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Genes that Control the Fidelity of Endoplasmic Reticulum to Golgi Transport Identified as Suppressors of Vesicle Budding Mutations

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Although convergent evidence suggests that proteins destined for export from the endoplasmic reticulum (ER) are separated from resident ER proteins and are concentrated into transport vesicles, the proteins that regulate this process have remained largely unknown. In a screen for suppressors of mutations in the essential COPII gene *SEC13*, we identified three genes (*BST1*, *BST2/EMP24*, and *BST3*) that negatively regulate COPII vesicle formation, preventing the production of vesicles with defective or missing subunits. Mutations in these genes slow the secretion of some secretory proteins and cause the resident ER proteins Kar2p and Pdi1p to leak more rapidly from the ER, indicating that these genes are also required for proper discrimination between resident ER proteins and Golgi-bound cargo molecules. The *BST1* and *BST2/EMP24* genes code for integral membrane proteins that reside predominantly in the ER. Our data suggest that the *BST* gene products represent a novel class of ER proteins that link the regulation of vesicle coat assembly to cargo sorting.

INTRODUCTION

Protein transport through the secretory pathway of eukaryotic cells consists of a series of vesicle budding, targeting, and fusion steps that move protein cargo from one membrane-bound organelle to the next (Palade, 1975). These processes have been studied mainly by biochemical dissection of the cytosolic components required for mammalian and yeast cell-free transport assays and by the analysis of yeast mutants that completely block the flow of cargo through the secretory pathway (reviewed in Pryer *et al.*, 1992; Rothman and Orci, 1992). Such approaches have identified many of the essential structural components of vesicles, but much less is known about how cargo molecules are segregated into vesicles. Cargo that is to be carried forward must be packaged into vesicles while permanent residents of an organelle must, to at least some degree, be kept out of the transport vesicles to maintain the integrity of individual organelles (reviewed in Pfeffer and Rothman, 1987).

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The first known sorting step occurs as proteins exit the endoplasmic reticulum (ER). Short peptide sequences carried by resident ER proteins act as signals for the retrieval to the ER of the small fraction of these proteins that have escaped to the Golgi (reviewed in Pelham, 1995). However, when the known retrieval signals are removed from ER resident proteins, secretion to the cell surface is still much slower than for actual secretory proteins, implying that additional retention mechanisms exist (Nilsson *et al.*, 1989; Hardwick *et al.*, 1990). Additionally, the concentration of at least some secretory proteins into regions of vesicle budding (Mizuno and Singer, 1993; Balch *et al.*, 1994) and the selective packaging of α-factor into COPII-coated but not COPI-coated vesicles that bud from the ER (Bednarek *et al.*, 1995) argue that sorting functions may act not only to restrict the progression of resident proteins but also to concentrate some cargo molecules into vesicles.

Cargo selectivity and the fidelity of vesicle assembly have been difficult to study because biochemical assays for these processes have not yet been developed. Furthermore, informative mutations that reduce the fidelity of transport vesicle function have not yet been

isolated, presumably because the phenotype of such mutants would be too subtle to have been recognized in screens for secretory mutants. An opportunity to identify genes that control the accuracy of vesicle formation presented itself when we found that recessive mutations in three genes can efficiently bypass the secretion block that results from null mutations of the essential vesicle coat protein gene *SEC13*. We reasoned that a negative regulatory mechanism may prevent the completion of defective vesicles, resulting in the block in vesicle transport exhibited by *sec13* mutants. The bypass suppressors could then represent mutations that inactivate this vesicle quality control system, allowing vesicles to form without Sec13p.

Bypass suppressors of *sec13*Δ were unexpected because Sec13p is one of the seven key proteins (Sec12p, Sec13p, Sec16p, Sec23p, Sec24p, Sec31p, and Sar1p) required for budding of transport vesicles from the ER in *Saccharomyces cerevisiae* (Nakano *et al.*, 1988; Nakano and Muramatsu, 1989; Kaiser and Schekman, 1990; Hicke *et al.*, 1992; Pryer *et al.*, 1993; Salama *et al.*, 1993; Espenshade *et al.*, 1995). For six of these proteins, involvement in vesicle formation has been demonstrated for ER-to-Golgi transport reconstituted in vitro (d'Enfert *et al.*, 1991; Oka *et al.*, 1991; Hicke *et al.*, 1992; Salama *et al.*, 1993), and all but Sec12p are found as components of the protein coat of vesicles produced in vitro (Barlowe *et al.*, 1994; Espenshade *et al.*, 1995). The elements of this coat are collectively known as COPII, and the assembly of this structure is thought to drive vesicle formation (Barlowe *et al.*, 1994). An analogous structure exists in mammalian cells, because homologs of Sec13p and Sec23p are localized to the transitional ER of mammalian cells (Orci *et al.*, 1991; Shaywitz *et al.*, 1995). These results demonstrate the central and evolutionarily conserved role played by Sec13p in the process of vesicle formation.

This report describes the suppressors of *sec13*Δ mutations that lie in three genes called *BST* for bypass of *SEC13*. The *BST1* gene was cloned and encodes a membrane protein situated in the ER. The *BST2* gene was also cloned and found to be identical to *EMP24*, the product of which was shown previously to be an ER protein and a membrane component of COPII vesicles (Schimmöller *et al.*, 1995). In addition to bypassing the need for Sec13p in ER-to-Golgi transport, the *bst* mutations were found to increase the rate by which resident ER proteins (Kar2p and Pdi1p) and retained proteins (s11-invertase) leave the ER and to decrease the rate by which the secretory protein invertase is transported from the ER. These genes, therefore, define a new type of ER function that specifies both the fidelity of cargo sorting and the cytosolic protein requirements for vesicle assembly. A simple explanation for the phenotypes of the *bst* mutants is that the processes of vesicle assembly and cargo loading are coupled. The loss of a quality control mechanism for vesicle assembly could allow inappropriate or premature formation of vesicles that have not yet properly segregated cargo away from ER-resident proteins.

MATERIALS AND METHODS

Media and Yeast Strains

Growth and maintenance of strains, preparation of standard media (Difco, Detroit, MI), crosses, and other genetic manipulations were performed as described in Kaiser *et al.* (1994). *S. cerevisiae* strains used in this study are listed in Table 1. The *sec13Δ1* allele is a deletion of the entire coding sequence of *SEC13* (R. Gimeno, unpublished observations), and the *sec13Δ2::LEU2* allele is a deletion/disruption as described in Pryer *et al.* (1993). The *SUC2-s11* allele is an Ala-to-Ile substitution at position −1 of the signal peptidase cleavage site that blocks peptidase cleavage (Bohni *et al.*, 1987). In strain construction, the *suc2*Δ allele was scored by invertase assay and the *KAR2ΔHDEL* allele was followed by the dominant Kar2p secretion phenotype it confers. *bst* alleles were scored by assaying suppression of *sec13*Δ and Kar2p secretion. The former trait often was examined by testing our ability to generate viable transformants of a strain with the *SEC13* knockout construct pCK1316 (Pryer *et al.*, 1993). This assay was used in addition to crosses to *bst sec13*Δ because the low viability (between 50 and 70%) of *bst sec13*Δ ascospores often rendered test crosses alone inconclusive. Isogenic *KAR2ΔHDEL, bst1*Δ *KAR2ΔHDEL,* and *bst2*Δ *KAR2ΔHDEL* strains were constructed from CKY190 by disruption of the *BST* genes using the plasmids pME1165 and pME262, respectively. Isogenic *suc2*Δ and *bst1*Δ *suc2*Δ strains were constructed similarly from CKY343.

Plasmids and DNA Manipulations

DNA manipulations were performed as described in Sambrook *et al.* (1989). pRS306-2 μ is an episomal derivative of pRS306 (Sikorski and Hieter, 1989) (D. Miller and G.R. Fink, unpublished observations). pCK1390 is a *GAL1*-promoted *SEC13* plasmid as described in Shaywitz *et al.* (1995). pCK1391 is *SEC13* in pRS306-2 μ (C. Kaiser, unpublished observations). pEHB29 is *SUC2-s11* in pRS316 (Sikorski and Hieter, 1989) (E. Hong, unpublished observations). pME11 is a YCp50 library (Rose *et al.*, 1987) plasmid containing *BST1*. pME1113 was constructed by recircularization of *Sal*I-digested pME11. pME1101 was constructed by ligating the 3.8-kb *Cla*I-*Kpn*I fragment from pME11 into the *Cla*I-*Kpn*I sites of pRS316. pME1120 and pME1121 are two plasmids from a series of nested deletions generated from pME1101 according to the method of Henikoff (1984). These plasmids have deletions of ~700 and 900 bp, respectively (see Figure 5A). pME1108 is the 3.5-kb *Cla*I-*Xho*I fragment of pME11 in pRS316. pME1165 was constructed by ligating the 1.3-kb *Spe*I-*Xho*I fragment of pME1101 into the *Spe*I-*Xho*I sites of pRS305 (Sikorski and Hieter, 1989). pME1170 was constructed in two steps. First, a *Not*I site was inserted at a position between codons 86 and 88 of *BST1* by site-directed mutagenesis according to Kunkel *et al.* (1987). The sequence of the oligonucleotide used was 5′-GAT GGA AAC ATA TAG ATG CGG CCG CTT TCA CAC TGA GGA GCA TCT GC-3′. A 117-bp *Not*I fragment from pGTEPI (Tyers *et al.*, 1993) containing three tandem repeats of the hemagglutinin (HA) epitope (Kolodziej and Young, 1991) was then ligated into the newly created *Not*I site to give pME1170. pME21 is a YCp50 library (Rose *et al.*, 1987) plasmid containing *BST2/EMP24*. pME253 was constructed by ligating the 3.7-kb *Hin*dIII fragment of pME21 into the *Hin*dIII site of pRS316. pME262 was constructed by first ligating the 0.5 kb *Eco*RI-*Sac*I fragment from pME253 into the *Eco*RI-*Sac*I sites of pRS306 to generate pME260.

Table 1. Strain List

Strain	Genotype	Source
CKY8	*MATα ura3-52 leu2-3,112*	Kaiser lab collection
CKY10	*MATa ura3-52 leu2-3,112*	Kaiser lab collection
CKY13	*MATα his4-619 lys2-801*	Kaiser lab collection
CKY14	*MATa his4-619 lys2-801*	Kaiser lab collection
CKY40	*MATa sec12-4 ura3-52*	Kaiser lab collection
CKY46	*MATa sec13-1 ura3-52 his4-619*	Kaiser lab collection
CKY53	*MATa sec16-1 ura3-52 leu2-3,112*	Kaiser lab collection
CKY55	*MATa sec17-1 ura3-52 his4-619*	Kaiser lab collection
CKY59	*MATa sec18-1 ura3-52 his4-619*	Kaiser lab collection
CKY69	*MATa sec21-1 ura3-52 his4-619*	Kaiser lab collection
CKY79	*MATa sec23-1 ura3-52 leu2-3,112*	Kaiser lab collection
CKY100	*MATa sec27-1 ura3-52 leu2-3,112*	Kaiser lab collection
CKY190	*MATα KAR2ΔHDEL ura3 leu2 his4 suc2*	M. Rose (Princeton)
CKY321	*MATα sec13Δ1 ade2 ade3 ura3-52 leu2-3,112* (pCK1390)	This study
CKY322	*MATα sec13Δ1 bst1-2 ade2 ade3 ura3-52 leu2-3,112*	This study
CKY323	*MATα sec13Δ1 bst2-2 ade2 ade3 ura3-52 leu2-3,112*	This study
CKY324	*MATα sec13Δ1 bst3-2 ade2 ade3 ura3-52 leu2-3,112*	This study
CKY325	*MATa sec13Δ2::LEU2 bst1-1 ura3-52 leu2-3,112 his4-619*	This study
CKY326	*MATa sec13Δ2::LEU2 bst2-1 ura3-52 leu2-3,112 his4-619*	This study
CKY327	*MATa sec13Δ1 bst3-1 ura3-52 leu2-3,112 his4-619*	This study
CKY330	*MATα bst1-1 ura3-52 leu2-3,112*	This study
CKY331	*MATa bst1::LEU2 ura3-52 leu2-3,112*	This study
CKY332	*MATα bst2-1 ura3-52 leu2-3,112*	This study
CKY333	*MATa bst2::URA3 ura3-52 leu2-3,112*	This study
CKY334	*MATα bst3-2 ura3-52 leu2-3112 ade2 ade3*	This study
CKY335	*MATα bst1::LEU2 KAR2ΔHDEL ura3 leu2 his4 suc2*	This study
CKY336	*MATα bst2::URA3 KAR2ΔHDEL ura3 leu2 his4 suc2*	This study
CKY337	*MATα bst3-2 KAR2ΔHDEL ura3 leu2 his4*	This study
CKY339	*MATa bst1::LEU2 bst2::URA3 ura3-52 leu2-3,112*	This study
CKY340	*MATα bst1::LEU2 bst3-2 ura3-52 leu2-3,112 ade2 ade3*	This study
CKY341	*MATα bst2::URA3 bst3-2 ura3-52 leu2-3,112 ade2 ade3*	This study
CKY343	*MATa suc2Δ9 ura3-52 leu2-3,112* (pEHB29)	This study
CKY344	*MATa bst1::LEU2 suc2Δ9 ura3-52 leu2-3,112* (pEHB29)	This study
CKY345	*MATa bst2-1 suc2Δ9 ura3-52 leu2-3,112* (pEHB29)	This study
CKY346	*MATα bst3-2 suc2Δ9 ura3-52* (pEHB29)	This study
CKY348	*MATa/MATα ura3-52/ura3-52 leu2-3,112/leu2-3,112*	This study
CKY349	*MATα bst1-1 sec12-1 ura3-52 leu2-3,112*	This study
CKY352	*MATa bst2-1 sec12-1 ura3-52*	This study
CKY355	*MATa bst1-1 sec13-1 ura3-52 leu2-3,112*	This study
CKY358	*MATα bst2-1 sec13-1 ura3-52 leu2-3,112*	This study
CKY361	*MATa bst1-1 sec16-1 ura3-52 leu2-3,112*	This study
CKY364	*MATa bst2-1 sec16-1 ura3-52 leu2-3,112*	This study
CKY367	*MATa bst1-1 sec17-1 ura3-52 leu2-3,112*	This study
CKY370	*MATa bst2-1 sec17-1 ura3-52 leu2-3,112*	This study
CKY373	*MATa bst1-1 sec18-1 ura3-52 leu2-3,112*	This study
CKY376	*MATα bst2-1 sec18-1 ura3-52 his4-619*	This study
CKY379	*MATa bst1-1 sec21-1 ura3-52 his4-619*	This study
CKY382	*MATa bst2-1 sec21-1 ura3-52 leu2-3,,12*	This study
CKY385	*MATα bst1-1 sec23-1 ura3-52 leu2-3,112*	This study
CKY388	*MATα bst2-1 sec23-1 ura3-52 leu2-3,112*	This study
CKY391	*MATα bst1-1 sec27-1 ura3-52 leu2-3,112*	This study
CKY394	*MATa bst2-1 sec27-1 ura3-52 leu2-3,112*	This study
CKY395	*MATa ura3-1 leu2-3,112 his3-11,15 trp1-1 ade2-1 pdi1::TRP1 PDI1ΔHDEL*	C. Shamu, UCSF

The 1.4-kb *Eco*RI-*Sal*I fragment from pME253 was then ligated into the *Eco*RI-*Sal*I sites of pME260.

Isolation and Complementation Testing of bst Mutants

One hundred colonies of CKY321 were cultured for 3 d in liquid YEP (2% galactose). For each culture, 8×10^6 viable cells were plated onto an SC (2% glucose) 5-FOA plate at 24°C. After 3 d of growth, a single 5-FOA resistant colony from each plate was chosen for further analysis.

Initially, complementation tests were done by crossing a *bst sec13Δ* mutant isolate to several other outcrossed *bst sec13Δ* mutant strains of the opposite mating type and with complementary auxotrophies. Mating mixtures were grown on rich medium (YPD) overnight, then replica-plated to minimal medium (SD) selective for the growth of diploids. Growth of the *sec13Δ/sec13Δ bst/bst* diploids indicated failure of the recessive *bst* mutations to complement. Once initial testing indicated three complementation groups, test strains representing each group (CKY325, CKY326, or CKY327) were used to classify the remaining mutants.

Protein Gels, Immunoblotting, and Quantitation

Protein extracts were prepared from 1–5 OD_{600} units of cells by boiling and lysis with glass beads in 30 μl of sample buffer (80 mM Tris, pH 6.8, 2% SDS, 10% glycerol, 100 mM DTT, and 10% bromophenol blue). Proteins from the culture medium were precipitated by adding 100% TCA to a final concentration of 10% and incubating at 0°C for 30 min. The precipitates were pelleted in a microfuge, washed with 100% acetone (−20°C for 30 min), and resuspended in sample buffer. Samples were heated at 95°C for 3 min before being resolved by SDS-PAGE. Proteins were transferred to nitrocellulose in a semidry transfer apparatus (Owl Scientific Plastics, Cambridge, MA) at 500 mV for 45 min. Blots were blocked and then incubated for 1 h with primary antibody in TBS-T (20 mM Tris, pH 7.5, 150 mM NaCl, and 0.05% Tween 20), 2% milk at the following dilutions: affinity-purified rabbit anti-Sec13p antibody was used at 1:1000 dilution; affinity-purified rabbit anti-KAR2p antibody (a gift from M. Rose, Princeton University) was used at 1:10,000 dilution; anti-Pdi1p antibody (a gift from C. Shamu, University of California, San Francisco) was used at 1:2000 dilution; and anti-HA antibody (12cA5 ascitic fluid; BABCO, Richmond, CA) was used at 1:1000 dilution. Blots were then washed three times for 10 min each in TBS-T, incubated with a 1:10,000 dilution of either goat anti-rabbit IgG-HRP or sheep anti-mouse IgG-HRP (both from Amersham, Arlington Heights, IL) in TBS-T, 1% BSA for 1 h, washed three times for 10 min each in TBS-T, developed for chemiluminescence using the ECL system (Amersham), and exposed to film.

For quantitation of Western blots, a serial dilution of sample first was immunoblotted as described above and quantitated on an LKB 2202 Ultroscan laser densitometer (LKB, Bromma, Sweden) to determine the range in which a linear response was observed. Within this range, samples were loaded in duplicate and all values reported are the average of the duplicate samples scanned three times each.

An immunoblotting assay was used to score Kar2p secretion for the cloning of *BST1* and *BST2/EMP24* (Figure 4A). Colonies on solid medium were covered with a wetted nitrocellulose filter (S&S BA85 0.45-μm circles, Keene, NH) and grown for 16–24 h (YPD) or 40–48 h (synthetic medium). Cells were washed from the filter with 10 mM Tris-HCl (pH 7.5) and 0.5 M NaCl, and the filters were processed by immunoblotting as described above.

Radiolabeling and Immunoprecipitation

Radiolabeling and immunoprecipitations were carried out essentially as described in Gimeno *et al.* (1995). The IP buffer used was 50 mM Tris-HCl (pH 7.4), 150 mM NaCl, 1% Triton X-100. One microliter of anti-CPY or 2 μl of anti-invertase antibody was used per OD_{600} unit of labeled cell extract. For the reimmunoprecipitation of s11-invertase with anti-α1,6 antibody, the precipitated primary immune complexes were disrupted by boiling in 30 μl of sample buffer (as above). A volume of 20 μl was removed and diluted in 1 ml of IP buffer. A volume of 0.5 μl of anti-α1,6 antibody was then used to reimmunoprecipitate the α1,6-modified protein. A volume of 10 μl of each sample was separated by SDS-PAGE. Gels were soaked in 1 M sodium salicylate for 30 min before being dried and exposed to a phosphorimaging screen. Images were analyzed using a 445si PhosphorImager and ImageQuant software (Molecular Dynamics, Sunnyvale, CA).

Cloning of BST1 and BST2/EMP24

BST1 and *BST2* were cloned by complementation of the Kar2p secretion phenotype. Plasmids were rescued and shown to complement both the Kar2p secretion phenotype of the corresponding *bst* mutant strain and the suppression of *sec13Δ* mutations. The latter was tested in transformation experiments in which our inability to generate viable transformants of the corresponding *sec13Δ bst* mutant strain was scored as complementation of the suppression phenotype. Controls for these experiments included showing that the *sec13Δ bst* mutant strain could be transformed with a control plasmid, and that the clone being tested could transform a control strain.

Once clones were obtained, they were subcloned into pRS316 (Sikorski and Hieter, 1989), and the minimal complementing region was identified using the transformation assay described above. For *BST1*, a series of nested deletions was generated from both directions as described in Henikoff (1984). These clones were used to sequence both strands by the dideoxy method following the Sequenase protocol (USB, Cleveland, OH). Sequence generated in this manner was used to search both DNA and protein databases for homologies. The *BST1* gene sequence is found on Chromosome VI as YFL025C (The *Saccharomyces* Genomic Information Resource [http://genome-www.stanford.edu/]). For *BST2*, sequence analysis identified the complementing region as the previously identified *EMP24* locus (Schimmöller *et al.*, 1995).

Bst1p Localization

To analyze the subcellular distribution of Bst1p, cells (CKY10) expressing either (HA) epitope-tagged *BST1* (pME1170) or untagged *BST1* (pME1101) were examined by Western analysis using anti-HA antibody, which detected a single diffuse band that was only present in strains containing the epitope-tagged *BST1*. This protein migrated with an apparent molecular mass of 142–146 kDa (Figure 6). Cell fractionation was carried out on these strains as described in Espenshade *et al.* (1995). $EndoH_f$ treatments were performed by diluting 3.5-fold into 20 mM sodium citrate (pH 5.5), adding 8 units of $EndoH_f$ (New England Biolabs, Beverly, MA), and incubating at 37°C for 3 h. Indirect immunofluorescence of Bst1p-HA was carried out essentially as described in Espenshade *et al.* (1995). A yeast strain (CKY10) expressing *BST1-HA* (pME1170) was grown selectively and then transferred to YPD for 2 h before fixation. The fixed cells were washed once in 0.1% SDS for 5 min before application to the slide to enhance the detection of the protein. Bst1p-HA was visualized using a 1:200 dilution of anti-HA antibody (12cA5 ascitic fluid; BABCO) and a 1:200 dilution of fluorescein-conjugated sheep anti-mouse secondary antibody (Amersham). Kar2p was visualized using a 1:5000 dilution of affinity-purified anti-Kar2p antibody (a gift from M. Rose, Princeton University) and a 1:200 dilution of rhodamine-conjugated goat anti-rabbit secondary antibody (Boehringer Mannheim, Indianapolis, IN). For the double-label immunofluorescence experiments, an additional blue excitation filter (Zeiss, #467974, Thornwood, NY) was used to eliminate crossover fluorescence.

Genetic Interaction between bst and sec Mutations

To minimize the possible variation attributable to genetic background, *sec13Δ bst* mutant isolates were outcrossed and the *bst* mutant spore clones were sequentially backcrossed four times to either CKY8, CKY10, CKY13, or CKY14. These strains are members of a set of isogenic strains derived from S288C (D. Botstein, Stanford University). In the final backcrosses, the Kar2p secretion and suppression phenotypes cosegregated 2:2 in the tetrads analyzed. Two such *bst* mutant strains (CKY330 and CKY332) were then crossed to the *sec* mutant strains listed in Table 1. Tetrads were tested for growth at 24, 28, 30, 33, 36, and 38°C. Genetic interactions (either partial suppression or synthetic enhancement of the temperature sensitivity) were observed in crosses in which 2:2, 3:1, and 4:0 ascospore viability patterns were seen at temperatures above or below the "cutoff temperature" for a given *sec* mutant. The suppression or synthetic effects (seen when in combination with a *sec* mutation) completely cosegregated with the Kar2p secretion phenotype of the *bst* mutation in the cross, as assayed by the immunoblotting filter assay described above. In crosses in which the *sec* mutant involved also secretes Kar2p (*sec17* and *sec18*; Semenza *et al.*, 1990), only NPD tetrads were considered. In representative tetrads, the segregation of the *bst* mutation in the cross was then confirmed by scoring the *sec13Δ* suppression phenotype. Isogenic *sec* and *sec bst* strains were constructed by transforming the *sec bst* double-

mutant strains from Table 1 with the corresponding *BST* gene on a CEN plasmid (pME1101 or pME253) or with a CEN vector (pRS316) control plasmid. The growth of these isogenic pairs of strains was compared on synthetic complete medium lacking uracil.

Invertase Assays

Invertase assays were performed on either whole cells (external) or cell lysates (total) prepared in the following manner. Exponentially growing cultures in either YPD or SD + casamino acids (2% glucose) were pelleted and resuspended at a density of 1 OD_{600} unit/ml in medium with 0.1% glucose. Cells were then incubated for 2 h. One OD_{600} unit of cells was removed to an equal volume of 20 mM Tris (pH 7.5), 20 mM NaN_3 at 0°C, washed twice in ice-cold 10 mM Tris (pH 7.5), and resuspended in either 1 ml of 10 mM Tris (pH 7.5) for assaying external invertase or 30 μl of 10 mM Tris (pH 7.5), 1% Triton X-100 for assaying total invertase activity. For assay of total activity, the cells were lysed by vigorous vortexing with glass beads for 3–5 min and then diluted to 1 ml in 10 mM Tris (pH 7.5). Invertase was assayed as described in Gascón *et al.* (1968) in duplicate for each strain tested.

RESULTS

Isolation and Complementation Testing of bst sec13Δ Mutant Strains

SEC13 is an essential gene, because the conditional allele *sec13-1* is inviable above 30°C and spores bearing a deletion of the gene (*sec13Δ*) never germinate on rich medium (Pryer *et al.*, 1993). To examine the phenotype of cells lacking Sec13p, we constructed a strain with *sec13Δ* on the chromosome and a plasmid-borne copy of *SEC13* under *GAL1* promoter control. Under conditions in which Sec13p expression is repressed by growth on glucose, this strain does not grow, yet rare revertant colonies arise on glucose plates. To explore the genetic basis of this reversion, a collection of revertants was isolated as follows. The strain CKY321 has a chromosomal deletion of the *SEC13* coding sequence and, therefore, was dependent on the *GAL1*-promoted *SEC13-URA3*-containing plasmid pCK1390 for viability. (This strain was designed to minimize the likelihood of reversion by plasmid integration or gene conversion: the plasmid carries a centromere and only shares homology with the chromosome in the 3′ region of the *SEC13* locus.) Cultures of CKY321 were plated on medium containing 5-FOA to select for cells that had lost the *SEC13-URA3* plasmid. Robust colonies arose on these plates after 3 d of growth at 24°C at an average frequency of 2×10^{-6} colonies per viable cell. After longer incubations (up to 7 d), additional colonies arose at a 10-fold higher frequency. These additional colonies comprise a second class of suppressors, weaker than those discussed in this report, that are currently under investigation. After 3 d of growth, 100 independent revertants were chosen for further analysis.

Preliminary experiments suggested that most of the revertants contained unlinked suppressor mutations that segregated as single genes on backcrossing. To distinguish suppressor-containing strains from other possible types of revertants, a number of independent tests were performed. Transformation with *URA3*-containing plasmids restored 98 of the isolates to Ura^+, indicating that in these cases 5-FOA resistance was probably attributable to plasmid loss, as expected. The two isolates that remained Ura^- in this test were not considered further because they still contained Sec13p detected by Western blotting and probably resulted from *ura1* mutation. Southern analysis on representatives of the 98 isolates confirmed that the deletion at the *SEC13* locus was still intact. Finally, cell extracts from representative isolates did not contain Sec13p by Western blotting. Figure 1A shows the absence of Sec13p in three such representatives that were subsequently identified as isolates from the three major complementation groups (see below). Together these findings show that reversion had occurred be-

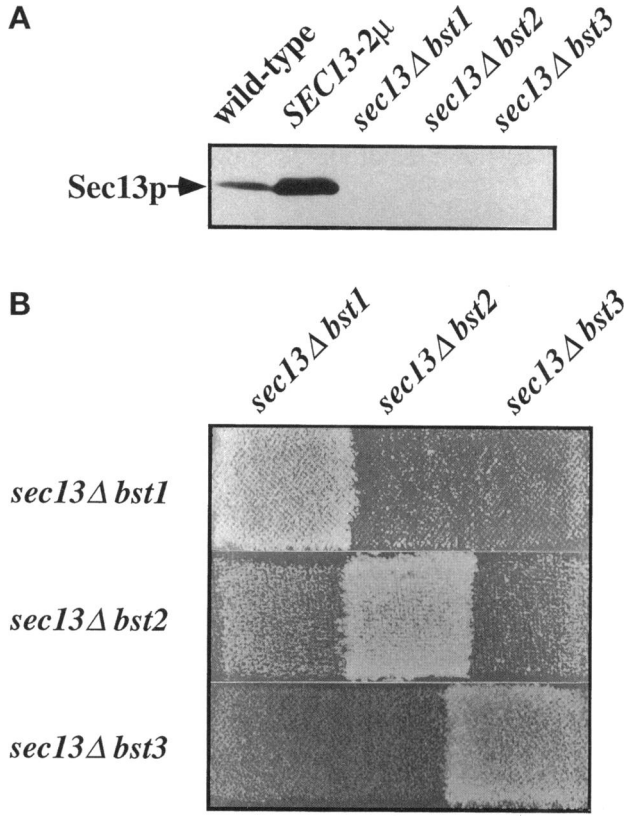

Figure 1. Analysis of *sec13Δ* revertants. (A) A wild-type strain CKY10, carrying either the empty vector pRS306-2 μ (lane 1) or the *SEC13*-containing high-copy plasmid pCK1391 (lane 2), and three *sec13Δ* revertant strains (CKY322, CKY323, and CKY324) carrying pRS306-2 μ (lanes 3–5) were grown in selective medium at 24°C. Sec13p was detected by immunoblotting with affinity-purified anti-Sec13p antibody. (B) Complementation analysis of *bst sec13Δ* mutant isolates. Strains of the indicated genotype (CKY322, CKY323, CKY324, CKY 325, CKY326, and CKY327) were patch-mated, and diploids were selected.

cause of suppressor mutations that allowed growth in the absence of Sec13p. We therefore called these *bst* mutants (for bypass of *sec* thirteen).

To determine the number of genes represented by this collection of mutants, *bst sec13Δ* strains that were appropriate for complementation testing were constructed by backcrossing. The test strains were mated to the 98 isolates and diploids were selected using complementary auxotrophic mutations. For all 98 isolates, the resulting diploids were found to be inviable for many of the test crosses. This showed that the suppressor mutations were recessive and could be organized into complementation groups as follows. In crosses in which the parental *bst* mutations are in different genes, complementation in the diploid should prevent suppression of the homozygous *sec13Δ* mutation, giving an inviable diploid; viable diploids should be recovered in crosses in which both suppressor mutations are in the same gene. This analysis allowed the mutants to be organized into three major complementation groups, designated *BST1* (52 isolates), *BST2* (24 isolates), and *BST3* (19 isolates). The remaining three isolates grew poorly and mated inefficiently and, therefore, gave ambiguous results in the complementation test. An example of complementation tests among the three groups is shown in Figure 1B.

ER-to-Golgi Transport Is Restored in bst sec13Δ Mutants

The strains with suppressed *sec13Δ* grow almost as well as wild-type: the doubling times in rich medium at 24°C for *bst1 sec13Δ*, *bst2 sec13Δ*, and *bst3 sec13Δ* are 170, 165, and 155 min, respectively, as compared with 150 min for wild-type. The ability of *bst sec13Δ* strains to grow well indicated a functional secretory pathway. To examine secretion more closely, the maturation of the vacuolar protease carboxypeptidase Y (CPY) was followed as a marker for early events in the secretory pathway. CPY is found in three distinct forms—P1, P2, and mature—that represent progression to the ER, Golgi, and vacuole, respectively (Stevens *et al.*, 1982). In the *bst sec13Δ* strains, CPY was targeted and transported normally at 24°C (Figure 2A), although somewhat more slowly than in the wild-type control (Figure 2C).

The transport of secreted invertase in *bst sec13Δ* strains was also examined. These strains showed normal levels of extracellular invertase activity, indicating that there was no block to invertase secretion. A more detailed examination of the kinetics of invertase secretion was performed by pulse-chase analysis. As with CPY, secreted invertase matured normally in *bst sec13Δ* strains at 24°C, although more slowly than in the wild-type control (Figure 2, B and C); this kinetic defect was more pronounced in *bst2 sec13Δ* and *bst3 sec13Δ* mutants than in *bst1 sec13Δ*. The acquisition by invertase of extensive outer-chain addition at later

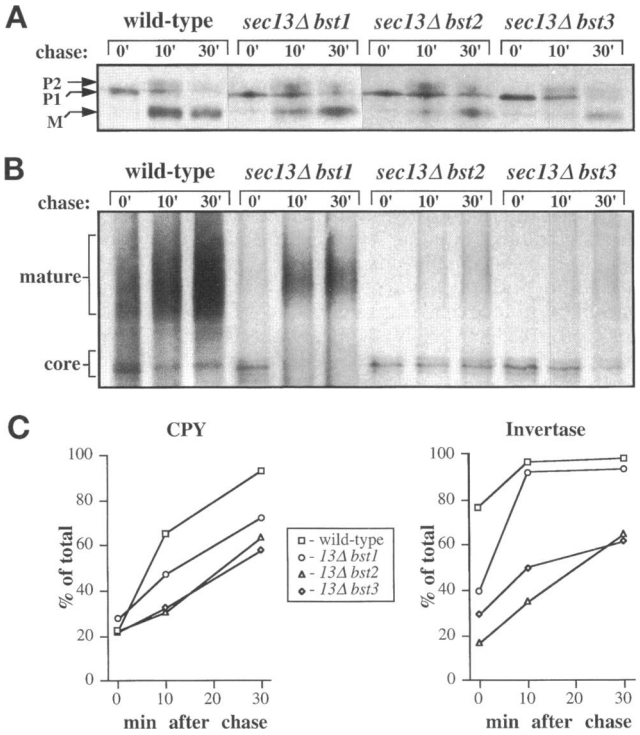

Figure 2. ER-to-Golgi transport in *bst sec13Δ* mutant strains. A wild-type strain (CKY10) and three *bst sec13Δ* mutant strains (CKY322, CKY323, and CKY324) were grown in supplemented minimal medium at 24°C. (A) Cultures were pulse-labeled with ^{35}S *trans*-label for 5 min and chased for the times indicated. CPY was immunoprecipitated from the labeled extracts, resolved by SDS-PAGE, and imaged on a phosphorimager (for quantitation) and by autoradiography. (B) Cultures were shifted to supplemented minimal medium containing 0.1% glucose to induce the expression of invertase, labeled, and chased as in A. Invertase was immunoprecipitated from the labeled extracts and treated as in A. (C) The individual forms of CPY and invertase were quantitated and expressed as a percentage of the total counts for each time point. Shown graphically are the average values for the appearance of the mature, fully processed form of each protein from two experiments, which varied by <10%.

times showed that transport through the Golgi occurred normally in the mutant strains. Thus, the bypass that occurs in *bst sec13Δ* strains does not completely circumvent any of the normal transport steps to the cell surface or vacuole.

ER-to-Golgi Transport in bst Mutant Strains

To understand better the role of the *BST* genes in the secretory process, the *bst* mutations were introduced into an otherwise wild-type genetic background. To construct these strains, *bst* mutant segregation was followed in crosses to wild-type by subsequent test crosses to *bst sec13Δ* strains and by transformation experiments to test whether individual segregants could be transformed with an integrating plasmid that

knocks out the *SEC13* gene. The growth characteristics and secretion phenotypes of these *bst* mutant strains were then analyzed. The *bst1*, *bst2*, and *bst3* strains (CKY330, CKY332, and CKY334, respectively) grew as well as wild-type at all temperatures. All of the parental *bst sec13*Δ strains were unable to grow at temperatures above 30°C. This temperature sensitivity now can be ascribed to a temperature dependence of the bypass process because the *bst* mutations themselves do not cause temperature sensitivity.

The transport of CPY and invertase in *bst* strains was analyzed by pulse-chase experiments at 30°C. Invertase matured normally in the *bst1* strain, but showed a significant delay in transport in *bst2* and *bst3* mutants (Figure 3, B and C). CPY matured normally in all three mutants (Figure 3, A and C). Thus, *bst2* and *bst3* mutations slowed invertase transport from the ER, but otherwise the *bst* mutations did not have a marked effect on secretory protein transport.

Cloning and Analysis of BST1

We also examined the effect of *bst* mutations on the retention of the resident ER protein Kar2p. The test was conducted by growing strains in contact with a nitrocellulose filter and then probing the filters with antibody to detect Kar2p released into the extracellular space. Both *bst sec13*Δ and *bst* strains showed a dramatic increase in the amount of Kar2p detectable by this assay compared with the wild-type control (Figure 4A). In tests of diploids, Kar2p secretion was recessive for *bst1*, *bst2*, and *bst3* mutations and therefore provided a plate assay for cloning the genes by complementation.

The *BST1* gene was cloned by transforming the *bst1-1* strain CKY331 with a genomic library in YCp50 and screening 6000 transformants for those that no longer secreted excess Kar2p. Three independent isolates of the same library clone were identified, and the minimum complementing region within this plasmid was determined by subcloning (Figure 5A). The 3.8-kb *Cla*I-*Kpn*I fragment of pME1101 was shown to be sufficient to complement both the Kar2p secretion (see Figure 4A) and *sec13*Δ suppression phenotypes of *bst1* mutant strains (our unpublished results). The latter property was tested in transformation experiments in which complementation of *bst1* was scored by the failure of a plasmid carrying the complementing gene to form viable transformants of a *bst1 sec13*Δ strain. The nucleotide sequence at the *Cla*I site was found to lie within the *STE2* locus, which is adjacent to the complementing region of the clone (Figure 5A). This observation places the *BST1* locus on the left arm of Chromosome VI.

The 3.3 kb of pME1101 adjacent to the *STE2* locus was sequenced and contains a single open reading frame predicted to encode a protein of 118 kDa. Analysis of the hydrophobicity of this sequence, using the algorithm of Kyte and Doolittle (1982), predicts multiple potential membrane spanning domains in the C terminus and a single transmembrane domain near the N terminus. Additional features are 14 potential N-linked glycosylation sites throughout the protein sequence and a double-Arg motif at the fourth and fifth amino acids from the N terminus. These aspects of the sequence are diagrammed in Figure 5B.

To confirm that this open reading frame corresponds to the *BST1* gene, a disruption of the gene was generated by subcloning the 1-kb *Spe*I-*Xho*I fragment that lies within the coding sequence into the *LEU2*-marked integrating vector pRS305 (Sikorski and Hieter, 1989). The resulting plasmid, pME1165, was linearized within the insert at the *Pst*I site and integrated into the wild-type diploid CKY348 by transformation. Integration results in two truncated copies of the gene separated by plasmid sequences, and neither truncated copy should be functional according to the complementation behavior of subclones. The resulting

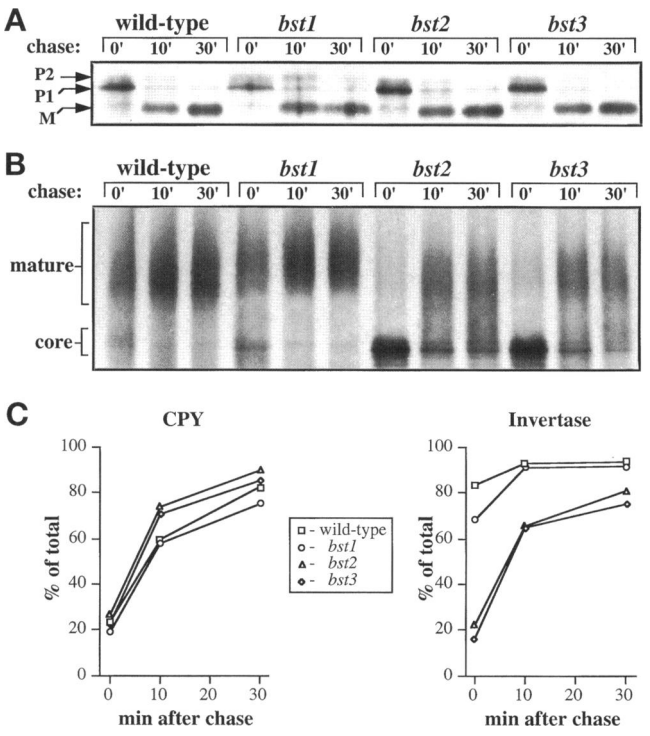

Figure 3. ER-to-Golgi transport in *bst* mutant strains. A wild-type strain (CKY10) and three *bst* mutant strains (CKY330, CKY332, and CKY334) grown in supplemented minimal medium at 30°C were shifted to medium with 0.1% glucose to induce the expression of invertase, pulse-labeled with ^{35}S *trans*-label for 5 min, and chased for the times indicated. (A) Immunoprecipitated CPY. (B) Immunoprecipitated invertase. (C) The individual forms of CPY and invertase were quantitated and expressed as a percentage of the total counts for each time point. Shown graphically are the average values for the appearance of the mature, fully processed form of each protein from two experiments, which varied by <10%.

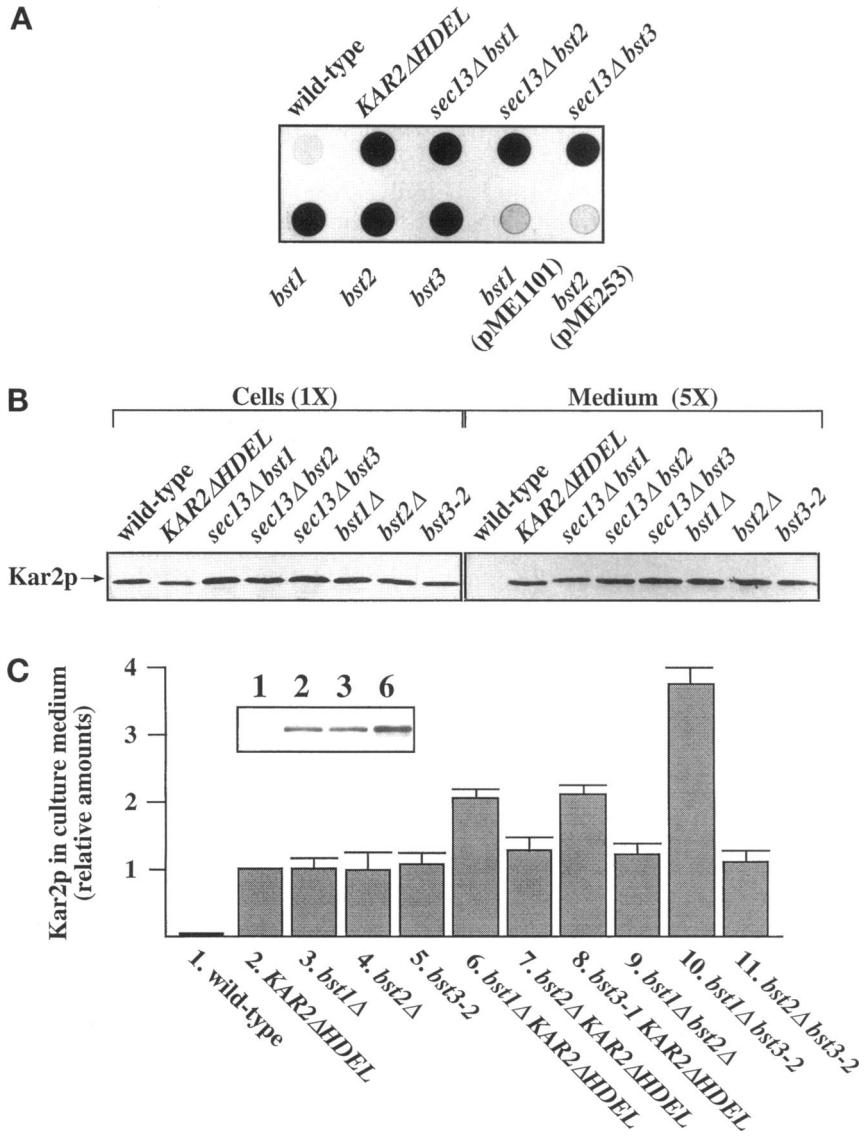

Figure 4. *bst* mutations cause defects in Kar2p retention that are independent of the HDEL retrieval pathway. Strains of the indicated genotype used were CKY10, CKY190, CKY322–CKY324, CKY330–CKY337, and CKY339–CKY341. (A) A wild-type strain, a *KAR2ΔHDEL* strain, three *bst sec13Δ* strains, and three *bst* strains carrying the empty vector pRS316, a *bst1* strain carrying the *BST1*-containing plasmid pME1101, and a *bst2* strain carrying the *BST2*-containing plasmid pME253 were spotted onto selective medium, covered with a nitrocellulose filter, and grown for 48 h at 24°C. Kar2p secreted onto the filter was detected by immunoblotting using anti-Kar2p antibody. (B) Exponentially growing cultures were washed, suspended in fresh medium, incubated for 3 h at 24°C, and split into cell and medium samples. Extracts from 0.5 OD_{600} units of cells and medium samples from 2.5 OD_{600} unit equivalents were resolved by SDS-PAGE. Kar2p was detected by Western analysis with anti-Kar2p antibody. (C) Samples from experiments as described in B were quantitated by densitometry. The value for the *KAR2ΔHDEL* control in each experiment was the baseline for comparison; shown are the average values from three experiments for the relative amounts of Kar2p detected in the culture medium. Error bars, 1 SD. The inset is a Western blot showing a sample of the data used to generate these values for the correspondingly numbered strain in the graphic below.

heterozygous diploid was sporulated, and all tetrads examined had four viable ascospores (n = 30), demonstrating that *BST1* is not essential. In each tetrad, the *LEU2* marker segregated 2:2 and showed complete linkage to excess Kar2p secretion as determined by the immunoblotting filter assay. The Leu^+ spore clones also exhibited the ability to suppress *sec13Δ* both in crosses to *bst1 sec13Δ* strains and in transformation experiments in which the *SEC13* gene was disrupted directly. Finally, in crosses between the Leu^+ spore clones and *bst1* mutant strains, complete linkage was demonstrated by 4:0 segregation of the Kar2p secretion phenotype (n = 36). We therefore refer to this gene as *BST1*. This analysis also demonstrated that the alleles of *bst1* isolated as suppressors of *sec13Δ* have the same phenotype as the disrupted allele.

Bst1p Is an Integral Membrane Glycoprotein that Resides in the ER

The predicted amino acid sequence of Bst1p indicated an integral membrane glycoprotein. To examine the protein, a 30-amino-acid epitope was inserted near the N terminus of the protein (see Figure 5) to produce a tagged version of the gene, *BST1*-HA. A centromere plasmid carrying *BST1*-HA (pME1170) complemented all of the phenotypes of *bst1* mutant strains, showing that insertion of the epitope did not disrupt function. Cells expressing *BST1*-HA were converted to spheroplasts, lysed gently, and fractionated by differential centrifugation. Bst1p-HA detected by immunoblotting was found exclusively in the 500 × g and 10,000 × g pellets, suggesting membrane association (Figure 6A).

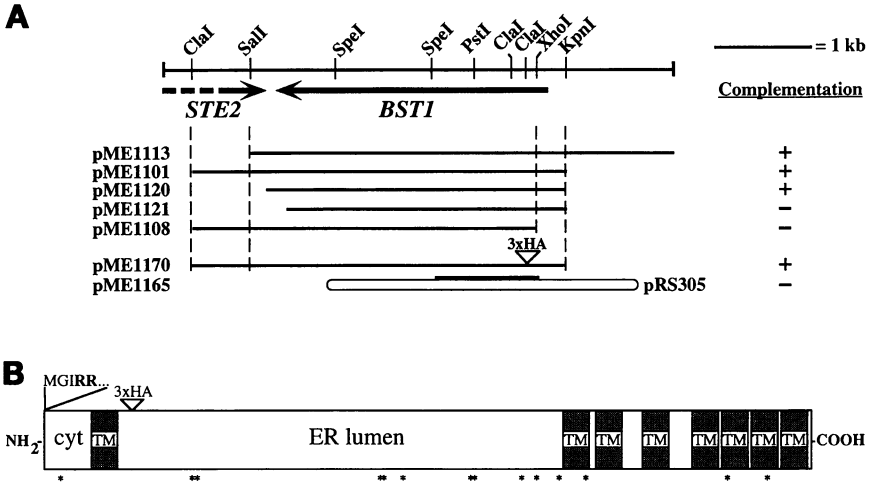

Figure 5. Physical map of *BST1*. (A) The plasmid subclones of the the *BST1* locus are shown along with their ability to complement the *bst1-1* mutation. The large solid arrow represents the predicted open reading frame. (B) Schematic representation of Bst1p. The shaded regions represent potential membrane-spanning regions, and (*) represents the location of potential N-linked glycosylation sites. The first five amino acids are shown to highlight the double-Arg motif. The location of the epitope tag in *BST1-HA* is indicated. The predicted topology shown is based on the number of glycosylation sites that apparently are used (see text).

Cytosolic invertase in these samples was found almost entirely in the 150,000 × *g* supernatant, demonstrating that the cells were efficiently lysed. After cell lysates were incubated in 1% Triton X-100 and centrifuged at 150,000 × *g*, much of the Bst1p-HA became soluble (Figure 6B), consistent with the behavior expected of an integral membrane protein. Treatment of cell lysates with 0.5 M NaCl, 2.5 M urea, or carbonate buffer, pH 11.5, did not solubilize Bst1p (our unpublished results).

To determine whether Bst1p is a glycoprotein, cell extracts were treated with EndoH_f to remove N-linked oligosaccharide chains. Treatment with EndoH_f resulted in an increase in the mobility of Bst1p-HA, consistent with the removal of six or seven core oligosaccharide chains from the protein (Figure 6B). This result demonstrated that Bst1p is a glycoprotein and indicated that the large domain of the protein between the single N-terminal and multiple C-terminal transmembrane sequences resides in the lumen of the ER (diagrammed in Figure 5B).

The demonstration that Bst1p is an integral membrane glycoprotein limited its subcellular distribution to the organelles of the secretory pathway. Additionally, the predicted sequence of the protein shows an N-terminal double-Arg motif at positions 4 and 5, which has been shown to be an ER retention signal for type II integral membrane proteins in mammalian cells (Schutze *et al.*, 1994). To determine whether Bst1p-HA progresses beyond the ER, we examined the extent of glycosylation of the protein in a *sec18-1* mutant blocked for ER-to-Golgi transport. Western blots of extracts from this strain grown either under permissive conditions or shifted to nonpermissive temperatures for 2 h showed no detectable difference in the amount or mobility of the Bst1p-HA. Most of the protein, therefore, is not subject to post-ER glycosylation and presumably resides in the ER (Figure 6B). As a control to demonstrate the efficacy of the *sec18* block, CPY was shown to remain in the P1 form in the *sec18* strain at the restrictive temperature.

The localization of Bst1p was examined further by indirect immunofluorescence microscopy. To enhance the detection of the protein in cells expressing *BST1-HA* (from the centromere plasmid pME1170), fixed cells were treated with SDS in a manner similar

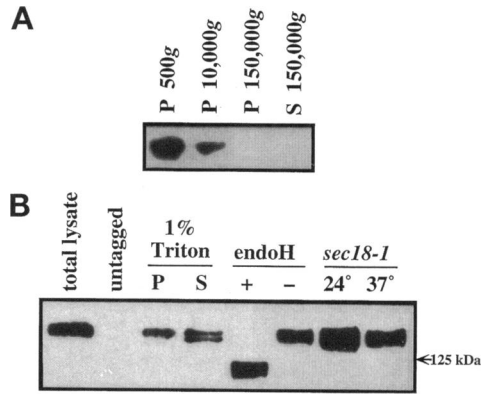

Figure 6. Bst1p is an integral membrane glycoprotein. (A) A cell lysate of a wild-type strain (CKY10) expressing Bst1p-HA from a CEN plasmid (pME1170) was subjected to a series of centrifugation steps resulting in 500 × *g*, 10,000 × *g*, and 150,000 × *g* pellets (P) and a 150,000 × *g* supernatant (S). An equal number of cell equivalents were loaded in each lane. (B) Lanes 1 and 2, Cell lysates of a wild-type strain (CKY10) carrying the *BST1-HA*-containing plasmid pME1170 (as in A) or the *BST1*-containing plasmid pME1101, respectively. Lanes 3–6, A cell lysate from the *BST1-HA*-containing strain in A was incubated in 1% Triton X-100 at 4°C for 1 h and centrifuged at 150,000 × *g* to give pellet (P) and supernatant (S) fractions (Lanes 3 and 4), or was treated (or mock-treated) with endoH_f (Lanes 5 and 6). Lanes 7 and 8, Cell lysates from a *sec18-1* mutant strain (CKY59) containing *BST1-HA* (pME1170) grown continuously at 24°C or shifted to 37°C for 2 h. An equal number of cell equivalents was used for each treatment. Bst1p-HA was detected by SDS-PAGE and Western analysis with the 12cA5 monoclonal antibody.

to that described for the optimal detection of Sec62p (Deshaies and Schekman, 1990). Cells treated in this manner and stained with anti-HA antibody showed a continuous band of concentrated staining surrounding the DAPI-stained nucleus. This staining pattern is characteristic of ER proteins (Rose *et al.*, 1989; Deshaies and Schekman, 1990) and was not seen in cells expressing untagged *BST1*. In double-staining experiments, the Bst1p staining was coincident with that of the luminal ER protein Kar2p (Figure 7), consistent with an ER localization for Bst1p.

BST2 Is Identical to EMP24

The *BST2* gene was cloned using the same strategy as that used to isolate *BST1*. Approximately 10,000 YCp50 library transformants of a *bst2-1* mutant were screened to find two overlapping clones that were shown to complement both the Kar2p secretion and *sec13*Δ suppression phenotypes of *bst2* mutant strains. Subclones were tested to find the minimal complementing region, and the sequence of this region showed it to be the *EMP24* locus. *EMP24* encodes a 24-kDa integral membrane protein that resides in the ER and in COPII vesicles that bud from the ER (Schimmöller *et al.*, 1995). A mammalian homolog of EMP24 was found to be enriched in COPI vesicles (Stamnes *et al.*, 1995). Disruption of *EMP24* was shown to decrease the transport of invertase and Gas1p from the ER, consistent with the transport defect of the *bst2* mutants described here (Schimmöller *et al.*, 1995).

To confirm that *BST2* and *EMP24* are identical, a disruption of the *EMP24* coding sequence was constructed by replacing an internal *Sac*I-*Sal*I fragment of *EMP24* with the *URA3*-marked integrative plasmid pRS306 in the wild-type diploid CKY348. Sporulation of the resulting diploid gave four viable spores in which the *URA3* marker segregated 2:2 ($n = 29$), confirming that *EMP24* is a nonessential gene. The disrupted allele was shown to confer the Kar2p secretion and *sec13*Δ suppression phenotypes of *bst2* mutant strains and to be linked to the isolated *bst2* mutations ($n = 40$), as described above for *BST1*. The *bst2* null alleles behaved identically to the *bst2* mutations isolated as suppressors of *sec13*Δ.

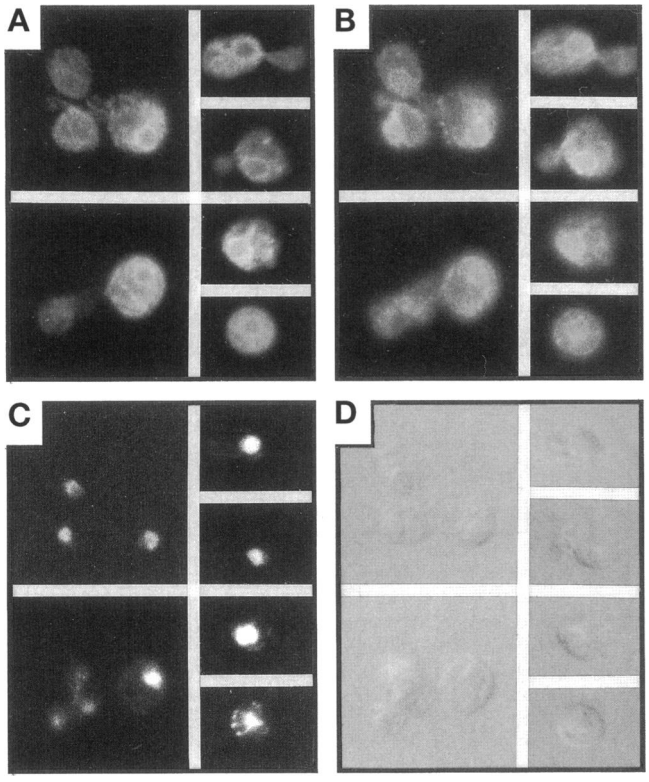

Figure 7. Bst1p colocalizes with the luminal ER protein Kar2p. Cells (CKY10) expressing *BST1-HA* from the centromere plasmid pME1170 were fixed, spheroplasted, washed with 0.1% SDS, and processed for double-label, indirect immunofluorescence. (A) Indirect immunofluorescence of Bst1p-HA. (B) Indirect immunofluorescence of Kar2p. (C) DAPI-stained nuclear DNA. (D) Cell bodies visualized using Nomarski optics. All panels are ×1250 magnification.

bst Mutations Cause Leakage of the Resident ER Proteins Kar2p and Pdi1p from the ER

The most striking phenotype of *bst* mutants is their defect in the retention of Kar2p. To investigate the nature and magnitude of this defect, we assayed the quantity of Kar2p released from cells by TCA precipitation of the growth medium and quantitation of Kar2p by Western blotting. Comparison of the amount of Kar2p in the medium to the amount in the corresponding cell extracts gave the fraction of Kar2p secreted. Both *bst sec13*Δ and *bst* mutant strains secreted ~17% of the total Kar2p within 3 h of transfer to fresh culture medium, whereas the wild-type strain secreted <1% of the total Kar2p (Figure 4B). Probing the medium for the presence of CPY (with anti-CPY antibody) showed that the *bst sec13*Δ and *bst* mutant strains had the same low levels of CPY in the medium as the wild-type control (P2 form only), ruling out the possibility that the Kar2p in the medium was from cell lysis (our unpublished results).

Excessive secretion of Kar2p into the culture medium is a hallmark of *erd* mutants, which are defective in the retrieval from the Golgi of resident ER proteins that, like Kar2p, have the motif HDEL at their C terminus (Hardwick *et al.*, 1990; Semenza *et al.*, 1990). Mutants of *erd2* secrete as much Kar2p into the medium as do strains bearing an allele of *KAR2* that lacks the HDEL retention signal (*KAR2*Δ*HDEL*) (Semenza *et al.*, 1990). In both cases, the rate of Kar2p secretion is slow compared with the rate of secretion for actual secretory proteins such as invertase. The slow secre-

tion of Kar2p$^{\Delta HDEL}$ implies that mechanisms independent of HDEL-dependent recycling contribute to retention of Kar2p in the ER.

The amount of Kar2p secreted by *bst* mutants was equivalent to that secreted by strains expressing the *KAR2ΔHDEL* allele (Figure 4B), suggesting that the *bst* mutations might affect the HDEL-dependent retrieval of Kar2p. Alternatively, the mutations could affect an HDEL-independent retention mechanism (i.e., the rate at which Kar2p exits the ER). To distinguish between these possibilities, we examined the extent of Kar2p$^{\Delta HDEL}$ secretion in *bst KAR2ΔHDEL* double-mutant strains to determine whether the effect of *bst* mutations on Kar2p secretion depended on HDEL. Removal of HDEL from Kar2p should completely eliminate the *ERD2*-dependent retrieval of this protein; if a *bst* mutation affects a retention mechanism that does not depend on HDEL, then a *bst KAR2ΔHDEL* double mutant should secrete more Kar2p$^{\Delta HDEL}$ than should either single mutant alone. Quantitative Western blotting of Kar2p$^{\Delta HDEL}$ from both the culture medium and the cell extracts revealed that whereas all of the mutants maintained wild-type levels of internal Kar2p, the *bst1Δ KAR2ΔHDEL* and *bst3-2 KAR2ΔHDEL* mutants consistently secreted approximately twice as much Kar2p$^{\Delta HDEL}$ into the culture medium (in a fixed amount of time) as did the single-mutant strains (Figure 4C), suggesting that *bst1* and *bst3* mutations affect a retention mechanism that is independent of the HDEL-dependent retrieval pathway.

The similarity of the phenotypes of *bst1*, *bst2*, and *bst3* mutants suggested that these genes might perform similar functions. To address the degree to which the functions of the *BST* genes overlap, the effect of double mutants on the extent of Kar2p secretion was examined. Neither the *bst1Δ bst2Δ* nor the *bst2Δ bst3-2* double mutants showed a significant increase in Kar2p secretion over that of the single mutants, indicating that loss of *BST2* does not exacerbate the defect already present in either *bst1* or *bst3* mutants. In contrast, the *bst1Δ bst3-2* double mutant secreted three- to fourfold more Kar2p into the culture medium than did either single mutant (Figure 4C). The additive effects of these mutations show that even when Bst3p is absent, the *BST1* gene product performs a function that contributes to the retention of Kar2p.

The effect of *bst1* and *bst3* mutations on the retention of Kar2p$^{\Delta HDEL}$ suggested that the *bst* mutations might affect the escape of other resident proteins from the ER. We therefore examined the retention of the luminal resident ER protein Pdi1p in *bst* mutants by TCA precipitation of the culture medium and quantitative Western analysis as was done for Kar2p. All three *bst* mutants secreted ~50% of the total Pdi1p into the culture medium during a 3 h incubation, whereas the wild-type strain secreted no detectable Pdi1p (Figure 8). Pdi1p, like Kar2p, contains a C-terminal HDEL retrieval signal, and removal of this sequence results in the secretion of a large fraction of the protein (LaMantia and Lennarz, 1993) (Figure 8). Because such a large fraction of the Pdi1p that lacks the HDEL was secreted from the cells, the double-mutant test that we applied to Kar2p was not feasible. The secretion of both Kar2p and Pdi1p in *bst* mutants indicates a general defect in the retention of ER resident proteins.

bst Mutations Also Cause Leakage of Retained Proteins from the ER

As an independent test for a defect in the proper retention of proteins in the ER, we examined the fate of invertase that is retained in the ER because of the presence of an uncleaved signal sequence. The *SUC2-s11* allele of invertase is an Ala-to-Ile substitution at the signal peptide cleavage site that blocks cleavage by signal peptidase and dramatically slows the exit of s11-invertase from the ER, presumably because the protein has the qualities of a misfolded protein and is recognized by the ER quality control system (Bohni *et al.*, 1987). s11-invertase is retained in the ER in an enzymatically active state, and the small fraction that is secreted acquires carbohydrate modifications that allow the progression of the protein through the secretory pathway to be monitored. The effect of *bst* mutations on the secretion of s11-invertase was examined by assaying the fraction of active invertase at the cell surface. The ratio of external to total invertase activity 2 h after induction of *SUC2-s11* showed that a *bst1::LEU2* mutant secreted 1.7 times and a *bst3-2* mutant secreted 1.4 times the amount of s11-invertase as did the wild-type control (Table 2). The *bst2-1* mutant did not significantly increase secretion of s11-invertase. These data were consistent with the effects of *bst* mutations on retention of Kar2p$^{\Delta HDEL}$ and demonstrated the generality of the retention defect of *bst1* and *bst3* mutants.

The rate of s11-invertase transport from the ER to the Golgi was measured directly in *bst1::LEU2*, *bst2-1*, *bst3-2*, and *BST*$^{+}$ strains. Pulse-chase experiments

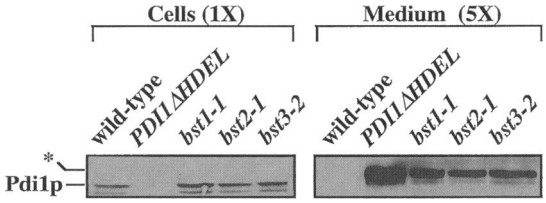

Figure 8. *bst* mutants secrete the luminal ER protein Pdi1p. Samples from a wild-type strain (CKY10), a *PDIΔHDEL* strain (CKY395), *bst1-1*, *bst2-1*, and *bst3-2* mutant strains (CKY330, CKY332, and CKY334) were made as described for Figure 4B. Pdi1p was detected by Western analysis with anti-Pdi1p antibody. (*) represents the glycosylated form of Pdi1p that is secreted from cells.

were performed by shifting cultures to low-glucose medium for 30 min to induce the expression of s11-invertase, labeling the cells for 20 min with [^{35}S]methionine and then chasing with unlabeled methionine. s11-invertase was first immunoprecipitated with anti-invertase antibody, and then a portion of each sample was reimmunoprecipitated with antibodies directed against the Golgi-specific modification α1,6 mannose to determine the fraction of the protein that had reached the Golgi. The rate at which s11-invertase acquired α1,6 mannose modifications in the *bst1::LEU2* mutant was approximately twice that of the isogenic *BST*$^+$ control (Figure 9). Thus, an increase in the rate of exit from the ER accounts for the increased secretion of s11-invertase in the *bst1* mutant. Similarly, for the *bst3-2* mutant the rate was ~1.5-fold that of wild-type, whereas that of the *bst2-1* mutant was indistinguishable from wild-type. Because the rate of transport for wild-type invertase from the ER in *bst2* and *bst3* mutants is threefold slower than it is in a wild-type strain (Figure 3, B and C), the rates observed for s11-invertase transport in the *bst2* and *bst3* mutants probably understate the effect these mutants have on the escape of s11-invertase from the ER.

Genetic Interactions between bst Mutants and sec Mutants

The bypass of the cellular requirement for Sec13p by *bst* mutations suggested that these mutations might also bypass the requirement for other *SEC* genes. We tested the ability of the *bst* mutations to suppress null alleles of other *SEC* genes in crosses segregating both a *bst* mutation and a null *SEC* gene allele. Null alleles of *SEC12*, *SEC23*, and *SEC31* were tested, and none was suppressed by *bst1*, *bst2*, or *bst3* mutations. In addition, selections for suppressors similar to the one used to isolate the *bst* mutations were tried with null alleles of *SEC12*, *SEC23*, and *SEC16*, and all failed to yield extragenic suppressor mutations (R. Gimeno and P. Espenshade, personal communication). Thus, *bst* mutations do not bypass the need for other essential ER vesicle proteins.

Table 2. Secretion of s11-invertase in *bst* mutants

Strain	Relevant genotype	External activity*
CKY343	*suc2Δ [suc2-s11]*	13.9 ± 0.5%
CKY344	*bst1::LEU2 suc2Δ [suc2-s11]*	24.0 ± 1.0%
CKY345	*bst2::URA3 suc2Δ [suc2-s11]*	15.8 ± 0.2%
CKY346	*bst3-2 suc2Δ [suc2-s11]*	19.3 ± 0.6%

*Intact cells and cell lysates were assayed to determine the fraction of total invertase activity that was extracellular. Three determinations on each of two independent transformants were averaged, and standard deviations are shown.

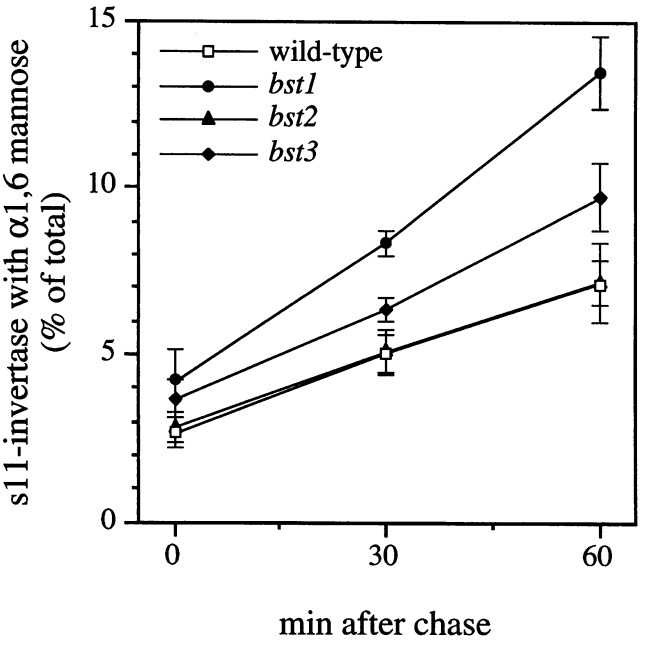

Figure 9. *bst* mutants increase the rate at which s11-invertase leaks from the ER. Isogenic *suc2Δ* (CKY343) and *bst1Δ suc2Δ* (CKY344) strains, a *bst2-1 suc2Δ* (CKY345), and a *bst3-2 suc2Δ* (CKY346) expressing s11-invertase from a plasmid (pEHB29) were grown in supplemented minimal medium at 30°C, pulse-labeled with ^{35}S *trans*-label for 20 min, and chased for the times indicated. s11-invertase was immunoprecipitated and then boiled in SDS to disrupt the immune complexes, and a fraction was reimmunoprecipitated with α1,6-specific antibody. Samples were treated with endoH_f, resolved by SDS-PAGE, visualized, and quantitated on a phosphorimager. Shown is the average value from three experiments for the fraction of the total s11-invertase that was recovered in the α1,6 precipitation. Error bars, 1 SD.

Suppression of temperature-sensitive alleles of *SEC13* was also examined. A *sec13-1* strain was inviable at temperatures above 30°C, whereas isogenic *bst1 sec13-1* and *bst2 sec13-1* double mutants grew at temperatures up to 36°C (Figure 10). Thus, *bst* mutants can partially suppress *sec13-1*, and the inability to suppress at temperatures above 36°C was presumably attributable to the failure of suppression at high temperatures that was observed for suppression of *sec13Δ*.

The ability to detect suppression of temperature-sensitive alleles allowed us to extend our evaluation of suppression to other *SEC* genes that are involved in ER-to-Golgi transport. The *bst1* and *bst2* mutant strains CKY330 and CKY332 were crossed to each of the *sec* mutant strains listed in Table 1. Tests of the temperature sensitivity of double-mutant segregants showed that both *bst* mutations could suppress to at least some degree the temperature sensitivity of all of the COPII vesicle formation mutants (*sec12*, *sec13*, *sec16*, and *sec23*). In contrast, an exacerbation of the temperature sensitivity of vesicle fusion mutants (*sec17* and *sec18*) and COPI mutants (*sec21* and *sec27*)

occurred when these mutations were combined with either *bst1* or *bst2* mutations.

To avoid possible effects of genetic background in tetrad analysis, *sec bst* strains were transformed with either the corresponding *BST* gene on a centromere plasmid or with a control plasmid. The growth of these isogenic pairs at different temperatures confirmed the suppression and synthetic interactions observed by tetrad analysis (Figure 10). Similar results were also seen with the *bst3* mutation in crosses to representative *sec* mutants, but a more rigorous confirmation of these results using truly isogenic strains must await cloning of the *BST3* gene. CPY pulse-chase experiments on isogenic pairs of *sec* and *sec bst* mutant strains confirmed that the temperature-sensitive growth was always accompanied by a corresponding ER-to-Golgi transport defect (our unpublished results). Thus, the *bst* mutations cause ER-to-Golgi transport to becomes less dependent on the COPII vesicle formation genes and more dependent on vesicle fusion genes and COPI genes.

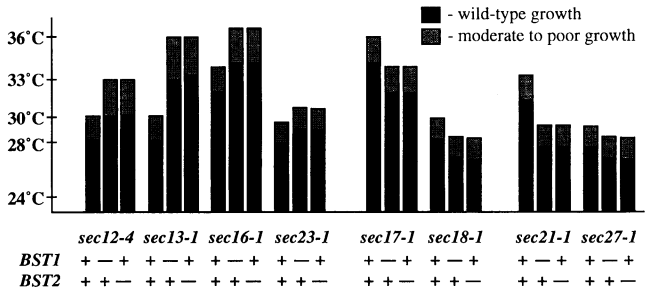

Figure 10. Genetic interactions between *bst* mutants and *sec* mutants. Each of the *bst sec* double-mutant strains listed in Table 1 carrying either the vector pRS316 or the corresponding *BST* gene-containing plasmid (pME1101 or pME253) was assayed in streak-outs on selective medium for growth at temperatures over the range indicated. Shown is a graphic summary of the data.

DISCUSSION

In this report, we describe the identification of three genes—*BST1*, *BST2/EMP24*, and *BST3*—that when mutated suppress the lethal secretion defect caused by deletion of the *SEC13* gene. Sec13p is known to be essential for formation of transport vesicles both in vivo, where *sec13* mutations cause a defect in ER vesicle formation (Kaiser and Schekman, 1990), and in vitro, where Sec13p is one of the five cytosolic COPII proteins that are necessary and sufficient for vesicle formation from ER membranes that have been depleted of peripheral proteins (Kaiser and Schekman, 1990; Pryer *et al.*, 1993; Salama *et al.*, 1993). It was surprising, therefore, to find that the cellular requirement for Sec13p could be bypassed by second-site mutations. A key observation concerning the mechanism of suppression is that the suppressor mutations are genetically recessive. In particular, we show that null alleles of *BST1* and *BST2/EMP24* are suppressors. Because loss of *BST* gene function gives suppression, we deduce that the *BST* proteins act negatively to prevent transport from the ER to the Golgi. Furthermore, Sec13p apparently is needed to overcome the transport block caused by the *BST* gene products, perhaps in a late step in vesicle assembly. Thus, the function of the *BST* genes in vesicular transport is formally like the inhibitory checkpoints that block the progression of the mitotic cell cycle in response to the incomplete assembly of the mitotic spindle or the incomplete replication of chromosomes.

There are two prominent possibilities for processes that could be inhibited by the *BST* gene products (diagrammed in Figure 11). The possibility we favor is that the *BST* gene products directly block completion of incorrectly assembled COPII vesicles. Thus, when Sec13p is absent, the observed secretory block would result from the action of the *BST* proteins. In addition, under semipermissive conditions, mutations in other COPII genes would also bring about a *BST*-dependent block, because loss of *BST* gene function renders less restrictive mutations in all COPII genes tested. The purpose of such a negative regulatory mechanism

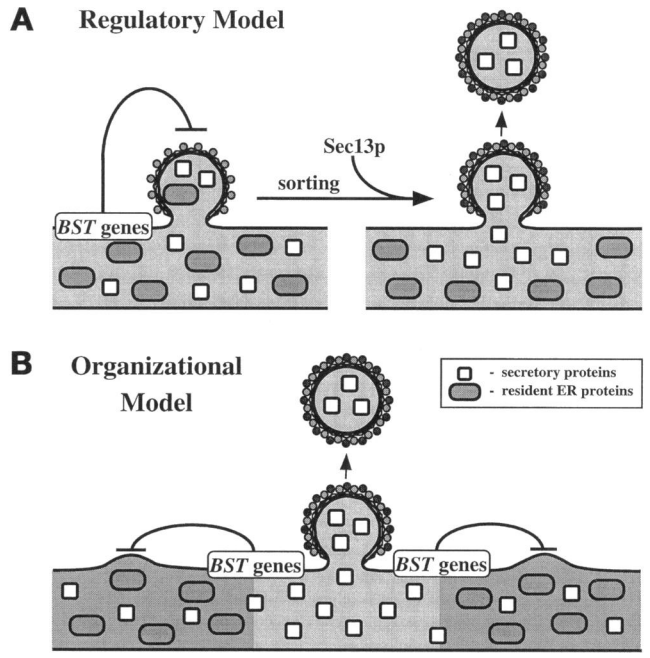

Figure 11. Models for the function of the *BST* genes. (A) Regulatory model in which the *BST* genes inhibit the completion of transport vesicle formation, allowing cargo sorting to occur. (B) Organizational model in which the *BST* genes maintain distinct subcompartments in the ER from which vesicle formation occurs. Resident proteins are excluded from these regions, and vesicle budding is inhibited outside these regions.

could be to improve the fidelity of vesicle assembly by preventing the pinching off of vesicles that have not been completely assembled.

An alternative possibility is that the *BST* proteins perform an organizational function to maintain a distinct subcompartment within the ER from which vesicle formation can take place. Disruption of this organization by a *bst* mutation might then either allow COPII-driven vesicle formation in the absence of Sec13p or give access to a new pathway out of the ER, perhaps utilizing COPI rather than COPII coat proteins (Bednarek *et al.*, 1995). A predicted consequence of the opening of a COPII-independent process for transport from the ER is that complete elimination of other COPII proteins would also be bypassed by *bst* mutations. Because *bst* mutations do not bypass null alleles of the COPII genes *SEC12*, *SEC31*, or *SEC23*, complete bypass of the COPII step is unlikely. We cannot rule out, however, the possibility that the COPII proteins other than *SEC13* are required for an essential process, in addition to ER-to-Golgi transport, that cannot be suppressed by *bst* mutations.

Cargo Segregation in bst Mutant Strains

When *bst* mutations are placed in a wild-type strain background, the most pronounced effect of the mutations is to cause secretion of the resident ER proteins Kar2p and Pdi1p into the extracellular space. It was by complementation of this recessive Kar2p secretion trait that we cloned the *BST1* and *BST2* genes. For *bst1* and *bst3* mutations, an increase in the rate of Kar2p secretion, even when HDEL-dependent retrieval of Kar2p from the Golgi has been inactivated, indicates that the increased Kar2p secretion is the result of an increased flux of Kar2p out of the ER. Further, invertase that in a wild-type background is retained because of an uncleaved signal sequence more readily escapes the ER in *bst1* and *bst3* mutants. Thus, these mutants cause the ER to exhibit a general reduction in the ability to retain proteins, and this property suggests that these mutants may have general utility for overriding the normal inhibitions for the secretion of misfolded proteins. The magnitude of these effects can be considerable: Kar2p secretion for all three mutants is the same as that for a derivative of Kar2p lacking the C-terminal HDEL retrieval sequence, and roughly half of the total Pdi1p is secreted from these mutants in 3 h of growth. In *bst1 bst3* double mutants, the Kar2p secretion phenotype is even more pronounced: ~35% of the total Kar2p is secreted into the medium during 3 h of growth, which is the most pronounced, nonlethal ER retention defect yet documented.

Not only do *bst* mutations increase the leakage of resident proteins from the ER, but they also decrease the rate of transport of a subset of secretory cargo. This effect can be seen in the selective effects of *bst2/emp24* and *bst3* mutations on the rate of invertase transport shown here and previously reported for *bst2/emp24* mutant strains (Schimmöller *et al.*, 1995). Taken together, the effect of disruption of the *BST* genes on the sorting of luminal proteins is generally to decrease the rate of secretion of proteins that are normally secreted (invertase) and to increase the rate of export of proteins that are normally retained in the ER (Kar2p, Pdi1p, and invertase with an uncleaved signal sequence). Thus, loss of *BST* gene function reduces the capacity of the ER to discriminate between secreted and retained proteins, thereby causing secretion in *bst* mutants to resemble a condition known as bulk flow in which cargo leaves the ER at a rate corresponding to its concentration in the vicinity of the forming vesicle.

The existence of mutations with the properties of the *bst* mutations suggests a general model for how cargo sorting could be coupled to vesicle coat assembly. The prevailing view of how cargo is selected by vesicles is that the vesicle coat forms an affinity matrix, and that a given type of cargo molecule is partitioned into the budding vesicle according to its affinity for the coat (in the case of membrane proteins) or for coat-associated receptors (in the case of luminal proteins). To accomplish this partitioning, the coat would have to exist for a time in a partially assembled state similar to that of clathrin-coated pits that are thought to be the precursors of clathrin-coated endocytic vesicles (reviewed in Pearse and Robinson, 1990). The negative effect of *BST* proteins on the formation of transport vesicles could simply provide a delay in the pinching off of vesicles that would give time for sorting to take place. In the absence of the restriction imposed by the *BST* checkpoint, vesicles could form prematurely before cargo sorting is completed. The cargo content of the vesicles thus formed would reflect reduced discrimination between resident proteins and secretory proteins. This putative *BST* checkpoint on vesicle completion may also be imposed in response to incomplete or improper assembly of the vesicle coat, so that in the absence of *BST* quality control, crippled but functional vesicles could form when the coat structure is compromised by mutation of COPII proteins.

Molecular analysis of the *BST1* and *BST2/EMP24* genes shows that these proteins reside primarily in the ER membrane, a location consistent with their proposed role in regulating the fidelity of vesicle assembly and cargo loading. The double-Arg motif at the N terminus in the predicted sequence of Bst1p may function to govern the retrieval of this protein in a manner similar to that observed for KKXX-containing membrane proteins (Gaynor *et al.*, 1994; Schutze *et al.*, 1994).

It is also noteworthy that Bst2p/Emp24p is a member of a family of proteins that have been found in COPII-coated vesicles (Schimmöller *et al.*, 1995) and COPI-coated vesicles (Stamnes *et al.*, 1995). An appreciation of the reduced rate of invertase and Gas1p

transport in *emp24* mutants led to the suggestion that these proteins are cargo receptors for a subset of proteins (Schimmöller *et al.*, 1995). However, this suggestion does not explain the effects of these mutations on Kar2p and Pdi1p release from the ER and suppression of *sec13* mutations that we describe here.

Interaction between BST Genes and Other sec Genes

Although the *bst* mutations fully or partially suppress mutations in COPII genes, they exacerbate mutations in COPI genes and vesicle fusion genes. These interactions can be explained in light of the proposed participation of *BST* genes in ensuring the fidelity of vesicle assembly. The exacerbation of the temperature-sensitive defects of *sec17-1* and *sec18-1* mutants may reflect structural defects in the vesicles formed in the absence of *BST* function: if proteins required for stability or for the targeting and fusion of the vesicles (V-SNARES) are not assembled as efficiently into the budding vesicles, this could cause the enhancement of the defect seen in *sec17-1* and *sec18-1* mutants. Additionally, the greatly increased flux of Kar2p and possibly other resident proteins from the ER in *bst* mutants may produce an unusually great load on the systems that retrieve ER proteins from the Golgi. The COPI vesicle proteins Sec21p, Sec27p, and Ret1p are required for the retrieval of type I ER membrane proteins bearing the retention signal KKXX at their cytosolic C termini (Letourner *et al.*, 1994). If COPI-coated vesicles are needed to retrieve from the Golgi a variety of ER-resident proteins, then the increased load on the retrieval system in *bst* mutants may be lethal when combined with the COPI *sec* mutants that reduce the capacity of the retrieval system.

Relationship to Other Genes Involved in the Retention of Resident ER Proteins

A number of genes have been reported to influence the retention and retrieval of resident ER proteins in yeast. The *rer* mutants were identified in screens for mutants that mislocalize hybrid transmembrane proteins normally retained in the ER (Nishikawa and Nakano, 1993; Boehm *et al.*, 1994). The defect in *rer1* and *rer3* mutants is for transmembrane proteins only, because both show normal retention of the soluble Kar2p (Boehm *et al.*, 1994). The *rer2* mutant is defective in the retention of Kar2p and may perform, like the *bst* mutants, a more general retention function (Nishikawa and Nakano, 1993). A more general retention defect has been observed for strains in which the *CNE1* gene has been deleted (Parlati *et al.*, 1995), but secretion of Kar2p was not examined for this mutant—nor was the capacity to suppress COPII mutations tested—so the relationship of *CNE1* to the *bst* mutants is not known.

The isolation of the *bst* mutants has given us a new insight into how cargo sorting could be related to the assembly of vesicle coats. The key questions now are how the *BST* proteins operate at a molecular level: how they negatively regulate vesicle transport, and what the inputs are that trigger this regulation. The isolation of the *BST* genes and their products provides a way to address these questions by studying the structure of the *BST* proteins, their possible association with one another, and their proximity to proteins of the vesicle coat and to cargo molecules.

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REFERENCES

Balch, W.E., McCaffery, J.M., Plutner, H., and Farquhar, M.G. (1994). Vesicular stomatitis virus glycoprotein is sorted and concentrated during export from the endoplasmic reticulum. Cell *76*, 841–852.

Barlowe, C., Orci, L., Yeung, T., Hosobuchi, M., Hamamoto, S., Salama, N., Rexach, M.F., Ravazzola, M., Amherdt, M., and Schekman, R. (1994). COPII: a membrane coat formed by Sec proteins that drive vesicle budding from the endoplasmic reticulum. Cell *77*, 895–907.

Bednarek, S.Y., Ravazzola, M., Hosobuchi, M., Amherdt, M., Perrelet, A., Schekman, R., and Orci, L. (1995). COPI- and COPII-coated vesicles bud directly from the endoplasmic reticulum in yeast. Cell *83*, 1183–1196.

Boehm, J., Ulrich, H.D., Ossig, R., and Schmitt, H.D. (1994). Kex2-dependent invertase secretion as a tool to study the targeting of transmembrane proteins which are involved in ER >Golgi transport in yeast. EMBO J. *13*, 3696–3710.

Bohni, P.C., Schauer, I., Tekamp-Olson, P., and Schekman, R. (1987). Signal peptide cleavage mutants of yeast invertase. In: Proteases in Biological Control and Technology. New York: Alan R. Liss, Inc. 255–264.

d'Enfert, C., Barlowe, C., Nishikawa, S.-I., Nakano, A., and Schekman, R. (1991). Structural and functional dissection of a membrane glycoprotein required for vesicle budding from the endoplasmic reticulum. Mol. Cell. Biol. *11*, 5727–5734.

Deshaies, R.J., and Schekman, R. (1990). Structural and functional dissection of Sec62p, a membrane-bound component of the yeast endoplasmic reticulum protein import machinery. Mol. Cell. Biol. *10*, 6024–6035.

Espenshade, P., Gimeno, R.E., Holzmacher, E., Teung, P., and Kaiser, C.A. (1995). Yeast *SEC16* gene encodes a multidomain vesicle coat protein that interacts with Sec23p. J. Cell Biol. *131*, 311–324.

Gascón, S., Neumann, N.P., and Lampen, J.O. (1968). Comparative study of the properties of the purified internal and external invertases from yeast. J. Biol. Chem. *243*, 1573–1577.

Gaynor, E.C., te Heesen, S., Graham, T.R., Aebi, M., and Emr, S.D. (1994). Signal-mediated retrieval of a membrane protein from the Golgi to the ER in yeast. J. Cell Biol. *127*, 653–665.

Gimeno, R.E., Espenshade, P., and Kaiser, C.A. (1995). *SED4* encodes a yeast endoplasmic reticulum protein that binds Sec16p and participates in vesicle formation. J. Cell Biol. *131*, 325–338.

Hardwick, K.G., Lewis, M.J., Semenza, J., Dean, N., and Pelham, H.R.B. (1990). *ERD1*, a yeast gene required for the retention of luminal endoplasmic reticulum proteins, affects glycoprotein processing in the Golgi apparatus. EMBO J. *9*, 623–630.

Henikoff, S. (1984). Unidirectional digestion with exonuclease III creates targeted breakpoints for DNA sequencing. Gene *28*, 351–359.

Hicke, L., Yoshihisa, T., and Schekman, R. (1992). Sec23p and a novel 105-kDa protein function as a multimeric complex to promote vesicle budding and protein transport from the endoplasmic reticulum. Mol. Biol. Cell *3*, 667–676.

Kaiser, C., Michaelis, S., and Mitchell, A. (1994). Methods in Yeast Genetics, Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press.

Kaiser, C.A., and Schekman, R. (1990). Distinct sets of *SEC* genes govern transport vesicle formation and fusion early in the secretory pathway. Cell *61*, 723–733.

Kolodziej, P.A., and Young, R.A. (1991). Epitope tagging and protein surveillance. Methods Enzymol. *194*, 508–519.

Kunkel, T.A., Roberts, J.D., and Zakour, R.A. (1987). Rapid and efficient site-specific mutagenesis without phenotypic selection. Methods Enzymol. *154*, 367–382.

Kyte, J., and Doolittle, R.F. (1982). A simple method for displaying the hydropathic character of a protein. J. Mol. Biol. *157*, 105–132.

LaMantia, M., and Lennarz, W.J. (1993). The essential function of yeast protein disulfide isomerase does not reside in its isomerase activity. Cell *74*, 899–908.

Letourner, F., Gaynor, E.C., Hennecke, S., Démollière, C., Dunden, R., Emr, S.D., Riezman, H., and Cosson, P. (1994). Coatamer is essential for retrieval of dilysine-tagged proteins to the endoplasmic reticulum. Cell *79*, 1199–1207.

Mizuno, M., and Singer, S.J. (1993). A soluble secretory protein is first concentrated in the endoplasmic reticulum before transfer to the Golgi apparatus. Proc. Natl. Acad. Sci. USA *90*, 5732–5736.

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.

Nakano, A., and Muramatsu, M. (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.

Nilsson, T., Jackson, M., and Peterson, P.A. (1989). Short cytoplasmic sequences serve as retention signals for transmembrane proteins in the endoplasmic reticulum. Cell *58*, 707–718.

Nishikawa, S.-I., and Nakano, A. (1993). Identification of a gene required for membrane protein retention in the early secretory pathway. Proc. Natl. Acad. Sci. USA *90*, 8179–8183.

Oka, R., Nishikawa, S.-I., and Nakano, A. (1991). Reconstitution of GTP-binding Sar1 protein function in ER to Golgi transport. J. Cell Biol. *114*, 671–679.

Orci, L., Ravazzola, M., Meda, P., Holcomb, C., Moore, H.-P., Hicke, L., and Schekman, R. (1991). Mammalian Sec23p homologue is restricted to the endoplasmic reticulum transitional cytoplasm. Proc. Natl. Acad. Sci. USA *88*, 8611–8615.

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

Parlati, F., Dominguez, M., Bergeron, J.J.M., and Thomas, D.Y. (1995). *Saccharomyces cerevisiae CNE1* encodes an endoplasmic reticulum (ER) membrane protein with sequence similarity to calnexin and calreticulin and functions as a constituent of the ER quality control apparatus. J. Biol. Chem. *270*, 244–253.

Pearse, B.M.F., and Robinson, M.S. (1990). Clathrin, adaptors, and sorting. Annu. Rev. Cell Biol. *6*, 151–171.

Pelham, H.R.B. (1995). Sorting and retrieval between the endoplasmic reticulum and Golgi apparatus. Curr. Opin. Cell Biol. *7*, 530–535.

Pfeffer, S.R., and Rothman, J.E. (1987). Biosynthetic protein transport and sorting by the endoplasmic reticulum and Golgi. Annu. Rev. Biochem. *56*, 829–852.

Pryer, N.K., Salama, N.R., Schekman, R., and Kaiser, C.A. (1993). Cytosolic Sec13p complex is required for vesicle formation from the endoplasmic reticulum in vitro. J. Cell Biol. *120*, 865–875.

Pryer, N.K., Wuestehube, L.J., and Schekman, R. (1992). Vesicle-mediated protein sorting. Annu. Rev. Biochem. *61*, 471–516.

Rose, M.D., Misra, L.M., and Vogel, J.P. (1989). *KAR2*, a karyogamy gene, is the yeast homolog of the mammalian BiP/GRP78 gene. Cell *57*, 1211–1221.

Rose, M.D., Novick, P., Thomas, J.H., Botstein, D., and Fink, G.R. (1987). A *Saccharomyces cerevisiae* genomic plasmid bank based on a centromere-containing shuttle vector. Gene *60*, 237–243.

Rothman, J.E., and Orci, L. (1992). Molecular dissection of the secretory pathway. Nature *355*, 409–415.

Salama, N.R., Yeung, T., and Schekman, R.W. (1993). The Sec13p complex and reconstitution of vesicle budding from the ER with purified cytosolic proteins. EMBO J. *12*, 4073–4082.

Sambrook, J., Fritsch, E.F., and Maniatis, T. (1989). Molecular Cloning: A Laboratory Manual. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press.

Schimmöller, F., Singer-Krüger, B., Schröder, S., Krüger, U., Barlowe, C., and Riezman, H. (1995). The absence of Emp24p, a component of ER-derived COPII-coated vesicles, causes a defect in transport of selected proteins to the Golgi. EMBO J. *14*, 1329–1339.

Schutze, M.-P., Peterson, P.A., and Jackson, M.R. (1994). An N-terminal double-arginine motif maintains type II membrane proteins in the endoplasmic reticulum. EMBO J. *13*, 1696–1705.

Semenza, J., Hardwick, K., Dean, N., and Pelham, H. (1990). *ERD2*, a yeast gene required for the receptor-mediated retrieval of luminal ER proteins from the secretory pathway. Cell *61*, 1349–1357.

Shaywitz, D.A., Orci, L., and Kaiser, C.A. (1995). Human SEC13Rp functions in yeast and is located on transport vesicles budding from the endoplasmic reticulum. J. Cell Biol. *128*, 769–777.

Sikorski, R.S., and Hieter, P. (1989). A system of shuttle vectors and yeast host strains designed for efficient manipulation of DNA in *Saccharomyces cerevisiae*. Genetics *122*, 19–27.

Stamnes, M.A., Craighead, M.W., Hoe, M.H., Lampen, N., Geromanos, S., Tempst, P., and Rothman, J.E. (1995). An integral membrane component of coatamer-coated transport vesicles defines a family of proteins involved in budding. Proc. Natl Acad. Sci. USA *92*, 8011–8015.

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.

Tyers, M., Tokiwa, G., and Futcher, B. (1993). Comparison of the *Saccharomyces cerivisiae* G1 cyclins: Cln3 may be an upstream activator of Cln1, Cln2, and other cyclins. EMBO J. *12*, 1955–1968.