A High Copy Suppressor Screen Reveals Genetic Interactions Between BET3 and a New Gene: Evidence for a Novel Complex in ER-to-Golgi Transport

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ABSTRACT

The BET3 gene in the yeast *Saccharomyces cerevisiae* encodes a 22-kD hydrophilic protein that is required for vesicular transport between the ER and Golgi complex. To gain insight into the role of Bet3p, we screened for genes that suppress the growth defect of the temperature-sensitive bet3 mutant at 34°C. This high copy suppressor screen resulted in the isolation of a new gene, called BET5. BET5 encodes an essential 18-kD hydrophilic protein that in high copy allows growth of the bet3-1 mutant, but not other ER accumulating mutants. This strong and specific suppression is consistent with the fact that Bet3p and Bet5p are members of the same complex. Using PCR mutagenesis, we generated a temperature-sensitive mutation in BET5 (bet5-1) that blocks the transport of carboxypeptidase Y to the vacuole and prevents secretion of the yeast pheromone α-factor at 37°C. The precursor forms of these proteins that accumulate in this mutant are indicative of a block in membrane traffic between the ER and Golgi apparatus. High copy suppressors of the bet5-1 mutant include several genes whose products are required for ER-to-Golgi transport (BET1, SEC22, USO1 and DSS4) and the maintenance of the Golgi (ANP1). These findings support the hypothesis that Bet5p acts in conjunction with Bet3p to mediate a late stage in ER-to-Golgi transport. The identification of mammalian homologues of Bet3p and Bet5p implies that the Bet3p/Bet5p complex is highly conserved in evolution.

In eukaryotic cells, the secretory pathway consists of a series of distinct membrane-bound compartments. The transport of proteins and lipids between these compartments is mediated by vesicles that dock and fuse with their acceptor membrane. In the neuron, membrane proteins called SNAREs (synaptobrevin, syntaxin and SNAP-25) are key players in these events (Söllner et al. 1993a). The SNAREs, which are evolutionarily conserved (Ferro-Novick and Jahn 1994), bind to each other to form a stable ternary complex that then becomes larger when the soluble factors NSF and α-SNAP bind (Söllner et al. 1993b). During membrane fusion (Söllner et al. 1993a), or after fusion, this complex is disassembled by NSF (Mayer et al. 1996) to prime the SNAREs for a new round of vesicular transport (Mayer et al. 1996). The recent finding that a v-SNARE can interact with more than one t-SNARE (Fischer von Mollard et al. 1997) implies that the SNAREs are not sufficient to target vesicles to the right compartment.

In the yeast *Saccharomyces cerevisiae*, genetic and biochemical studies have led to the identification of a number of genes whose products function in the docking and fusion of ER-derived transport vesicles with the Golgi apparatus. Among them are the homologues of NSF and α-SNAP, which are encoded by the SEC18 and SEC17 genes, respectively (Wilson et al. 1989; Griff et al. 1992), as well as analogues of the neuronal SNAREs, synaptobrevin, syntaxin and SNAP-25. The synaptobrevin-like proteins Bos1p (Lian and Ferro-Novick 1993) and Sec22p (Lian et al. 1994; Segarra et al. 1994) are classified as v-SNAREs (vesicle SNAREs), while a third SNARE, Bet1p, contains a domain that is homologous to SNAP-25 (Stone et al. 1997). The interactions of Bet1p with Bos1p and Sec22p are regulated by the small GTP-binding protein Ypt1p (Lian et al. 1994; Stone et al. 1997). Sed5p, a syntaxin related protein (Hardwick and Pelham 1992), is the t-SNARE (target SNARE) that acts at this stage of the secretory pathway (Segarra et al. 1994; Sacher et al. 1997).

We recently identified Bet3p, a small hydrophilic protein that appears to mediate a late stage of ER-to-Golgi transport in yeast (Rossi et al. 1995). The importance of Bet3p in membrane traffic is marked by its essential role in this process and its genetic interactions with YPT1 and known v-SNAREs (Rossi et al. 1995). Despite these interactions, however, Bet3p is not part of the SNARE complex that accumulates at 37°C in sec18 mutant cells when ER-to-Golgi transport is blocked (Rossi et al. 1995). In an attempt to understand the role of Bet3p in intracellular membrane traffic, we screened for genes that suppress the temperature-sensitive (ts) growth defect of the bet3-1 mutant. This screen resulted in the identification of a new gene, BET5. BET5 encodes an
essential 18-kD hydrophilic protein that is required for ER-to-Golgi membrane traffic. Here we show that Bet3p and Bet5p are members of a complex that is found in a variety of organisms from yeast to humans.

MATERIALS AND METHODS

Strains, plasmids and media: Yeast cells were grown in either YP or minimal medium that was supplemented with the appropriate nutrients (20 μg/ml of histidine, 40 μg/ml of uracil). The growth medium contained either 2% glucose or 0.5% galactose and 2% raffinose as a carbon source.

Isolation of the BET5 gene: The BET5 gene was isolated by screening a 2-μm yeast genomic library (Carlson and Botstein 1982) for genes that suppress the growth defect of the ts bet3-1 mutant. Plasmid DNA was transformed into the bet3-1 mutant strain, and Ura+ transformants were selected on minimal medium lacking uracil. After 3 days at 25°C, transformants were replica plated onto YPD plates and then incubated overnight at 30°C. Of the 11,146 Ura+ transformants examined, 166 grew at 30°C. These transformants were then tested for growth at 34°C. Plasmids from the 8 transformants that grew at 34°C were retrieved and reintroduced into the bet3-1 mutant to confirm suppression. Restriction analysis indicated that two of these plasmids contained the BET3 gene, while the other six shared a common region of DNA that conferred suppression. The plasmid with the smallest insert (4.3 kb) was subcloned further and tested for activity. This analysis indicated that the region that includes the Xhol site is critical for suppression. A 1.0-kb Bsu36I-Smal genomic fragment, which fully suppressed bet3-1, was sequenced. The open reading frame (ORF) that spanned the Xhol site was named BET5.

Disruption of the BET5 gene and construction of SFNY576: A chromosomal deletion of BET5 was created by replacing all but the last 12 amino acids of the coding sequence with the URA3 gene. Plasmid pSFNB387 (Figure 2), which contains a disruption of the BET5 gene, was constructed as described below. First, a 1.6-kb KpnI-SacI DNA fragment containing BET5 was subcloned into pBlueScript. (The KpnI and SacI sites were generated by PCR.) The URA3 gene was then inserted between the BsaI and Xhol sites that encode the first 140 amino acids of BET5 to yield pSFNB387. A diploid strain, with one disrupted copy of BET5, was created by digesting pSFNB387 with KpnI and SacI and transforming the linear fragment into SFNY562 (Table 1). The transformants were sporulated, and tetrad analysis was performed. After 3 days at 25°C, the 46 tetrads examined displayed 2:2 segregation for viability. All viable spores were Ura+.

SFNY576 (Table 1) was constructed in several steps. First, BET5 was fused to the GAL1 promoter in plasmid pNB527 (GAL1, LEU2) by cloning the ORF into the BamHI site adjacent to GAL1. The resulting construct was linearized with AflII and transformed into SFNY570 (Table 1). As the AflII site is internal to LEU2, the linearized plasmid was expected to integrate at the LEU2 locus. The transformants were sporulated and tetrads were dissected on YP plates that contained 0.5% galactose and 2% raffinose as a carbon source. Colonies that were Ura+ and Leu+ (SFNY576) contained BET5 under the control of the GAL1 promoter.

Construction of yeast strains containing epitope-tagged BET5: A strain (SFNY583; see Table 1) in which the sole copy of BET5 is tagged with c-myc was constructed as described below. Briefly, BET5 was cloned into an integrating vector (pRS305, LEU2; Sikorski and Hieter 1989), and site-directed mutagenesis was used to replace the last codon in the gene with two c-myc epitopes (Kunkel et al. 1987). The tagged BET5 gene encodes a protein containing the following sequence at the C-terminus: AEQKLISEEDLAEQKLIISEDAL-S-STOP. After confirming that the mutagenesis was done correctly by DNA sequence analysis, the plasmid was digested with AflII and transformed into SFNY570 (Table 1), a strain in which the BET5 gene is disrupted by URA3. The transformants were sporulated and tetrad analysis was performed. Colonies that contained c-myc-tagged Bet5p as the sole copy were selected (SFNY583) by screening for Ura+ and Leu+ transformants.

Isolation of the bet5-1 mutant: A ts mutation in BET5 (bet5-1) was generated by PCR mutagenesis (Muhlrad et al. 1992). Briefly, SFNY712 (Table 1) was transformed with a gapped plasmid (pSFN404) and mutagenized BET5 DNA (Muhlrad et al. 1992). Plasmid pSFN404 (BET5, CEN, LEU2) was gapped by excising a Bsu36I-BsBl fragment that contains the BET5 gene. Transformants, selected on minimal plates containing glucose but lacking leucine, were screened for their inability to grow on YPD plates at 37°C. Plasmid pSFN470 (GAL1-BET5, URA3, CEN) was then displaced from the transformants on 5-FOA (orotidine-5'-phosphate decarboxylase) plates at 25°C. One transformant, whose ts growth defect was complemented by plasmid pSFN469 (BET5, URA3, CEN), was analyzed further. The mutated plasmid was retrieved from the host strain and subcloned into an integrating vector (pRS305) that contained the LEU2 gene. The resulting plasmid was then linearized.
with EoRV and integrated at the LEU2 locus of SFNY712. A 
Leu<sup>+</sup> transformant was incubated on a plate containing 5-
FOA to displace pSFN470. The resulting strain was SFNY713 (Table 1). DNA sequence analysis revealed that the bet5-1 
mutant contained nine mutations (C13R; I32V; M47T; K57R; 
N65D; D66A; R68P; F88L; S98P).

Preparation of anti-Bet5p serum: Bet5p antiserum was 
raised against recombinant Bet5p that contained a six-histi-
dine (His<sub>6</sub>) tag at its amino terminus. The His-tagged con-
struct was made by cloning a PCR product, that contained 
the BET5 ORF, into a T7 expression vector (pET15b from 
Novagen, Madison, WI). The cloning sites (NcoI/BamHI) of 
the PCR product were generated so that, after ligation, the 
initial ATG of BET5 was fused in-frame with the coding se-
quence for the His<sub>6</sub> tag. Bet5p was expressed in BL21 (DE3) 
cells and gel purified from a cell extract. The immunization 
protocol used was described before (Louvard et al. 1982).

In vivo labeling and immunoprecipitation: Yeast cells were 
grown at 25<sup>°</sup>C to early exponential phase in minimal medium 
containing 2% glucose. The cells were harvested and resus-
pended in 1 ml of medium to an OD<sub>600</sub> = 1.00 before they 
were radiolabeled for 30 min with 200 μCi of 35S Trans label.
SFNY576 was incubated for 15 hr at 25<sup>°</sup>C in glucose containing 
medium prior to the addition of label. Subsequent to this 
incubation, the cells were labeled for 30 min at 25<sup>°</sup>C. The bet1-1, 
sec18-1, and bet5-1 (Table 1) mutants were preincubated for 
30 min at 37<sup>°</sup>C and then labeled at the same temperature. 
Radiolabeled cells were washed with 10 mM sodium azide, 
converted to spheroplasts, and lysed as described before (Shim 
et al. 1991). CPY and α-factor were precipitated from clarified 
electrodes as extract as described by Shim et al. (1991).

For the Bet3p/Bet5p coprecipitation studies, yeast cells were 
grown to early exponential phase in YPD medium at 25<sup>°</sup>C. 
Eight OD<sub>600</sub> units of cells were collected, washed with cold 
medium, and resuspended in 2 ml of spheroplast buffer (1.4 
M sorbitol, 50 mM potassium phosphate, pH 7.5, 10 mM 
sodium azide, 50 mM β-mercaptoethanol and 10 μg/ml zymolase/ 
OD unit of cells). Spheroplasts formed during a 60-min incu-
bation at 37<sup>°</sup>C were lysed in 250 μl of lysis buffer (20 mM HEPES, 
PH 7.4, 500 mM KCl, 1 mM DTT, 1 mM MgCl<sub>2</sub>, 2% Triton 
X-100). ρ × Protease Inhibitor Cocktail also called PIC (see 
Ruohola et al. 1988) and centrifuged for 15 min at 3000 g in 
an Eppendorf centrifuge. The supernatants were removed and 
diluted with 750 μl of Buffer A (20 mM HEPES (pH 7.4), 100 
mM KCl, 1 mM DTT, 1 mM MgCl<sub>2</sub>, 0.5% Triton X-100, 1× PIC). 
Affinity-purified anti-Bet3p antibody (320 μg), preimmune 
serum (20 μl), or anti-c-myc antibody (20 μl) was added to 
samples before they were incubated overnight at 4<sup>°</sup>C. The anti-
gen antibody complexes were precipitated onto protein A 
Sepharose beads during a 90-min incubation at 4<sup>°</sup>C and 
the beads were washed twice with Buffer B (20 mM HEPES 
(pH 7.4), 500 mM KCl, 1 mM DTT, 1 mM MgCl<sub>2</sub>, 0.5% Triton 
X-100, 1× PIC), six times with Buffer A and once with 20 
mM TrisCl (pH 7.0). The antigen-antibody complexes were 
solubilized by heating the beads for 5 min in the presence of 
100 μl of sample buffer. The beads were pelleted by centrifuga-
tion and 30 μl of the supernatant was electrophoresed on a 
15% SDS polyacrylamide gel. Western blot analysis was 
performed using anti-Bet3p antiserum (1:1000 dilution).

Cloning the human BET5 homologue: Using sequence 
AA203173, primers were constructed to amplify the full-length 
coding region for human Bet5p from a CLONTECH (Palo 
Alto, CA) fetal liver cDNA library (catalog no. 7403-1). As 
for human Bet3p, plasmid clones were sequenced at the Keck 
Foundation at Yale University. Sequence analysis and the data-
base search were performed using the Wisconsin Genetics 
Computer Group (GCG) software version 8.1.
performed. Of the 48 tetrads examined, all displayed 2:2 segregation for viability. In addition, the viable spores were found to be Ura−, suggesting that spores containing a disrupted copy of BET5 could not give rise to viable colonies. Microscopic examination revealed that growth ceased in the disrupted cells after 3–4 cell divisions (8–12 cells). Thus, BET5 is an essential gene whose product is required for the vegetative growth of yeast cells.

**Yeast cells depleted of Bet5p fail to transport proteins between the ER and Golgi complex:** Previous studies have shown that Bet3p is required for vesicular transport between the ER and Golgi complex. As a suppressor of bet3-1, we hypothesized that Bet5p may also play a role in membrane traffic at this stage of the secretory pathway. To test this notion, we determined the consequences of depleting yeast cells of Bet5p. This was done by constructing a strain (SFNY 576) in which the sole copy of BET5 was placed under the control of the regulatable GAL1 promoter. In glucose containing growth medium, the expression of BET5 is repressed in this strain. Growth of SFNY 576 was reduced subsequent to a 13-hr incubation in YPD medium and ceased after 15 hr. At the 15-hr time point, no Bet5p was detected (data not shown).

To determine if Bet5p is required for intracellular
Because it harbors a copy of BET5 genomic copy of introducing mutagenized bet5.

In membrane traffic, we examined the transport of the vacuolar protease carboxypeptidase Y (CPY) and the yeast pheromone α-factor in SFNY576. CPY, which is initially synthesized as a 59-kD precursor, is processed to a 67-kD species (p1CPY) in the ER and then modified in the Golgi complex (p2CPY), before it is proteolytically cleaved in the vacuole to yield the mature form (mCPY) ([for a review see Jones et al. 1997]). As shown in Figure 3A, when Bet5p-depleted cells were radiolabeled with 35S Trans label for 30 min, p1CPY was observed (lane 4). This form of CPY was also present in bet1-1 and sec18-1, two mutants that fail to support ER-to-Golgi transport at 37°C (lanes 2 and 3). The mature form of CPY was only found in wild-type cells (lane 1).

Bet5p-depleted cells also fail to secrete α-factor. A precursor form of α-factor is converted to a 26-kD species (called pro-α-factor) in the ER before it is processed in the Golgi and post-Golgi secretory vesicles to a mature form that is secreted into the medium. As shown in Figure 3B, wild type failed to accumulate α-factor (lane 1), while the 26-kD species accumulated in bet1-1 (lane 2), sec18-1 (lane 3) and Bet5p-depleted cells (lane 4). Thus, in the absence of Bet5p, CPY and α-factor are blocked in transit between the ER and Golgi complex.

Isolation and characterization of the bet5-1 mutant:

To address the role of Bet5p more directly, we used PCR mutagenesis (Muhlrad et al. 1992) to construct a ts allele of bet5. The bet5-1 mutant was constructed by introducing mutagenized BET5 DNA and a gapped plasmid (CEN, LEU2) into a strain (SFNY712) in which the genomic copy of BET5 was disrupted by HIS3. SFNY712 grows in the absence, but not the presence, of glucose because it harbors a copy of BET5 that is under the control of the inducible GAL1 promoter (pSFN470). Leu" transformants that grew on YPD plates at 25°C, but not 37°C, were selected. The growth defect of one transformant was fully complemented at 37°C by pSFN469 (BET5, CEN, URA3), indicating that this strain was ts for growth because it contained a mutant copy of bet5.

The bet5-1 mutant was retrieved from the mutagenized plasmid and subcloned into a vector that was integrated at the leu2 locus of SFNY712. Plasmid pSFN470 (GAL1-BET5, URA3, CEN) was then displaced on 5-FOA plates to yield SFNY713.

SFNY713 was assayed for its ability to secrete α-factor and transport CPY to the vacuole. Mutant and wild-type cells, grown at 25°C, were shifted to 37°C for 30 min before they were incubated with 35S Trans label. As shown in Figure 4, at 37°C, the bet5-1 mutant failed to process CPY (Figure 4B, lane 4) and accumulated the 26-kD ER form of α-factor (also called pro-α-factor), as well as a partially glycosylated ER form (Figure 4A, compare lane 1 with lane 4). These forms were also found in sec18-1 (Figure 4A, lane 2) and bet1-1 (Figure 4A, lane 3) mutant cells. Like sec18-1 (Figure 4B, lane 2) and bet1-1 (Figure 4B, lane 3), the CPY that accumulated in bet5-1 was the ER
or p1 form (Figure 4B, compare lane 1 with lane 4). These findings indicate that, upon a short incubation at the restrictive temperature, bet5-1 fails to transport proteins from the ER to the Golgi complex. These findings clearly indicate that Bet5p plays a role in membrane traffic at this stage of the secretory pathway.

**Bet5p coprecipitates with Bet3p:** To analyze the product of the BET5 gene, we raised polyclonal antibody to a recombinant form of Bet5p that contains a six-histidine epitope tag at its amino terminus. Anti-Bet5p antibody recognized an 18-kD polypeptide (Figure 5A, lane 3) that was overproduced in a strain that overexpresses BET5 (lane 4). In contrast, preimmune serum failed to identify this band (lanes 1 and 2). To further characterize this antibody, we also constructed an epitope-tagged version of Bet5p (SFNY583) that contains two carboxy-terminal c-myc tags. Anti-Bet5p antibody, as well as anti-c-myc antibody, recognized this form of Bet5p which migrates at 21 kD (Figure 5B, lane 6).

Previous studies have shown that the overexpression of YPT1, BET1, SEC22, and BOS1 suppressed the growth defect of bet3-1 at 30°C (Rossi et al. 1995), but not at 34°C as BET5 does. However, overexpression of Bet5p failed to suppress other ER-to-Golgi mutants. These findings indicate that the genetic interactions between BET5 and BET3 are strong and specific, implying a stable physical association between the products of these genes. To test this notion, we quantitatively precipitated a yeast cell extract with affinity-purified anti-Bet3p antibody and analyzed proteins of the precipitate with anti-Bet5p serum. As is shown in Figure 5B, Bet5p was detected in the anti-Bet3p precipitates (lane 3). Quantitation of this precipitate revealed that all of the cellular Bet5p was present. When this experiment was performed with a strain (SFNY583) in which the sole copy of Bet5p contained two c-myc tags, the 18-kD Bet5p band was shifted to 21 kD (lane 6). Bet5p did not coprecipitate with Bet3p if preimmune serum replaced anti-Bet3p antibody (lanes 2 and 5), or if the extract was denatured prior to the addition of anti-Bet3p antibody (lanes 1 and 4). Bet3p also coprecipitated with Bet5p if a cell lysate, prepared from SFNY583, was precipitated with anti-c-myc antibody (compare lane 8 with control in lane 7). These findings conclusively demonstrate that Bet5p and Bet3p associate with each other to form a complex.

**High copy suppressors of the bet5-1 mutant:** We previously demonstrated that BET3 interacts genetically with BOS1, BET1, SEC22, and YPT1 (Rossi et al. 1995). This observation suggested that Bet3p functions in con-
TABLE 2
Summary of high copy suppression of bet5-1

<table>
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<th>35°</th>
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junction with v-SNAREs and the GTPase that regulates their activity (Lian et al. 1994; Rossi et al. 1995). Since Bet5p and Bet3p are components of a stable complex, we anticipated that BET5 would display a similar pattern of genetic interactions. Indeed, as shown in Table 2, the overexpression of BOS1, BET1, SEC22, and YPT1 suppressed the growth defect of the bet5-1 mutant at 35° and 37°. YPT1 and BOS1 were the strongest suppressors of bet5-1 at 35°, while YPT1 displayed the strongest suppression at 37°. To confirm that the suppression we observed was due to the restoration of intracellular membrane traffic, we examined the transport of α-factor and CPY in bet5-1 mutant cells overexpressing YPT1 or BOS1. The overexpression of either gene partially restored the maturation of CPY (Figure 4B, compare lane 4 with lanes 5 and 6) and the secretion of α-factor, and as a consequence, less of the 26-kD form of α-factor accumulated within mutant cells (Figure 4A, compare lane 4 with lanes 5 and 6).

To identify additional genes that interact genetically with BET5, we screened for high copy suppressors of the bet5-1 mutant. Of the 20,000 transformants examined, 31 grew at 37°. Plasmids retrieved from these transformants suppressed bet5-1, indicating that suppression was linked to the plasmid. The 12 plasmids that suppressed bet5-1 best were analyzed further, and the genes contained within them were identified by sequencing the ends of each insert and comparing the sequence with known sequences in the yeast database. As anticipated, BET1, SEC22, and BET5 were among the suppressors. Several genes whose products have been implicated in membrane traffic either directly (USO1) or indirectly (DSS4 and ANP1) were also isolated. To demonstrate that suppression was conferred by BET1, SEC22, USO1, DSS4, and ANP1, these genes were cloned by PCR into high copy expression vectors. The resulting plasmids were transformed into the bet5-1 mutant and found to retain full suppression activity (Table 2), indicating that the identified genes were indeed the suppressors. It is noteworthy that we did not isolate either YPT1 or BOS1, which are strong suppressors of bet5-1. Thus, our screen has not yet reached saturation.

Bet5p is highly conserved: We previously reported that Bet3p is homologous to a Caenorhabditis elegans protein of unknown function (Rossi et al. 1995), suggesting that Bet3p may be highly conserved. To identify homologues of Bet5p, we searched the dbEST database with TBLASTN using the yeast protein. Fifty sequences with a probability score (P value) of less than 0.01 were identified. Two of these sequences contained full-length coding regions for human (AA041907) Bet5p. Pairwise alignment in Figure 6 shows that the mammalian proteins (145 amino acids) are shorter than the yeast protein (159 amino acids). In addition, the human and mouse coding regions differ only by two amino acids, while the yeast protein is 53.8% similar and 29% identical to the human homologue. These findings indicate that Bet3p and Bet5p are highly conserved proteins that are present in a variety of species.

DISCUSSION

We previously identified BET3 by its interactions with BET1 (Rossi et al. 1995), a gene whose SNARE-like prod...
uct acts in ER-to-Golgi transport (Stone et al. 1997). The observation that a block in ER-to-Golgi transport immediately ensues upon inactivation of Bet3p implies that this protein plays a critical role in this process (Rossi et al. 1995). In an effort to gain insight into the function of Bet3p, we isolated high copy suppressors of the bet3-1 mutant. This screen resulted in the identification of BET5, a novel gene whose product is required for ER to Golgi transport. The strong and specific interactions observed between BET3 and BET5 implies that their products physically interact with each other. This hypothesis was confirmed by the demonstration that Bet5p coimmunoprecipitates with Bet3p. More recently we have shown that Bet3p and Bet5p are members of a large complex (~800 kD) that includes at least eight other proteins (Sacher et al. 1998).

Previous studies indicate that cells lacking functional Bet3p accumulate ER and small vesicles, suggesting that this gene product acts in vesicle targeting or fusion (Rossi et al. 1995). This finding was corroborated by the isolation of USO1, BET1, SEC22, and DSS4 as high copy suppressors of the bet3-1 mutant. These genes all encode proteins that act at a late stage of ER-to-Golgi transport. USO1 is a large globular cytosolic protein (206 kD) that has been proposed to tether transport vesicles to a putative receptor on the Golgi (Sapperstein et al. 1996; Barlowe 1997), while BET1 and SEC22 encode SNAREs (Sacher et al. 1997; Stone et al. 1997). Dss4p may activate the small GTP-binding protein Ypt1p by promoting nucleotide displacement (Collins et al. 1997). Finally, ANP1, which was also isolated as a high copy suppressor of bet3-1, encodes a 58-kD type II membrane protein that resides on the ER and is required to maintain a functional Golgi apparatus (Chapman and Munro 1994). Taken together, these findings imply that the Bet3p/Bet5p complex mediates a late stage of ER-to-Golgi transport and that the maintenance of the Golgi may be important for its function.

Although Bet3p and Bet5p are unrelated to previously identified proteins required for membrane traffic, they are highly conserved. In an earlier study (Rossi et al. 1995), we found that Bet3p is 36% identical to a C. elegans protein of unknown function. A more recent search of the dbEST database has led to the identification of a human homologue of Bet3p (Sacher et al. 1998) and Bet5p (Figure 6). Human Bet3p (Hbet3p) is 180 amino acids and is highly homologous (54% identity, 72% similarity) to its yeast counterpart, while human and mouse homologues of Bet5p are ~29% identical to the yeast protein. This finding reinforces the notion that the role of the Bet3p/Bet5p complex in membrane traffic is highly conserved. Further characterization of Bet3p and Bet5p in both yeast and mammalian cells will enable us to determine precisely how these proteins function together to mediate a late stage in ER-to-Golgi transport.

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