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Factors Mediating the Late Stages of ER-to-Golgi Transport in Yeast

Y. JIANG,[1] M. SACHER,[1] B. SINGER-KRÜGER,[1] J.P. LIAN,[2], S. STONE,[1] AND S. FERRO-NOVICK[1]

[1]*Department of Cell Biology and Howard Hughes Medical Institute, Yale University School of Medicine, New Haven, Connecticut 06536;* [2]*Center for Radiological Research, Columbia University Medical School, New York, New York 10032*

The movement of proteins and lipids between membranous compartments within a cell is mediated by small membrane-bound vesicles (Palade 1975). In addition to their cargo, these vesicles contain proteins on their surfaces that direct the vesicle to its proper target. The SNARE (soluble NSF attachment protein receptor) hypothesis states that a protein on the vesicle (v-SNARE) pairs with a protein on the target membrane (t-SNARE), allowing the general fusion factor NSF and SNAPs to bind and fuse the two membranes (Söllner et al. 1993b). The v-SNAREs show homology with the synaptic vesicle protein VAMP/synaptobrevin, and t-SNAREs are homologous to either syntaxin or SNAP-25 (*syn*aptosomal *a*ssociated *p*rotein of *25* kD). Indeed, biochemical evidence has shown that the recognition step is mediated by the sequential interactions of these and other proteins at the target membrane in a SNARE complex (Söllner et al. 1993a; Søgaard et al. 1994). However, a molecular description of this event, as well as the subsequent fusion of the two membranes, remains to be determined. Furthermore, components of the SNARE complex may be regulated by small GTP-binding proteins that act prior to the assembly of this complex (Lian et al. 1994).

The yeast *Saccharomyces cerevisiae* has proven to be fruitful in elucidating the molecular aspects of membrane traffic. It has been shown that many of the proteins involved in this process in yeast have homologs in higher eukaryotic organisms, including mammals (Ferro-Novick and Jahn 1994), indicating that the mechanism of membrane traffic is highly conserved. Highlighting this point is the ability of yeast proteins to functionally replace their mammalian counterparts (Wilson et al. 1989; Griff et al. 1992). The genetically tractable nature of yeast has allowed the identification of many mutants in the yeast secretory pathway, which in turn has allowed the identification of related proteins in mammalian cells.

Our laboratory has taken a combined genetic and biochemical approach to decipher the mechanism by which endoplasmic reticulum (ER)-to-Golgi transport vesicles target and fuse with the *cis*-Golgi compartment. To achieve our goal, we have employed a variety of genetic screens to identify new genes whose products function in this aspect of the secretory pathway. These screens have led to the identification of *BET1*, *BET2*, *BET3*, *BOS1*, and several uncharacterized genes. An analysis of the *BET* and *BOS* gene products in a cell-free in vitro assay that faithfully reproduces ER-to-Golgi transport (see below) has shown that these proteins play a key role in vesicle targeting and fusion.

MATERIALS AND METHODS

Immunoprecipitation of the SNARE complex. Yeast cells were grown in YPD medium to early log phase and converted to spheroplasts as described previously (Ruohola et al. 1988; Groesch et al. 1992). The spheroplasts were regenerated for 1 hour at 37°C and lysed. After the lysate was spun at 120,000*g* for 1 hour, the supernatants were collected and diluted to a protein concentration of 2 mg/ml with Buffer A (20 mM HEPES, pH 7.4, 100 mM KCl, 1 mM DTT, 2 mM EDTA, 0.5 mM ATP, 0.5% Triton, and 1x Pic). For immunoprecipitation, 40 μg of affinity-purified anti-Bos1p antibody was added to 1 ml of lysate and incubated for 2 hours. The antigen/antibody complexes were precipitated with protein-A-Sepharose, and the beads were washed extensively and solublized in 1x sample buffer as described by Lian et al. (1994). Samples were electrophoresed and analyzed by Western blot analysis using anti-Sec22p and anti-Sed5p antibodies.

Immunofluorescence. Immunofluorescence was performed according to the method of Pringle et al. (1991). Briefly, wild-type yeast cells were grown at 25°C in YPD to early exponential phase and fixed with 3.7% formaldehyde. The fixed cells were converted to spheroplasts and permeabilized with 0.5% SDS. The permeabilized cells were treated with affinity-purified anti-Sec22p antibody (1:5 dilution) or anti-Kar2p serum (1:10,000 dilution) followed by staining with fluorescein (DTAF)-conjugated donkey anti-rabbit IgG (Jackson Immunoresearch Laboratory).

In vitro transport assay. In performing the transport assay, donor permeabilized yeast cells (PYCs), wild-type cytosol, and acceptor Golgi membranes were prepared as described previously (Ruohola et al. 1988; Groesch et al. 1992). PYCs made from Sec22p-depleted cells were always prepared from cells that were grown at 30°C. Each transport reaction was performed in a total volume of 60 μl that contained 60 μg

of PYCs, 1 mg of an S1 fraction, and 5 μl of energy mix (12 mM ATP, 1.2 mM GTP, 240 mM creatine phosphate, and 2.4 mg/ml creatine kinase). 5 μl of apyrase (400 units/ml) or 2 μg of anti-Sec22p antibody was added as indicated in Figures 2 and 3. Reactions were incubated on ice for 20 minutes, then at 20°C for 90 minutes before the addition of concanavalin A-Sepharose. Quantitation of the released pro-α-factor was performed as described by Lian and Ferro-Novick (1993).

RESULTS AND DISCUSSION

Reconstitution of ER-to-Golgi Transport In Vitro

To study the mechanism of vesicle targeting and fusion at a biochemical level, we developed an in vitro assay that reconstitutes transport between the ER and Golgi apparatus (Ruohola et al. 1988; Groesch et al. 1990). In this assay, yeast cells are first converted to spheroplasts and resuspended in a hypotonic buffer. This permeabilizes the cells (referred to as PYC), washes out soluble factors required for transport, and renders the endogenous Golgi inactive for vesicle consumption. The marker protein used in this assay is the yeast mating pheromone α-factor. A precursor form of α-factor, prepro-α-factor, is translated in vitro in a yeast translation lysate as a protein that migrates at 19 kD on SDS-PAGE. This protein is posttranslationally translocated into the ER of the PYCs where, following signal sequence cleavage and the addition of three N-linked oligosaccharides, it then migrates at 26 kD. When an S1 fraction (supernatant of a yeast lysate centrifuged at 1000*g*) and an ATP-regenerating system are provided to the PYCs, vesicles that bud from the ER are released from the cells to target and fuse with exogenously added Golgi. Addition of high-mannose side chains to the 26-kD form of pro-α-factor in an early Golgi compartment leads to, initially, a 28-kD form of the protein, which is then converted to a heterogeneous high-molecular-weight smear on SDS polyacrylamide gels (see Fig. 3). The S1 can be further divided by centrifugation at 120,000*g* into a high-speed supernatant (HSS), which supplies the factors necessary for transport, and a high-speed pellet (HSP). The HSP provides the Golgi necessary for vesicle consumption. Incubation of PYCs with the HSS leads to the production of functional ER-to-Golgi transport vesicles that are readily separated from the PYCs by centrifugation.

Bet1p: An Integral ER Membrane Protein Required for ER-to-Golgi Transport

The *bet1-1* mutant was originally identified using a [^{3}H]mannose suicide selection (Newman and Ferro-Novick 1987). It was subsequently shown that *SLY12*, a gene cloned as a high-copy suppressor of the loss of *YPT1*, a small Ras-related GTP-binding protein (Dascher et al. 1991), was in fact *BET1* (Newman et al. 1992). *BET1* encodes a type II integral membrane protein of 142 amino acids with a membrane-spanning domain at its extreme carboxyl terminus. This mutant displays defects in secretion and accumulates ER forms of secretory proteins as well as carboxypeptidase Y (CPY), a vacuolar protein. Morphologically, *bet1-1* exhibits dilated ER and shows a modest accumulation of small (50 nm) vesicles thought to represent intermediates in ER-to-Golgi transport. On a genetic level, *BET1* displays interactions with *BOS1* and *SEC22* (Newman et al. 1990), two other genes encoding ER membrane proteins, as well as *YPT1*. In fact, *BOS1* was identified as a gene that could suppress the growth defect associated with the *bet1-1* mutant in a gene-dosage-dependent fashion (Newman et al. 1990). In addition, both *BET1* and *BOS1* are capable of suppressing the *sec22-3* mutant, and a *bet1 sec22* double mutant is inviable. These interactions indicate that the aforementioned gene products act either on the same pathway or on parallel pathways in ER-to-Golgi transport.

Subcellular fractionation has shown that Bet1p copurifies with the ER (Newman et al. 1992), a site which is well suited for it to influence ER-to-Golgi transport. However, since Bos1p resides on the ER as well as the ER-to-Golgi transport vesicles (see below), several studies were undertaken to examine whether Bet1p was also a constituent of these vesicles. In one study, PYC were incubated with a HSS to produce functional transport vesicles. The vesicles were immunoisolated using an anti-Bos1p antibody and the components were examined by Western blot analysis. Although Bos1p, Sec22p, and Ypt1p were all found on the transport vesicles, Bet1p was conspicuous by its absence (Lian and Ferro-Novick 1993). A subsequent study, in which nonfunctional transport vesicles were produced using Ypt1p-deficient cytosol, showed that Bet1p was a component of these vesicles (Rexach et al. 1994), whereas the mutant Ypt1p was not. These vesicles could be rendered competent for fusion by the addition of wild-type cytosol, which provides functional Ypt1p. These results suggest that the localization of Bet1p may be dependent on the activity of Ypt1p.

The Role of Bos1p and Sec22p in ER-to-Golgi Transport

Bos1p and Sec22p are two small membrane proteins that are structurally related to synaptobrevin, the v-SNARE in nerve terminals (Jessell and Kandel 1993). *BOS1* was originally isolated by its ability to suppress the growth and secretion defects of the *bet1-1* and *sec22-3* mutants (Newman et al. 1990). It encodes a 27-kD type II membrane protein that is essential for growth (Shim et al. 1991). At the amino acid level, Bos1p is 23% identical to synaptobrevin, and 27% identical to the *SNC1* gene product that is the proposed v-SNARE for post-Golgi transport vesicles (Gerst et al. 1992; Protopopov et al. 1993). Cells de-

pleted of Bos1p fail to transport pro-α-factor and CPY to the Golgi apparatus and accumulate ER and ER-to-Golgi transport vesicles (Shim et al. 1991), indicating that Bos1p is a constituent of the transport machinery that acts at an early stage of the secretory pathway. Recently, the role of Bos1p in ER-to-Golgi transport has been dissected in detail (Lian and Ferro-Novick 1993; Lian et al. 1994). On the basis of functional studies and its similarity with synaptobrevin, it has been proposed to be the v-SNARE of ER-to-Golgi transport vesicles (Ferro-Novick and Jahn 1994). Three lines of evidence support this hypothesis. First, Bos1p resides on the ER as well as the ER-to-Golgi transport vesicles (Newman et al. 1992; Lian and Ferro-Novick 1993). Second, Bos1p specifically functions on vesicles and not on other compartments. Its activity is required for the docking and fusion of ER-derived transport vesicles with the Golgi apparatus, but not for the release of carrier vesicles from the ER (Lian and Ferro-Novick 1993). Third, Bos1p was found to be a component of a SNARE complex that includes Sed5p and several other proteins (Lian et al. 1994; Søgaard et al. 1994). Sed5p, a homolog of syntaxin residing on the *cis*-Golgi stack, is the putative t-SNARE in ER-to-Golgi transport (Banfield et al. 1994). These results imply that Bos1p, the v-SNARE, modulates the docking and fusion of ER-derived vesicles with the Golgi apparatus through specific pairing with its cognate t-SNARE, Sed5p.

SEC22 was identified in a screen for temperature-sensitive mutants defective in secretion (Novick et al. 1980). It encodes a 25-kD transmembrane protein with a similar topology to Bos1p, and it contains a region just before its transmembrane-spanning domain that is significantly homologous to synaptobrevin. Unlike *BOS1*, *SEC22* is dispensable for cellular growth. Cells lacking Sec22p are cold-sensitive and temperature-sensitive, but grow well at 30°C (Dascher et al. 1991; Lian et al. 1994). As such, Sec22p is unlikely to play a critical role in intracellular vesicle transport. However, genetic studies have revealed that *SEC22* displays strong interactions with several genes required for transport between the ER and Golgi apparatus, including *BET1*, *BOS1*, *BET3*, and *YPT1* (Newman et al. 1990; Lian et al. 1994; Rossi et al. 1995), suggesting that Sec22p acts in concert with the products of these genes to mediate vesicular transport. The observation that Ypt1p appears to regulate the association of Bos1p and Sec22p on the ER-derived transport vesicles reinforces this notion (Lian et al. 1994).

To understand the role of Sec22p on the secretory pathway, it is necessary to know at which stage its activity is required and how this activity corresponds with the function of other proteins that interact with Sec22p. For this purpose, we analyzed the intracellular distribution of Sec22p, its interactions with other proteins, and its activity in vesicle budding and fusion events.

In wild-type yeast cells where intracellular transport is rapid and constitutive, vesicle budding and fusion occur efficiently. Under such conditions, the ER-derived v-SNAREs only associate with the Golgi-bound t-SNARE transiently. The majority of the v-SNAREs dwell on the ER, unbound to the t-SNARE, which resides on the *cis*-Golgi complex. Therefore, as a v-SNARE of the ER-to-Golgi transport vesicles, Sec22p would be expected to reside mainly on the ER. We used indirect immunofluorescence to examine the localization of Sec22p in wild-type yeast cells. We found that Sec22p (Fig. 1A,B) colocalizes with Kar2p (Fig. 1C,D), an ER-resident protein, indicating that Sec22p, like Bos1p, resides on the ER. However, it is

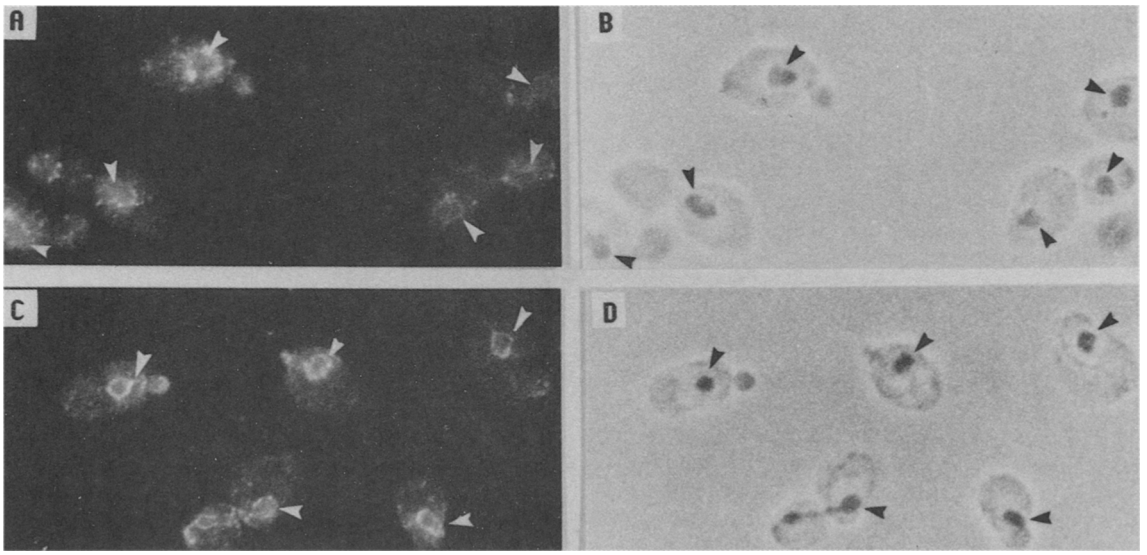

Figure 1. Localization of Sec22p in wild-type yeast cells. (*A*) Cells stained with affinity-purified anti-Sec22p antibody; (*C*) cells stained with anti-Kar2p serum; (*B*,*D*) phase contrast images of cells in *A* and *C*.

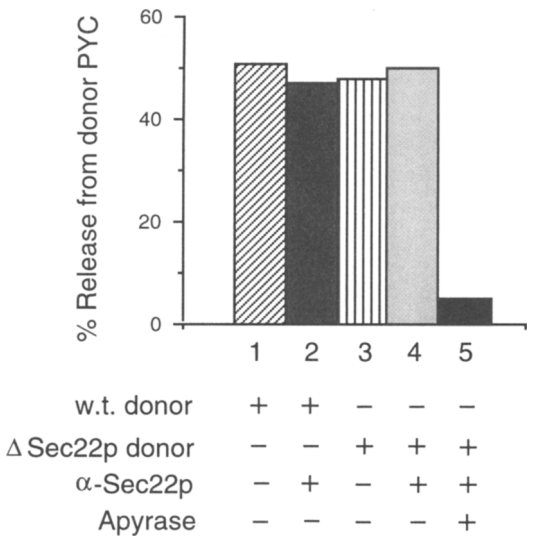

Figure 2. The activity of Sec22p is not required for vesicle budding. Vesicle-budding activity was measured as the percentage of pro-α-factor released from the PYCs. The S1 fraction used in these assays was prepared from wild-type cells. (Lane *1*) Wild-type donor PYCs; (lane *2*) wild-type donor PYCs in the presence of anti-Sec22p antibody; (lane *3*) PYCs from Sec22p-depleted cells; (lane *4*) PYCs from Sec22p-depleted cells in the presence of anti-Sec22p antibody; (lane *5*) PYCs from Sec22p-depleted cells in the presence of anti-Sec22p antibody and apyrase.

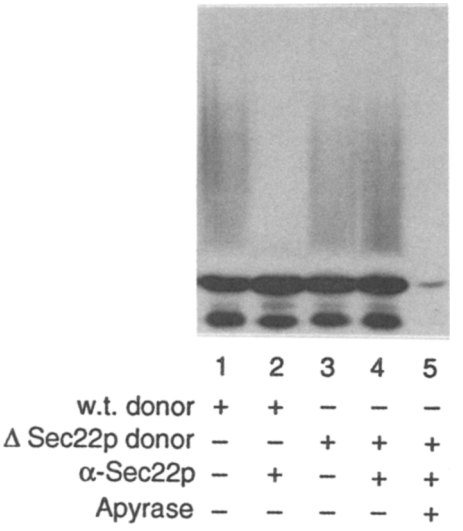

Figure 3. Sec22p is involved in the late stages of ER-to-Golgi transport. The docking and fusion activity of vesicles derived from different PYCs was measured with anti-outer-chain antibody. The S1 fraction used in these assays was prepared from wild-type cells. (Lane *1*) Wild-type PYCs; (lane *2*) wild-type PYCs in the presence of anti-Sec22p antibody; (lane *3*) Sec22p-depleted PYCs; (lane *4*) Sec22p-depleted PYCs in the presence of anti-Sec22p antibody; (lane *5*) Sec22p-depleted PYCs in the presence of anti-Sec22p antibody and apyrase.

worthwhile to note that even though Bos1p and Sec22p colocalize on the ER, they do not interact with each other on this compartment (Lian et al. 1994). This interaction takes place only during or after the carrier vesicle is released from the ER and is believed to play an important role in subsequent docking and fusion events.

To test the role of Sec22p in ER-to-Golgi transport, donor PYCs prepared from wild-type or Sec22p-depleted cells (grown at 30°C) were assayed for their ability to release pro-α-factor. As shown in Figure 2, PYCs from Sec22p-depleted cells formed vesicles as efficiently as wild type (Fig. 2, compare lane 1 with lane 3). Furthermore, addition of anti-Sec22p antibody in the assay had no effect on vesicle budding (Fig. 2, compare lane 1 with lanes 2 and 4). This result indicates that Sec22p is not required for the budding of pro-α-factor-containing vesicles from donor PYC. We then investigated the role of Sec22p in the subsequent docking and fusion event. As shown in Figure 3, the PYCs from Sec22p-depleted cells are capable of transporting pro-α-factor to the Golgi complex to an extent similar to that of wild type (Fig. 3, compare lane 1 with lane 3), indicating that vesicle docking and fusion are fully active in the absence of Sec22p. However, when anti-Sec22p antibody was added to the assay, it completely blocked the outer chain extension of pro-α-factor in wild-type, but not in Sec22p-depleted PYCs (Fig. 3, compare lanes 2 and 4). One possible explanation for these apparently contradictory results is that Sec22p does not play a critical role in the docking and fusion event, but still is a part of the SNARE complex. As such, anti-Sec22p antibody would be targeted to the SNARE complex, and therefore, in some way, interfere with docking and/or fusion. The fact that anti-

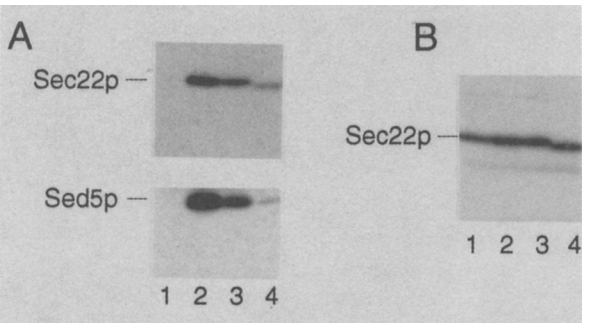

Figure 4. Active Sec22p is required for efficient formation of the SNARE complex. (*A*) Precipitates prepared from wild type (lane *1*), *sec18-1* (lane *2*), *sec17-1* (lane *3*), and *sec22-3* (lane *4*) mutant extracts were analyzed for the presence of Sec22p (*upper panel*) and Sed5p (*lower panel*). (*B*) TCA precipitates of lysates prepared from wild-type (lane *1*), *sec18-1* (lane *2*), *sec17-1* (lane *3*), and *sec22-3* (lane *4*) extracts were blotted with anti-Sec22p antibody. The findings show that a constant amount of Sec22p is present in all of the extracts that were analyzed.

Sec22p antibody fails to block outer chain addition in the absence of Sec22p supports this hypothesis (Fig. 3, lane 4).

To demonstrate that Sec22p is a component of the ER-to-Golgi SNARE complex, detergent-treated yeast-cell extracts were precipitated with anti-Bos1p antibody, and the presence of Sec22p was examined by Western blot analysis. If Sec22p is a component of this docking/fusion complex, then it should coprecipitate with Bos1p under conditions in which the complex accumulates. Previous studies have shown that the SNARE complex accumulates in *sec18* mutant cells when fusion is blocked (Søgaard et al. 1994). We examined *sec18-1* and two other temperature-sensitive mutants, *sec17-1* and *sec22-3*, that are blocked in vesicle targeting and fusion at 37°C for the accumulation of the SNARE complex. Figure 4 shows the results of this analysis. In wild-type cells where vesicle fusion occurs efficiently and SNARE complexes fail to accumulate to a significant extent, Sec22p and Sed5p do not coprecipitate with Bos1p (Fig. 4, lane 1). However, Sec22p coprecipitates with Bos1p and Sed5p in *sec18-1* after a shift to 37°C, which blocks vesicle fusion (Fig. 4, lane 2). This complex also accumulates in the *sec17-1* mutant (Fig. 4, lane 3), but to a lesser extent than in *sec18-1*, suggesting that the SNARE complex is formed but not stable when Sec17p is inactive. Some accumulation of Sec22p in the SNARE complex is also observed in *sec22* mutant cells (Fig. 4, lane 4). However, this amount is significantly reduced (Fig. 4, lane 4).

Apparently, Sec22p is not absolutely required for vesicle docking and fusion. However, the above results imply that it acts to facilitate the assembly of the SNARE complex. The accessory role of Sec22p is also supported by genetic results. First, Sec22p is dispensable, which makes it unlikely to be the sole v-SNARE for ER-to-Golgi transport (Dascher et al. 1991). Second, overproduction of *BOS1* dramatically restores the growth defect of Sec22p-depleted cells at 34°C, whereas overexpression of *SEC22* cannot substitute for the loss of *BOS1* (Newman et al. 1990; Lian et al. 1994). It is likely that Sec22p participates in the formation of the SNARE complex, perhaps by stabilizing the pairing between Bos1p and Sed5p. In the absence of Sec22p, the SNARE complex assembles inefficiently.

Isolation and Characterization of the *bet3-1* Mutant

In a search for additional factors that might act in concert with Bet1p, Sec22p, Ypt1p, and Bos1p, a screen was performed to identify mutants that are synthetically lethal in combination with the *bet1-1* mutant. Synthetic lethality is frequently observed when the corresponding gene products act in a similar transport step or are redundant. This approach has led to the identification of a novel temperature-sensitive secretory mutant that is blocked in ER-to-Golgi transport, *bet3-1* (Rossi et al. 1995). As previously reported for *bet1-1* and other ER-accumulating mutants, in *bet3-1* the secretion of invertase is rapidly blocked upon a shift to the nonpermissive temperature, and invertase accumulates as the 80-kD ER form. Similar blocks were observed when the transport of α-factor and CPY was followed (Rossi et al. 1995). As was found for *bet1-1*, when secretion is blocked in *bet3-1* mutant cells, dilated ER membranes build up (Newman and Ferro-Novick 1987). Additionally, the accumulation of smaller (~60 nm) and larger (~110 nm) vesicles was apparent (Rossi et al. 1995). Whereas the smaller profiles appeared similar in size to the vesicles that accumulate in other ER-to-Golgi-blocked mutants (Kaiser and Schekman 1990), the simultaneous appearance of the larger vesicles was reminiscent of the morphology of two other mutants that function at more than one step in the yeast secretory pathway, *bet2-1* and *sec19-1* (Rossi et al. 1991; Garrett et al. 1994).

Besides the secretion defect and the morphological alteration observed in *bet1-1* and *bet3-1*, another striking similarity between the two mutants is the nature of their genetic interactions. Like *bet1-1*, *bet3-1* could be partially suppressed by the overproduction of *BET1*, *SEC22*, *YPT1*, or *BOS1*. Furthermore, haploid double mutants carrying mutations in *BET3* and *BET1*, or *SEC21*, *SEC22*, *YPT1*, respectively, were inviable at a temperature (25°C) at which each of the single mutants was viable (Rossi et al. 1995), supporting the idea that Bet1p and Bet3p may act at a similar step.

Cloning of the *BET3* gene by complementation of the *bet3-1* temperature-sensitive growth phenotype revealed an open reading frame that encodes a hydrophilic protein of 22 kD (Rossi et al. 1995). Bet3p also displays significant sequence similarity (36% identity) to a *Caenorhabditis elegans* protein of unknown function, indicating that it is highly conserved. Gene disruption analysis in yeast has shown that *BET3* is an essential gene (Rossi et al. 1995).

Since genetic and morphology studies, as well as in vivo transport experiments, suggested a role for Bet3p in the late stages of ER-to-Golgi transport, we determined whether Bet3p is a component of the ER-to-Golgi SNARE complex. As discussed above, this multiprotein complex becomes apparent when vesicle fusion is blocked in the *sec18* mutant. It contains Bos1p, Sec22p, Sed5p, and several unidentified proteins (Søgaard et al. 1994). To determine whether Bet3p is part of this complex, anti-Bos1p antibody was used to immunoprecipitate the SNARE complex. Although Sec22p and Sed5p were found to coimmunoprecipitate with Bos1p, Bet3p did not. Interestingly, although the SNARE complex accumulates in *sec18* mutant extracts, it was not found in the *bet3-1* mutant (Rossi et al. 1995), suggesting that Bet3p might be required for its formation. Additionally, the Bos1p/Sec22p complex, which accumulates in secretory mutants that accumulate ER-to-Golgi transport vesicles (Lian et al. 1994), could not be detected in *bet3-1* mutant cells (Rossi et

al. 1995). These findings are consistent with the idea that Bet3p may act prior to the formation of the SNARE complex.

Whereas the phenotypic characterization of the *bet3-1* mutant clearly suggests an important role for Bet3p in ER-to-Golgi transport, as mentioned before, genetic and morphological studies indicate that Bet3p fulfills a more general function in the secretory pathway. For example, both ER-to-Golgi and post-Golgi secretory vesicles appear to accumulate in the *bet3-1* mutant at 37°C. Furthermore, *BET3* interacts genetically with several late-acting *SEC* genes (*sec2-41* and *sec4-8*). Due to the largely hydrophilic nature of the Bet3 protein sequence, it is likely that Bet3p is a soluble protein. Therefore, it could play a role in multiple stages of intracellular membrane traffic events.

Currently, we are investigating whether endocytotic membrane traffic to the vacuole is impaired in *bet3-1* mutant cells. Using ^{35}S-labeled α-factor (a mating pheromone) as a marker for receptor-mediated endocytosis (Riezman 1993), we followed its internalization and endocytotic delivery to the vacuole in *bet3-1* and wild-type cells. Whereas internalization of the pheromone can be measured by analyzing its protection after an acid wash, transport to the vacuole is followed by α-factor degradation. Upon binding of α-factor to cells at 4°C, internalization and degradation were studied in cells incubated at 30°C, the nonpermissive growth temperature for *bet3-1* cells. This revealed that both pheromone internalization and degradation occurred with similar kinetics in mutant and wild-type cells. Preincubating mutant cells for 20 minutes at 30°C before α-factor binding and internalization did not result in a delay or inhibition of α-factor degradation (B. Singer-Krüger and S. Ferro-Novick, unpubl.). These findings suggest that Bet3p is not required for endocytosis.

Prenylation Is Required for the Function of Rab Proteins

In yeast, Ypt1p and Sec4p are two members of the Rab family of small GTP-binding proteins. They are involved in the regulation of the exocytotic pathway in which Ypt1p functions at an early stage and Sec4p acts later in post-Golgi transport (Ferro-Novick and Novick 1993). Like their mammalian homologs, both proteins are found to associate with membrane compartments, but neither of them possesses a transmembrane domain. The ability to associate with membranes is conferred by the addition of 20-carbon geranylgeranyl groups onto their carboxy-terminal cysteine residues. This event, called geranylgeranylation, is catalyzed by the type-II geranylgeranyl transferase (GGTase-II) (Rossi et al. 1991; Seabra et al. 1992).

GGTase-II modifies Rab proteins that terminate in either a CC or CXC motif. It catalyzes the covalent attachment of geranylgeranyl groups onto terminal cysteine residues, thereby providing a membrane anchor for otherwise soluble proteins. GGTase-II is one of three prenyltransferases that have been identified in eukaryotic cells. The other two prenyltransferases, farnesyltransferase (FTase) and type-I geranylgeranyltransferase (GGTase-I), modify proteins ending in a CXXX motif. Both enzymes are heterodimers that contain α and β subunits (Schafer and Rine 1992). GGTase-II differs from the other two transferases not only in its substrate specificity, but also in its subunit composition. It is composed of three subunits, an α/β heterodimer and an escort protein (previously called component A) (Seabra et al. 1992; Jiang and Ferro-Novick 1994). The α/β heterodimer comprises the catalytic component. However, unlike the FTase and GGTase-I, which binds and transfers an isoprenoid group onto its protein substrate efficiently (Seabra et al. 1991), the α/β heterodimer of GGTase-II binds geranylgeranyl diphosphate (GGPP), but only displays limited transferase activity in the absence of the escort protein (Jiang et al. 1993). The escort protein of the GGTase-II acts as an accessory protein that binds and presents protein substrate to the catalytic component (Andres et al. 1993). After modification, it delivers the Rab protein to its membrane compartment (Alexandrov et al. 1994). In yeast, the β subunit of the enzyme is encoded by the *BET2* gene. It is a 36.6-kD hydrophilic protein that shares approximately 34% amino acid identity with the β subunits of the other two transferases (Rossi et al. 1991; Andres et al. 1993; Jiang et al. 1993). Another yeast gene, *BET4* (originally called *MAD2*), encodes the α subunit, which is a homolog to the α subunit of the other transferases (Li et al. 1993). The third subunit, the escort protein, is the product of the *MRS6* gene (Jiang and Ferro-Novick 1994) and shares about 30% identity with Rab GDI (GDP dissociation inhibitor), a GTP/GDP exchange factor (Garrett et al. 1994). The similarity between these proteins reflects the fact that they both bind to Rabs. All three genes are essential for cellular growth.

In wild-type cells, about 50% of the Ypt1p and 80–90% of the Sec4p are bound to membranes, and the remainder is found in the soluble fraction (Rossi et al. 1991). The cellular distribution of these proteins is a prerequisite for maintaining their function. When the distribution of Ypt1p and Sec4p was examined in *bet2* mutant cells, the majority was found in the soluble fraction (Rossi et al. 1991), indicating a defect in membrane association. Like *ypt1-1* mutant cells, *bet2-1* is blocked in ER-to-Golgi transport (Rossi et al. 1991), suggesting that the defect in membrane association impedes the function of Ypt1p. Because Ypt1p acts at an earlier stage than Sec4p in the secretory pathway, the block in membrane traffic in *bet2-1* is a consequence of a malfunction of Ypt1p. The same transport defect was also observed in the *bet4-2* mutant and in cells that are depleted of Mrs6p (Li et al. 1993; Jiang and Ferro-Novick 1994). These findings suggest that prenylation plays an important role in mediating the function of Ypt1p and Sec4p.

Unlike integral membrane proteins that reside permanently on membranes, membrane-bound prenylated proteins, such as Ypt1p and Sec4p, are released from membranes. In wild-type cells, a fraction of these small GTP-binding proteins is always found in the cytosol. The cytosolic forms are believed to associate with a soluble protein, called GDI (Garrett et al. 1994). Studies with Sec4p have suggested a cycle of localization in which the soluble fraction binds to newly formed vesicles on donor membranes, and is subsequently transferred to the acceptor compartment before it is recycled back through the cytoplasm to bind to a new round of vesicles (Novick et al. 1993). It is not clear how this reversible membrane association corresponds to the function of these proteins. However, the cycle of localization is found to be coupled to the cycle of GTP binding, hydrolysis, and nucleotide exchange, and therefore is likely to play a critical role in regulating the function of these small GTP-binding proteins.

CONCLUDING REMARKS

The preceding sections have summarized our recent work on the role of the *BET* and *BOS* gene products in ER-to-Golgi transport in yeast. Recent findings from our laboratory and other laboratories, on the mechanism by which vesicles target and fuse with their acceptor compartment, have greatly propelled our understanding of this process at a molecular level. The rapid pace of discovery in this field serves to heighten our awareness of the complexities of secretion and stimulates our curiosity in this process. Our approach has proven powerful in this endeavor and will continue to be instrumental in furthering our knowledge of secretion.

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