Cell, Vol. 61, 723–733, May 18, 1990, Copyright © 1990 by Cell Press

Distinct Sets of *SEC* Genes Govern Transport Vesicle Formation and Fusion Early in the Secretory Pathway

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Summary

A vesicular intermediate in protein transport from the endoplasmic reticulum is detected in a subset of temperature-sensitive mutants blocked early in the yeast secretory pathway. By electron microscopy three of the mutants, *sec18*, *sec17*, and *sec22*, accumulate 50 nm vesicles at the nonpermissive temperature. Vesicle accumulation is blocked by the mutations *sec12*, *sec13*, *sec16*, and *sec23* as shown by analysis of double-mutant strains. Thus the early *SEC* genes can be divided into vesicle forming and vesicle fusion functions. Synthetic lethal interactions between *sec* mutations define two groups of *SEC* genes, corresponding to the groups involved in vesicle formation or fusion. Mutations in two of the genes involved in vesicle fusion, *SEC17* and *SEC18*, are lethal in combination, and five of six possible pairwise combinations of mutations in genes required for vesicle formation, *SEC12*, *SEC13*, *SEC16*, and *SEC23*, are lethal. These interactions suggest cooperation between different *SEC* gene products in vesicle budding and vesicle fusion processes.

Introduction

In eukaryotic cells, secreted proteins pass through a series of membrane-bounded compartments en route to the cell surface (Palade, 1975). At each step in the transport pathway, secreted proteins are thought to be carried by transport vesicles that bud from one compartment and then fuse with the appropriate acceptor compartment. In the case of transport between the endoplasmic reticulum (ER) and the Golgi apparatus, several lines of evidence indicate the existence of specific vesicle carriers. An intermediate vesicle stage between the ER and Golgi apparatus was first suggested by the appearance in the electron microscope of vesicles budding from the transitional region of the ER, located between the rough ER and the Golgi complex (Saraste and Kuismanen, 1984). Membrane preparations with properties suggestive of vesicles specific for transport between the ER and Golgi have been identified by fractionation of tissue culture cells (Lodish et al., 1987) and have been produced in vitro by incubation of isolated ER membranes with ATP and cytosol (Paulik et al., 1988). Recently, NSF (N-ethylmaleimide–sensitive fusion protein), a protein factor required for transport within the Golgi apparatus in vitro (Orci et al., 1989), was found also to act in ER to Golgi transport in vivo (Wilson et al., 1989) and in vitro (Beckers et al., 1989). Thus, ER to Golgi transport may have a mechanism similar to the vesicle-mediated process known to carry out transport between Golgi cisternae (Orci et al., 1986). The idea that carrier vesicles mediate all transport between organelles is attractive in its simplicity, but other possible mechanisms for ER to Golgi transport are consistent with the available data. Tubular connections could be transiently formed between the ER and Golgi, or regions of the ER may be directly transformed into the *cis* Golgi compartment.

If vesicles mediate protein transport between the ER and the Golgi complex, a key to understanding their function will be the identification of the proteins that govern their formation and fusion. One avenue for identifying the proteins required for vesicle transport is the analysis of *SEC* genes in yeast. Temperature-sensitive mutations that disrupt secretory protein transport between the ER and the Golgi have been isolated in 12 Saccharomyces cerevisiae genes (Novick et al., 1980; Newman and Ferro-Novick, 1987; Schmitt et al., 1988; Segev et al., 1988). At the nonpermissive temperature, these mutants accumulate excess ER-like membrane structures filled with core-glycosylated forms of secretory and vacuolar proteins (Novick et al., 1980; Esmon et al., 1981; Stevens et al., 1982; Julius et al., 1984). A subset of the mutants accumulate small membrane-enclosed vesicles that may represent transport intermediates (Novick et al., 1981). Extracts from some of these mutants are also defective for an in vitro reaction that reproduces transport between the ER and the Golgi complex (Baker et al., 1988; Ruohola et al., 1988; M. Rexach, N. Pryer, and R. S., unpublished data). These genes may, therefore, encode proteins that are components of secretory vesicles or that act transiently to promote vesicle formation or fusion.

Here we present a morphological and genetic characterization of early *sec* mutations that shows seven *SEC* genes play a role in either the formation or consumption of a vesicle intermediate in transport from the ER to the Golgi apparatus.

Results

Changes in Membrane Structure Induced by *sec* Mutations

Under favorable conditions, intermediates in an assembly pathway can be identified as structures that accumulate when the pathway is blocked by mutation. To investigate in detail the substeps in protein transport from the ER to the Golgi apparatus, we looked for subtle differences in the membrane morphology produced by mutations in genes required for transport of secretory proteins from the ER. Each of nine mutant strains (*sec12*, *sec13*, *sec16*, *sec17*, *sec18*, *sec20*, *sec21*, *sec22*, *sec23*) was grown at the permissive temperature (24°C) and then shifted to the nonpermissive temperature (37°C). Cells were fixed rapidly with glutaraldehyde, postfixed with osmium, and prepared for electron microscopy (Byers and Goetsch, 1975).

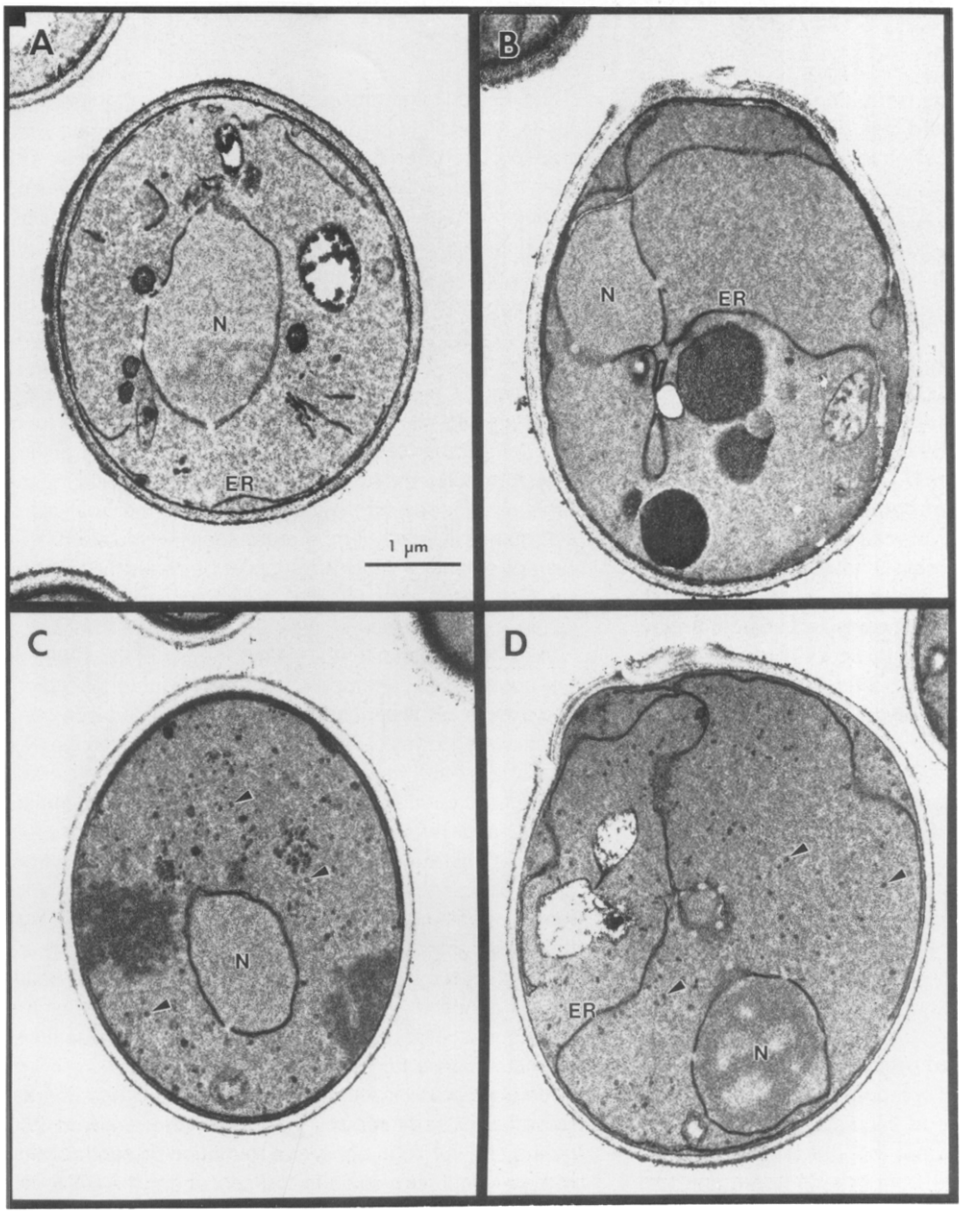

Figure 1. Thin-Section Electron Micrographs of Yeast Cells Fixed with Potassium Permanganate

(A) Wild type (RSY255) grown at 30°C. (B) *sec12-4* (RSY263) grown at 24°C and then for 1 hr at 37°C. (C) *sec17-1* (RSY269) grown at 17°C and then for 1 hr at 37°C. (D) *sec22-3* (RSY279) grown at 17°C and then for 1 hr at 37°C. N: nucleus. ER: endoplasmic reticulum. Arrows in (C) and (D) point to representative 50 nm vesicles

As found previously (Novick et al., 1980), all of the mutants had accumulated ER membranes after 1 hr at 37°C; in the three mutant strains *sec17*, *sec18*, and *sec22*, small membrane-bounded vesicles had proliferated. Thus, growth at the nonpermissive temperature for 1 hr can produce significant accumulation of the small vesicles while minimizing the potential for gross alterations in membrane distribution that occur as the ER membranes are progressively amplified within the cell at the nonpermissive temperature.

The small vesicles were difficult to detect against the dense background of cytoplasmic ribosomes. To count individual vesicles reliably, we employed potassium permanganate as a fixative and stain. Fixation with permanganate selectively deposits manganese oxide at cellular membranes (Riemersma, 1970), revealing small membrane-bounded vesicles as electron-dense rings or disks (Figures 1 and 2). Accumulation of small vesicles was quantified by counting vesicles in random cell sections and normalizing the number to cell volume (Table 1). The nine mutants were grouped into two general classes. The number of small vesicles (smaller than 80 nm in diameter) in wild-type cells was used as a standard for comparison, although not all such vesicles in a wild-type cell can be assumed to be derived from the ER. Class I mutants *sec12*,

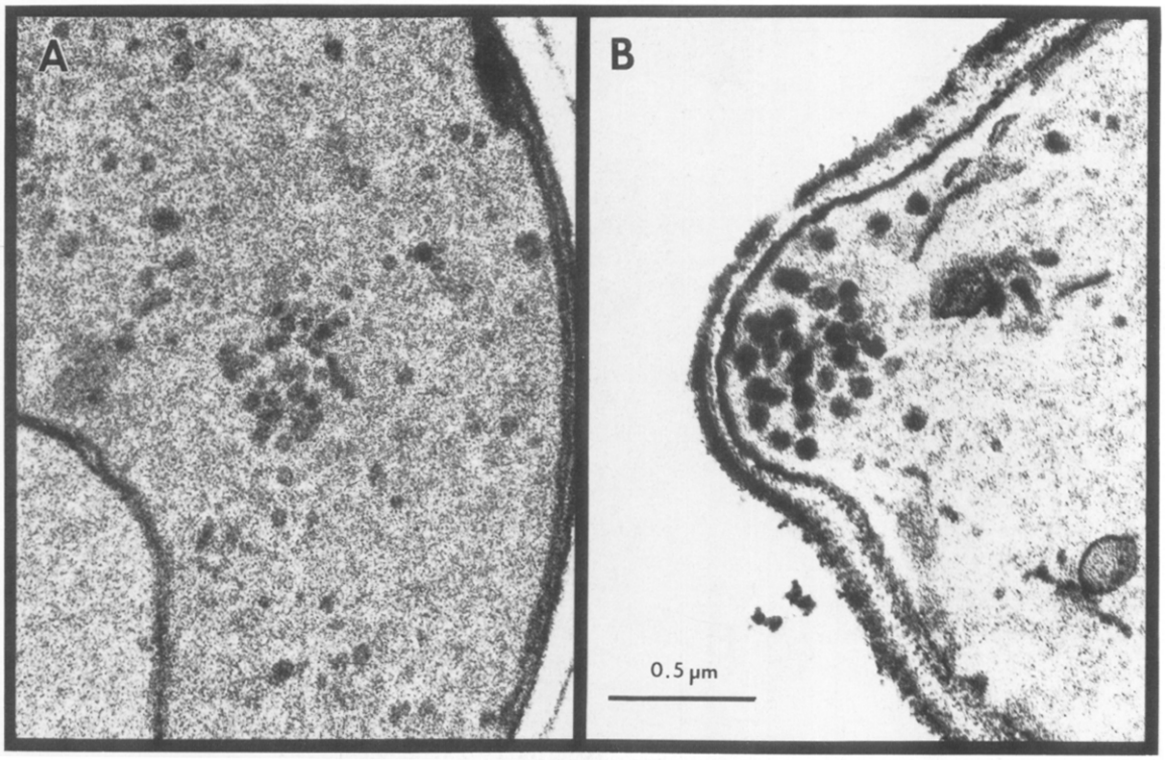

Figure 2. Thin-Section Electron Micrographs of Yeast Cells Illustrating the Difference in Morphology between 50 nm Vesicles and Mature Secretory Vesicles

(A) Enlarged view of 50 nm vesicles in the *sec17-1* cell shown in Figure 1C.

(B) Late secretory vesicles in the growing bud of a wild-type cell (RSY255) grown at 30°C.

sec13, *sec16*, and *sec23* contained approximately the same number of vesicles as wild type. Class II mutants *sec17*, *sec18*, and *sec22* contained about five times the number of vesicles as wild type. Two mutants, *sec20-1* and *sec21-1*, contained an intermediate number of vesicles. In *sec20-1* (RSY275), the accumulated vesicles displayed a unique spatial distribution. Unlike the other class II mutants, in *sec20* many of the vesicles were located between the peripheral ER membranes and the plasma membrane (not shown). This mutant may therefore represent a third phenotypic class.

Table 1. 50 nm Vesicle Accumulation after 1 hr at 37°C

Strain	Allele	Vesicles per μm^3 of Cell Volume
RSY269	*sec17-1* (37°C)	61.9 ± 1.8
RSY269	*sec17-1* (17°C)	13.0 ± 0.8
RSY387	*sec17-2* (37°C)	58.5 ± 3.4
RSY271	*sec18-1* (37°C)	47.9 ± 3.4
RSY319	*sec18-2* (37°C)	41.0 ± 2.5
RSY319	*sec18-2* (17°C)	12.2 ± 1.6
RSY321	*sec22-1* (37°C)	38.5 ± 2.6
RSY279	*sec22-3* (37°C)	55.3 ± 3.0
RSY277	*sec21-1* (37°C)	22.2 ± 1.5
RSY275	*sec20-1* (37°C)	17.4 ± 1.2
RSY255	*SEC*$^+$ (37°C)	7.7 ± 0.7
RSY255	*SEC*$^+$ (17°C)	11.3 ± 0.8
RSY309	*sec12-1* (37°C)	5.9 ± 0.6
RSY263	*sec12-4* (37°C)	7.3 ± 0.9
RSY265	*sec13-1* (37°C)	7.8 ± 0.7
RSY313	*sec13-3* (37°C)	6.4 ± 0.8
RSY317	*sec16-1* (37°C)	9.8 ± 1.1
RSY281	*sec23-1* (37°C)	11.6 ± 1.2

Vesicle density values are given as mean ± standard error of the mean. The standard error is the sample standard deviation divided by the square root of the sample size (n = 32).

Different alleles of a given gene accumulated similar numbers of vesicles, implying that the accumulated vesicle density was a property of a given gene and was not allele specific (Table 1). The considerable breadth of the distribution of vesicles per cell section, especially for class II mutants, was presumably the expected consequence of random sampling, nonuniform distribution of vesicles within the cell body, and variation among individual cells within a culture.

Occasionally, the vesicles appeared in clusters (Figure 1), but most often they were dispersed throughout the cytoplasm. For example, in 32 cell sections of *sec17*, 16% (195 of 1248) of the vesicles were in groups of ten or more vesicles. *sec18* and *sec22* had similarly low frequencies of grouped vesicles. No obvious association of the vesicles with other organelles was noticed other than a tendency of clusters of vesicles to be found in proximity to peripheral ER membranes.

Staining by potassium permanganate produces a thick electron-dense deposit at the vesicle membranes, making accurate measurement of the vesicle diameter difficult. Measurement of 20 vesicles in which the membranes were clearly visible gave mean outer diameters of the vesicles of 46 ± 8, 46 ± 8, and 48 ± 9 nm for *sec17-1*, *sec18-*

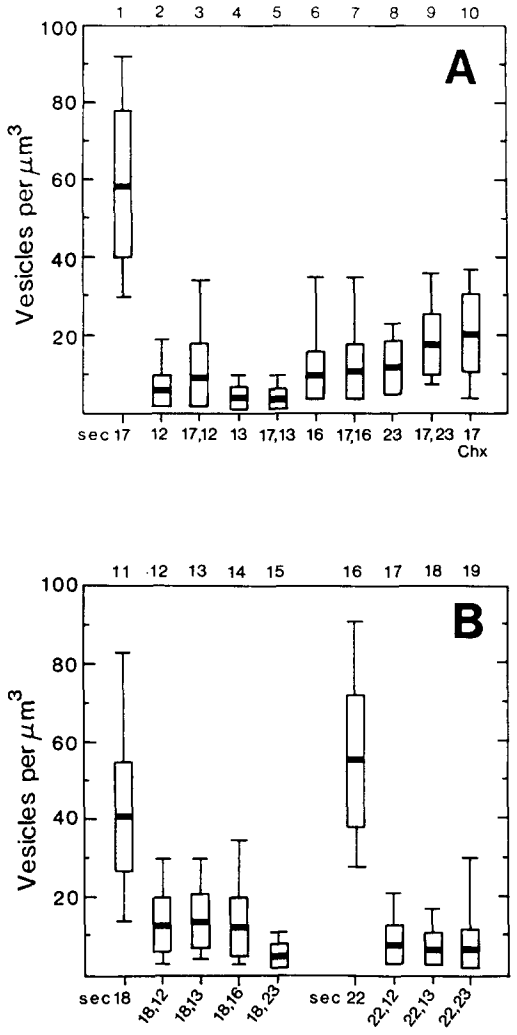

Figure 3. The Influence of *sec* Mutations on Accumulation of 50 nm Vesicles

Numbers of vesicles counted in electron micrographs are expressed as the density of vesicles per cell volume. The distributions of vesicle densities in at least 32 cell sections for each strain are represented by box plots. The bar in the middle of each box indicates the mean vesicle density; the limits of the box indicate one sample standard deviation on each side of the mean; and the lines extend to indicate the extremes of the distribution. The following strains were used: lane 1, RSY269; lane 2, RSY263; lane 3, RSY434; lane 4, RSY265; lane 5, RSY435; lane 6, RSY317; lane 7, RSY327; lane 8, RSY281; lane 9, RSY393; lane 10, RSY269; lane 11, RSY319; lane 12, RSY331; lane 13, RSY329; lane 14, RSY410; lane 15, RSY408; lane 16, RSY281; lane 17, RSY362; lane 18, RSY402; lane 19, RSY363. Chx indicates the cycloheximide-treated sample.

2, and *sec22-3*, respectively. These vesicles were distinctly smaller than the mature secretory vesicles, which are about 80 nm in diameter and appear at the growing bud of wild-type cells (Figure 2). Henceforth we will assume that the small vesicles accumulated in the different *sec* mutants are the same organelle, and we will refer to them collectively as 50 nm vesicles.

Epistatic Relationship between the Two Mutant Classes

Accumulation of 50 nm vesicles in the class II mutants at the nonpermissive temperature suggested that the vesicles represent an intermediate stage in the transport of proteins from the ER to the Golgi apparatus and that the products of the class II genes are required for the consumption of the vesicles. In this view, the class I genes might be required for vesicle formation. To address the order of function of the *SEC* genes, we performed epistasis tests by counting vesicles in strains carrying both class I and class II mutations. Double mutants were constructed by genetic crosses, and vesicles were counted in cells incubated for 1 hr at 37°C prior to fixation. All of the double mutants contained about the same density of vesicles as the respective class I single mutants (Figure 3). The vesicles that accumulate at the nonpermissive temperature in the class II mutants must therefore depend on the function of the class I genes for their formation. That the 50 nm vesicles depended on at least four genes (class I) for formation and at least three genes (class II) for their consumption implied that transport from the ER results from the sequential action of the class I genes to produce 50 nm vesicles followed by the action of the class II genes to consume this vesicle intermediate.

During the course of these experiments, we found that even at 24°C the class II mutants exhibited a phenotype different from that of wild type. For example, the density of 50 nm vesicles in *sec17* grown at 24°C was 23.7 ± 1.4 vesicles per μm^3 (mean ± SEM) as compared with 11.1 ± 0.6 vesicles per μm^3 in wild type. Furthermore, genetic data that we describe later indicated that 24°C was not a completely permissive growth condition for either *sec17* or *sec18*, though neither mutant exhibited a detectable growth defect at 24°C. These findings raised the concern that some of the vesicles counted in the double mutants could have accumulated prior to the shift to 37°C because of a partial defect in the class II mutants at 24°C. For this reason, cultures used for vesicle quantitation were grown at 17°C, a more permissive growth temperature than 24°C, and then shifted to 37°C for 1 hr before fixation.

The mutant *sec21* exhibited an unusual epistatic relationship to *sec17*. The *sec21 sec17* double mutant accumulated as many vesicles as *sec17* alone (not shown). This finding suggests that *sec21* may act at a different stage of the transport pathway than the class I mutations. Alternatively, the *sec21-1* allele may not completely block vesicle formation. Of the ER-accumulating *sec* mutants, *sec21-1* is the weakest in its ability to accumulate incompletely glycosylated invertase (Novick et al., 1980), and unlike other *sec* mutations, this allele does not significantly inhibit growth at temperatures below 34°C (Table 2). Clarification of the stage at which this gene acts will require the isolation of alleles of *sec21* that are more restrictive.

Cycloheximide Limits Vesicle Accumulation

The conventional means of assessing the function of the secretory pathway is to follow the posttranslational modifications of newly synthesized secretory proteins. A test for involvement of protein synthesis in the transport process that does not depend on protein synthesis for the assay method is to determine the requirement for protein synthesis in the accumulation of 50 nm vesicles. *sec17* mutant

Table 2. Growth of *sec* Alleles

Strain	Allele	Growth at 24°C	30°C	37°C
RSY309	*sec12-1*	+	−	−
RSY263	*sec12-4*	+	−	−
RSY265	*sec13-1*	+	−	−
RSY315	*sec13-4*	+	+	−
RSY317	*sec16-1*	+	+/−	−
RSY267	*sec16-2*	+	+/− −	−
RSY269	*sec17-1*	+	+	−
RSY387	*sec17-2*	+	+	−
RSY271	*sec18-1*	+	−	−
RSY319	*sec18-2*	+	+	−
RSY275	*sec20-1*	+	+/− −	−
RSY277	*sec21-1*	+	+	−
RSY321	*sec22-1*	+	+/−	−
RSY279	*sec22-3*	+	−	−
RSY281	*sec23-1*	+	−	−

Growth is represented in decreasing order by: + > +/− > +/− − > −.

cells were grown at 17°C and then shifted to 37°C in either the presence or absence of cycloheximide. The formation of vesicles at 37°C was reduced 3-fold in the presence of cycloheximide (Figure 3). Thus, accumulation of the 50 nm vesicles is limited by the availability of one or more proteins.

Genetic Interactions between *sec* Mutations

Independent evidence for two groups of genes acting at distinct stages of the transport pathway between the ER and the Golgi apparatus was provided by the simple growth phenotype of strains with different combinations of *sec* mutations. Certain combinations of *sec* mutations produced inviability at 24°C, a permissive growth temperature for all of the *sec* mutant alleles (Table 2). The crosses of all possible combinations of mutations in each of the nine genes under examination gave two different outcomes. In most of the crosses, more than 95% of the spores were viable and the double mutants grew well at 24°C. All of the strains used in the morphological epistasis tests were from crosses of this type. For a few of the crosses, about one fourth of the spores did not produce visible colonies after 5 days at 24°C (Table 3). Analysis of the viable meiotic products from these crosses by complementation tests showed that the inviable spores were double mutants.

This phenomenon of mutations in different genes producing lethality when in combination is known as synthetic lethality and can be an indication of functional interaction between gene products (Huffaker et al., 1987). The pattern of *SEC* gene combinations that produce inviability indicates functional relationships between gene products in agreement with the functional distinctions made on the basis of epistasis tests. The combination of *sec17* and *sec18* was lethal, and five of the six possible pairwise combinations of the four genes *sec12*, *sec13*, *sec16*, and *sec23* were inviable. These groups exactly coincided with the two classes of mutations defined by vesicle accumulation. The specificity of these mutant interactions suggests close functional connections within subsets of the class I and class II genes.

Effect of Alternative Alleles on Genetic Interactions

The interactions between *sec* mutations were specific for certain genes. To analyze whether the interactions were also allele specific, two mutant alleles were analyzed for all of the relevant genes except *SEC23*. Alleles of the same gene were taken to be different either because they could recombine with one another or because they were isolated from different mutagenized cultures. The different allelic combinations were constructed by genetic crosses, and their growth properties are shown in Table 4. For those combinations that were lethal, the genotypes of the dead spores were inferred from the genotypes of the viable members of the same tetrad as determined by complementation tests. In most cases the lethality of double-mutant combinations was produced by both alleles of a given gene, arguing strongly that the synthetic lethal phenotypes observed here resulted primarily from the *sec* mutations themselves as opposed to other differences in genotype.

Some alleles displayed subtle phenotypic differences in double-mutant combinations. These differences were observed by microscopic examination of doubly mutant cells that did not form colonies visible by eye. For example, *sec18-1 sec17-1* spores remained as single cells for at least 5 days at 24°C, whereas *sec18-2 sec17-1* spores formed microcolonies at 24°C and grew at 17°C. This apparent specificity exhibited by different *SEC18* alleles could sim-

Table 3. Growth of Double Mutants at 24°C

	sec13-1	*sec16-1*	*sec17-1*	*sec18-1*	*sec20-1*	*sec21-1*	*sec22-1*	*sec23-1*
sec12-1	−	−	+	+	+	+	+	+
sec13-1		−	+	+	+	+	+	−
sec16-1			+	+	+	+	+	−
sec17-1				−	+	+	+	+
sec18-1					+	+	+	+
sec20-1						+	+	+
sec21-1							+	+
sec22-3								+

−: double mutant does not form a visible colony after 5 days at 24°C. +: double mutant grows as well as wild type at 24°C.

Table 4. Growth of Allelic Combinations at 17°C and 24°C

	sec17-1		*sec17-2*	
	17°C	24°C	17°C	24°C
sec18-1	nt	–	nt	–
sec18-2	+/–	+/– –	+/–	+/– –

	sec12-1		*sec12-4*	
	17°C	24°C	17°C	24°C
sec13-1	+	+/– –	+	+
sec13-4	+	+	+	+
sec16-1	+/–	+/– –	+	+/–
sec16-2	+/–	+/– –	+	+/–
sec23-1	+	+	+	+

	sec16-1		*sec16-2*		*sec23-1*	
	17°C	24°C	17°C	24°C	17°C	24°C
sec13-1	nt	–	nt	–	+/– –	+/– –
sec13-4	nt	–	+/–	+/– –	+	+
sec23-1	nt	–	+	+/– –		

+ : growth comparable to wild type. +/– : growth slower than that of wild type. +/– – : microcolony after 5 days of growth. – : fewer than eight cells after 5 days of growth. nt: not tested.

ply be explained by assuming that the *sec18-2* defect is generally less severe than that of *sec18-1*. In combination with either *sec17-1* or *sec17-2*, *sec18-1* was more restrictive than *sec18-2*; when the two single mutants were compared, *sec18-2* grew at 30°C whereas *sec18-1* did not (Table 2). The differences between alleles of the other *sec* genes could be similarly explained by assuming that *sec13-1* is more restrictive than *sec13-4* and *sec12-1* is more restrictive than *sec12-4*. *SEC16* had a more complicated behavior. Temperature-sensitive growth of *sec16-2* appeared to be more restrictive than that of *sec16-1* (Table 2), but in combination with either *sec23-1* or *sec13-4*, *sec16-1* was more restrictive than *sec16-2* (Table 4). The interaction of *sec16* with *sec13* and *sec23* may be based on aspects of protein function different from those that govern the severity of the single *sec16* mutation defect at 24°C.

Lethal Gene Combinations Block Protein Transport

The *SEC* genes could participate in other cellular processes besides intracellular transport. It was therefore of interest to test whether the lethality of double-mutant combinations resulted directly from a block in protein transport. The double mutants *sec16-1 sec23-1* and *sec17-1 sec18-2* grew at 17°C but grew very slowly at 24°C (Table 4). Strains carrying these combinations of *sec* mutations proved defective in transport from the ER at 24°C, as shown by following the maturation of carboxypeptidase Y from the core-glycosylated ER form of the proenzyme (p1), to the Golgi form with extended carbohydrate chains (p2), and then to the proteolytically processed vacuolar form (m) (Figure 4). Cells were grown at 17°C, shifted to 25°C for 20 min, then pulse-labeled with [^{35}S]methionine for 5 min to label the p1 form in all strains. After a 20 min chase at 25°C, the wild-type strain and the single mutants *sec17-1*, *sec18-2*, *sec16-2*, and *sec23-1* showed conversion of most of the carboxypeptidase Y to the p2 and m forms. In contrast, the double mutants had a pronounced secretory defect at 25°C: both *sec17-1 sec18-2* and *sec16-1 sec23-1* showed no conversion of carboxypeptidase Y to p2 or m forms within 20 min. The transport defect in *sec17-1* at 37°C is shown (Figure 4, lanes 3 and 4) for comparison. Since the single mutants showed little or no transport defects at 25°C, the extreme defect exhibited by the double mutants was too severe to be accounted for by the added effects of each of the single mutations. Thus at 25°C these two pairs of mutations have defects that combine synergistically to block ER to Golgi transport.

Discussion

Protein transport between organelles is likely to be governed by a large number of gene products. At least 12 genes whose products function in transport between the ER and Golgi complex have been identified by mutation in yeast. Here we classify nine of these genes with respect to their roles in either the formation or consumption of 50 nm vesicles.

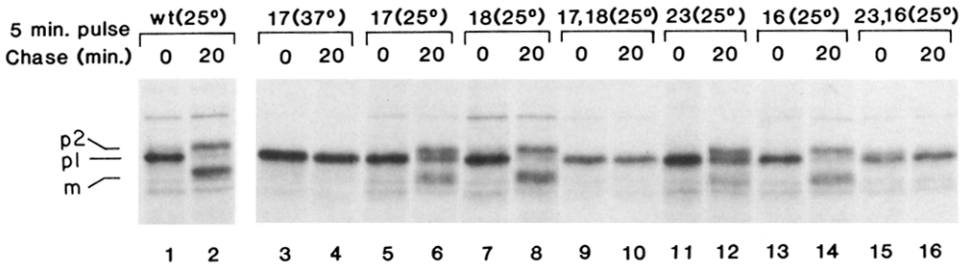

Figure 4. Certain Combinations of *sec* Mutations Block Transport of Carboxypeptidase Y from the ER at 25°C

Cells grown in minimal medium at 17°C were shifted to either 25°C (lanes 1, 2, 5–16) or 37°C (lanes 3 and 4) for 20 min and then pulse-labeled with [^{35}S]methionine for 5 min followed by a 20 min chase initiated by addition of excess unlabeled methionine. Carboxypeptidase Y was isolated from cell extracts by immunoprecipitation and resolved by SDS–PAGE. p1: core-glycosylated carboxypeptidase Y. p2: outer-chain glycosylated carboxypeptidase Y. m: mature carboxypeptidase Y. Lanes 1 and 2, RSY255; lanes 3–6, RSY269; lanes 7 and 8, RSY319; lanes 9 and 10, RSY433; lanes 11 and 12, RSY281; lanes 13 and 14, RSY267; lanes 15 and 16, RSY399.

A Vesicular Intermediate between the ER and Golgi Apparatus

On incubation at the nonpermissive temperature, class II mutants (*sec17*, *sec18*, or *sec22*) accumulate about 3000 of the 50 nm membrane-bounded vesicles per cell body. A significant fraction of the total cellular membrane is thus incorporated into these vesicles, as the total surface area of 3000 spheres 50 nm in diameter is equivalent to about one third the area of the plasma membrane, assuming a yeast cell to be a smooth sphere 5 μm in diameter.

If the 50 nm vesicles are indeed transport intermediates, they should contain secretory and vacuolar protein precursors. Histochemical staining of acid phosphatase in a *sec18* mutant at the nonpermissive temperature shows staining of both ER cisternae and small vesicles (Esmon et al., 1981). Therefore at least some of the vesicles that appear in permanganate-stained cell profiles contain secretory proteins.

Identification of the vesicles that accumulate in class II mutants as derived from the ER is based on three lines of evidence. First, vesicle accumulation is dependent on incubation at the restrictive temperature (Table 1). Second, secretory glycoprotein intermediates that accumulate in class I and II mutants are exclusively core-glycosylated (Novick et al., 1981; Esmon et al., 1981; Stevens et al., 1982; Julius et al., 1984), whereas secretory glycoproteins caught in transit within the Golgi apparatus have at least partial outer-chain carbohydrate (Franzusoff and Schekman, 1989). Finally, double-mutant analysis demonstrates that the action of the class I *SEC* genes is required to produce the vesicles that accumulate in class II mutants (Figure 3). Detailed analysis of the contents of these vesicles requires their isolation in pure form.

Initial morphological characterization of the *sec* mutants revealed small vesicles in *sec17*, *sec18*, and *sec22* (Novick et al., 1980). A double mutant, *sec18 sec20*, was also examined, and since vesicles appeared to accumulate in this strain it was concluded that the vesicles were not part of the secretory pathway and were perhaps the result of fragmentation of the ER (Novick et al., 1981). The quantitative data presented here indicate that a high density of vesicles would be expected in this double mutant. Our examination here of additional epistatic relationships between *sec* mutations shows that formation of the vesicles depends on at least four *SEC* genes, implying that vesicle formation is an aspect of the normal secretory process.

It is possible that the class II genes act repeatedly in the secretory pathway. Since the mammalian homolog of Sec18p acts in both intercisternal Golgi transport (Wilson et al., 1989) and endocytosis (Diaz et al., 1989) as well as in transport from the ER (Beckers et al., 1989), a *sec18* mutant might be expected to block transport of Golgi-derived vesicles and mature secretory vesicles in addition to those budding from the ER. However, since transport from the ER precedes the other events, no step subsequent to this one can be monitored in this group of mutants. By similar reasoning we propose that the class I genes are involved in multiple vesicle budding events elsewhere in the pathway.

Cycloheximide reduces the number of vesicles that accumulate in *sec17* cells upon incubation at the nonpermissive temperature by about 3-fold. This reduction in vesicle accumulation implies that during incubation for 60 min at the nonpermissive temperature, the extent of vesicle formation is limited by the availability of one or more proteins. The limiting factor(s) could be either soluble or membrane-bound vesicle components, or, if vesicle assembly is driven by protein cargo, the limiting factor could be secretory proteins in the ER lumen. The class II mutants accumulate ER membranes in addition to 50 nm vesicles. Assuming that the class II mutations primarily disrupt vesicle fusion with the Golgi apparatus, the accumulation of ER membranes in class II mutants suggests that in the steady state, cells are limited in one or more components necessary to vesiculate all of the excess ER. Thus even when protein synthesis is not blocked, cells appear to have a limited capacity to produce vesicles. In this case the limiting factor is probably not the secretory protein cargo, since histochemical staining of *sec18* after incubation at 37°C shows the accumulated ER cisternae to contain the secreted protein acid phosphatase (Esmon et al., 1981).

Gene Relationships Indicated by Genetic Interactions

All of the *sec* mutant alleles examined in this study grow well at 24°C, yet particular combinations of *sec* mutations produce very slow growth or complete inviability at 24°C. These observations imply that mutations in *SEC12*, *SEC13*, *SEC16*, *SEC17*, *SEC18*, and *SEC23* produce partial defects at 24°C, and although these defects are insufficient on their own to affect cell growth overtly, the particular combinations of mutations in one cell can lead to extreme growth defects. One explanation for the apparent synergism between some *sec* mutations would be that each mutation causes cells to be sickly at 24°C because of a partial defect in protein secretion or membrane organization. In certain double mutants, these defects would add to one another to the point of lethality. This explanation based on additive effects of general sickness is not supported by our data. The tendency of a mutation to be lethal in combination with other mutations was not correlated with the severity of the mutation as judged by growth of single mutants at 30°C (Table 2). Instead, the synthetic lethality of mutant combinations is quite specific for certain gene combinations. Consider the four genes *SEC16*, *SEC23*, *SEC17* and *SEC18*. For all alleles tested, the combinations *sec17 sec18* and *sec16 sec23* grow very slowly or are dead at 24°C. If these synergistic effects were simply the result of addition of general growth defects, then some or all of the other four pairwise combinations of these genes should similarly produce inviability. The other gene combinations, such as *sec17 sec16* and *sec18 sec23*, are healthy at 24°C, indicating specific functional relationships of *SEC17* with *SEC18* and *SEC16* with *SEC23*.

In sum, the combined genetic and morphological data imply that the products of *SEC12*, *SEC13*, *SEC16*, and *SEC23* cooperate in the formation of transport vesicles and that the products of *SEC17* and *SEC18* cooperate to execute vesicle fusion. Two additional arguments support

the idea of functional connections between these *SEC* genes. First, in the two cases tested, the lethal interaction between genes at 24°C appeared to be directly related to a defect in protein transport from the ER. Second, a specific functional connection between *SEC12* and *SEC16* is supported by the finding that the temperature sensitivity of both the *sec12* and *sec16* mutations is suppressed by overproduction of the *SAR1* gene product, a GTP binding protein (Nakano and Muramatsu, 1989).

Synthetic lethal mutant combinations have been found in yeast for mutations in α-tubulin and β-tubulin (Huffaker et al., 1987), for mutations in *SEC4* and other late *SEC* genes involved in the function of mature secretory vesicles (Salminen and Novick, 1987), and for mutations that block translocation of secretory proteins into the ER (Rothblatt et al., 1989). The identification of lethal mutant combinations may, like the isolation of extragenic suppressor mutations, point the way to significant in vivo interactions between gene products long before the tools are available to define the interactions biochemically. How then should these genetic interactions be interpreted? The gene-specific interactions described above indicate a functional unit of a higher order of complexity than that of one gene product. The products of the genes that interact could be components of a multisubunit complex, and the lethal phenotype at 24°C would imply, in this case, that the complex could function with one but not two partially defective subunits. However, direct physical contact between gene products is not required by the data. The interacting genes might encode components of a multimeric protein "machine" that are not in direct physical contact, one gene product might depend on the other to be active, or both could carry out parallel steps in an assembly process such that the overall assembly rate depends on both activities.

In contrast to the discussion here, other cases of synergism between mutations have been taken as an indication that the mutations are *not* on the same pathway. For example, for yeast mutants defective in DNA repair, if the double-mutant combination is more defective than either single mutation alone, the two genes are assumed to act in different repair pathways (Haynes and Kunz, 1978). This type of analysis requires the assumption that the mutations completly block their respective pathways. In contrast, the temperature-sensitive products of mutant alleles of *SEC12*, *SEC18*, *SEC23*, and *SEC16* must have at least partial activity at 24°C since null mutations in these genes are lethal at all temperatures (Nakano et al., 1988; Eakle et al., 1988; Hicke and Schekman, 1989; C. K. and R. S., unpublished data). Nevertheless, the logic used to classify repair genes can account for the genetic interactions that we observe, if we assume that there are two sequential substeps in the overall ER to Golgi transport pathway. In one substep, *SEC13* and *SEC16* would each act in pathways parallel to a pathway that requires the action of both *SEC12* and *SEC23*. In the other substep, *SEC17* and *SEC18* would act in parallel pathways. From the point of view of protein interactions, the formalism of gene products acting on parallel pathways is consistent with these proteins cooperating in the same macromolecular assembly process. Thus, the two alternative modes of interpreting the genetic data that we have presented, although superficially quite different, generate essentially the same model for functional relationships between *SEC* gene products (Figure 5).

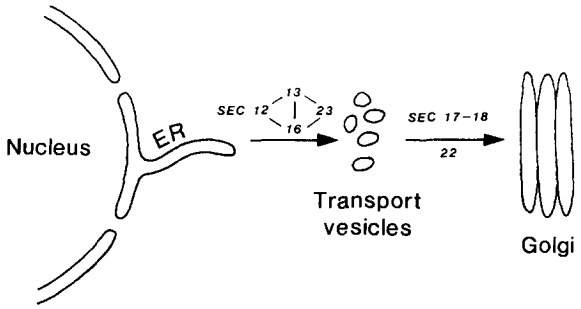

Figure 5. Role of Early *SEC* Genes in Either the Formation or Consumption of a Vesicular Intermediate in Transport between the ER and Golgi Apparatus

Genes whose temperature-sensitive mutant alleles exhibit synthetic lethal interactions are connected by lines.

SEC Gene Product Function

The combined morphological and genetic characterization of the *SEC* genes provides a basis for considering the biochemical functions of their gene products. Protein transport between the cisternae of the mammalian Golgi apparatus is mediated by transport vesicles of about the same size as the vesicles accumulated in yeast class II mutants (Orci et al., 1986). Fusion of Golgi-derived vesicles with their target membranes has been reconstituted in vitro and proceeds in two distinct stages (Orci et al., 1989). The two stages are defined by successive blocks in the fusion process that can be imposed by chemical treatment of the transport reaction. The first block, imposed by the nucleotide analog GTPγS, causes coated transport vesicles to accumulate (Orci et al., 1989). These vesicle coats are neither morphologically nor immunologically related to clathrin coats (Orci et al., 1986). Uncoated vesicles are accumulated at the second, later stage if the transport factor NSF is inactivated by treatment with an alkylating agent (Malhotra et al., 1988). As Sec18p is the yeast homolog of NSF, the vesicles that accumulate in a *sec18* mutant should be of an uncoated variety. It would be of interest to classify the other class II mutants with respect to the presence or absence of a vesicle coat. Unfortunately, the permanganate fixation used to visualize vesicles would probably destroy a proteinacious vesicle coat.

As discussed above, the genetic interaction between *sec18* and *sec17* suggests that their products may function in the same biochemical step. Two additional proteins are known to interact physically with NSF. One is SNAP (soluble NSF attachment protein), which facilitates NSF binding to a Golgi apparatus membrane preparation; the other is a membrane-bound receptor for NSF (Weidman et al., 1989). Biochemical evidence indicates that Sec17p is the yeast homolog of SNAP (Clary et al., 1990).

The products of *SEC12*, *SEC13*, *SEC16*, and *SEC23* are

Table 5. Yeast Strains

Strain	Genotype	Source
RSY255	*MATα ura3-52 leu2-3,112*	D. Botstein
RSY257	*MATa ura3-52 leu2-3,112*	D. Botstein
RSY248	*MATα his4-619*	D. Botstein
RSY269	*MATα sec17-1 ura3-52 his4-619*	
RSY271	*MATα sec18-1 ura3-52 his4-619*	
RSY319	*MATα sec18-2 leu2-3,112*	
RSY321	*MATα sec22-1 ura3-52 leu2-3,112*	
RSY279	*MATα sec22-3 ura3-52 his4-619*	
RSY275	*MATα sec20-1 ura3-52 his4-619*	
RSY309	*MATα sec12-1 leu2-3,112*	
RSY263	*MATα sec12-4 ura3-52 leu2-3,112*	
RSY265	*MATα sec13-1 ura3-52 his4-619*	
RSY313	*MATα sec13-3 leu2-3,112*	
RSY317	*MATα sec16-1 leu2-3,112*	
RSY277	*MATα sec21-1 ura3-52*	
RSY281	*MATα sec23-1 ura3-52 his4-619*	
RSY315	*MATα sec13-4 leu2-3,112*	
RSY267	*MATα sec16-2 ura3-52 his4-619*	
RSY387	*MATα sec17-2 ura3-52 leu2-3,112 ade2 lys2*	
RSY434	*MATa sec12-4 sec17-1 ura3-52 his4-619*	
RSY435	*MATa sec13-1 sec17-1 ura3-52 his4-619*	
RSY327	*MATα sec16-1 sec17-1 leu2-3,112*	
RSY393	*MATα sec17-1 sec23-1 ura3-52 his4-619*	
RSY331	*MATα sec12-4 sec18-2 leu2-3,112*	
RSY329	*MATα sec13-1 sec18-2 ura3-52*	
RSY410	*MATα sec16-1 sec18-2 leu2-3,112*	
RSY408	*MATα sec18-2 sec23-1 leu2-3,112*	
RSY362	*MATα sec12-4 sec22-3 ura3-52 his4-619*	
RSY402	*MATa sec13-1 sec22-3 ura3-52*	
RSY363	*MATa sec22-3 sec23-1 ura3-52 his4-619*	
RSY433	*MATa sec17-1 sec18-2 leu2-3,112*	
RSY399	*MATα sec16-2 sec23-1 ura3-52 leu2-3,112 his4-619*	

Unless indicated otherwise, strains were from this study.

required for vesicle formation or some ER function that precedes the vesicle forming step. The genetic data suggest that the products of these genes might therefore be components of a multisubunit complex. Two of the gene products in this group have been characterized and both are associated with membranes. Sec12p is an integral membrane protein (Nakano et al., 1988), and Sec23p is peripherally associated with the cytoplasmic face of a membrane (Hicke and Schekman, 1989). Sec12p, Sec13p, Sec16p, and Sec23p could be structural components of the 50 nm vesicles. Alternatively, these proteins could be components of the ER membrane that act transiently in vesicle assembly.

Experimental Procedures

Genetic Methods and Media

Yeast strains used in this study are listed in Table 5. The origins of the different *sec* alleles used are as follows: *sec12-1* and *sec12-4* were isolated from different mutagenized cultures (Novick et al., 1980). *sec13-1* and *sec13-4* are from the same mutagenized culture (Novick et al., 1980) but must be different alleles since they exhibit different temperature sensitivities and they mitotically recombine (C. K. and R. S., unpublished data) (Ts^+ recombinants are produced at a high frequency when heteroallelic diploids are exposed to UV light [Roman and Jacob, 1958]). *sec13-3* and *sec13-1* may be the same allele since both were isolated from the same mutagenized culture, they exhibit similar temperature sensitivities, and mitotic recombination between them is undetectable. *sec16-1* and *sec16-2* are from the same mutagenized culture but mitotically recombine. *sec17-1* was isolated previously (Novick et al., 1980), and *sec17-2* was isolated independently from a collection of 500 temperature-sensitive yeast strains we screened for temperature-sensitive mutations that did not complement one of the known ER to Golgi *sec* mutations (C. K. and R. S., unpublished data). *sec18-1* and *sec18-2* show different temperature sensitivities and mitotically recombine. *sec22-1* and *sec22-3* may be the same allele since both were isolated from the same mutagenized culture, they exhibit similar temperature sensitivities, and mitotic recombination between them is undetectable.

To minimize the genetic variation between strains, each *sec* allele was crossed two to three times against either RSY255, RSY257, or RSY248. These strains (obtained from D. Botstein, MIT) are members of an essentially isogenic set of strains derived from S288C. In the last backcross, each of the strains showed uniform 2:2 segregation of the mutant phenotype. The temperature-sensitive phenotype of each strain was characterized by relative growth at 24°C, 30°C, and 37°C and by the frequency of reversion to Ts^+ at 30°C and 37°C.

Yeast crosses were performed by standard methods (Sherman et al., 1974), and the genotypes of all double mutants were confirmed by complemention tests against parental alleles. Mitotic recombination tests were performed by exposing 10^8 diploids spread on a plate to 200 erg/mm^2 shortwave UV light followed by incubation at 24°C for 4 hr in the dark and then at 37°C for 3 days. Mitotic recombination between loci was indicated by a much higher frequency of Ts^+ clones produced by the doubly heterozygous diploid than by either homozygous diploid after treatment with UV light.

YEP liquid medium is 10 g per liter yeast extract (Difco Laboratories, Detroit, Mich.) and 20 g per liter Bacto-Peptone (Difco). Solid medium contains 20 g per liter Bacto-Agar (Difco). SD liquid medium is 6.7 g per liter Yeast Nitrogen Base without amino acids (Difco) and 2% glucose suplemented with the required amino acids at 100 μg/ml. Cell density was measured in a 1 cm quartz cuvette in a Zeiss spectrophotometer.

Electron Microscopy

Yeast cultures were grown to exponential phase (about 10^7 cells per ml) in YPD medium at either 17°C or 24°C. The cultures were transferred to 37°C with aeration for 1 hr to impose a *sec* mutant block. Where appropriate, protein synthesis was inhibited by the addition of 100 μg/ml cycloheximide (Sigma). Fixation was achieved by mixing 100 ml of culture with 2 ml of 50% aqueous glutaraldehyde (Ted Pella, Biological Grade) and immediately centrifuging the cells at 5000 × g for 10 min (4°C). All subsequent manipulations were carried out in 15 mm × 100 mm glass tubes, and solutions were changed by centrifugation at 2000 × g (24°C). Cells were prepared for electron microscopy using either osmium (Byers and Goetsch, 1975) or permanganate fixation (Stevens and White, 1979). The details of permanganate fixation are as follows: Cells were washed once in 5 ml of distilled water, then postfixed in 5 ml of fresh 4% aqueous $KMnO_4$ and incubated for 2–4 hr at 4°C with gentle mixing. Cells were washed five times with distilled water, then suspended in 2% aqueous uranyl acetate and incubated for 12–18 hr at 4°C. Fixed cells were dehydrated in a graded series of ethanol washes and were infiltrated in a 1:1 mixture of ethanol and Spurr's resin. Embedding was completed in Spurr's resin. Pale-gold sections (about 90 nm) were cut and stained with Reynold's lead citrate for 2 min to increase the contrast of stained membranes. Sections were examined in a Philips 300 electron microscope at 80 kV. To count vesicles, well-stained cell sections were selected at random at a low magnification such that the vesicles themselves could not be seen. Selected cells were then photographed at a magnification of 11,000×, and vesicles seen on the negative were counted. Vesicles were scored as disks or rings of electron density between 30 and 70 nm in diameter. In cases where vesicles were clustered, only those vesicles that could clearly be seen in outline were counted. Vesicle counts were normalized for cell volume calculated by measuring the area of the cell section (measured from the plasma membrane) and a section thickness of 90 nm. For each determination, at least 32 cell sections were counted.

Radiolabeling and Immunoprecipitation

Yeast cultures were grown to an A_{600} of 1.0 in SD medium at 17°C. To impose the *sec* mutant block, cultures were shifted to either 25°C or 37°C for 20 min. Cultures (2 ml) were labeled by addition of 200 μCi

of a ^{35}S-labeled mixture of methionine and cysteine (Tran^{35}S-label from ICN Biomedicals). After 5 min the chase was initiated by addition of methionine and cysteine to a final concentration of 0.5 mg/ml. Samples of 1 ml were removed at the beginning of the chase and after 20 min. Cells were sedimented in a microcentrifuge and then suspended in 20 μl of 1% SDS, 50 mM Tris (pH 7.5), 10 mM dithiothreitol, and incubated at 95°C for 2 min. Extracts were produced by agitation in the presence of 0.1 g of glass beads for 1 min followed by further incubation at 95°C for 2 min. Carboxypeptidase Y was isolated from the extracts by immunoprecipitation and was resolved by gel electrophoresis (Rothblatt and Schekman, 1989).

Acknowledgments

We thank Peter Novick for providing singly backcrossed *sec* alleles, John Abelson for the collection of random temperature-sensitive mutants, Robin Wright for assistance with electron microscopy, and Ray Deshaies, Linda Hicke, Nancy Pryer, and Jasper Rine for comments on the manuscript. This work was supported by grants from the National Institutes of Health (NIGMS) and fellowships from Merck and the Helen Hay Whitney Foundation to C. K.

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Received November 14, 1989; revised March 30, 1990.

References

Baker, D., Hicke, L., Rexach, M., Schleyer, M., and Schekman, R. (1988). Reconstitution of *SEC* gene product-dependent intercompartmental protein transport. Cell *54*, 335–344.

Beckers, C. J. M., Block, M. J., Glick, B. J., Rothman, J. E., and Balch, W. E. (1989). Vesicular transport between the endoplasmic reticulum and the Golgi stack requires the NEM-sensitive fusion protein. Nature *339*, 397–398.

Clary, D. O., Griff, I. C., and Rothman, J. E. (1990). SNAPs, a family of NSF attachment proteins involved in intracellular membrane fusion in animals and yeast. Cell *61*, this issue.

Byers, B., and Goetsch, L. (1975). Behavior of spindles and spindle plaques in the cell cycle and conjugation of *Saccharomyces cerevisiae*. J. Bacteriol. *124*, 511–523.

Diaz, R., Mayorga, L. S., Weidman, P. J., Rothman, J. E., and Stahl, P. D. (1989). Vesicle fusion following receptor-mediated endocytosis requires a protein active in Golgi transport. Nature *339*, 398–400.

Eakle, K. A., Bernstein, M., and Emr, S. D. (1988). Characterization of a component of the yeast secretion machinery: identification of the *SEC18* gene product. Mol. Cell. Biol. *8*, 4098–4109.

Esmon, B., Novick, P., and Schekman, R. (1981). Compartmentalized assembly of oligosaccharides on exported glycoproteins in yeast. Cell *25*, 451–460.

Franzusoff, A., and Schekman, R. (1989). Functional compartments of the yeast Golgi apparatus are defined by the *sec7* mutation. EMBO J. *8*, 2695–2702.

Haynes, R. H., and Kunz, B. A. (1978). DNA repair and mutagenesis in yeast. In The Molecular Biology of the Yeast Saccharomyces, Volume 1, J. N. Strathern, E. W. Jones, and J. R. Broach, eds. (Cold Spring Harbor, New York: Cold Spring Harbor Laboratory), pp. 371–414.

Hicke, L., and Schekman, R. (1989). Yeast Sec23p acts in the cytoplasm to promote protein transport from the endoplasmic reticulum to the Golgi complex *in vivo* and *in vitro*. EMBO J. *8*, 1677–1684.

Huffaker, T. C., Hoyt, M. A., and Botstein, D. (1987). Genetic analysis of the yeast cytoskeleton. Annu. Rev. Genet. *21*, 259–284.

Julius, D., Schekman, R., and Thorner, J. (1984). Glycosylation and processing of yeast prepro-α-factor through the yeast secretory pathway. Cell *36*, 309–318.

Lodish, H. F., Kong, N., Hirani, S., and Rasmussen, J. (1987). A vesicular intermediate in the transport of hepatoma secretory proteins from the rough endoplasmic reticulum to the Golgi complex. J. Cell Biol. *104*, 221–230.

Malhotra, V., Orci, L., Glick, B. S., Block, M. R., and Rothman, J. E. (1988). Role of an N-ethylmaleimide-sensitive transport component in promoting fusion of transport vesicles with cisternae of the Golgi stack. Cell *54*, 221–227.

Nakano, A., and Muramatsu, M. (1989). A novel GTP-binding protein Sar1p is involved in transport from the ER to the Golgi apparatus. J. Cell Biol. *109*, 2677–2691.

Nakano, A., Brada, D., and Schekman, R. (1988). A membrane glycoprotein, Sec12p, required for protein transport from the endoplasmic reticulum to the Golgi apparatus in yeast. J. Cell Biol. *107*, 851–863.

Newman, A. P., and Ferro-Novick, S. (1987). Characterization of new mutants in the early part of the yeast secretory pathway isolated by a [^{3}H]mannose suicide selection. J. Cell Biol. *105*, 1587–1594.

Novick, P., Field, C., and Schekman, R. (1980). Identification of 23 complementation groups required for post-translational events in the yeast secretory pathway. Cell *21*, 205–215.

Novick, P., Ferro, S., and Schekman, R. (1981). Order of events in the yeast secretory pathway. Cell *25*, 461–469.

Orci, L., Glick, B. S., and Rothman, J. E. (1986). A new type of coated vesicular carrier that appears not to contain clathrin: its possible role in protein transport within the Golgi stack. Cell *46*, 171–184.

Orci, L., Malhotra, V., Amherdt, M., Serafini, T., and Rothman, J. E. (1989). Dissection of a single round of vesicular transport: sequential intermediates for intercisternal movement in the Golgi stack. Cell *56*, 357–368.

Palade, G. (1975). Intracellular aspects of the process of protein secretion. Science *189*, 347–358.

Paulik, M., Nowack, D. D., and Morre, D. J. (1988). Isolation of a vesicular intermediate in cell-free transfer of membrane from transitional elements of the endoplasmic reticulum to Golgi apparatus cisternae of rat liver. J. Biol. Chem. *263*, 17738–17748.

Riemersma, J. C. (1970). Chemical effects of fixation on biologicl specimens. In Some Biological Techniques in Electron Microscopy, D. F. Parson, ed. (New York: Academic Press), pp. 69–100.

Roman, H., and Jacob, F. (1958). A comparison of spontaneous and ultraviolet-induced allelic recombination with reference to the reconstitution of outside markers. Cold Spring Harbor Symp. Quant. Biol. *23*, 155–160.

Rothblatt, J., and Schekman, R. (1989). A hitchhiker's guide to analysis of the secretory pathway in yeast. Meth. Cell Biol. *32*, 3–36.

Rothblatt, J., Deshaies, R. J., Sanders, S. L., Daum, G., and Schekman, R. (1989). Multiple genes are required for proper insertion of secretory proteins into the endoplsmic reticulum in yeast. J. Cell Biol. *109*, 2641–2652.

Ruohola, H., Kabcenell, A. K., and Ferro-Novick, S. (1988). Reconstitution of protein transport from the endoplasmic reticulum to the Golgi complex in yeast: the acceptor Golgi compartment is defective in the *sec23* mutant. J. Cell Biol. *107*, 1465–1476.

Salminen, A., and Novick, P. J. (1987). A *ras*-like protein is required for a post-Golgi event in yeast secretion. Cell *49*, 527–538.

Saraste, J., and Kuismanen, E. (1984). Pre- and post-Golgi vacuoles operate in the transport of Semliki forest virus membrane glycoproteins to the cell surface. Cell *38*, 535–549.

Schmitt, H. D., Puzicha, M., and Gallwitz, D. (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., Mulholland, J., and Botstein, D. (1988). The yeast GTP-binding YPT1 protein and a mammalian counterpart are associated with the secretion machinery. Cell *52*, 915–924.

Sherman, F., Fink, G., and Lawrence, C. (1974). Methods in Yeast Genetics (Cold Spring Harbor, New York: Cold Spring Harbor Laboratory).

Stevens, B. J., and White, J. G. (1979). Computer reconstruction of mitochondria from yeast. Meth. Enzymol. *56*, 718–728.

Stevens, T., Esmon, B., and Schekman, R. (1982). Early stages in the yeast secretory pathway are required for transport of carboxypeptidase Y to the vacuole. Cell *30*, 439–448.

Weidman, P. J., Melançon, P., Block, M. R., and Rothman, J. E. (1989). Binding of an N-ethylmaleimide-sensitive fusion protein to Golgi membranes requires both a soluble protein(s) and an integral membrane receptor. J. Cell Biol. *108*, 1589–1596.

Wilson, D. W., Wilcox, C. A., Flynn, G. C., Chen, E., Kuang, W.-J., Henzel, W. J., Block, M. R., Ullrich, A., and Rothman, J. E. (1989). A fusion protein required for vesicle-mediated transport in both mammalian cells and yeast. Nature *339*, 355–359.