duplication to suppress a null allele of sec15. A diploid strain (NY648) homozygous for the ura3-52 and the leu2-3, 112 mutations, was transformed to Leu+ with the Hind III-Bam HI fragment from plasmid pNB291. This fragment contains the SEC15 gene, disrupted with the LEU2 gene. A diploid strain (NY648) was transformed with a linear Hind III-Bam HI fragment from plasmid pNB291. This fragment contains the SEC15 gene disrupted with the LEU2 gene, and upon transformation replaces one chromosomal copy with the disrupted one.

SEC4Duplication Cannot Suppress a Null Allele of sec15

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SEC15 Sequence

After localizing the SEC15 gene to the 3.35-kb fragment we determined the nucleotide sequence of this region. The sequence contains one long open reading frame starting from nucleotide 1 and ending at nucleotide 2,733 (Fig. 3). This predicts a protein product of 105 kD in molecular mass. The predicted amino acid sequence is hydrophilic (54.5% polar, 45.5% nonpolar), and does not contain a hydrophobic domain long enough to span the lipid bilayer. 26% of the amino acids are charged, giving the protein a net negative charge (excess of 26 negative charges; pI 5.95) at neutral pH. We screened the PIR protein database for identical sequences, but found no sequence with substantial homology to the SEC15 sequence. The upstream region of the sequence from the presumed ATG start codon contains another, in-frame, start codon at nucleotide position -158-156, this reading frame is terminated by a UGA stop codon 27 nucleotides upstream from the predicted start. Since UGA is not a strong stop codon in yeast, we determined if the upstream region carried essential information or could be replaced by another promoter. The SEC15 gene was cloned behind the inducible GAL1 promoter, so that only nine nucleotides were left from the upstream region (see Materials and Methods). NY64 (sec15-1, ura3-52) was then transformed to Ura+ with pNB300, a centromere based plasmid containing this construction. Transformants, selected on minimal glucose plates at 25°C, were streaked to single colonies on minimal glucose or minimal galactose plates which were incubated at 25°C for 5 h and shifted to 37°C. Growth was detected at 37°C on plates containing galactose, but not on glucose plates. Thus galactose induced the synthesis of functional SEC15 gene product capable of complementing the sec15-1 defect. This suggested that the wild-type SEC15 gene is translated from

Figure 2. Cloned sequence integrates into the SEC15 locus. (A) Plasmid pNB192 was cleaved at the unique Pvu II site. Wild-type strain (NY15) was transformed with the linear plasmid DNA. Upon transformation the linear plasmid integrates into the genome and generates a duplication of the gene with the URA3 gene between the two copies. (B) Plasmid pNB193, which contains the internal Eco RI–Cla I fragment of the SEC15 gene, was cleaved with Pvu II and the linearized plasmid DNA was used to transform a diploid strain (NY363). Integration of this plasmid into the diploid genome replaces one chromosomal copy with a duplication in which both copies of the gene are truncated. (C) Southern blot analysis verifies the integration and disruption events. Total DNA from parental strains and from transformants derived from NY15 (NY467, 468) and from NY363 (NY-
Figure 3. Nucleotide sequence of the 3.35-kb complementing region. SEC15 gene starts at the nucleotide position 1 and ends at the position 2733. Predicted amino acid sequence of the Sec15p is shown as single letter code under the DNA sequence.
the start codon at nucleotide position 1–3. This conclusion was supported by the observation that the gene product encoded by the GALI-SEC15 construction had the identical mobility, in an SDS gel, to the SEC15 gene product transcribed by its own promoter (not shown).

**Generation of Antisera against the Sec15p**

To address the biochemical properties of the Sec15p we have raised polyclonal rabbit antibodies against two nonoverlapping portions of the Sec15 sequence (amino acids 1–241 and 257–676), each fused to the bacterial TrpE protein (see Materials and Methods). As shown above, the nucleotide sequence of SEC15 predicts a protein product of 105 kD. However, by Western blot analysis, these antibodies recognized a protein in a wild-type lysate that migrated at 116 kD apparent molecular mass (Fig. 4). To confirm that this band represented the SEC15 gene product we cloned the SEC15 gene into a yeast multicopy plasmid. A wild-type strain was transformed with this plasmid (pNB148) and transformants were selected and maintained in minimal medium (SD). Immunoblot analysis of lysates from wild-type (NY15) and plasmid containing cells (NY440) showed a 12-fold increase of the 116-kD immunoreactive band in the plasmid containing cells (NY440). This proved that the antibody recognized the Sec15p. Some additional bands were also seen. Of these the 100- and 85-kD bands were considered degradation products, since they are amplified in lysates from cells overproducing SEC15. The band around 50 kD comigrates with a major protein band in the lysate and probably represents nonspecific binding of the antibody. In fractionation experiments this band shows a distribution independent of Sec15p (not shown). Analysis of a sec15-1 strain (NY64) showed that the mutant protein was shifted to a higher mobility and was present at a reduced level relative to the wild-type protein, supporting our identification of the 116-kD band as Sec15p. The nature of the sec15-1 mutation is not known at this time. It may result in either premature truncation of the protein or cause the mutant translation product to be subject to proteolysis and therefore unstable.

**Duplication of SEC4 Does Not Alter Expression or Stability of Sec15-1 Protein**

To address the mechanism by which duplication of SEC4 suppresses sec15-1 we performed Western blot analysis using anti-Sec15p antibody on sec15-1 strains containing one or two copies of SEC4 (NY64 and NY376, respectively). At 25°C the two strains have approximately equal levels of Sec15-1 protein, although it is reduced in abundance and increased in its mobility with respect to the Sec15 protein in wild-type cells (NY15) (Fig. 4). When NY64 and NY376 cells are shifted to the restrictive temperature, 37°C, the amount of the Sec15-1 protein drops to 20% of the wild-type level at 25°C, however it remains approximately equal in both mutant strains (Fig. 4). Wild-type cells (NY15) exhibit a decrease of lesser magnitude in the level of Sec15 protein after growth at 37°C. The apparent loss of Sec15 and Sec15-1 protein upon a shift to 37°C is seen with antibodies directed against either the amino terminus or the internal region of the protein. The Sec15-1 protein in NY64 and NY376 shows slight, but reproducible shifts to lower mobility upon a shift to the restrictive temperature. The nature of these shifts is unknown. In total, these results indicate that duplication of SEC4 does not increase either the expression or the stability of the Sec15-1 mutant protein.

**Solubility of the Sec15p Is pH Dependent**

The sequence of the SEC15 gene predicts a hydrophilic protein. We have investigated the solubility properties of the Sec15p in cell fractionation experiments (see Materials and Methods). NY467 cells were grown in rich medium (YPD) at 25°C, collected by centrifugation and converted to spheroplasts. Spheroplasts were lysed osmotically and the crude lysate spun at 450 g to remove unlysed cells. Aliquots were prepared for gel electrophoresis by boiling in sample buffer. 175 μg of samples per lane were electrophoresed on a 2-μm circle based multicopy plasmid, were grown in SD minimal medium containing histidine at 25°C. 30 A600 units of cells were broken by agitation with glass beads at +4°C, and the lysates were suspended in 1.5 ml of ice-cold lysis buffer at pH 7.2 (see Materials and Methods). Lysates were removed from the glass beads to separate tubes, and centrifuged 450 g to remove unbroken cells. Aliquots were prepared for gel electrophoresis by boiling in sample buffer. 175 μg of samples per lane were electrophoresed on a 2-μm circle based multicopy plasmid, were grown in SD minimal medium containing histidine at 25°C. 30 A600 units of cells were broken by agitation with glass beads at +4°C, and the lysates were suspended in 1.5 ml of ice-cold lysis buffer at pH 7.2. Analysis of the lysates from cells overproducing SEC15. The band around 50 kD comigrates with a major protein band in the lysate and probably represents nonspecific binding of the antibody. In fractionation experiments this band shows a distribution independent of Sec15p (not shown). Analysis of a sec15-1 strain (NY64) showed that the mutant protein was shifted to a higher mobility and was present at a reduced level relative to the wild-type protein, supporting our identification of the 116-kD band as Sec15p. The nature of the sec15-1 mutation is not known at this time. It may result in either premature truncation of the protein or cause the mutant translation product to be subject to proteolysis and therefore unstable.

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pelletable structure.

Centrifuged at 100,000 g to generate supernatant

elements were extracted from this fraction by 1% Triton X-100. 5 M urea

proceedures. In these experiments we have extracted the

Secl5p was found in the supernatant. The opposite

by adding 2-(N-morpholino)ethane sulfonic acid (MES) buf-

phenomenon was also observed when the pH 7.2 lysate was
equal volumes of samples (200 µg of total protein of S1) were elec-
trophoresed in the gel and transferred to nitrocellulose. The filter
was probed with αSecl5257-676 antibodies and iodinated protein A.

Figure 5. Analysis of the solubility properties of the Sec15 protein.
The top section of the figure shows the effect of pH on the solubility
of the Sec15p. The bottom section shows the solubility of the Sec15p
extracted from the pH 6.5 pellet (P2) by various reagents (see Results).
Lysates were prepared from strain NY467, which carries a duplication of the $SEC15$ gene integrated into the chromosome
(also see Fig. 2). Cells were grown in YPD at 25°C to $A_600$ of 2.0.
Lysates were prepared as described in Materials and Methods and
centrifuged at 100,000 g to generate supernatant (S2) and pellet (P2)
fractions. Aliquots of all samples were boiled in sample buffer.

Equal volumes of samples (200 µg of total protein of S1) were elec-
trophoresed in the gel and transferred to nitrocellulose. The filter
was probed with αSecl5517-676 antibodies and iodinated protein A.

lysate was prepared at pH 8.0, and then centrifuged as above,
all of the Sec15p was found in the supernatant. The opposite
was observed when the pH of the lysate was adjusted to 6.5
by adding 2-(N-morpholino)ethane sulfonic acid (MES) buf-
der directly to the pH 7.2 lysate; all of the Sec15p was found
in the membrane fraction (Fig. 5, top). The solubility of
the Sec15p was also affected by other parameters. When the lysate,
prepared at pH 7.2, was incubated on ice for 4 h before centrifugation,
we observed a shift of the Sec15p from the membrane fraction to the soluble fraction (Fig. 5). A similar
phenomenon was also observed when the pH 7.2 lysate was
diluted twofold with lysis buffer to 1.4 mg/ml and spun im-
immediately (not shown).

The nature of the interaction between the Sec15p and the
membrane fraction was tested by using various extraction
procedures. In these experiments we have extracted the
Sec15p from both the SI supernatant, adjusted to pH 6.5, and
from the pH 6.5, P2 pellet and obtained essentially the same
results in either case. Samples were treated with various re-
agents, spun at 100,000 g and the Sec15p was visualized by
Western blot analysis. As shown in Fig. 5, bottom, the Sec15p
is associated with the membrane fraction at pH 6.5. It is not
extracted from this fraction by 1% Triton X-100. 5 M urea
readily solubilizes Sec15p from the membrane fraction. Partial
solubilization was observed when the lysate was treated
with 1 M NaCl. These findings suggest an ionic interaction
rather than a hydrophobic interaction of Sec15p with a
pelletable structure.

Sec15p Associates with the Microsomal Fraction

We have studied the distribution of the Sec15p upon subcel-
ular fractionation by differential centrifugation. In these ex-
periments the SI (450 g) supernatants derived from wild-
type cells (NY 451) and cells that overproduce the Sec15p
from the GALI promoter (NY724) were used. NY724 was
constructed by transforming the NY451 wild-type strain with
the GALI-SEC15 integrating plasmid pNB304 (see Materials
and Methods). Supernatants were centrifuged at 10,000 g to
yield S2 and P2 and the S2 fractions were spun at 100,000 g
to yield S3 and P3. Aliquots of the different supernatants
(S1, S2, S3) and pellets (P1, P2, P3) generated during the
differential centrifugation were analyzed by Western blot as
above. Antibodies generated against the TrpE-Sec15 fusion
protein containing the aminoterminal portion of the Sec15,
αSec151-246, were used in this and the following experi-
ments. These antibodies allowed improved detection of the
Sec15p and also confirmed our earlier results.

Since we had observed a pH effect on the solubility of the
Sec15p we did these spins at both pH 7.2 and 6.5, to study
the possible difference in distribution. At pH 7.2 very little
Sec15p is seen in the P2 pellet, whereas S3 and P3 contain
the Sec15 protein distributed equally between these fractions
(Fig. 6). This distribution is consistent with our solubility
studies described above (Fig. 5, top). When the lysate is first
adjusted to pH 6.5 and then spun, Sec15p is found primarily
in the P3 fraction with no apparent soluble pool. A small
pool of Sec15p is seen in the P2 pellet. In similar centrifuga-
tion experiments we have found most of the endoplasmic
reticulum, plasma membrane, vacuolar, and mitochondrial
markers in the P2 fraction and the secretory vesicle marker,
invertase, in the P3 pellet fraction (Walworth and Novick,
1987; Goud et al., 1988). If Sec15p is associated with an or-
ganelle, possible candidates include secretory vesicles and
the Golgi apparatus.

When the Sec15p is overproduced from the GALI pro-

Figure 6. Differential centrifugation of lysates derived from wild-
type (NY451) and Sec15p overproducing cells (NY724). Cells were
grown in YP lactate overnight and then induced by addition of
galactose. After 5 h of induction, cells were harvested and lysates
(type) (NY 451) and Secl5p overproducing cells (NY724). Cells were
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Figure 7. Immunofluorescence localization of the Sec15 and Sec4 proteins. (a) NY451, SEC*, grown in galactose and labeled with αSec15^1-24 antibody; (b) NY724, SEC*, GAL-SEC15, grown in glucose and labeled with αSEC15^1-24 antibody; (c) NY724, SEC*, GAL-SEC15 grown in galactose and processed without primary antibody; (d) NY15, SEC* grown in glucose and labeled with αSec15^1-24 antibody. Excluding the top row of the figure, cells were grown in YP lactate-galactose medium for 15 h at 25°C, and fixed for immunofluorescence (e,f,i,j,m, and n) or were shifted to 37°C for 2 h before fixation (g, h, k, l, o, and p). (e) NY724, SEC*, GAL-SEC15, 25°C, labeled with αSec15^1-24 antibody; (f) NY724, SEC*, GAL-SEC15, 25°C, labeled with αSec4 antibody; (g) NY724, SEC*, GAL-SEC15, 37°C, labeled with αSec15^1-24 antibody; (h) NY724, SEC*, GAL-SEC15, 37°C, labeled with αSec4 antibody; (i) NY725, sec4-8, GAL-SEC15, 25°C, labeled with αSec15^1-24 antibody; (j) NY725, sec4-8, GAL-SEC15, 25°C, labeled with αSec4 antibody; (k) NY725, sec4-8, GAL-SEC15, 37°C, labeled with αSec15^1-24 antibody; (l) NY725, sec4-8, GAL-SEC15, 37°C, labeled with αSec4 antibody; (m) NY742, sec6-4, GAL-SEC15, 25°C, labeled with αSec15^1-24 antibody; (n) NY742, sec6-4, GAL-SEC15, 25°C, labeled with αSec4 antibody; (o) NY742, sec6-4, GAL-SEC15, 37°C, labeled with αSec15^1-24 antibody; (p) NY742, sec6-4, GAL-SEC15, 37°C, labeled with αSec4 antibody.

Overproduction of Sec15p Results in the Formation of a Patch of Sec15p, a Patch of Sec4p, and a Cluster of Vesicles

Immunolocalization of Sec15p in wild-type cells was problematic due to the low level of Sec15p under normal conditions. Wild-type cells (NY15) were grown in YPD at 25°C. Cells were fixed and prepared for immunofluorescence. Af-
finity-purified anti-Secl5p antibody was applied (see Materials and Methods) and the slides were visualized. Only a faint diffuse staining was observed in this situation (Fig. 7 d). This staining was somewhat brighter than was observed when no first antibody was used (Fig. 7 c).

To determine if overproduction of Secl5p would allow us to localize the protein, we analyzed cells which express Secl5 protein from the GALI promoter. NY724 cells were grown in YP lactate-galactose medium at 25°C for 15 h and fixed. The Secl5p signal was greatly enhanced in these cells and the most striking observation was the presence of a brightly staining concentrated patch. This structure was located either in the bud, or adjacent to an emerging bud (Fig. 7 b). Observation in a confocal microscope established that the staining was intracellular rather than cortical (not shown). This signal was not detected in wild-type Gal+ cells, grown in YP lactate-galactose (NY451; Fig. 7 a). Neither was it detected in NY724 cells grown on YP glucose (Fig. 7 b), nor in galactose induced NY724 cells without the first antibody (Fig. 7 c), indicating that the patch corresponds to Secl5p overproduced by expression from the GALI promoter.

We pursued the nature of the patch structure by thin section electron microscopy. Wild-type Gal+ (NY451) and Secl5p overproducing (NY724) cells were grown in YP lactate-galactose medium as described above. Cells were fixed and prepared for electron microscopy as described earlier (Salminen and Novick, 1987). These EM results demonstrate an accumulation of 100-nm vesicles in a concentrated array (Fig. 8, b and d). This vesicle cluster is often located towards the bud end of the cell. Wild-type cells grown in the same conditions did not accumulate vesicles (Fig. 8 a). It was not evident that this patch was held together by any apparent structure (Fig. 8 c).

In a previous study we have shown that Sec4p is associated with secretory vesicles (Goud et al., 1988). The observation of a cluster of vesicles in Secl5p overproducing cells therefore predicts the formation of a patch of Sec4p in these cells. As shown in Fig. 7 f, a patch of similar size and position to that seen with anti-Secl5p antibody was observed with affinity purified anti–Sec4p antibody, confirming that prediction. Furthermore, the result suggests that both Sec4p and Sec15p may be associated with the same vesicular structure.

Double label immunofluorescence experiments are precluded by the fact that both antisera were produced in rabbits, yet immunoelectron microscopic analysis is underway to test the association of Sec15p with the cluster of vesicles.

These data suggest that the increased amount of the Sec15p, overproduced from the strong GALI promoter, in some fashion interferes with the transport of the secretory vesicles at a stage between the Golgi apparatus and the plasma membrane, causing the vesicles to aggregate. Interference with the secretory pathway is generally associated with a growth defect (Novick et al., 1980). Cells induced to overproduce the Sec15p (NY724) grow at wild-type rate for 6 h, after which the growth rate slows by a factor of two. This slower growth rate is, nevertheless, maintained for up to 25 h. The slower growth rate may be an adjustment to the effect of accumulating vesicles, since we have observed by immunofluorescence with anti–Sec15p antibody and by thin section microscopy that the cluster of vesicles forms between 2 and 5 h of induction (not shown). Overproduction of the Sec15-I mutant protein does not cause a slowing of the growth rate and does not lead to a patch by staining with anti–Sec15p antibody (not shown).

**Sec15p Patch Formation Requires Function of Sec2 and Sec4**

The strong genetic interactions seen between SEC4 and SEC15, as well as several other SEC genes, suggests that their gene products may functionally interact on a dependent pathway (Salminen and Novick, 1987). These findings led us to test the effects of mutations in the various late-acting sec genes on the ability of overproduced Sec15 protein to form a patch. We transformed Gal+ derivatives of each of the 10 vesicle accumulating mutants with the integrating plasmid pNB304 to overproduce the Sec15p in these mutant cells. Resulting transformants were grown at steady state in YP lactate–galactose at 25°C, aliquots were shifted to grow at the restrictive temperature 37°C for 2 h, and cells were fixed for immunofluorescence.

Initial studies were done with sec4-8 and sec6-4 strains. The analysis of NY725 (sec4-8) cells with anti–Sec15p antibody showed that the patch-like Sec15p signal was much reduced in the sec4-8 mutant background. Even at 25°C, only a very small dot was seen at the tip of the cells reminiscent of the patch structure (Fig. 7 i). The staining of the NY725 cells grown at 25°C with anti–Sec4p antibody showed the presence of a weakly staining patch (Fig. 7 j). The lower intensity of the Sec4p signal can be explained by the fivefold reduction in the amount of the Sec4p present in the sec4-8 mutant cells (Goud et al., 1988). When the NY725 cells were shifted to the restrictive temperature and analyzed with both antibodies we found that the Sec15p and the Sec4p signals were more diffuse (Fig. 7, k and l). This suggested that the loss of Sec4 function at 37°C causes a dissociation of the residual patch structure. In SEC+ cells (NY724) shifted to 37°C the elevated temperature did not by itself affect the formation of the patches, as was seen with anti–Sec15p and anti–Sec4p antibodies (Fig. 7, g and h). In the sec6-4 mutant background at 25°C patches were seen with both anti–Sec15p and anti–Sec4p antibodies (Fig. 7, m and n). At the higher temperature the patches were still the predominant stained structures in these cells (Fig. 7, o and p) although there was some increased staining of the cytoplasm as well and a higher frequency of cells with multiple patches. These results suggest that the sec 6-4 mutation had a lesser effect on the formation of the patch resulting from the overproduction of Sec15p than did the sec4-8 mutation.

We have extended these studies to include mutants in all of the late acting genes. The mutants were grown at 25°C in noninducing media, 2% lactate, induced for 4 h at 25°C by the addition of 1% galactose, and then shifted to 37°C for 1 h, before fixation in formaldehyde. In each of the mutants, with the exception of sec2-4I and sec4-8, overproduction of Sec15 protein led to the formation of a patch or patches, as revealed by anti–Sec15p antibody (Fig. 9). In the case of sec2-4I (Fig. 9 c), general staining of the cell was seen with only a slight tendency towards local bright spots. Consistent with our previous findings, sec4-8 cells showed a nearly complete lack of patch formation (Fig. 9 e). A construction was made which overexpressed the sec15-I product from the GALI promoter in addition to the wild-type gene product from the SEC15 promoter (NY755) (Fig. 10, lane J0). Overexpression of the mutant protein did not lead to formation of a patch (Fig.
Figure 8. Electron microscopic analysis of NY724 cells overproducing the Sec15 protein from the GAL1 promoter. Cells were grown in YP lactate–galactose medium for 15 h before processing for microscopy. (A) Wild-type, NY451. (B, C, and D) NY724 cells show a distinct patch of aggregated secretory vesicles. Bars: (A, B, and D) 1 μm; (C) 2 μm.
Figure 9. Immunofluorescence localization of Sec15 protein in sec mutants overproducing the Sec15 protein from the GAL1 promoter. Cells were grown in YP lactate medium at 25°C then induced by addition of galactose. After 4 h of induction the cultures were shifted to 37°C for one additional hour then fixed and labeled with αSec15-24t antibody. (A) NY724, SEC+, GAL-SEC15; (B) NY748, sec1-1 GAL-SEC15; (C) NY755, sec2-41, GAL-SEC15; (D) NY751, sec3-2, GAL-SEC15; (E) NY725, sec4-8, GAL-SEC15; (F) NY749, sec5-24, GAL-SEC15; (G) NY742, sec6-4, GAL-SEC15; (H) NY750, sec8-9, GAL-SEC15; (I) NY752, sec9-4, GAL-SEC15; (J) NY753, sec10-2, GAL-SEC15; (K) NY754, SEC+, GAL-sec15-1.

Discussion

We have presented a characterization of SEC15 and its protein product. Our specific goal in this analysis has been to understand the physical basis underlying the strong genetic interaction seen between SEC15 and SEC4. Both of these genes are essential for growth. This suggests that both gene products play distinct and separate roles in the vesicular transport process. In support of this, we have extended the genetic analysis of the interaction between these genes by showing that duplication of the SEC4 gene can suppress the sec15-1 mutation but cannot suppress the deletion of the SEC15 gene from the genome. Thus partial, but indispensable, function is provided by the temperature-sensitive mutant protein, Sec15-1p, even at the restrictive temperature. The fact that the sec15-1 mutation was originally identified as incompletely restrictive with respect to invertase secretion (Novick et al., 1980) may reflect this partially functional mutant phenotype. We have also shown that the presence of a second copy of the SEC4 gene does not significantly alter the amount of the Sec15-1p in the cells, ruling out increased synthesis or stability of the mutant protein as the mechanism of suppression. We favor a model in which Sec4p acts as an upstream activator of Sec15p function. An increase in the level of Sec4p may stimulate the residual Sec15-1p to provide sufficient function. Our studies on the effects of the sec4-8 mutation on localization of Sec15 protein are consistent with this model.

The nucleotide sequence of SEC15 predicts a hydrophilic protein product of 105 kD, containing no hydrophobic stretches capable of spanning the lipid bilayer. Nevertheless, Sec15p is associated with the microsomal fraction by ionic interactions. This association presumably reflects an interaction with a protein component of a small organelle, possibly secretory vesicles. Efforts to pursue the fractionation studies further through the use of sucrose density gradients and gel filtration have been hampered by the sensitivity of Sec15p attachment to high molarities of sucrose, prolonged incubation, and high dilution. However, studies presented here
are consistent with an interaction of Sec15p with either secretory vesicles or the Golgi apparatus.

We initially cloned the SEC15 gene behind the strong GALI promoter to overproduce the Sec15p, and thus enhance the signal in immunofluorescence. Upon induction of synthesis, a concentrated patch of Sec15p became apparent. Thin section analysis indicated that a cluster of vesicles forms in response to Sec15p overproduction suggesting a physical association of Sec15p with secretory vesicles. Immunoelectron microscopy will be necessary to prove this point, however results from cell fractionation experiments are consistent with an association of Sec15p with vesicles in wild-type cells, and with the vesicle cluster in a Sec15p overproducer. In differential centrifugation experiments with wild-type cells the Sec15p was primarily found in the 100,000-g pellet, while in the Sec15 overproducer a substantial pool was also found to pellet at 10,000 g. This shift in distribution could reflect the formation of vesicular aggregates which pellet at the lower speed.

Sec15p is apparently not associated with the plasma membrane in wild-type cells, since Sec15p is not found in the 10,000-g pellet, yet most of the plasma membrane marker enzyme does pellet at this speed (Walworth et al., 1987; Goud et al., 1988). If Sec15p is associated with secretory vesicles, but not the plasma membrane, then it must either dissociate before vesicle fusion or very soon after fusion. Such a transient association fits well with a model of vesicular transport which can accommodate both the formation of vesicular aggregates which pellet at the lower speed and the association of Sec15p with secretory vesicles. The phenotype of the Sec15p overproducer has revealed a new, albeit aberrant function of the Sec15 protein, the ability to form a cluster of vesicles and a patch of Sec15p. We have used this property to ask if Sec15p function is dependent upon the function of any other SEC gene product. The mutations fall into two clear groups: sec4-8 and sec2-41 prevent formation of the Sec15p patch, while the other mutations do not. If we postulate that the secretion pathway consists of a linear series of dependent events, then we can conclude that Sec2p and Sec4p must function upstream of Sec15p. The other gene products may function downstream from Sec4p, or on an independent pathway. Given the somewhat artificial nature of the experiment, these conclusions must remain tentative at this time. Nonetheless, this result is consistent with the observation, discussed above, that duplication of SEC4 can suppress a partial loss of Sec15p function, but not total loss, and supports our model of Sec4p as an upstream activator of Sec15p function. Such a model is also consistent with the known functions of other GTP binding proteins. In general, these proteins do not, by themselves, catalyze enzymatic reactions other than the very slow hydrolysis of GTP, but through their interaction with downstream effectors they serve to control a broad range of cellular functions. Sec15p could be the immediate downstream effector of Sec4p, or there may be one or more protein intermediaries between Sec4p and Sec15p function. In fact, our finding that the sec2-41 mutation prevents patch formation by overproduction of Sec15p implicates Sec2p as a possible intermediary in such a chain of protein function. However, we cannot distinguish this from an alternative model in which Sec2p acts upstream of Sec4p.

While it may be premature to propose a specific model for Sec15 function, we can attempt to extrapolate from the vesicle aggregating phenotype of the overproducer to the normal function of the protein. Sec15 protein may, at normal concentration, serve to attach vesicles bearing Sec4 protein onto the appropriate target, the plasma membrane of the bud. At excess concentration, such a vesicle docking protein could lead to vesicle aggregation. Since a close structural homolog of Sec4, the Ypt I protein, appears to play a critical role in an earlier stage of the yeast secretory pathway (Segev et al., 1988) and an as yet unidentified GTP binding protein may function in transport through the mammalian Golgi (Melancon et al., 1987), we can speculate that there may be analogs of Sec15p. They may function, in response to their respective GTP binding protein, to attach the appropriate vesicle to the appropriate target membrane and thereby maintain the specific identity of the vesicular transport mechanism.

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