New Mutants of *Saccharomyces cerevisiae* Affected in the Transport of Proteins From the Endoplasmic Reticulum to the Golgi Complex

Linda J. Wuestehube, Rainer Duden, Arlene Eun, Susan Hamamoto, Paul Korn, Rachna Ram and Randy Schekman

Department of Molecular and Cell Biology and Howard Hughes Medical Institute, University of California, Berkeley, California 94720

Manuscript received June 24, 1995

Accepted for publication October 31, 1995

ABSTRACT

We have isolated new temperature-sensitive mutations in five complementation groups, *sec31–sec35*, that are defective in the transport of proteins from the endoplasmic reticulum (ER) to the Golgi complex. The *sec31–sec35* mutants and additional alleles of previously identified *sec* and vacuolar protein sorting (*vps*) genes were isolated in a screen based on the detection of α -factor precursor in yeast colonies replicated to and lysed on nitrocellulose filters. Secretory protein precursors accumulated in *sec31–sec35* mutants at the nonpermissive temperature were core-glycosylated but lacked outer chain carbohydrate, indicating that transport was blocked after translocation into the ER but before arrival in the Golgi complex. Electron microscopy revealed that the newly identified *sec* mutants accumulated vesicles and membrane structures reminiscent of secretory pathway organelles. Complementation analysis revealed that *sec32-1* is an allele of *BOS1*, a gene implicated in vesicle targeting to the Golgi complex, and *sec33-1* is an allele of *RET1*, a gene that encodes the α subunit of coatomer.

THE secretory pathway of eucaryotic cells consists of a series of distinct membrane-bound organelles through which exported proteins pass en route to the cell surface (PALADE 1975). Genetic analysis of the secretory pathway in the yeast *Saccharomyces cerevisiae* has allowed the identification of essential gene products that function in this pathway, many of which are conserved (reviewed in PRYER *et al.* 1992; WUESTEHUBE and SCHEKMAN 1993). A large number of temperature-sensitive mutations that disrupt secretory protein transport upon shift to the nonpermissive temperature have been isolated (NOVICK *et al.* 1980). These secretory (*sec*) mutations were shown to block secretion at three phenotypically distinguishable stages of the pathway using both morphological and biochemical criteria. The first class of *sec* mutants produced an extended network of endoplasmic reticulum (ER) membrane and accumulated core-glycosylated protein precursors. The second class formed stacked membrane structures reminiscent of the mammalian Golgi complex and accumulated heterogeneously glycosylated protein precursors. The third *sec* mutant class accumulated 80–100 nm secretory vesicles and nearly fully glycosylated precursors. Epistatic relationships were established by examining the morphology of double mutants (NOVICK *et al.* 1981). The mutants that displayed the ER-accumulating phenotype were epistatic to those that displayed the stacked membrane and vesicle-accumulating phenotypes, and mutants that displayed the stacked membrane phenotype were epistatic to those that displayed the vesicle-accu-

mulating phenotype. Thus, the temporal and spatial organization of the secretory pathway has been defined by phenotypic characterization of the *sec* mutants.

The first stage of the secretory pathway, that of transport from the ER to the Golgi complex, has been the focus of intense study. Temperature-sensitive mutations have been isolated in 13 genes involved in this step, including *SEC12/SED2*, *SEC13*, *SEC16*, *SEC17*, *SEC18*, *SEC20*, *SEC21*, *SEC22/SLY2*, *SEC23*, *BET1/SLY12*, *BET2*, *YPT1*, *USO1* (NOVICK *et al.* 1980; NEWMAN and FERRO-NOVICK 1987; SCHMITT *et al.* 1988; SEGEV *et al.* 1988; BACON *et al.* 1989; NAKAJIMA *et al.* 1991). Other genes important for ER to Golgi transport have been identified by high copy suppression screens, including *BOS1*, *SAR1*, *SLY1*, and *SED5* (NAKANO and MURAMATSU 1989; NEWMAN *et al.* 1990; OSSIG *et al.* 1991; HARDWICK and PELHAM 1992). Biochemical analysis of the products encoded by these genes has been facilitated by the development of cell free assays that reconstitute ER to Golgi transport (reviewed in PRYER *et al.* 1992). Several gene products have been shown to function *in vitro* as multisubunit complexes. In addition to known *SEC* gene products, these complexes were composed of other proteins required for ER to Golgi transport that had not been previously identified by genetic means (HICKE *et al.* 1992; PRYER *et al.* 1993; SALAMA *et al.* 1993).

The *SEC* genes required for protein traffic between the ER and Golgi complex have been subdivided into interacting groups using epistasis tests and synthetic-lethal genetic interactions (KAISER and SCHEKMAN 1990). One group of *SEC* genes functions in the formation of ER-derived transport vesicles and includes

Corresponding author: Randy Schekman, Department of Molecular and Cell Biology, University of California, Berkeley, CA 94720.

SEC12/SED2, *SEC13*, *SEC16*, *SEC23*, *SEC24*, *SAR1* (reviewed in BARLOWE *et al.* 1994). A second group of genes is involved in the targeting and fusion of ER-derived transport vesicles with the Golgi complex, including *SEC17*, *SEC18*, *SEC22/SLY2*, *YPT1*, *USO1*, *BOS1*, *BET1/SLY12*, and *SED5* (reviewed in ROTHMAN 1994). A third group of interacting genes, *SEC21*, *SEC26*, *SEC27* and *RET1*, encode the γ -, β -, β' - and α -subunits, respectively, of the conserved nonclathrin protein complex known as coatomer (HOSOBUCHI *et al.* 1992; DUDEN *et al.* 1994; LETOURNEUR *et al.* 1994). This grouping of *SEC* genes appears to be consistent with what is known about the biochemical activity of the gene products as demonstrated *in vitro* using both yeast and mammalian components (reviewed in PRYER *et al.* 1992; ROTHMAN 1994).

There are many genes that function in ER to Golgi transport for which no conditional yeast mutants have yet been isolated. To better understand the molecular events involved in ER to Golgi transport, we devised a new screen to isolate novel temperature-sensitive mutations that affect the early stages of the secretory pathway. This screen was based on detection of precursor forms of the secreted protein, α -factor, in yeast colonies replicated to and lysed on nitrocellulose filters. In this report, we present the isolation and phenotypic characterization of mutations in five complementation groups, *sec31–sec35*, that block transport from the ER to the Golgi complex. Similar to previously characterized ER-Golgi mutants, the *sec31–sec35* mutant cells accumulated ER-modified precursor forms of secretory proteins and exhibited morphological defects as visualized with electron microscopy. *sec31-1* exhibited low vesicle densities, indicating that this mutation may affect the formation of ER-derived transport vesicles. *sec32-1*, the first temperature-sensitive allele of the *BOS1* gene, accumulated a tubular network structure. *sec33-1* was shown to be an allele of the *RET1* gene, encoding the α subunit of coatomer. *sec34-1* and *sec35-1* accumulated high vesicle densities and may affect the targeting or fusion stage of ER-Golgi transport.

MATERIALS AND METHODS

Strains and culture methods: Genotypes of the yeast strains used in this study are listed in Table 1. Yeast crosses, complementation tests and meiotic segregation analyses were performed using standard genetic techniques (ROSE *et al.* 1989). For mutant isolation, wild-type strain RSY255 was mutagenized to 50% viability by spreading ~7500 cells from each of 10 independent stationary cultures onto YPD-agar plates (150 mm diameter) and irradiating with UV light. After UV light exposure mutagenized cells were shielded from light for ≥ 24 hr. Each mutant was crossed two or three times against the congenic wild-type strain RSY259 to minimize genetic variation between strains. Strains of a mating type for complementation studies were obtained as segregants of the mating RSY259 $\times$ *sec* mutant strain. Double mutants were obtained as segregants of the mating *sec* mutant $\times$ *sec* mutant, and their

genotypes were checked by complementation analysis against each parental allele to ensure the retention of both alleles.

Media for growth of yeast were prepared as described (SHERMAN 1991). YP medium contained 1% Bacto-yeast extract and 2% Bacto-peptone (Difco Laboratories, Detroit, MI); YPD medium was the same with 2% glucose. YPD-agar was supplemented with 2% Bacto-agar (Difco). Minimal medium contained 0.67% Bacto-yeast nitrogen base without amino acids or ammonium sulfate and 2% glucose. The optical density at 600 nm (OD_{600}) of dilute cell suspensions was measured in 1-cm quartz cuvettes in a Zeiss PMQII spectrophotometer (Carl Zeiss, Inc., Thornwood, NY). One OD_{600} U of cells corresponds to $\sim 1 \times 10^7$ cells or 0.15 mg dry weight.

Colony immunoblotting for α -factor precursor: Elevated levels of α -factor precursor were detected by immunoblotting of yeast colonies transferred to nitrocellulose filters. Colonies were grown on YPD-agar plates at 24° to ≤ 1 mm in diameter then replica plated to nitrocellulose filters (0.45 mm, Schleicher & Schuell, Keene, NH) that were placed onto fresh YPD-agar plates and shifted to 38° for 3 hr. Cells were lysed by placing the nitrocellulose filters onto Whatman paper soaked with 0.1% SDS, 0.2 M NaOH, 35 mM dithiothreitol (DTT) for 30 min. After removal of lysed cells by rapidly flowing distilled water, nitrocellulose filters were treated with 0.1 M acetic acid for 30 min to inactivate endogenous alkaline phosphatase, rinsed with 50 mM Tris pH 7.4, 150 mM NaCl, and then were incubated in milk buffer (50 mM Tris pH 7.4, 150 mM NaCl, 0.1% NP-40, 2% nonfat dry milk) for ≥ 30 min to block nonspecific protein binding sites. Nitrocellulose filters then were incubated with a 1:1000 dilution of affinity-purified anti- α -factor antibody in milk buffer for 2 hr at 24°C, washed three times with milk buffer, and subsequently incubated in 1:3000 alkaline phosphatase-conjugated anti-rabbit IgG (Bio-Rad, Richmond, CA) for 1 hr at 24°. Filters were washed three times with milk buffer, once with 50 mM Tris pH 7.4, 150 mM NaCl, and were developed by incubation in 150 μ g/ml 5-bromo-4-chloro-3-indolyl phosphate, 300 μ g/ml Nitro blue tetrazolium, 0.1 M NaHCO₃, 1 mM MgCl₂, pH 9.8, for 10–30 min at 24°. Colonies exhibiting elevated levels of α -factor precursor appeared as purple deposits on the filters. Antibody to α -factor precursor (ROTHBLATT and MEYER 1986) was affinity-purified as described (WUESTEHUBE and SCHERMAN 1993).

Carboxypeptidase Y (CPY) secretion: Secreted CPY was detected using an immunoblotting technique in which patches of yeast cells grown at 24° were overlaid with a prewetted nitrocellulose filter (ROTHMAN *et al.* 1986; ROBERTS *et al.* 1991). For this analysis, antibody to CPY was first adsorbed against a column containing lysate proteins of cells lacking CPY. The cell lysate was prepared from stationary phase cultures of *PRC1* deletion mutant cells and coupled to cyanogen bromide-activated Sepharose 4B (Pharmacia, Piscataway, NJ). As a control for cell lysis during blotting, replicate filters were probed with antibody against the cytosolic protein, phosphoglycerate kinase, kindly provided by J. THORNER (this department). Filters were probed with goat-anti-rabbit conjugated to alkaline phosphatase and color development reagents from Bio-Rad as described above.

Invertase assays: Yeast cells were grown at 24° in YPD medium to an OD_{600} of 0.5–1.0 and then shifted to YP medium containing 0.1% glucose at either 24° or 38° to induce secreted invertase (PERLMAN and HALVORSON 1981; CARLSON and BOTSTEIN 1982). Invertase activity in periplasm and spheroplast fractions was measured to assess secreted and internal invertase, respectively (GOLDSTEIN and LAMPEN 1975). Cells (1 OD_{600} U) were sedimented in a clinical centrifuge, washed once with distilled H₂O, resuspended in YP medium containing 0.1% glucose, and then incubated for 3 hr at either 24° or 38°. An equal volume of ice-cold 20 mM Na₃ was

TABLE 1
Yeast strains

Strain	Genotype	Source or reference
Strains for crosses		
RSY255	<i>MATα ura3-52 leu2-3,-112</i>	D. BOTSTEIN
RSY259	<i>MATα lys2-801</i>	D. BOTSTEIN
Strains for complementation analysis		
RSY783	<i>MATα sec1-1 leu2-3,-112 lys2-801</i>	This study
RSY787	<i>MATα sec2-56 lys2-801 his4-619</i>	This study
RSY793	<i>MATα sec3-2 lys2-801 his4-619</i>	This study
sf264-1D	<i>MATα sec4-2</i>	NOVICK <i>et al.</i> (1980)
RSY795	<i>MATα sec5-24 lys2-801 his4-619</i>	This study
sf267-1D	<i>MATα sec6-4</i>	NOVICK <i>et al.</i> (1980)
AFY89	<i>MATα sec7-4 trp1-1 ura3-1</i>	A. FRANZUSOFF
sf270-5D	<i>MATα sec8-6</i>	NOVICK <i>et al.</i> (1980)
sf271-2D	<i>MATα sec9-4</i>	NOVICK <i>et al.</i> (1980)
sf239-2C	<i>MATα sec10-2</i>	NOVICK <i>et al.</i> (1980)
sf273-2C	<i>MATα sec11-7</i>	NOVICK <i>et al.</i> (1980)
RSY940	<i>MATα sec12-1 lys2-801</i>	This study
RSY266	<i>MATα sec13-1 ura3-52 his4-619</i>	KAISER and SCHEKMAN (1990)
sf292-1	<i>MATα sec14-3</i>	NOVICK <i>et al.</i> (1980)
sf279-5C	<i>MATα sec15-1</i>	NOVICK <i>et al.</i> (1980)
RSY268	<i>MATα sec16-2 ura3-52</i>	KAISER and SCHEKMAN (1990)
RSY270	<i>MATα sec17-1 ura3-52 his4-619</i>	KAISER and SCHEKMAN (1990)
RSY272	<i>MATα sec18-1 ura3-52 his4-619</i>	KAISER and SCHEKMAN (1990)
RSY274	<i>MATα sec19-1 ura3-52 his4-619</i>	KAISER and SCHEKMAN (1990)
RSY276	<i>MATα sec20-1 ura3-52 his4-619</i>	KAISER and SCHEKMAN (1990)
PC70	<i>MATα ret1-1 ura3 leu2 trp1</i>	LETOURNEUR <i>et al.</i> (1994)
RSY278	<i>MATα sec21-1 ura3-52 his4-619</i>	KAISER and SCHEKMAN (1990)
RSY942	<i>MATα sec22-3 ura3-52 lys2-801</i>	This study
RSY426	<i>MATα sec23-1 ura3-52 ade his</i>	This lab
RSY23	<i>MATα sec53-6 ura3-52 his4-593 suc2-432</i>	FERRO-NOVICK <i>et al.</i> (1984)
RSY26	<i>MATα sec59-1 ura3-52 lys2-801 suc2-432</i>	FERRO-NOVICK <i>et al.</i> (1984)
RSY533	<i>MATα sec61-2 ura3-52 leu2-3,-112 ade2 pep4-3</i>	DESHAIES and SCHEKMAN (1987)
RSY534	<i>MATα sec62 ura3-52 suc2-Δ9 prc1::LEU2</i>	ROTHBLATT <i>et al.</i> (1989)
RSY155	<i>MATα sec63-1 ura3-52 leu2-3,-112 ade2-1 pep4-3</i>	ROTHBLATT <i>et al.</i> (1989)
RSY458	<i>MATα sec65 ura3-52 leu2-3,-112 ade2 his3 trp1-1</i>	STIRLING <i>et al.</i> (1992)
RSY475	<i>MATα kar2-159 ura3-52 leu2-3,-112</i>	M. ROSE
RSY944	<i>MATα bet1-1 lys2-801 ura3-52</i>	This study
SFNY81	<i>MATα bet1-1 ura3-52 leu2-3,-112 his4-619 BOS1::URA3</i>	S. FERRO-NOVICK
RSY946	<i>MATα bet2-1 lys2-801</i>	This study
RSY948	<i>MATα uso1-1 lys2-801 ura3-52</i>	This study
RSY950	<i>MATα YPT1::ypt1^{ts}LEU his3 leu2 ura3-52</i>	This study
RSY952	<i>MATα sec31-1 lys2-801</i>	This study
RSY954	<i>MATα sec32-1 lys2-801 leu2-3,-112</i>	This study
RSY956	<i>MATα sec33-1 lys2-801</i>	This study
RSY958	<i>MATα sec34-1 lys2-801</i>	This study
RSY960	<i>MATα sec34-2 lys2-801</i>	This study
RSY962	<i>MATα sec35-1 lys2-801</i>	This study

added, and cells were placed on ice for 10 min. Cells then were sedimented in a clinical centrifuge, resuspended in 200 μ l 25 mM potassium phosphate pH 7.5, 1.4 M sorbitol, 10 mM Na₂S₂O₈, 80 mM β -mercaptoethanol containing 100 U recombinant β -1,3 glucanase (SHEN *et al.* 1991) and incubated for 45–60 min at 30°. The periplasm fraction, containing secreted invertase, was collected after sedimenting spheroplasts at 3000 $\times g$ for 10 min. β -mercaptoethanol in the periplasm fraction was inactivated with 160 mM N-ethylmaleimide. The spheroplasts were resuspended in 200 μ l 50 mM potassium phosphate pH 7.5, 10 mM Na₂S₂O₈, 0.1% Triton X-100 and lysed by vigorous vortexing in the presence of glass

beads (425–600 mm, Sigma, St. Louis, MO). The spheroplast lysate, containing intracellular invertase, was clarified by centrifugation at 17,000 $\times g$ for 15 min. Aliquots (3 μ l, 0.015 OD₆₀₀ U cell equivalent) of the spheroplast and periplasm fractions were assayed at 37° for 10 min. N-ethylmaleimide, 200 μ M, was added to the second stage of the reaction to prevent interference by residual β -mercaptoethanol. One unit of invertase activity was defined as the amount of enzyme at pH 5.1 that hydrolyzes sucrose to produce 1 μ mole glucose per minute at 37°. The percentage of invertase secreted was calculated as follows: [U invertase periplasm : (U invertase periplasm + U invertase spheroplasts)] $\times$ 100.

Radiolabeling and immunoprecipitation: Yeast cultures were grown to a density of 0.1–0.4 OD₆₀₀ U/ml at 24° in minimal medium supplemented with 0.2% casamino acids (Difco), appropriate nutrients and 100 μ M (NH₄)₂SO₄. An aliquot of cells was sedimented in a clinical centrifuge, washed once and resuspended to 5 OD₆₀₀ U/ml in minimal medium containing appropriate nutrients, 100 μ M (NH₄)₂SO₄, 0.2 mg/ml bovine serum albumin (BSA), 0.1 mg/ml α 2-macroglobulin, and incubated at 38° for 1 hr. Radiolabeling was performed in the same medium without (NH₄)₂SO₄ using Express ³⁵S protein labeling mix (I. E. Du Pont, Wilmington, DE) at a level of 30–60 μ Ci/OD₆₀₀ cells for 10 min at 38° and chased with 5 mM cysteine, 5 mM methionine, 0.2% yeast extract, 100 μ M (NH₄)₂SO₄ at 38°. Reactions were terminated by addition of an equal volume of ice-cold 50 mM Tris-HCl pH 7.5, 40 mM NaN₃, 40 mM NaF, 2 M sorbitol, and samples were chilled on ice for 10 min. Radioactive cells were converted to spheroplasts by treatment with 10 mM DTT for 10 min at 30° followed by 30 U/OD₆₀₀ cells of recombinant β -1,3 glucanase (SHEN *et al.* 1991) for 30 min at 30°. Samples then were fractionated into spheroplast and periplasm/media fractions by sedimentation in a clinical centrifuge. Spheroplasts were resuspended to 50 μ l/OD₆₀₀ cell equivalent with sample buffer (1% SDS, 10% sucrose, 1 mM EDTA, 30 mM DTT, 40 μ g/ml Bromophenol Blue, 10 mM Tris-HCl pH 8.0) and lysed by vigorous vortexing with glass beads. Secreted proteins in the periplasm/media fractions were precipitated by incubation with 10% trichloroacetic acid (TCA) for $\geq$ 20 min on ice, washed twice in ice-cold acetone with agitation in a bath sonicator in the presence of glass beads, and resuspended to 50 μ l/OD₆₀₀ cell equivalent with sample buffer. Samples were heated at 70° for 10 min.

Aliquots of spheroplast and periplasm/media samples were diluted in 1 ml IP buffer (50 mM Tris-HCl pH 7.4, 150 mM NaCl, 5 mM EDTA, 1% Triton X-100, 0.1% SDS) containing 1 mM phenylmethyl sulfonyl fluoride (PMSF) and 1 mg/ml BSA, incubated with 50 μ l/OD₆₀₀ cell equivalent of IgG Sorb (The Enzyme Center, Boston, MA) for 45 min at room temperature and clarified by centrifugation for 10 min in a microcentrifuge (Oscar Fisher Co., Inc., Newburgh, NY). The supernatant (0.8 ml) was removed and incubated for 1 hr at room temperature with saturating amounts of antiserum against α -factor precursor, CPY or dipeptidyl aminopeptidase B (DPAP B). The amount of antiserum per OD₆₀₀ cell equivalent used was as follows: 4 μ l of anti- α -factor, 5 μ l of anti-CPY, 3 μ l of anti-DPAP B. Protein A-Sepharose (Pharmacia Fine Chemicals, Piscataway, NJ) was added (1 μ l beads/ μ l antiserum), and incubation was continued for 1 hr at room temperature. Immune complexes were sedimented in a microcentrifuge and washed as described (ROTHBLATT and SCHEKMAN 1989). The washed Protein A-Sepharose pellet with bound antigen was resuspended in 50 μ l/OD₆₀₀ cell equivalent of sample buffer and heated for 10 min at 70°. After addition of 1 ml IP buffer, samples were clarified by centrifugation for 10 min in a microcentrifuge, and the immunoprecipitation procedure was repeated. To detect the presence of outer chain carbohydrate structures, precipitated CPY was reprecipitated with the anti- α 1,6-Mannose serum as described (FRANZUSOFF and SCHEKMAN 1989). Bound antigens were dissociated from the final Protein A-Sepharose pellet by heating at 70° for 10 min in sample buffer, and aliquots were analyzed by SDS-PAGE (LAEMMLI 1970). The gels were fixed in 10% acetic acid containing 25% isopropyl alcohol, dried, and exposed to a Phosphorimaging screen. Results were visualized using a PhosphorImager from Molecular Dynamics (Sunnyvale, CA). Antisera to α -factor precursor (ROTHBLATT and MEYER 1986), to CPY (STEVENS *et al.* 1982), and to α 1,6-Mannose (BALLOU 1970) have been described previously. Antiserum to DPAP B

(ROBERTS *et al.* 1989) generously was provided by Dr. TOM STEVENS (University of Oregon, Eugene, OR).

Electron microscopy: Yeast cultures were grown to 1–2 OD₆₀₀ U of cells/ml in YPD at 24° and then were shifted to 38° to impose a *sec* mutant block. The permanganate fixation protocol used was as previously described (KAISER and SCHEKMAN 1990) except that smaller volumes of cells (25–30 OD₆₀₀ U of cells) were used. The smaller cell pellets seemed to stain more evenly, perhaps due to better solution exchanges, and were found to be sufficient for several sample blocks. For serial sections, sample block faces were trimmed to a wide thin rectangle. Ribbons of sections were picked up on a formvar-coated 75 mesh or slotted grids and coated with carbon after lead citrate staining. Vesicle accumulation was determined on $\geq$ 30 cell sections, and vesicle density values were calculated as the mean $\pm$ SE of the mean as previously described (KAISER and SCHEKMAN 1990). In this study the incubation at the nonpermissive temperature was extended from 1 to 3 hr to ensure the maximal secretory block. For some mutants, the longer time of incubation at the nonpermissive temperature may account for increased vesicle density values. As a step beyond general descriptive terms but without employing rigorous morphometric analysis, the amount of membrane was estimated by comparing the total length of intracellular membranes seen in cross sectional view (segments connected to nucleus, in cytoplasm and at cell periphery) to the cell circumference. Since much of the intracellular membrane underlies the plasma membrane at the cell periphery, a fair approximation of membrane lengths relative to cell circumference is obtained. Cell sections were scored using a five-level scale of membrane length:cell circumference ratio as follows: level 1 = <0.50, level 2 = 0.50–0.75, level 3 = 0.75–1.00, level 4 = 1.00–1.25, level 5 = >1.25. A total of 60 random cell sections were scored that consisted of the 30 cell sections that were assessed for vesicle accumulation and 30 additional cell sections from random fields photographed at a lower magnification (4000–9000 $\times$). Contact prints of the first set of 30 cell sections and 8 $\times$ 10 prints of the second set of 30 cell sections were found to be suitable for this purpose. Although this method provided qualitative, rather than strict quantitative, data, it permitted a more objective comparison of the amount of membrane in wild-type and *sec* mutant strains.

Suppression and tetrad analysis: Suppression conferred by genes involved in ER to Golgi transport was examined. The *sec31–35* mutants were transformed with either single-copy or 2 μ -plasmids encoding Sar1p, Sed5p, Sly1p or Bos1p (and control plasmids). In addition, *sec33-1* was transformed with plasmids harboring the *RET1*, *SEC21*, *SEC26* and *SEC27* genes. Plasmids were introduced into *sec* strains by the lithium acetate method (ITO *et al.* 1983), and transformants were grown on selective minimal plates at 24°. Single colonies were restreaked onto selective minimal plates to maintain selection for the plasmid, and growth at 24°, 30°, 34° and 37° was scored after 3–4 days.

The allelic nature of some genes was tested using tetrad analysis. To determine whether *SEC32* was allelic to *BOS1*, an isolate of *sec32-1* (strain JBY1, genotype: *MATa*, *sec32-1*, *ura3-52*, *leu2-3,-112*) was crossed to a strain in which the *BOS1* chromosomal locus was marked by integration of the *URA3* plasmid marker (strain JBY2, genotype: *MATa*, *ura3-52*, *leu2-3,-112*, *his4-619*, *BOS1::URA3*). This marked strain was constructed by backcrossing an isolate of *bet1-1* (strain SFNY81, genotype: *Mat α* , *bet1-1*, *ura3-52*, *his4-619*, *BOS1::URA3*) to a wild-type strain (strain RSY257, genotype: *Mat α* , *ura3-52*, *leu2-3,-112*) to eliminate the *bet1-1* mutation and obtain the desired auxotrophic markers. To test whether *SEC33* and *RET1* were allelic, *sec33-1* (strain RSY956, genotype: *MATa*,

lys2) was crossed with *ret1-1* (strain PC70, genotype: *Mat α* , *ura3*, *leu2*, *trp1*). Diploids resulting from JBY1 $\times$ JBY2 and RSY956 $\times$ PC70 crosses were selected on minimal medium and then sporulated. Ascospores were dissected onto YPD-agar plates, and resulting colonies were analyzed for growth on selection plates at 24° and on YPD-agar plates at 24°, 30°, 34° and 37°. Strain SFNY81 was kindly provided by Dr. SUSAN FERRO-NOVICK (Yale University School of Medicine, New Haven, CT), and strain PC70 was obtained from Dr. PIERRE COS-SON (Basel Institute of Immunology, Switzerland).

RESULTS

Detection of α -factor precursor by colony immunoblotting: The glycosylation and processing events that occur as the yeast pheromone precursor prepro- α -factor traverses the secretory pathway in yeast have been well documented (JULIUS *et al.* 1984; reviewed in BRAKE *et al.* 1990). Initially synthesized as a long precursor containing four copies of the mature peptide within it, α -factor precursor is glycosylated in the ER and Golgi complex and cleaved into four peptides by the Kex2 protease in a late Golgi compartment (FRANZUSOFF *et al.* 1991; REDDING *et al.* 1991). Previous studies have shown that α -factor precursor, but not mature α -factor peptide, absorbs to nitrocellulose (WILSBACH and PAYNE 1993). We reasoned that the detection of α -factor precursor on nitrocellulose would serve as a powerful primary screen for the isolation of mutants defective in the early stages of the secretory pathway in yeast, which would accumulate the uncleaved α -factor precursor. We therefore developed conditions to identify yeast mutants exhibiting elevated levels of α -factor precursor. In this procedure, yeast colonies were replicated to and lysed on nitrocellulose filters under conditions that allow absorption of solubilized proteins onto nitrocellulose (LYONS and NELSON 1984). The α -factor precursor then was visualized with affinity-purified antibodies using standard immunoblotting techniques. In this screen, we would expect to isolate mutations that block the secretory pathway before the Kex2p-containing late Golgi compartment, including mutations affecting translocation of proteins into the ER, transport from the ER to the Golgi complex, and transport within the Golgi complex. We also would expect to isolate mutations in genes affecting the sorting of proteins through the secretory pathway. For example, α -factor is secreted in a precursor form by some of the vacuolar-protein sorting (*vps*) mutants (ROBINSON *et al.* 1991; WILSBACH and PAYNE 1993). Since both accumulated as well as secreted α -factor precursor would be detected by the colony immunoblotting screen, mutations in *vps* genes also would be isolated.

As expected, we found that immunoblotting of yeast colonies for elevated levels of α -factor precursor was most sensitive in detecting mutations that affect the early stages of the secretory pathway (Figure 1). As compared to wild-type (*SEC*) strains, a strong signal was observed for mutants blocked in translocation into the

FIGURE 1.—Secretory mutants blocked in the early stages of protein transport were detected by immunoblotting of lysed yeast colonies for α -factor precursor. *MAT α* wild-type (*SEC*) and temperature-sensitive mutant strains were grown on YPD-agar plates (A) and replicated to nitrocellulose filters (B). After shifting to 38° for 3 hr, cells were lysed and filters were probed with affinity-purified anti- α -factor antibody and alkaline phosphatase-conjugated secondary antibody. Elevated levels of α -factor precursor were detected on colony immunoblots of secretory mutants blocked in translocation into the ER (*sec63*), ER to Golgi transport (*sec18*, *sec23*) and intra-Golgi transport (*sec7*). Kex2p-cleaved α -factor accumulated in mutants blocked in late transport steps (*sec1*) was not detected by this procedure.

ER (*sec63*) and in transport from the ER to the Golgi complex (*sec18*, *sec23*). A faint but reproducible signal, most readily seen in individual colonies, was exhibited by mutants defective in intra-Golgi transport (*sec7*). No signal was observed for mutants blocked late in the secretory pathway (*sec1*). In addition to α -factor precursor accumulated intracellularly in *sec* mutants, α -factor precursor that was secreted by yeast colonies also was detected by colony immunoblot. Several *vps* mutants as well as the clathrin light chain mutant reproducibly gave a signal in this assay (data not shown). These re-

TABLE 2

Unprocessed α -factor colony immunoblot screen for temperature-sensitive secretion mutants

Screening stage	Colonies	Percentage
Colonies tested	37,600	100
α -factor precursor	998	2.7
Temperature-sensitive growth	152	0.40
Genetic linkage	104	0.28
CPY secretion low or undetectable	73	0.19
Complement 34 previously isolated secretory mutants	57	0.15
Accumulation of CPY core-glycosylated p1 precursor	6	0.02

sults indicated that immunoblotting yeast colonies for elevated levels of α -factor precursor detected mutations affecting both transport and sorting of proteins through the secretory pathway.

Mutant isolation: Several steps were employed to isolate new temperature-sensitive mutants blocked in the secretory pathway (Table 2). Mutagenized cells were grown to colonies and then tested for elevated levels of α -factor precursor by the immunoblotting method described above. Colonies showing elevated levels of α -factor precursor were purified by streaking onto fresh YPD-agar plates, retested by immunoblotting for α -factor precursor to ensure heritability of this phenotype, and subsequently tested for temperature-sensitive growth. Genetic linkage between the elevated α -factor precursor and temperature-sensitive growth phenotypes then was determined by crossing all haploid mutants to a congenic wild-type strain and performing tetrad analysis. For 104 of the outcrossed mutant strains, a 2:2 segregation for temperature sensitivity to temperature insensitivity was observed in 10–20 tetrads, and in all *MAT α* segregants examined, the α -factor precursor phenotype cosegregated with temperature sensitivity. These results indicated that the elevated levels of α -factor precursor and temperature-sensitive growth phenotypes were the result of a single nuclear lesion in each mutant. Dominance tests were performed by crossing mutants derived from RSY255 to a wild-type strain (RSY259). The growth properties of the resultant heterozygous diploids at 38° indicated that all but one of the mutations was recessive.

The mislocalization of soluble vacuolar proteins to the cell surface is a hallmark phenotype of *vps* mutants (reviewed in KLIONSKY *et al.* 1990). To identify mutations that confer a *vps* phenotype, we screened directly for the secretion of the soluble vacuolar protease, CPY (Figure 2). A wild-type strain (*SEC*), two secretory mutants (*sec18* and *ypt1ts*) and a *vps* mutant (*vps18*) were included as controls. The *vps18* mutant gave a very strong signal in this assay. By contrast, the secretory mutants gave a low or nearly undetectable signal, similar to that observed for wild-type cells. In all, we found

FIGURE 2.—Mutants secreting CPY were detected by colony overlay immunoblotting. Patches of yeast cells were grown on YPD-agar plates (left) and then overlaid with a prewetted nitrocellulose filter (right) at 24° for 12 hr. Cells were removed by gentle rinsing, and the filters were probed with antibody against CPY and alkaline-phosphatase-conjugated secondary antibody to visualize secreted CPY. A strong signal was observed for the vacuolar protein sorting mutant (*vps18*). Low or nearly undetectable signals were obtained for the secretory mutants (*sec18* and *ypt1ts*) and wild-type cells (*SEC*). A subset of the newly isolated mutants gave a strong signal, comparable to that observed for *vps18*.

that 31 of the mutants we isolated secreted CPY at levels comparable to that secreted by previously isolated *vps* mutants (Table 2). Complementation analysis of the temperature-sensitive growth phenotype demonstrated that ≥ 20 of these mutants fall into six previously identified *VPS* complementation groups including *VPS11*, *VPS15*, *VPS16*, *VPS18*, *VPS33*, and *VPS34*. The remaining 73 mutants that did not secrete significant amounts of CPY were thus phenotypically distinct from the *vps* mutants and were studied further.

Mutations in known secretory genes that were isolated in our screen were identified by complementation analysis. Mutants were crossed with each of 34 previously isolated secretory mutants (listed in Table 1) and the resulting diploids were examined for growth at 24° and 38°. To confirm the allelic nature of mutations that failed to complement, linkage analysis was performed by sporulating the diploids and examining the growth properties of the meiotic segregants. As expected, our screen detected mutations in previously identified genes that function at different early stages of the secretory pathway including ER translocation and processing (*KAR2*, *SEC53*, *SEC59*), ER to Golgi transport (*SEC13*, *SEC16*, *SEC23*, *USO1*, *YPT1*), and intra-Golgi transport (*SEC7*). It is likely that the alleles isolated in this screen affect transport at the same stage of the secretory pathway as previously isolated alleles because many of the mutants accumulate the same incompletely processed precursors of secretory proteins and exhibit similar ultrastructural features (data not shown). Unexpectedly, mutations in genes affecting late stages in the secretory pathway (*SEC2*, *SEC3*, *SEC5*) also were isolated in this screen. It is possible that these

mutations represent new alleles that are blocked in secretion before the Kex2p-containing compartment. However, this appears unlikely since these mutants accumulated an abundance of 80–100 nm vesicles, a phenotype characteristic of post-Golgi blocked secretory mutations (NOVICK *et al.* 1981). Alternatively, the mutant alleles of the late-acting secretory genes isolated in this screen may impede the pathway in such a way that incompletely processed α -factor precursor accumulates in these strains.

Mutations affecting ER to Golgi transport: The stage in the secretory pathway at which *sec* mutant cells are arrested was monitored by analyzing the extent of modification and processing of CPY. CPY is synthesized as a 59-kD polypeptide that acquires 10 kD of N-linked carbohydrate (HASILIK and TANNER 1978) as it transits through the secretory pathway to the vacuole (STEVENS *et al.* 1982). A 59-kD unglycosylated form of CPY that is cytosolically exposed accumulates in mutants that block translocation into the ER lumen (DESHAIES and SCHEKMAN 1987). An incompletely glycosylated 67-kD form (p1 CPY) accumulates in mutants blocked in transport from the ER, whereas a fully glycosylated 69-kD form (p2 CPY) also accumulates in mutants defective in transport within the Golgi complex (STEVENS *et al.* 1982).

We focused on those mutations that affected the transport of proteins from the ER to the Golgi complex by screening for mutants that accumulated the 67-kD form of CPY that lacked outer chain mannose residues indicative of arrival in the Golgi complex (Figure 3). Mutant cells were radiolabeled at the nonpermissive temperature and spheroplast lysates were immunoprecipitated with antibody to CPY. To detect addition of outer chain mannoses, the radiolabeled CPY was reprecipitated either with anti-CPY (Figure 3A) or with anti- α 1,6-Mannose (Figure 3B) sera. Wild-type cells and *sec18* mutant cells, which are blocked in ER-Golgi transport, were included as controls. The mature form of CPY observed in wild-type cells (Figure 3A, lane 1) contained outer chain carbohydrates and was precipitated with anti- α 1,6-Mannose serum (Figure 3B, lane 1). In contrast, the p1 form of CPY accumulated in *sec18* mutant cells (Figure 3A, lane 2), lacked outer chain carbohydrates and was not precipitated with anti- α 1,6-Mannose serum (Figure 3B, lane 2). Six mutants, which defined five complementation groups (*SEC31–SEC35*), were found to accumulate a form of CPY that comigrated with the p1 form of CPY accumulated in *sec18* mutants (Figure 3A, lanes 3–7). The remaining 51 mutants either accumulated other precursor forms of CPY or mature CPY indicating that they were blocked elsewhere in the pathway or were unaffected in CPY transport (data not shown). The form of CPY accumulated in the *sec31–sec35* mutants could not be precipitated with the anti- α 1,6-Mannose serum and thus lacked outer chain carbohydrate modifications (Figure 3B, lanes 3–7). Also, CPY could not be precipitated from

FIGURE 3.—The p1 precursor form of CPY accumulated in *sec* mutants. Wild-type and mutant cells including *SEC* (RSY255, lane 1), *sec18* (RSY271, lane 2), *sec31* (RSY953, lane 3), *sec32* (RSY955, lane 4), *sec33* (RSY957, lane 5), *sec34* (RSY959, lane 6), and *sec35* (RSY963, lane 7) were radiolabeled for 10 min after a preshift to 38° for 1 hr. CPY was precipitated from spheroplast lysates first with anti-CPY serum; then equal aliquots (0.5 OD₆₀₀ U cell equivalents) were reprecipitated with either anti-CPY serum (A) or α 1,6-Mannose serum (B), and precipitates were resolved on 8% SDS gels. The relative mobilities of the core-glycosylated form (p1) and the processed mature form (m) of CPY are noted on the left.

the media/periplasm fraction of the *sec31–sec35* mutants confirming that CPY was not secreted aberrantly (data not shown). These results indicated that the *sec31–sec35* mutants were blocked in the transport of proteins from the ER to the Golgi complex.

Transport of soluble and membrane proteins is blocked in the *sec31–sec35* mutants: The severity and inducibility in the secretory block in the *sec31–sec35* mutants was examined by measuring the amount of the soluble protein invertase accumulated upon shift to the nonpermissive temperature (Figure 4). Wild-type cells, a restrictive mutant (*sec18-1*), and a less restrictive mutant (*sec21-1*) were included as controls. Most of the mutants secreted invertase at nearly wild-type levels at 24°. However, upon shifting to the nonpermissive temperature, the mutants accumulated intracellular in-

FIGURE 4.—Invertase secretion was affected in *sec* mutants. Yeast cells were grown at 24° in YPD and shifted to YP containing 0.1% glucose at either 24° (black bars) or 38° (gray bars) for 3 hr. Invertase activity was measured in spheroplast and periplasm fractions to determine the percentage of invertase secreted. These data were from one experiment that was representative of three independent experiments. The average value of duplicate measurements are shown. The measurements for each sample varied by <2%.

vertase, indicating that a block in the secretion of this protein was induced. The *sec32-1* mutant exhibited a particularly restrictive and inducible block in invertase secretion, comparable to that observed for *sec18-1*. The *ypt1-3* allele isolated in this screen also showed a restrictive block in invertase secretion. Less restrictive blocks in invertase secretion, comparable to that of *sec21-1*, were observed in *sec31-1* and *sec35-1* mutants. The block in invertase secretion was nearly identical for *sec34-1* and *sec34-2*; and the *sec33-1* mutant was deficient for invertase secretion at both temperatures.

The effect of the *sec31-sec35* mutations on trafficking of integral membrane proteins was assessed by monitoring the transport of the vacuolar membrane protein, DPAP B (Figure 5). DPAP B is an integral membrane protein that is localized to the vacuole through the early stages of the secretory pathway (ROBERTS *et al.* 1989). The mature form of DPAP B was observed in wild-type cells (lane 1). A precursor form of DPAP B that migrated with a faster mobility than the mature form accumulated in the *sec18-1* mutant (lane 2) and the *sec31-sec35* mutants (lanes 3–7). These results indicated that *sec31-sec35* mutations effectively blocked the transport of both membrane proteins and soluble proteins through the secretory pathway.

Membranes and vesicles accumulated in *sec* mutants:

The *sec* mutants were examined by electron microscopy to assess morphological changes in the membrane compartments of the secretory pathway (Figure 6). We found that various membrane structures were accumulated in the *sec* mutants that were present to a lesser degree in wild-type cells (Figure 6A). As had been observed previously for mutations such as *sec12-4* (Figure 6C) that block the transport of proteins from the ER

FIGURE 5.—The precursor form of the integral membrane vacuolar protein, DPAP B, accumulated in *sec* mutants. The same spheroplast lysates (2.5 OD₆₀₀ U cell equivalents) that were used for the experiment described in Figure 3 were treated with DPAP B serum, and precipitates were resolved on 6% SDS gels. Wild type (*SEC*, lane 1) contained the fully processed mature form (m) of DPAP B. The precursor form (p) of DPAP B was accumulated in *sec18* (lane 2), *sec31* (lane 3), *sec32* (lane 4), *sec33* (lane 5), *sec34* (lane 6), and *sec35* (lane 7) mutant strains.

(NOVICK *et al.* 1980), several of the mutants isolated in our screen exhibited an extensive proliferation of ER membrane including *sec23-5* and *sec33-1*. However, this classic morphology was not observed in all of the mutants blocked in transport of proteins from the ER. The phenotype of the *sec32-1* mutant (Figure 6B) was particularly intriguing. Although the *sec32-1* mutant was very restrictive both for growth and for secretion of invertase at the nonpermissive temperature, this mutant clearly did not accumulate the level of membranes characteristic of the mutants previously classified as defective in transport from the ER. We considered that some ultrastructural features of *sec32-1* may not have been readily apparent in the examination of random cross sections that was used to assess vesicle density and membrane accumulation. We therefore employed limited serial cross section analysis to explore the nature of the membrane structures of the *sec32-1* mutant in greater detail (Figure 7). Indeed, *sec32-1* mutant cells exhibited a distinctive structure, a network of anastomosed membranous tubules with mesh spacing of 80–150 nm. These tubular networks often were seen close to the nucleus and connections to the nuclear membrane were observed (Figure 7, large arrowheads). When tubular network structures were found elsewhere in the cytoplasm, it was more difficult to trace their connections. Once clear views of this tubular network structure were obtained from serial sections, it was possible to identify these structures in random nonserial sections. The tubular network structure was observed in nearly half the *sec32-1* mutant cells. These tubular network structures also were seen occasionally in wild-type cells that indicated that they were not aberrantly formed

FIGURE 6.—*sec12 sec32* double mutants exhibited a morphology similar to the parental *sec12* mutant. Yeast cells were grown in YPD medium at 24°, then shifted to 38° for 3 hr. Cells were prepared for electron microscopy using permanganate fixation. (A) Wild type (RSY255), (B) *sec32* (RSY955), (C) *sec12* (RSY263), (D) *sec12 sec32* (RSY996). Bar, 1 μ m.

and likely corresponded to normal structures of the secretory pathway.

The density of 50-nm vesicles has been used in earlier studies (KAISER and SCHEKMAN 1990) to group *sec* mutants blocked in the transport of proteins from the ER into two phenotypic classes: the class I mutants contain a low density of vesicles similar to that of wild-type cells, and the class II mutants contain three to five times the number of vesicles as wild-type cells. We quantified the density of vesicles in wild-type and *sec* mutant strains isolated in this screen (Table 3). A low density of vesicles, comparable to that of wild type, was observed in *sec31-1* and *sec23-5* mutants. A high density of vesicles, comparable to that of the *sec18-1* mutant, was observed in *sec34-1* and *sec35-1* mutants. The remaining mutants, *sec32-1*, *sec33-1*, and *ypt1-3* accumulated a moderate number of vesicles.

The level of membrane accumulation in wild-type and *sec* mutant strains also was assessed by comparing the length of intracellular membrane to the cell circumference in random cross sections. Wild-type cells (*SEC*) and two previously isolated ER-blocked secretory mutants (*sec12-4* and *sec18-1*) were included as controls. We found that the *sec* mutants varied in the degree to which they accumulated membranes (Figure 8). The *sec33-1* and *sec23-5* mutants showed a high level of membrane accumulation, slightly less than that observed for the *sec12-4* mutant. A moderate level of membrane accumulation, comparable to that seen in the *sec18-1* mutant, was observed in *sec31-1*, *sec35-1*, and *ypt1-3* mutants. A slight increase in membrane accumulation relative to wild-type cells was observed in the *sec32-1* mutant, while no significant increase was noted in the *sec34-1* mutant.

Test of epistasis: The epistatic relationships between

FIGURE 7.—Serial sections of *sec32* showed an extensive network of anastomosed membranous tubules. A tubular network structure located close to the nucleus showed several connections to the nuclear envelope (large arrowheads). Other small tubular network structures were located away from the nucleus (small arrowheads). At the cell periphery, the constant cross-sectional view of the ER throughout the serial sections (*) indicated a sheet-like conformation of the membrane. Another membrane structure, possibly a single Golgi cisterna, had several vesicles nearby (arrow). Bar, 0.5 μm .

the morphological phenotype observed in the *sec32-1* mutant and the phenotypes of other secretory mutants were determined by double mutant analysis. The *sec32-1* mutant was particularly well suited for this analysis as this mutant exhibited a very restrictive block both for growth and invertase secretion upon shift to the non-permissive temperature. Whereas *sec32-1* mutants exhibited tubular network structures (Figures 6B and 8),

TABLE 3
50-nm vesicle accumulation in *sec* mutants

Strain	<i>sec</i> group	Vesicles per μm^3 of cell volume
Previously isolated mutations		
RSY255	<i>SEC</i>	11.7 ± 0.6
RSY263	<i>sec12-4</i>	19.9 ± 1.4
RSY271	<i>sec18-1</i>	42.5 ± 2.3
Mutations isolated in this study		
RSY953	<i>sec31-1</i>	15.1 ± 0.7
RSY955	<i>sec32-1</i>	24.1 ± 1.5
RSY957	<i>sec33-1</i>	32.7 ± 1.9
RSY959	<i>sec34-1</i>	36.3 ± 2.1
RSY963	<i>sec35-1</i>	42.0 ± 2.5
RSY975	<i>sec23-5</i>	13.2 ± 1.4
RSY977	<i>ypt1-3</i>	21.1 ± 1.2

sec12-4 mutants showed an extensive proliferation of ER membrane (Figure 6C), and *sec7-5* mutants accumulated stacked membranes. Previous studies have established that mutants that displayed the ER-accumulating phenotype were epistatic to those that displayed the stacked membrane-accumulating phenotype (NOVICK *et al.* 1981). We found that the *sec12 sec32* double mutant exhibited a phenotype similar to that observed in the *sec12-4* parental strain; these double mutants showed an extensive proliferation of ER membrane (Figures 6, C and D, and 7). Double mutants constructed from *sec32-1* and *sec7-5* resembled the *sec32-1* parental strain; these mutants exhibited tubular network structures but lacked stacked membranes. Thus, mutants that displayed the ER-accumulating phenotype were epistatic to those that displayed the tubular network-accumulating phenotype, and mutants that displayed the tubular network-accumulating phenotype were epistatic to those that displayed the stacked membrane-accumulating phenotype.

Identification of genes corresponding to *SEC32* and *SEC33*: Several genes implicated in ER-Golgi transport, including *BOS1*, *SAR1*, *SED5*, and *SLY1*, had been identified by high copy suppression approaches. To test whether *SEC31-SEC35* corresponded to any of these genes, the *sec31-sec35* mutants were transformed with plasmids encoding Sar1p, Sed5p, Sly1p, Bos1p, or control plasmids and the growth of transformants at permissive and restrictive temperatures was examined. No suppression of the temperature-sensitive growth phenotype of *sec31-sec35* by the *SAR1*, *SED5* and *SLY1* genes was observed, even when introduced on high copy plasmids. By contrast, the temperature-sensitive growth defect of *sec32-1*, but none of the other mutants, was fully suppressed at 37° by *BOS1* on a single-copy plasmid. These results suggested that the gene defective in *sec32-1* may be *BOS1*, and tetrad analysis was performed to test this hypothesis (see MATERIALS AND METHODS). In

FIGURE 8.—Analysis of membrane accumulation in *sec* mutants. The amount of membrane in wild-type and *sec* mutant strains was assessed by determining the length of intracellular membrane relative to the cell circumference in electron micrographs of permanganate-fixed yeast cells. For each yeast strain analyzed, 60 random cell sections were scored according to five levels of intracellular membrane:cell circumference ratio as follows: level 1 = 0.50, level 2 = 0.50–0.75, level 3 = 0.75–1.00, level 4 = 1.00–1.25, level 5 = <1.25. As compared to *SEC* cells (RSY255), a substantial increase in membrane was observed in *sec12* (RSY263) and *sec33* (RSY957) cells. Moderate amounts of membrane were seen in *sec18* (RSY271), *sec31* (RSY953), and *sec35* (RSY963) mutants. A slight increase was observed in *sec32* (RSY955), and no significant increase was observed in *sec34* (RSY959) mutants. *sec12 sec32* double mutants (RSY996) exhibited membrane accumulation levels comparable to that of the parental *sec12* mutant.

a total of 49 tetrads analyzed, only parental ditypes were observed demonstrating that the *sec32* and *BOS1* loci were closely linked and most likely identical.

We noticed that *sec21-1* and *sec33-1* displayed strong synthetic lethality, the pattern of spore viability in 33 tetrads analyzed suggested that the double mutant was inviable at 24°. Yet, no synthetic lethality was observed between *sec21-1* and *sec31-1*, *sec34-1* or *sec35-1* mutants. Since *sec21* encodes the γ subunit of coatamer (HOSOBUCHI *et al.* 1992), we decided to test whether *sec33* might also encode a coatamer subunit. *sec33-1* was transformed with plasmids harboring the *RET1*, *SEC21*, *SEC26* and *SEC27* genes, encoding α -, γ -, β - and β' coatamer subunits, respectively, or control plasmids and growth of the transformants was analyzed. A single-copy plasmid harboring the *RET1* gene, but not the other three genes, suppressed the temperature-sensitive growth of *sec33-1*, indicating that *SEC33* may be allelic to *RET1*. To test this possibility, haploid strains carrying the *sec33-1* and *ret1-1* mutations were crossed. The re-

sulting diploids exhibited temperature-sensitive growth, indicating that the two mutations fall into the same complementation group. Sporulation of the *sec33-1 ret1-1* diploid and dissection of 44 tetrads yielded only temperature-sensitive spores, demonstrating that the two loci are closely linked. Thus, the *sec33* locus likely is identical to the *RET1* locus encoding the α subunit of coatamer.

DISCUSSION

Screen for isolation of *sec* mutants: A complete understanding of the secretory pathway in eukaryotic cells will require the identification of all the genes that function in this pathway. Toward this end, we have developed a screen for the isolation of conditional yeast mutants affected in the transport and sorting of secretory proteins. This screen was based on the detection of unprocessed α -factor precursor in yeast colonies lysed on nitrocellulose filters. The ease and expediency of the colony immunoblotting technique made it a powerful tool for screening large numbers of colonies. Mutations in genes acting both early and late in the secretory pathway (Figure 1), as well as mutations affecting vacuolar protein sorting, were readily detected. Since only a single mutant allele was isolated for many of these genes, it is likely that mutations in additional genes that function in the transport and sorting of secretory proteins would be isolated by repeated application of this screen.

ER-Golgi-blocked *sec* mutants: We have focused on the characterization of novel temperature-sensitive mutants blocked in ER-to-Golgi transport that define five complementation groups, *SEC31–SEC35*. Like previously isolated ER-Golgi mutants, the *sec31–sec35* mutants accumulated ER-modified forms of both soluble and membrane proteins (Figures 3–5) and exhibited alterations in the morphology and quantity of membranes (Figures 6–8) and vesicles (Table 3). The *sec* mutants differed in levels of membrane accumulation and vesicle density. In some cases, the membrane accumulation and vesicle density were inversely correlated. For example, the *sec34-1* mutant showed a high vesicle density and a low level of membrane accumulation, while the *sec23-5* mutant showed a low vesicle density and a high level of membrane accumulation. However, this inverse correlation was not always observed. High vesicle densities were present in the *sec18-1* and *sec34-1* mutants, yet these mutants showed different levels of membrane accumulation. Similarly, the *sec23-5* and *sec31-1* mutants exhibited comparably low vesicle densities yet differed significantly in membrane accumulation levels. The variations noted in membrane accumulation and vesicle density between the *sec* mutants may be due to differences in the restrictiveness of the mutant alleles or to membrane structures not easily discerned or quantified by the methods used here. A more

interesting possibility to explain these variations, however, is that secretory protein transport and membrane proliferation may be uncoupled. In this regard it has been observed that yeast cells containing elaborated membrane structures, *e.g.*, around the nucleus (karmellae) do not necessarily exhibit profound defects in secretion or cell growth (WRIGHT *et al.* 1988).

SEC31 is involved in vesicle formation: The low vesicle density observed in the two mutants, *sec23-5* and *sec31-1*, is consistent with the previous designation of *sec23-1* as a class I mutation that affects the formation of ER-derived vesicles (KAISER and SCHEKMAN 1990) and suggested that *sec31-1* also may be defective in this transport step. Biochemical studies have shown that the formation of ER-derived vesicles requires at least three purified protein fractions: Sar1p, Sec23p/Sec24p complex, and Sec13p/p150 complex (SALAMA *et al.* 1993). These proteins cooperate on ER membranes to form an electron-dense coat, designated COP II, around ER-derived vesicles (BARLOWE *et al.* 1994). Our lab found that a genomic DNA clone encoding p150 fully suppressed the temperature-sensitive phenotype of *sec31-1*, and standard genetic methods confirmed that the cloned DNA corresponds to the *sec31* locus (N. SALAMA, J. CHUANG and R. SCHEKMAN, unpublished results). Thus, the *SEC31* gene product is the p150 subunit of the COP II membrane coat, involved in the formation of ER-derived vesicles. The *sec31-1* mutant should prove useful for further study of the function of this protein.

SEC32 is allelic to BOS1: Unlike the other ER-Golgi mutants, the *sec32-1* mutant did not show an extensive proliferation of ER membrane and showed only a moderate vesicle density. The intermediate number of vesicles displayed by *sec32-1* cannot be attributed to leakiness of the allele because *sec32-1*, like *ypt1-3*, was very restrictive for both invertase secretion and growth. Surprisingly, *sec32-1* mutant cells exhibited a network of membranous tubules (Figure 7), which was unlike the ER-accumulating phenotype of *sec12-4* or the stacked membrane phenotype of *sec7-5*. Double mutant analysis indicated that *sec12-4* was epistatic to *sec32-1*, and that *sec32-1* was epistatic to *sec7-5*. Although the precise nature of these membrane tubules remains unclear, they appear to be morphologically distinct from ER membrane (PREUSS *et al.* 1991) and Golgi cisternae (PREUSS *et al.*, 1992). Our genetic analysis has identified *sec32-1* as the first temperature-sensitive mutation in the *BOS1* gene to be reported. *BOS1* originally was isolated as a high copy suppressor of the *bet1-1* mutation (NEWMAN *et al.* 1990). *BOS1*, *BET1/SLY12* and *SEC22/SLY2* all encode membrane proteins thought to be involved in the fidelity of targeting of ER-derived vesicles to the Golgi complex (reviewed in LIAN and FERRO-NOVICK *et al.* 1993; ROTHMAN 1994). One possibility is that the *sec32-1* mutant forms vesicles that are competent for fusion but, due to inactivation of Bos1p, lack the targeting information that enables them to fuse specifically. If this were the

case, then vesicles may fuse with each other or with other membrane organelles resulting in a low or moderate vesicle density and the appearance of new membrane structures and/or an enhancement in existing membrane structure. Further analysis will be necessary to ascertain the function and identity of these novel membrane structures accumulated in *sec32-1* cells.

SEC33 is allelic to RET1, encoding the α subunit of coatamer: Coatamer is the cytosolic assembly unit of the nonclathrin coat that in mammalian cells is associated with the Golgi complex and Golgi-derived transport vesicles (reviewed in ROTHMAN 1994). In both yeast and mammalian cells, coatamer is a 700- to 800-kD protein complex consisting of seven stoichiometric subunits, designated α -, β -, β' -, γ -, δ -, ϵ -, and ζ -COP (WATERS *et al.* 1991; HOSOBUCHI *et al.* 1992). Several of the yeast genes encoding subunits of coatamer have been identified, including *RET1* (α -), *SEC26* (β -), *SEC27* (β' -) and *SEC21* (γ -). Coatamer has been shown to interact specifically with the KKXX-motif present on ER-resident membrane proteins (COSSON and LETOURNEUR 1994) that is required for the selective retrieval of escaped ER proteins from the Golgi to the ER (GAYNOR *et al.* 1994). A temperature-sensitive mutation in the *RET1* gene, *ret1-1*, was isolated in a genetic screen for mutants defective in KKXX-retrieval (LETOURNEUR *et al.* 1994). The *ret1-1* mutant displays no secretion defect at nonpermissive temperatures, although coatamer from *ret1-1* mutants incubated at nonpermissive temperatures has lost the ability to bind KKXX-motifs (LETOURNEUR *et al.* 1994).

Our genetic data demonstrated that *sec33-1* was a temperature-sensitive allele of the *RET1* gene that showed strong synthetic lethality with *sec21-1*. *sec33-1* accumulated a moderate number of vesicles at the nonpermissive temperature (Table 3) and thus can be placed into the "intermediate" class of mutants defined by KAISER and SCHEKMAN (1990), which also includes the coatamer subunit mutants *sec21-1* and *sec27-1*. In contrast to what has been reported for the first mutation isolated in *RET1*, *ret1-1* (LETOURNEUR *et al.* 1994), we found that *sec33-1* exhibited a clear secretion phenotype. It has been speculated that coatamer may act only in retrograde Golgi-to-ER traffic, and that its involvement in ER-to-Golgi transport may be indirect (PELHAM 1994). Another possibility, suggested by the phenotypes of the *sec33-1* and *ret1-1* mutants, is that the α subunit of coatamer may have a role both in forward transport as well as retrograde Golgi-to-ER traffic. Further investigation into the apparent phenotypic differences of the mutant alleles of *RET1* may yield additional insight into the function of coatamer in protein trafficking.

SEC34 and SEC35 may define components of the targeting/fusion machinery: The high vesicle densities observed in the *sec34-1* and *sec35-1* mutants suggests that these mutants may be similar to the class II mutations (KAISER and SCHEKMAN 1990) with defects in the tar-

geting or fusion of ER-derived vesicles with the Golgi complex. Our genetic analysis has shown that *SEC34* and *SEC35* did not correspond to any of the genes known to be involved in the targeting/fusion process, including *SEC17*, *SEC18*, *BET1/SLY12*, *SEC22/SLY2*, *BOS1*, *USO1*, *YPT1*, *SED5*, and *SLY1*, and thus may encode novel proteins involved at this stage of the secretory pathway. Analysis of *sec34-1* and *sec35-1* mutant phenotypes in *in vitro* assays, cloning of the genes, and biochemical characterization of the gene products will be necessary to define the role of *SEC34* and *SEC35*.

We thank Dr. JON BERTSCH for excellent technical assistance and Drs. DAVID BOTSTEIN, PIERRE COSSON, SCOTT EMR, SUSAN FERRO-NOVICK, ALEX FRANZUSOFF, DIETER GALLWITZ, PETER NOVICK, HUGH PELHAM, MARK ROSE, NAVA SEGEV, TOM STEVENS, JEREMY THORNER and MAKARI YAMASAKI for strains, plasmids and antibodies. We are grateful to Drs. GREG PAYNE and KATHLEEN WILSBACH for sharing data before publication and to Dr. BRUCE HORAZDOVSKY for advice on radiolabeling. We thank KRISTINA WHITFIELD for graphic artwork and Drs. TAMARA DOERING, PAUL HERMAN, MARTIN LATTERICH and NANCY PRYER for careful review of the manuscript. This work was supported by Postdoctoral Fellowships from the American Cancer Society, California Division to L.W. and R.D. and by grants from the Howard Hughes Medical Institute and the National Institutes of Health to R.S. L.W. dedicates this work to her children, Julia and Markus.

LITERATURE CITED

- BACON, R. A., A. SALMINEN, H. RUOHOLA, P. NOVICK and S. FERRO-NOVICK, 1989 The GTP-binding protein Ypt1p is required for transport *in vitro*: the Golgi apparatus is defective in *ypt1* mutants. *J. Cell Biol.* **109**: 1015–1022.
- BALLOU, C. E., 1970 A study of the immunochemistry of three yeast mannans. *J. Biol. Chem.* **245**: 1197–1203.
- BARLOWE, C., L. ORCI, T. YEUNG, M. HOSOBUCHI, S. HAMAMOTO *et al.*, 1994 COP II: a membrane coat formed by Sec proteins that drive vesicle budding from the endoplasmic reticulum. *Cell* **77**: 895–90751.
- BRAKE, A. J., R. S. FULLER and J. THORNER, 1990 Identification of human prohormone-processing endoproteases by homology to the yeast *KEX2* gene product, p. 197, in *Neuropeptides and Their Receptors*, edited by T. W. SCHWARTZ, L. M. HILSTED and J. F. REHFELD. Munksgard, Copenhagen, Denmark.
- CARLSON, M., and D. BOTSTEIN, 1982 Two differentially regulated mRNAs with different 5' ends encode secreted and intracellular forms of yeast invertase. *Cell* **28**: 145–154.
- COSSON, P., and F. LETOURNEUR, 1994 Coatamer interaction with di-lysine endoplasmic reticulum retention motifs. *Science* **263**: 1629–1631.
- DESHAIES, R. J., and R. SCHEKMAN, 1987 A yeast mutant defective at an early stage in import of secretory protein precursors into the endoplasmic reticulum. *J. Cell Biol.* **105**: 633–645.
- DUDEN, R., M. HOSOBUCHI, S. HAMAMOTO, M. WINEY, B. BYERS *et al.*, 1994 Yeast β - and β' -coat proteins (COP): two coatamer subunits essential for ER-to-Golgi traffic. *J. Biol. Chem.* **269**: 24486–24495.
- FERRO-NOVICK, S., W. HANSEN, I. SCHAUER and R. SCHEKMAN, 1984 Genes required for completion of proteins into the endoplasmic reticulum in yeast. *J. Cell Biol.* **98**: 44–53.
- FRANZUSOFF, A., and R. SCHEKMAN, 1989 Functional compartments of the yeast Golgi apparatus are defined by the *sec7* mutation. *EMBO J.* **8**: 2695–2702.
- FRANZUSOFF, A., K. REDDING, J. CROSBY, R. S. FULLER and R. SCHEKMAN, 1991 Localization of components involved in protein transport and processing through the yeast Golgi apparatus. *J. Cell Biol.* **112**: 27–37.
- GAYNOR, E. C., S. TE HEESSEN, T. R. GRAHAM, M. AEBI and S. D. EMR, 1994 Signal-mediated retrieval of a membrane protein from the Golgi to the ER in yeast. *J. Cell Biol.* **127**: 653–665.
- GOLDSTEIN, A., and J. O. LAMPEN, 1975 β -D-Fructofuranoside fructohydrolase from yeast. *Methods Enzymol.* **42**: 504–511.
- HARDWICK, K. G., and H. R. PELHAM, 1992 SED5 encodes a 39-kD integral membrane protein required for vesicular transport between the ER and the Golgi complex. *J. Cell Biol.* **119**: 513–521.
- HASILIK, A., and W. TANNER, 1978 Carbohydrate moiety of carboxypeptidase Y and perturbation of its biosynthesis. *Eur. J. Biochem.* **91**: 567–575.
- HICKE, L., T. YOSHIHISA and R. SCHEKMAN, 1992 Sec23p and a novel 105kDa protein function as a multimeric complex to promote vesicle budding and protein transport from the ER. *Mol. Biol. Cell* **3**: 667–676.
- HOSOBUCHI, M., T. E. KREIS and R. SCHEKMAN, 1992 SEC21 is a gene required for ER to Golgi protein transport that encodes a subunit of a yeast coatamer. *Nature* **360**: 603–605.
- ITO, H., Y. FUKUDA, K. MURATA and A. KIMURA, 1983 Transformation of intact yeast cells treated with alkali cations. *J. Bacteriol.* **153**: 163–168.
- JULIUS, D., R. SCHEKMAN and J. THORNER, 1984 Glycosylation and processing of prepro- α -factor through the yeast secretory pathway. *Cell* **36**: 309–318.
- KAISER, C., and R. SCHEKMAN, 1990 Distinct sets of SEC genes govern transport vesicle formation and fusion early in the secretory pathway. *Cell* **61**: 723–733.
- KLIONSKY, D. J., P. K. HERMAN and S. D. EMR, 1990 The fungal vacuole: composition, function, and biogenesis. *Microbiol. Rev.* **54**: 266–292.
- LAEMMLI, U. K., 1970 Cleavage of structural proteins during the assembly of the head of the bacteriophage T4. *Nature* **227**: 680–685.
- LETOURNEUR, F., E. C. GAYNOR, S. HENNECKE, C. DEMOLIERE, R. DUDEN *et al.*, 1994 Coatamer is essential for retrieval of dilysine-tagged proteins to the ER. *Cell* **79**: 1199–1207.
- LIAN, J. P., and S. FERRO-NOVICK, 1993 Bos1p, an integral membrane protein of the endoplasmic reticulum to Golgi transport vesicles is required for their fusion competence. *Cell* **73**: 735–745.
- LYONS, S., and N. NELSON, 1984 An immunological method for detecting gene expression in yeast colonies. *Proc. Natl. Acad. Sci. USA* **81**: 7426–7430.
- NAKAJIMA, H., A. HIRATA, Y. OGAWA, T. YONEHARA, K. YODA *et al.*, 1991 A cytoskeleton-related gene, *USO1*, is required for intracellular protein transport in *Saccharomyces cerevisiae*. *J. Cell Biol.* **113**: 245–260.
- NAKANO, A., and M. MURAMATSU, 1989 A novel GTP-binding protein, Sar1p, is involved in transport from the endoplasmic reticulum to the Golgi apparatus. *J. Cell Biol.* **109**: 2677–2691.
- NEWMAN, A. P., and S. FERRO-NOVICK, 1987 Characterization of new mutants in the early mutants in the early part of the yeast secretory pathway isolated by a [3 H]mannose suicide selection. *J. Cell Biol.* **105**: 1587–1594.
- NEWMAN, A. P., J. SHIM and S. FERRO-NOVICK, 1990 BET1, BOS1, and SEC22 are members of a group of interacting yeast genes required for transport from the endoplasmic reticulum to the Golgi complex. *Mol. Cell. Biol.* **10**: 3405–3414.
- NOVICK, P., C. FIELD and R. SCHEKMAN, 1980 Identification of 23 complementation groups required for post-translational events in the yeast secretory pathway. *Cell* **21**: 205–215.
- NOVICK, P., S. FERRO and R. SCHEKMAN, 1981 Order of events in the yeast secretory pathway. *Cell* **25**: 461–469.
- OSSIG, R., C. DASCHER, H. H. TREPTE, H. D. SCHMITT and D. GALLWITZ, 1991 The yeast SLY gene products, suppressors of defects in the essential GTP-binding Ypt1 protein, may act in endoplasmic reticulum-to-Golgi transport. *Mol. Cell. Biol.* **11**: 2980–2993.
- PALADE, G., 1975 Intracellular aspects of the process of protein synthesis. *Science* **189**: 347–358.
- PELHAM, H. R. B., 1994 About turn for the COPs? *Cell* **79**: 1125–1127.
- PERLMAN, D., and H. HALVORSON, 1981 Distinct repressible mRNAs for cytoplasmic and secreted invertase are encoded by a single gene. *Cell* **25**: 525–536.
- PREUSS, D., J. MULHOLLAND, C. A. KAISER, P. ORLEAN, C. ALBRIGHT *et al.*, 1991 Structure of the yeast endoplasmic reticulum: localization of ER proteins using immunofluorescence and electron microscopy. *Yeast* **7**: 891–911.
- PREUSS, D., J. MULHOLLAND, A. FRANZUSOFF, N. SEGEV and D.

- BOTSTEIN, 1992 Characterization of the *Saccharomyces cerevisiae* Golgi complex through the cell cycle by immunoelectron microscopy. *Mol. Biol. Cell* **3**: 789–803.
- PRYER, N. K., L. J. WUESTEHUBE and R. SCHEKMAN, 1992 Vesicle-mediated protein sorting. *Annu. Rev. Biochem.* **16**: 471–516.
- PRYER, N. K., N. R. SALAMA, R. SCHEKMAN and C. A. KAISER, 1993 Cytosolic Sec13p complex is required for vesicle formation from the endoplasmic reticulum *in vitro*. *J. Cell Biol.* **120**: 865–875.
- REDDING, K., C. HOLCOMB and R. FULLER, 1991 Immunolocalization of Kex2 protease identifies a late Golgi compartment in the yeast *Saccharomyces cerevisiae*. *J. Cell Biol.* **113**: 527–538.
- ROBERTS, C. J., G. POHLIG, J. H. ROTHMAN and T. H. STEVENS, 1989 Structure, biosynthesis, and localization of dipeptidyl aminopeptidase B, an integral membrane glycoprotein of the yeast vacuole. *J. Cell Biol.* **108**: 1363–1373.
- ROBERTS, C. J., C. K. RAYMOND, C. T. YAMASHIRO and T. H. STEVENS, 1991 Methods for studying the yeast vacuole. *Methods Enzymol.* **194**: 644–661.
- ROBINSON, J. S., T. R. GRAHAM and S. D. EMR, 1991 A putative zinc finger protein, *Saccharomyces cerevisiae*. Vps18p, affects late Golgi functions required for vacuolar protein sorting and efficient α -factor prohormone maturation. *Mol. Cell Biol.* **11**: 5813–5824.
- ROSE, M. D., F. WINSTON and P. HIETER, 1989 *Methods in Yeast Genetics*. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
- ROTHBLATT, J. A., and D. MEYER, 1986 Secretion in yeast: reconstitution of the translocation and glycosylation of α -factor and invertase in a homologous cell-free system. *Cell* **44**: 619–628.
- ROTHBLATT, J. A., and R. SCHEKMAN, 1989 A hitchhiker's guide to analysis of the secretory pathway in yeast. *Methods Cell Biol.* **32**: 3–36.
- ROTHBLATT, J. A., R. J. DESHAIES, S. L. SANDERS, G. DAUM and R. SCHEKMAN, 1989 Multiple genes are required for proper insertion of secretory proteins into the endoplasmic reticulum in yeast. *J. Cell Biol.* **109**: 2641–2652.
- ROTHMAN, J. E., 1994 Mechanisms of intracellular protein transport. *Nature* **372**: 55–63.
- ROTHMAN, J. H., C. P. HUNTER, L. A. VALLS and T. H. STEVENS, 1986 Overproduction-induced mislocalization of a yeast vacuolar protein allows isolation of its gene. *Proc. Natl. Acad. Sci. USA* **83**: 3248–3252.
- SALAMA, N. R., T. YEUNG and R. SCHEKMAN, 1993 The Sec13p complex and reconstitution of vesicle budding from the ER with purified cytosolic proteins. *EMBO J.* **12**: 4073–4082.
- SCHMITT, H. D., M. PUZICHA and D. GALLWITZ, 1988 Study of a temperature-sensitive mutant of the *ras*-related *YPT1* gene product suggests a role in the regulation of intracellular calcium. *Cell* **53**: 635–647.
- SEGEV, N., J. MULHOLLAND and D. BOTSTEIN, 1988 The yeast GTP-binding YPT1 protein and a mammalian counterpart are associated with the secretion machinery. *Cell* **52**: 915–924.
- SHEN, S.-H., P. CHRETIEN, L. BASTIEN and S. SLILATY, 1991 Primary sequence of the glucanase gene from *Oerskovia xanthinolytica*. *J. Biol. Chem.* **266**: 1058–1063.
- SHERMAN, F., 1991 Getting started with yeast. *Methods Enzymol.* **194**: 3–21.
- STEVENS, T., B. ESMON and R. SCHEKMAN, 1982 Early stages in the yeast secretory pathway are required for transport of carboxypeptidase Y to the vacuole. *Cell* **30**: 439–448.
- STIRLING, C. J., J. ROTHBLATT, M. HOSOBUCHI, R. DESHAIES and R. SCHEKMAN, 1992 Protein translocation mutants defective in the insertion of integral membrane proteins into the endoplasmic reticulum. *Mol. Biol. Cell* **3**: 129–142.
- WATERS, M. G., T. SERAFINI and J. E. ROTHMAN, 1991 'Coatamer': a cytosolic protein complex containing subunits of non-clathrin-coated Golgi transport vesicles. *Nature* **349**: 248–251.
- WILSBACH, K., and G. PAYNE, 1993 Vps1p, a member of the dynamin GTPase family, is necessary for Golgi membrane protein retention in *Saccharomyces cerevisiae*. *EMBO J.* **12**: 3049–3059.
- WRIGHT, R., M. BASSON, L. D'ARI and J. RINE, 1988 Increased amounts of HMG-CoA reductase induce "karmellae": a proliferation of stacked membrane pairs surrounding the yeast nucleus. *J. Cell Biol.* **107**: 101–114.
- WUESTEHUBE, L. J., and R. SCHEKMAN, 1993 Selection and screening for yeast secretory mutants. *Methods Mol. Genetics* **1**: 88–106.

Communicating editor: M. CARLSON