The BOS1 Gene Encodes an Essential 27-kD Putative Membrane Protein That Is Required for Vesicular Transport from the ER to the Golgi Complex in Yeast

Joseph Shim,* Anna P. Newman,† and Susan Ferro-Novick‡

*Department of Molecular Biophysics and Biochemistry and †Department of Cell Biology, Yale University School of Medicine, New Haven, Connecticut 06510

Abstract. We recently described the identification of BOS1 (Newman, A., J. Shim, and S. Ferro-Novick. 1990. Mol. Cell. Biol. 10:3405-3414.). BOS1 is a gene that in multiple copy suppresses the growth and secretion defect of bet1 and sec22, two mutants that disrupt transport from the ER to the Golgi complex in yeast. The ability of BOS1 to specifically suppress mutants blocked at a particular stage of the secretory pathway suggested that this gene encodes a protein that functions in this process. The experiments presented in this study support this hypothesis. Specifically, the BOS1 gene was found to be essential for cellular growth. Furthermore, cells depleted of the Bosl protein fail to transport pro-α-factor and carboxypeptidase Y (CPY) to the Golgi apparatus. This defect in export leads to the accumulation of an extensive network of ER and small vesicles. DNA sequence analysis predicts that Bosl is a 27-kD protein containing a putative membrane-spanning domain. This prediction is supported by differential centrifugation experiments. Thus, Bosl appears to be a membrane protein that functions in conjunction with Betl and Sec22 to facilitate the transport of proteins at a step subsequent to translocation into the ER but before entry into the Golgi apparatus.

The process of vesicle-mediated transport is complex and involves several events. Carrier vesicles, containing contents destined to traverse the secretory pathway, bud from a donor membrane. These vesicles are then targeted to their acceptor compartment where they bind to and fuse with this membrane to permit the mixing of contents (Palade, 1975). How a transport vesicle is formed and how it delivers its cargo to the correct acceptor organelle are pivotal questions regarding the mechanism of membrane traffic that remain unanswered. Although little is known about the molecular details underlying the budding, targeting, and fusion events, it is assumed that the regulation and specificity of these processes are ensured by proteins. Therefore, a prerequisite to addressing the mode of vesicular traffic is the identification and characterization of these components.

Classical genetic studies in the yeast Saccharomyces cerevisiae have defined 11 SEC and BET genes whose products are required for the transport of proteins from the lumen of the ER to the Golgi complex (Novick et al., 1980; Newman and Ferro-Novick, 1987). The SEC and BET gene products may participate in the budding of vesicular carriers from the ER or their subsequent binding and fusion with the Golgi apparatus. The recent development of assays that faithfully reproduce these events in vitro (Ruohola et al., 1988; Baker et al., 1988) should facilitate studies aimed at elucidating the function of the Sec and Bet proteins.

We have described the isolation of a new gene, BOS1 (betl one suppressor), that suppresses the growth and secretion defect of betl and sec22 (Newman et al., 1990). The ability of this gene to specifically suppress two different secretory mutants, that disrupt transport from the ER to the Golgi complex, suggests that its product functions in this process. In this report, we show that the BOS1 gene encodes a 27-kD protein containing a putative membrane spanning domain. To test the function of Bosl, we have constructed a strain in which the synthesis of this protein is under the control of the regulatable GALI promoter. Here we demonstrate that yeast cells depleted of Bosl fail to transport pro-α-factor and carboxypeptidase Y to the Golgi complex. This defect in transport leads to an extensive accumulation of ER and patches of small vesicles. These findings support the hypothesis that the BOS1 gene product is one of several proteins mediating transport at this stage of the secretory pathway.

Materials and Methods

Growth Conditions

Yeast cells were grown in YP medium (1% Bacto-yeast extract and 2% Bacto-peptone) on LB medium; YP, 1% Bacto-yeast extract and 2% Bacto-peptone.

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Bacto-peptone with 2% glucose or in Wickerham's minimal medium (Wickerham, 1946) supplemented with the appropriate nutrients (20 μg/ml of histidine, 40 μg/ml of uracil). For sulfate-free minimal medium, all sulfate salts were replaced with chloride salts. JSY1 was grown in YE or minimal medium with 0.5% galactose and 2% raffinose. To change growth medium, cells were pelleted in a clinical tabletop centrifuge at room temperature and resuspended in fresh medium. Cell densities were measured in a 1-cm quartz cuvette at a wavelength of 599 nm in an LKB Biochrom Ultro Spec Plus spectrophotometer (LKB Instruments, Gaithersburg, MD).

**Genetic Techniques and Constructions**

All plasmids constructed were transformed into *Escherichia coli* (DH1) to amplify DNA. Ampicillin resistant transformants were selected in LB plates containing ampicillin (100 μg/ml). Plasmid DNA was isolated from E. coli (Birnboim and Doly, 1979) and transferred into Ura- yeast strains using lithium alkali cation treatment (Ito et al., 1983). Several plasmid constructions were required to construct JSY1, a strain in which the BOSI gene was placed under the control of the regulatable *GALI* promoter. The scheme used to construct this strain is summarized in Fig. 5. Plasmid pYG1 was constructed as follows: pNRB187 (Table II) was cut with Bam HI, the 3' recessed end was filled in using Klenow and the vector fragment was gel purified and treated with alkaline phosphatase. A 0.26-kb Taq I-Ava II fragment, containing the amino terminus of BOSI, was excised from pFN8 and blunt end-ligated into the Bam HI site of pNRB187. To construct pYG2, a 1.05-kb Eco RI fragment from pYGI, containing the *GALI-BOSI* gene, was digested with Nhe I (blunt) site of pYG2 and partially digested with Eco RI to obtain the 4.0-kb Bgl II-Eco RI fragment. The 0.44-kb Sau3A fragment (nucleotide 329-769 of the BOSI gene) and the pATH10 vector were purified from an agarose gel and the purified fragments were ligated and used to transform E. coli (DH1). The TrpE-BOSI fusion protein contains 147 of the 244 amino acids encoded by the BOSI gene product.

**DNA Sequence Analysis and Homology Search**

To determine the DNA sequence of the Kpn I-Nco I fragment which contains the BOSI gene, restriction fragments were cloned into the polylinker site of M13 phage derivatives (mp18 and mp19; Dale et al., 1985) and the nucleotide sequence was determined by the dideoxy chain termination method (Sanger et al., 1977) using the Sequenase DNA sequencing kit (United States Biochemicals Corp., Cleveland, OH). For each sequencing reaction the replicated DNA was labeled with 5 μCi of [α-32P]dATP. All sequencing reactions were electrophoresed at 1,500 V on 8% polyacrylamide gels (40:1.3, acrylamide-bisacrylamide), containing 8 M urea, and the dried gels were exposed to Kodak XAR-5 film for 24 h. The BOSI sequence was compared with that of other proteins entered into the National Biomedical Research Foundation protein sequence library. The FASTA and

### Table I. Yeast Strains

<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotype</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>NY363</td>
<td>MATa/α, leu2-3, 112+/+, ura3-52/ura3-52, his4-619</td>
<td>P. Novick*</td>
</tr>
<tr>
<td>SFNY59</td>
<td>MATα, ura3-52, pBR307 (2 μm, URA3)</td>
<td>This study</td>
</tr>
<tr>
<td>SFNY61</td>
<td>MATα, ura3-52, pFN8B61 (2 μm, URA3, BOSI)</td>
<td>This study</td>
</tr>
<tr>
<td>SFNY26-6A</td>
<td>MATα, his4-619</td>
<td>This laboratory</td>
</tr>
<tr>
<td>NY447</td>
<td>MATα, ura3-52, GAL+, his4-619</td>
<td>This study</td>
</tr>
<tr>
<td>JSY1</td>
<td>MATα, GAL+, his4-619, GALI-BOSI</td>
<td>This study</td>
</tr>
<tr>
<td>ANY113</td>
<td>MATα, ura3-52, his4-619, bel1-1</td>
<td>This study</td>
</tr>
<tr>
<td>NY432</td>
<td>MATα, ura3-52, sec18-1</td>
<td>This study</td>
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<td>MATα, ura3-52, GAL+, bel1-1</td>
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<td>MATα, ura3-52, GAL+, sec22-3</td>
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<td>MATα his4-619, GAL+, bel1-1, GALI-BOSI</td>
<td>This study</td>
</tr>
<tr>
<td>SFNY88-5C</td>
<td>MATα, his4-619, SEC22-3, GALI-BOSI</td>
<td>This study</td>
</tr>
</tbody>
</table>

*Department of Cell Biology, Yale University School of Medicine.

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**Table II. Plasmids**

<table>
<thead>
<tr>
<th>Plasmid</th>
<th>Derivation</th>
</tr>
</thead>
<tbody>
<tr>
<td>pFN8</td>
<td>YCp50, BOSI; 4.2-kb SphI fragment from pAN105 (Newman et al., 1990) inserted in the Sph I site</td>
</tr>
<tr>
<td>pFN13</td>
<td>YCp50, BOSI; 2.2-kb Hinc II-Nco I fragment from pAN105 inserted in the Hind III (blunt) site of the vector</td>
</tr>
<tr>
<td>pFN14</td>
<td>YCp50, BOSI::URA3 gene disruption; 1.1-kb Hind III fragment (URA3) inserted in the internal Eco RI (blunt) site of BOSI in pFN8</td>
</tr>
<tr>
<td>pTB1</td>
<td>pATH10, TrpE-BOSI; fusion; 0.44-kb Sau3A fragment from pFN13 inserted in the Bam HI site</td>
</tr>
<tr>
<td>pRB8</td>
<td>pBR322; 1.1-kb Hind III fragment (URA3) inserted in the Hind III site</td>
</tr>
<tr>
<td>pNRB103</td>
<td>YCp50, 1.1-kb Hind III fragment (URA3) inserted in the Hind III site</td>
</tr>
<tr>
<td>pNRB187</td>
<td>YCp50, with GALI promoter; 0.82-kb Eco RI-Bam HI fragment inserted in the Eco RI-Bam HI site; expression under GALI control by cloning into the Bam HI site</td>
</tr>
<tr>
<td>pYG1</td>
<td>YCp50, GALI-BOSI; 0.26-kb Taq I-Ava II fragment (amino terminus of BOSI) from pFN13 inserted in the Bam HI (blunt) site of pNB187</td>
</tr>
<tr>
<td>pYG2</td>
<td>YCp50, GALI-BOSI; 1.05-kb Eco RI fragment from pYGI inserted in the Eco RI site of pNB103</td>
</tr>
<tr>
<td>pYG3</td>
<td>YCp50, GALI-BOSI; 1.7-kb Kpn I fragment (upstream region of BOSI) from pFN8 inserted into the Nhe I (blunt) site of pYG2</td>
</tr>
<tr>
<td>pFN8B61</td>
<td>2 μm, BOSI, 15-kb Sal I fragment from pAN105, in the Sal I site of vector, pBR307 (from the collection of D. Botstein, Stanford University)</td>
</tr>
</tbody>
</table>
FASTP programs were used in the ktp2-2 mode to perform this analysis (Lipman and Pearson, 1985).

Preparation of the TrpE-Bosl Fusion Protein, Rabbit Immunization, and Affinity Purification of the Anti-Bosl Antibody

SPNB16 (DH1 transformed with pTBI) was grown as described previously by Goud et al. (1988). Cells from a 200 ml culture (ODDp 90 = 0.5) were pelleted and washed with 50 ml of 20 mM Tris-HCl (pH 7.0). The pellet was resuspended in 5 ml of cracking buffer (10 mM sodium phosphate, pH 7.2, 1% SDS, 6 M urea, 1% β-mercaptoethanol) and incubated for 2 h at 37°C. DNA was precipitated from the sample by centrifuging the lysate for 30 min at 20,000 rpm in a TI 50 rotor (Beckman Instruments, Inc., Palo Alto, CA). The supernatant was mixed with concentrated sample buffer (Laemmli, 1970) and electrophoresed on a preparative 8% SDS polyacrylamide gel. The gel was stained for protein and destained. The fusion protein was excised from the gel and the gel stripe, containing the fusion protein, was washed several times with distilled water over the course of 2 h. The sample was chopped into small cubes and the hybrid protein was electroeluted from the gel at 100 V for 12 h onto dialysis membrane (Hunkapiller et al., 1983). This procedure yielded ~500 µg of fusion protein in 0.5 ml of dialysis buffer which was stored at ~20°C.

The immunization protocol used was essentially the same as described by Louvard et al. (1982). The serum was affinity purified on an Affigel column to which the TrpE-Bosl hybrid protein was cross-linked. The protocol used was described by Goud et al. (1988). This procedure yielded 1.5 mg of purified antibody in a volume of 2.5 ml.

In Vivo Labeling and Immunoprecipitation

Cells were grown to early exponential phase at 30°C in minimal medium containing 100 µM ammonium sulfate, 0.5% galactose, 2% raffinose, 40 µg/ml uracil, and 20 µg/ml L-histidine. To repress transcription from the GAlU promoter, JSY1 cells were pelleted and resuspended (ODDp 90 = 0.025) in medium containing 2% glucose as the sole carbon source. After 12 h of growth at 30°C (approximately six doublings), the ODs99 of JSY1 failed to increase. At this point, one ODs99 of wild type and JSY1 cells were pelleted and resuspended in 0.5 ml of minimal medium, which was supplemented with 25 µM ammonium sulfate, 2% glucose, nutrients, and 200 µCi of [35S]sulfate. The cells were incubated for 45 min at 30°C and lysates were prepared and processed for immunoprecipitation as described below.

The bet and sec mutants were grown to early exponential phase at 25°C in minimal medium supplemented with 100 µM ammonium sulfate and 2% glucose. One ODs99 of cells were pelleted, resuspended in 0.5 ml of the same medium containing 25 µM ammonium sulfate and incubated for 15 min at 37°C. After this incubation, [35S]sulfate (200 µCi) was added to the medium and the incubation was continued for 45 min. The radiolabeled cells were washed with 1 ml of cold 10 mM sodium azide, resuspended in spheroplast medium (1.4 M sorbitol, 50 mM potassium phosphate (pH 7.5), 10 mM sodium azide, and 36 mM β-mercaptoethanol) containing 1 U of zymolyase 100T and incubated for 1 h at 30°C. The spheroplasts were pelleted, lysed in 100 µl of 1% SDS, and heated to 100°C for 3 min. Lysates were diluted with 900 µl of dilution buffer (0.2 M sodium chloride, 12.5 mM potassium phosphate, pH 7.5, 2% Triton X-100) and centrifuged for 15 min in an appendor centrifuge at 4°C. The supernatant (800 µl) was removed and added to an Eppendorf tube containing 2 µl of the appropriate antibody. Protease inhibitor cocktail (Waters and Biobre, 1986) was added and the incubation was continued for 16 h at 4°C. The antigen–antibody complexes were precipitated with Protein A-Sepharose (60 µl of a 10% solution) during a 90-min incubation at 4°C and the beads were washed three times with urea wash buffer (2 M urea, 200 mM sodium chloride, 100 mM Tris pH 7.6 and 1% Triton X-100) and three times with 1% β-mercaptoethanol. The washed Protein A-Sepharose beads were heated in Laemmli sample buffer at 100°C for 3 min and subjected to SDS-PAGE (10 or 12.5%). The samples were normalized to compare strains; the amount of sample loaded onto the gel was based on the [35S]sulfate incorporated into the total lysate.

High molecular weight markers used for SDS-PAGE were 205-kD myosin, 116-kD β-galactosidase, 97.4-kD phosphorylase B, 66-kD BSA, 45-kD ovalbumin, 36-kD carbonic anhydrase, and 29-kD carbonic anhydrase. Low molecular weight markers were 66-kD BSA, 45-kD ovalbumin, 36-kD glyceraldehyde-3-phosphate dehydrogenase, 29-kD carbonic anhydrase, 24-kD trypsinogen, 20.1-kD trypsin inhibitor, and 14.2-kD α-lactoalbumin. Dried gels were exposed to preflashed Kodak XAR-5 film.

Results

Nucleotide Sequence of the BOS1 Gene

Our previous studies have shown that BOS1, BETI, and SEC22 are members of a group of interacting yeast genes (Newman et al., 1990). As a first step toward determining the function of Bosl, we cloned and sequenced the gene that encodes this protein. BOS1 was identified by its ability to partially suppress the bet1 mutant at 37°C. Yeast genomic fragments containing this gene were described previously (Newman et al., 1990) and a 2.2-kb Hinc II–Nco I genomic insert, that suppressed bet1 to the same extent as the original isolate, was the smallest reported clone. This insert was subcloned to a 1.4-kb Kpn I–Nco I fragment and the nucleotide sequence of this region was determined by the strategy shown in Fig. 1. The 244-amino acid (27-kD) protein (called Bosl), contained within this fragment, suppressed the bet1 and sec22 mutants (see Fig. 6). The BOS1 gene contains a putative 88-bp intron with 5' [GTATGT] and 3' consensus sequences for yeast RNA splicing (Langford et al., 1984; Padgett et al., 1986). Fig. 2 shows that this 5' consensus splice site follows the first codon of BOS1 and the yeast branch point box (TACTAAC) is 47 nucleotides downstream from the splice site. The predicted 3' splice site is 25 nucleotides downstream from the branch point box.

DNA sequence analysis predicts that the amino terminus of the Bosl protein is hydrophilic with a hydrophobic stretch of 18 amino acids 4 amino acids from its carboxy terminus. This hydrophobic region is flanked by basic residues that may associate with negatively charged head groups of a phospholipid bilayer to anchor Bosl to a membrane. The DNA sequence also predicts one potential N-linked glycosylation site (at amino acid position 99) preceding the hydrophobic carboxy terminus. Since the primary sequence of Bosl does not contain a structure resembling a classical signal peptide, this protein may not enter the lumen of the ER where it would become accessible to the glycosylation machinery. Thus, this site may not be used. Further experiments will be needed before any final conclusions can be drawn.

BOS1 Is an Essential Gene

Since many of the genes required for protein transport perform an essential function, we have determined if BOS1 is
needed for the vegetative growth of yeast cells. Plasmid pFN14 (described in Table II), containing a disrupted copy of the BOSI gene, was constructed in several steps. A 1.1 kb HindIII fragment, marked by the URA3 gene, was excised from pRB8 (described in Table II) and inserted into pFN8 (described in Fig. 5) at a unique Eco RI site (shown in Fig. 1) internal to the BOSI gene. A 5.4-kb Sph I fragment, containing the disrupted gene, was removed from pFN14 and introduced into NY 363 (Table I), a homozygous Ura− diploid yeast strain. This Sph I fragment recombined with one of two copies of the BOSI gene present in diploid cells, replacing one copy with the disrupted copy that contains URA3. Ura− cells (described in Fig. 5) were used, as shown in Table II, contained a disrupted copy of the BOSI gene, was constructed as described in the legend to Fig. 3.

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Figure 5. Construction of the GAL1-BOS1 gene fusion. The strategy for constructing JSY1, a strain in which the BOS1 gene is under the control of the regulatable GAL1 promoter, is illustrated. First, to construct pYG1, the BOS1 gene was placed behind the GAL1 promoter (Johnston and Davis, 1984; Yocum et al., 1984) by inserting a Taq1-AvalI fragment containing a portion of BOS1 into the BamHI site of pNRB187 (Table II). Then, an Eco RI fragment containing BOS1 was excised from pYG1 and inserted into the Eco RI site of pNRB103 to yield pYG2. A Kpn I fragment, containing the region upstream from BOS1 (checkered), was excised from pFN8 and inserted into the Kpn I site of pYG2 to yield pYG3. Finally, pYG3 was digested with Bgl II and partially digested with Eco RI. The resulting Eco RI-Bgl II fragment was transformed into NY447 and Ura+ transformants were selected. E, Eco RI; B, Bam HI; H, Hind III; S, Sph I; K, Kpn I.

Transformants were selected, sporulated, and twenty-two tetrads were dissected and germinated at 25°C. Two viable Ura+ spores were recovered from each tetrad, implying that the Ura+ spores were inviable. When examined under the light microscope, we noted that the inviable spores had initiated one round of cell division. These findings indicate that the BOS1 gene is essential for the mitotic growth of yeast cells.

Identification of the Bosl Protein and its Association with Microsomal Membranes

The nucleotide sequence of the BOS1 gene predicts a protein of 27-kD. This prediction was tested by raising polyclonal antibody to a portion of Bosl (amino acid 81–227) fused to the E. coli TrpE protein. Western blot analysis revealed that this antibody cross-reacts with a 27-kD species (Fig. 3, lanes 3 and 4) which was not recognized by preimmune serum (Fig. 3, lanes 1 and 2). Affinity-purified antibodies also cross-react with this species (Fig. 3, lanes 3 and 6). To confirm that the 27-kD species is the Bosl protein, we demonstrated that this immunoreactive protein was overproduced in a strain (SFNY61) harboring pFNB61, a high-copy plasmid containing the cloned BOS1 gene (Fig. 3, lanes 2, 4, and 6).

To determine if Bosl is a soluble or membrane-associated protein, differential centrifugation studies were used. These experiments were performed with wild type yeast spheroplasts that were lysed in a buffer that provides osmotic support. The method of cell lysis used in these studies released 95% or more of a cytoplasmic marker protein (cytoplasmic invertase activity; Goldstein and Lampen, 1975). Nonlysed cells were removed from the homogenate by low-speed centrifugation (450 g) and the 450 g supernatant was centrifuged at 100,000 g for 1 h. Western blot analysis revealed that the Bosl (Fig. 4, lane 4), residing in the lysate (Fig. 4, lane 1), sedimented during high-speed (100,000 g) centrifugation (Fig. 4, compare lanes 2 and 3). However, on occasion, small amounts of this protein were found in the high speed supernatant (not shown). Thus, Bosl appears to be associated with membranes. We have also performed extractions with sodium carbonate, a reagent known to solubilize proteins from the periphery of membranes (Howell and Palade, 1982). In an average of three experiments, we have found that 60% of the starting material remained in the pellet, 6% was solubilized, and the rest was not recovered, presumably because it was degraded. This observation supports the hypothesis that Bosl is a membrane protein.

Construction of JSY1

We have demonstrated that the BOS1 gene is essential for the vegetative growth of yeast cells. To determine the phenotypic consequences of depleting yeast cells of the Bosl protein, we constructed a strain (JSY1) in which the only copy of BOS1 was placed under the control of the regulatable GAL1 promoter. In growth medium containing 2% glucose, the synthesis of Bosl is repressed in this strain. To construct JSY1, we placed the BOS1 gene behind the GAL1 promoter and then inserted a piece of flanking DNA upstream from BOS1 to facilitate replacement of the genomic copy with the inducible copy of this gene. This region of DNA was excised from the vector and then recombined into the genome. The manipulations involved in the construction of JSY1 are summarized in Fig. 5. Briefly, a Taq I–Ava II fragment, containing the amino-terminal portion of the BOS1 gene, was inserted into pNRB187 (described in Table II) behind the GAL1 promoter. The resulting GAL1-BOS1 fusion was placed downstream from URA3 by inserting an Eco RI fragment isolated from pFNG5 into the Eco RI site of pNRB103. The region upstream of BOS1 was inserted in front of the URA3 gene by ligating the Kpn I fragment of pFN8 into the Nhe I site of pYG2. The resulting plasmid, pYG3, was digested with Bgl II and partially digested with Eco RI. The Eco RI–Bgl II fragment, containing the region upstream of BOS1 and the GAL1-BOS1 fusion, was transformed into NY447 and Ura+ transformants were selected.

The growth properties of one transformant, JSY1, were examined on agar plates containing different carbon sources.
JSY1 failed to grow on YPD plates (YP + 2% glucose) but did grow as well as wild type (NY447) on YP plates supplemented with 2% raffinose and 0.5% galactose. A second transformant displayed the same properties. This result was anticipated since the BOS1 gene is needed for the vegetative growth of yeast cells. To demonstrate that JSY1 expressed the Bosl protein in a regulatable manner, we constructed haploid strains containing the GALI-BOS1 gene fusion in combination with either the betl-1 (SFNY87-4D) or sec22-3 (SFNY88-5C) mutation. The ability of BOS1 to suppress the growth defect associated with each mutation was then tested. We observed that the Gall–Bosl fusion protein suppressed betl-1 (SFNY87-4D) on YPGal (2% galactose) plates at 35°C, whereas sec22-3 (SFNY88-5C) was suppressed at 30°C (Fig. 6). As expected, SFNY87-4D and SFNY88-5C failed to grow on YPD plates at 35°C and 30°C respectively (not shown). This suppression was specific to betl and sec22, as the Gall-Bosl fusion protein failed to suppress sec4 (not shown), a mutant blocked in post-Golgi secretion (Salminen and Novick, 1987).

Bosl-depleted Cells Fail to Transport Proteins to the Golgi Complex

The ability of the BOS1 gene to specifically suppress the growth and secretion defect of mutants blocked in transit from the ER to the Golgi complex suggests that this gene encodes a protein that functions in this process. If the Bosl protein is a necessary component of the yeast secretory apparatus, depletion of this gene product should block secretion. To test this prediction, we examined the transport of two different proteins in Bosl-depleted cells: a precursor of the yeast pheromone pro-α-factor and the vacuolar protease carboxypeptidase Y (CPY). To repress the synthesis of Bosl, ex-
ponentially grown JSY1 cells were shifted into fresh medium containing glucose. After a 12-h incubation at 30°C, the ODs99 of JSY1 failed to increase, whereas wild-type cells continued to grow (Fig. 7). Lysates prepared from JSY1 cells, treated in this way, did not contain detectable amounts of the 27-kD Bosl protein when analyzed on Western blots (not shown).

The transit of pro-α-factor through the secretory pathway can be assessed by monitoring several processing events. The secreted pheromone α-factor, initially synthesized as a large 19-kD precursor molecule, contains a signal sequence, a hydrophilic proregion and four tandem repeats of the mature peptide (Kurjan and Herskowitz, 1982). As a consequence of signal sequence cleavage and the addition of three N-linked core oligosaccharide units, prepro-α-factor is converted to a 26-kD species in the lumen of the ER. These N-linked oligosaccharide chains are extended when pro-α-factor is transported to the Golgi complex. Pro-α-factor is then processed to the mature form in the trans-Golgi network or in secretory vesicles enroute to the plasma membrane (Julius et al., 1984). To determine if the Bosl protein is required for pro-α-factor transport, JSY1 and wild-type cells (NY447) were incubated for varying periods

![Figure 9](https://example.com/figure9.png)

**Figure 9.** The pl form of CPY accumulates in JSY1 when synthesis of the Bosl protein is repressed. Cells were radiolabeled as described in Materials and Methods. The radiolabeled cells were converted to spheroplasts, lysed and the forms of CPY were immunoprecipitated and analyzed by SDS-PAGE (10%): (lane 1) wild type (NY447), (lane 2) BOS1 under the control of the GAL1 promoter (JSY1); (lane 3) bet1-1 (ANY 113); (lane 4) sec18-1 (NY432).

We also examined the processing of CPY in Bosl-depleted cells. ProCPY is synthesized as a zymogen with an 8-kD prorregion and a cleavable signal sequence (Blachly-Dyson and Stevens, 1987; Hemmings et al., 1981). This protein acquires N-linked core oligosaccharides in the lumen of the ER, which are extended in the Golgi complex, before CPY is activated by the PEP4 gene product in the vacuole (Hemmings et al., 1981; Stevens et al., 1982). Secretory mutants that disrupt protein export at or before the Golgi complex (Stevens et al., 1982; Newman and Ferro-Novick, 1987; Deshaies and Schekman, 1987; Toyn et al., 1988) fail to transport CPY to the vacuole. To determine if Bosl-depleted cells block CPY transport, lysates were prepared from cells radiolabeled with [35S]sulfate as described above. JSY1, wild-type, and mutants cells, that block export from the ER to the Golgi complex, were examined. The sec18 (Fig. 9, lane 4) and bet1 mutants (Fig. 9, lane 3) were found to accumulate the 67-kD pl form of CPY at 37°C (Stevens et al., 1982; Newman and Ferro-Novick, 1987). This form (Fig. 9, lane 2) was also synthesized in Bosl-depleted cells, whereas the mature form (61-kD) was synthesized in wild type cells (Fig. 9, lane 1). Therefore, in the absence of Bosl protein, CPY fails to be transported to the Golgi apparatus.

In the experiments described above, JSY1 cells were analyzed subsequent to a long incubation (12 h) in glucose containing growth medium. If the observed block in transport is a direct consequence of the loss of Bosl, the onset of this defect should correlate with the depletion of this protein. JSY1 and wild type cells were incubated for varying periods

![Figure 10](https://example.com/figure10.png)

**Figure 10.** The depletion of Bosl protein from JSY1 cells is coincident with the block in secretion. Cells were grown overnight at 30°C in minimal medium containing 10 µM ammonium sulfate, 0.5% galactose, 2% raffinose, 40 µg/ml uracil, and 20 µg/ml l-histidine. Early log phase cells were resuspended in the same medium, containing 2% glucose, as described in Materials and Methods. Aliquots of cells (one OD90 unit) were removed at 0 (lane 1), 2 (lane 2), 4 (lane 3), 6 (lane 4), 8 (lane 5), 10 (lane 6), and 12 (lane 7) h, analyzed for the presence of Bosl protein by Western blot analysis, and examined for a defect in pro-α-factor (p-α-f) transport, as described in the legend to Fig. 8 and Materials and Methods. A defect in pro-α-factor transport was detected at the 8-h time point, when cell viability was approximately the same as time zero. Cell viability was measured by plating an aliquot of cells onto YP plates, containing 0.5% galactose and 2% raffinose, and comparing the number of viable cells obtained to that of the zero time point.
of time (0, 2, 4, 6, 8, 10, 12 h) in glucose containing growth medium and the presence of Bosl protein was measured at each time point. At time zero, JSY1 cells contained approximately four times more Bosl protein than NY447 (not shown). Although the level of Bosl remained unaltered in wild type, this protein was depleted from JSY1 subsequent to 8 h in glucose containing medium (see Fig. 10 A, lane 5). During this incubation, aliquots of cells were removed at various times, radiolabeled, and pro-α-factor transport was monitored as described above. A secretion defect was first observed at 8 h (Fig. 10 B, lane 5), when the cells were viable and in exponential growth (Fig. 7). The same results were obtained when CPY transport was examined (not shown). Thus, the export defect observed in JSY1 cells correlates with the depletion of Bosl protein.

Bosl-depleted Cells Accumulate ER and Patches of Small Vesicles

The previously identified sec and bet mutants, that block transport from the ER to the Golgi complex, accumulate an extensive network of ER at their restrictive growth temperature. Vesicles, 40–60 nm in size, also accumulate in some of these mutants (Novick et al., 1980; Newman and Ferro-Novick, 1987). To determine the morphological consequences of depleting yeast cells of the Bosl protein, thin sections of cells lacking Bosl were examined by electron microscopy. In wild type, tubules of ER in contact with the nuclear envelope or at the periphery of cells were observed (Fig. 11 A). In contrast, JSY1 cells incubated for 10 h in glucose containing growth medium accumulated an extensive network of ER (Fig. 11 B). The lumen of the ER and the nuclear envelope were also dilated in these cells and patches of vesicles 50–60 nm in size were found in the cytoplasm (Fig. 11, B and C). These vesicles were comparable in size to the vesicles that accumulate in several other mutants (bet1, bet2, sec17, sec18, and sec22) blocked in transport from the ER to the Golgi complex (Novick et al., 1980; Newman and Ferro-Novick, 1987). Thus, thin section analysis confirms the findings discussed above and indicates that, in the absence of Bosl, protein transport is blocked before entry into the Golgi apparatus.

Discussion

In this report, we describe the characterization of BOS1, an essential gene identified as a stage-specific suppressor of ER-accumulating mutants (Newman et al., 1990). The ability of this gene to specifically suppress bet1 and sec22 suggests that its product acts in conjunction with the Bet1 and Sec22 proteins to perform an interrelated function. Thus, Bosl itself is likely to be required for ER to Golgi transport. To address this hypothesis, we have determined the phenotypic consequences of depleting yeast cells of the Bosl protein. This was achieved by constructing JSY1, a strain in which the sole copy of BOS1 was placed under the control of the regulatable GALI promoter. In growth medium containing glucose, JSY1 fails to synthesize Bosl. As a consequence, pro-α-factor transport is blocked and unprocessed precursors accumulate within the cell (Figs. 8 and 10). Cells depleted of the Bosl protein also fail to transport CPY to the vacuole (Fig. 9). The extent of precursor processing of proCPY and pro-α-factor is consistent with a block in ER to Golgi transport and

![Figure 11. EM analysis of BOS1-depleted yeast cells. Samples were prepared for electron microscopic analysis as described by Newman and Ferro-Novick (1987). Thin-section analysis of wild-type (A) and BOS1-depleted cells, JSY1 (B). C is a high magnification of the small vesicles observed in Bosl-depleted cells. er, endoplasmic reticulum; n, nucleus; sv, small vesicles.](https://jcb.rupress.org/content/113/1/62.full)
correlates with the loss of Bos1 protein (Fig. 10), supporting the notion that this gene product plays a direct role in secretion. Based on this observation, we anticipate that conditional lethal temperature-sensitive mutants in bos1 should display a secretion defect within minutes after a shift to the restrictive temperature. This hypothesis will be tested, in the future, by constructing mutations in this gene.

Although no significant homologies were revealed when Bos1 was compared with other proteins in the National Biomedical Research Foundation protein sequence library, DNA sequence analysis has revealed that BOS1 shares certain structural features with BET1. Both genes contain introns and encode small proteins that are hydrophilic at their amino-termini, with a stretch of hydrophobic amino acids at or near the carboxy end of the protein. The Bet1 protein is structurally similar to synaptobrevin, a constituent of synaptic vesicles (Newman, A., and S. Ferro-Novick, unpublished observations). Like synaptobrevin, Bet1 has significant alphahelical potential in the middle region of the protein. In addition, a sequence of four amino acids “klkr,” which is conserved in synaptobrevin homologues found in a variety of species, is also present in Bet1 at the appropriate distance from the hydrophobic carboxy terminus (Baumert et al., 1989; Sudhof et al., 1989). The BOS1 gene does not, however, contain this consensus sequence.

Selection schemes previously used in yeast have led to the isolation of a large number of secretory mutants that disrupt vesicular transport at various stages of the pathway (Novick et al., 1980; Newman and Ferro-Novick, 1987). The distribution of mutant alleles, conferring a block in ER to Golgi transport, suggested that additional genes whose products mediate transport at this stage of the pathway remained to be identified. The BOS1 gene was isolated (Newman et al., 1990) by its ability to suppress the growth defect of a known ER-accumulating mutant, bet1 (Newman and Ferro-Novick, 1987). The isolation of BOS1 and the characterization of its product has shown that suppression by overexpression is one means of identifying a gene whose product performs a function related to the gene bearing the original mutation. Several other examples have been reported whereby this tactic has led to the isolation of a gene that encodes a protein which functions in the same process (Salminen and Novick, 1987; Dietzel and Kurjan, 1987; Bender and Pringle, 1989; Nakano and Muramatsu, 1989). Thus, this approach provides a useful means of identifying new components that mediate secretion in yeast.

Does the 27-kD membrane protein encoded by the BOS1 gene product mediate vesicle budding or subsequent stages of ER to Golgi transport? A morphological analysis of Bos1-depleted cells may provide a clue. Cells depleted of this protein accumulate a network of dilated ER (Fig. 11) as well as 50-nm vesicles. Vesicles of this size also accumulate in sec17, sec18, and sec22, three mutants that disrupt transit from the ER to the Golgi complex (Novick et al., 1980). Recently, it was proposed that these 50 nm vesicles constitute an intermediate compartment that mediates transport at this stage of the pathway (Kaiser and Schekman, 1990). Thus, transport-incompetent vesicles, that fail to fuse with the Golgi apparatus, may bud from donor membranes in Bos1-depleted cells. Alternatively, Bos1 may control the attachment or fusion of intermediate transport vesicles with their acceptor compartment.

The yeast in vitro transport assay will enable us to address the role of the Bos1 protein in secretion. Transit from the ER to the Golgi complex is achieved in vitro when donor ER membranes are incubated with a yeast lysate in the presence of an ATP-regenerating system (Ruohola et al., 1988; Baker et al., 1988). This event is mediated by carrier vesicles, a short lived intermediate which is normally present in cells in low amounts. The ER to Golgi transport assay has enabled us to trap this intermediate in vitro, facilitating the isolation of this transient organelle (Groesch et al., 1990). Currently, we are purifying these vesicles to determine if Bos1 is a true constituent of this compartment. Now that we have developed an assay that permits the isolation of functional carrier vesicles, we can definitively determine whether the BOS1 gene product controls vesicle budding or later stages of transport.

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References


