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Yos1p Is a Novel Subunit of the Yip1p–Yif1p Complex and Is Required for Transport between the Endoplasmic Reticulum and the Golgi Complex

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Yeast Yip1p is a member of a conserved family of transmembrane proteins that interact with Rab GTPases. Previous studies also have indicated a role for Yip1p in the biogenesis of endoplasmic reticulum (ER)-derived COPII transport vesicles. In this report, we describe the identification and characterization of the uncharacterized open reading frame YER074W-A as a novel multicopy suppressor of the thermosensitive *yip1-4* strain. We have termed this gene *Yip One Suppressor 1* (*YOS1*). Yos1p is essential for growth and for function of the secretory pathway; depletion or inactivation of Yos1p blocks transport between the ER and the Golgi complex. *YOS1* encodes an integral membrane protein of 87 amino acids that is conserved in eukaryotes. Yos1p localizes to ER and Golgi membranes and is efficiently packaged into ER-derived COPII transport vesicles. Yos1p associates with Yip1p and Yif1p, indicating Yos1p is a novel subunit of the Yip1p–Yif1p complex.

INTRODUCTION

Transport of lipids and proteins through the eukaryotic secretory pathway is a highly regulated process mediated by vesicles and/or tubular elements. Distinct sets of protein factors catalyze the budding, targeting, and fusion of transport carriers with cognate acceptor membranes (Bonifacino and Glick, 2004). In this way, secretory cargos are directed to their correct cellular destination, whereas donor and acceptor organelles maintain their specific protein and lipid compositions. Specific sets of cytosolic coat proteins mediate the biogenesis of transport carriers, whereas conserved membrane fusion factors such as Rabs and soluble *N*-ethylmaleimide–sensitive factor attachment protein receptors (SNAREs) are believed to mediate site-specific fusion of transport vesicles with acceptor compartments.

Although many of the protein factors required for intracellular transport have been identified and characterized, it is less clear how these factors interact to coordinate the maintenance of the secretory pathway. In the budding yeast *Saccharomyces cerevisiae*, transport between the endoplasmic reticulum (ER) and the Golgi complex also relies on coat proteins, Rabs, and SNAREs. Specifically, the Rab protein Ypt1p is thought to play a key role in the tethering of ER-derived transport vesicles to the Golgi apparatus (Segev, 1991; Cao *et al*., 1998). Yeast two-hybrid approaches identified a Ypt1p-interacting protein (Yip1p) required for transport through the early secretory pathway (Yang *et al*., 1998). Yip1p is an essential protein of 27-kDa and is predicted to contain three transmembrane segments. Yip1p is conserved across species, and a family of Yip1p-like proteins has recently been described in humans (Tang *et al*., 2001; Shakoori *et al*., 2003). The NH_2 terminus of Yip1p projects into the cytosol and is proposed to be sufficient for interaction with Ypt1p (Yang *et al*., 1998). Yip1p localizes to Golgi and ER membranes as well as ER-derived COPII transport vesicles, indicating Yip1p cycles between these compartments (Heidtman *et al*., 2003).

Additional studies showed that Yip1p forms a heteromeric complex with a second multispanning integral membrane protein termed Yif1p (Matern *et al*., 2000). Yif1p shares sequence homology with Yip1p and also possesses a similar topology. Like Yip1p, Yif1p is able to bind Rab GTPases and is required for transport through the early secretory pathway (Matern *et al*., 2000). Yeast cells also have two nonessential proteins with homology to Yip1p and Yif1p. These proteins, termed Yip4p and Yip5p, were proposed to constitute additional members of a Yip1p-like family of proteins in yeast and may act at later stages of intracellular transport (Calero *et al*., 2002). Although much has been learned about Yip1p and related proteins, their precise mechanism of action in the early secretory pathway remains to be elucidated.

We recently investigated the function of Yip1p in transport between the ER and the Golgi complex by using biochemical, genetic, and morphological approaches (Heidtman *et al*., 2003). Using a cell-free assay driven by purified protein factors that recapitulates protein transport between the ER and the Golgi complex, we found that inhibitors of Yip1p blocked transport at the COPII-dependent budding stage. In addition, a strain bearing the thermosensitive *yip1-4* allele accumulated ER membranes but not transport vesicles at the restrictive temperature, corroborating a role for Yip1p in vesicle budding. We concluded that Yip1p plays a critical role in the biogenesis of ER-derived COPII transport vesicles (Heidtman *et al*., 2003).

To gain further insight into Yip1p function, we have taken a genetic approach. Using a YEp24 yeast genomic library, we selected for multicopy suppressors of the *yip1-4* allele. A

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Table 1. Yeast strains used in this study

Strain	Genotype	Source
RCY1764	*MAT***a** *ura3-52 leu-3112 YIP1ΔKAN^R* [*pRS315-yip1-4*]	Calero *et al*. (2003)
RCY1768	*MATα ura3-52 leu2-3112 YIP1ΔKAN^R* [*pRS315-YIP1*]	Calero *et al*. (2003)
FY833	*MAT***a** *his3Δ200 ura3-52 leu2Δ1 lys2Δ202 trp1Δ63*	Winston *et al*. (1995)
FY834	*MATα his3Δ200 ura3-52 leu2Δ1 lys2Δ202 trp1Δ63*	Winston *et al*. (1995)
CBY453	FY833 × FY834	Powers and Barlowe (1998)
CBY801	*MAT***a** *his3Δ200 ura3-52 leu2Δ1 lys2Δ202 trp1Δ63 YIF1–3×HA-HIS3MX6*	Otte *et al*. (2001)
CBY1388	RCY1764 with YEp24-*YIP1*	This study
CBY1485	RCY1768 with pRS426	This study
CBY1622	RCY1764 with pRS426	This study
CBY1665	RCY1764 with pRS426-*YOS1*	This study
CBY1670	CBY453 with *YOS1/yos1::his5$^+$*	This study
CBY1730	*MATα his3Δ200 ura3-52 leu2Δ1 lys2Δ202 trp1Δ63 HIS3MX6-PGAL1-YOS1*	This study
CBY1766	*MATα his3Δ200 ura3-52 leu2Δ1 lys2Δ202 trp1Δ63 yos1::his5$^+$* with pRS314-*YOS1*	This study
CBY1781	*MATα his3Δ200 ura3-52 leu2Δ1 lys2Δ202 trp1Δ63 yos1::his5$^+$* with pRS316-*YOS1*	This study
CBY1822	*MATα his3Δ200 ura3-52 leu2Δ1 lys2Δ202 trp1Δ63 yos1::his5$^+$* with pRS314-*yos1-1*	This study

multicopy suppression screen can be a tool to identify gene products that function in the same process as a gene of interest and can therefore provide important insights into function (Carlson and Botstein, 1982). Here, we describe the identification of the open reading frame YER074W-A as a novel multicopy suppressor of the *yip1-4* strain. We show that this gene encodes a conserved, essential integral membrane protein that localizes to Golgi and ER membranes as well as COPII transport vesicles. We have termed this gene *Y*ip *O*ne *S*uppressor 1 (*YOS1*) and demonstrate that Yos1p physically associates with the Yip1p–Yif1p complex. Cells depleted of Yos1p display secretory defects characteristic of a block in transport between the ER and the Golgi complex, indicating Yos1p is required for this process.

MATERIALS AND METHODS

Yeast Strains and Media

Yeast strains used in this study are listed in Table 1. Unless noted otherwise, cultures were grown at 25°C (for mutant strains) or 30°C (for wild-type strains) in rich medium (YPD, 1% Bacto-yeast extract, 2% Bacto-peptone, 2% dextrose) or in minimal medium (YMD, 0.67% yeast nitrogen base without amino acids, 2% dextrose, appropriate amino acid supplements) to maintain selective pressure on strains carrying plasmids. Standard yeast (Sherman, 1991) and cloning protocols (Ausubel *et al*., 1987) were used.

Multicopy Suppressor Screen

For the multicopy suppression screen of the *yip1-4* strain, 12,000 transformants of a yeast genomic YEp24 library (Carlson and Botstein, 1982) were grown on YMD-Ura plates at 25°C. Colonies were then replica plated to YPD plates. The replica YPD plates were incubated for 48 h at 34°C. Fifty-five colonies were observed to grow at the restrictive temperature. Cells from each temperature-resistant transformant were restuck to YMD-Ura plates to check that each of the isolates contained plasmid DNA. The putative suppressor strains were then grown overnight in selective medium, and plasmid DNA was isolated. The isolated plasmid DNA was retransformed into the *yip1-4* strain to check for plasmid-linked suppression. Of the 55 isolated plasmid preparations, 22 were able to confer temperature resistance to the *yip1-4* strain upon retransformation. The ends of the genomic inserts of these 22 suppressing plasmids were then sequenced with primers YEpF1–5′CTAGCGCTATATGCGTTG3′and YEpR3 5′GTGATGTCGGCGA TATAGG3′.

Plasmid Construction

Point mutations in *YOS1* were introduced by QuikChange mutagenesis (Stratagene, La Jolla, CA) by using primers containing the desired changes and plasmid pRS314-*YOS1* as the template. Mutant plasmids were sequenced using the primer SYMF1 (5′GCTGGACTGGGAGCCT TCTCAATAAG 3′). Mutant plasmids were transformed into strain CBY1781. Transformants were struck to YMD-Trp supplemented with 5-fluororotic acid (5-FOA) (0.1% final concentration) to cure the strain of wild-type (WT) *YOS1*.

Antibodies and Immunoblotting

Antibodies against Sec61p, Och1p, carboxypeptidase Y (CPY), Sec23p (Otte *et al*., 2001), Sec22p (Liu and Barlowe, 2002), α1,6-mannose linkages, Ypt1p (Cao *et al*., 1998), Yip1p (Heidtman *et al*., 2003), and Gas1p (Belden and Barlowe, 1996) have been described previously. Polyclonal antibodies were raised against a glutathione *S*-transferase (GST)-Yos1p (aa 33–66) fusion protein expressed from plasmid pGEX-2T-YOS1 in *Escherichia coli*. The fusion protein was purified according to the manufacturer's specifications (Pfizer, Inc., New York, NY) and used to immunize rabbits by standard procedures (Covance, Berkeley, CA). Anti-hemagglutinin (HA) monoclonal antibody (mAb) (HA.7) raised against the peptide YPYDVPDYA was obtained from Sigma-Aldrich (St. Louis, MO). For densitometric analysis of immunoblots, films were scanned and plotted using NIH Image 1.52.

Subcellular Fractionation

For experiments to determine the subcellular distribution of Yos1p and Yip1p, organelles were resolved on sucrose gradients according to Powers and Barlowe (1998). To characterize the membrane association of Yos1p, wild-type microsomes were treated with buffer 88 (20 mM HEPES, pH 6.8, 250 mM sorbitol, 150 mM KOAc, and 5 mM MgOAc), 0.1 M sodium carbonate, or 1% Triton X-100 in buffer 88. Samples were mixed and incubated on ice for 10 min, followed by centrifugation at 60,000 rpm (model TLA 100.3 rotor; Beckman Coulter, Fullerton, CA) for 12 min. Equivalent amounts of total samples before centrifugation, supernatant and pellet fractions were diluted in SDS-PAGE buffer, and resolved on a 15% acrylamide gel and analyzed by immunoblot.

Immunoprecipitations

For immunoprecipitation of Yif1p-3×HA or Yos1p, 300 μl of microsomes (320 μg of total membrane protein) was solubilized in an equal volume of 0.5% digitonin/buffer 88-8 (Kuehn *et al*., 1998) in the presence of 10 mM phenylmethylsulfonyl fluoride and 5 mM EDTA. After centrifugation at 14,000 rpm for 4 min at room temperature (RT) to remove unsolubilized material, the supernatant fluid (~500 μl) was transferred to a fresh tube on ice. The solubilized material was diluted with 1.5 volumes of 0.05% digitonin/buffer 88-8, and HA-tagged proteins were immunoprecipitated by addition of 2.0 μg of anti-HA mAb and 25 μl of 20% protein A-Sepharose beads. For immunoprecipitation of Yos1p, 0.5 μl of anti-Yos1p polyclonal antiserum was used in combination with 25 μl of 20% protein A-Sepharose beads. After binding for 2 h at 4°C, beads with bound protein were washed four times with 0.05% digitonin/buffer 88-8. Finally, the bound protein was released from the beads by the addition of 30 μl of SDS-PAGE sample buffer and heated at 75°C for 3 min. Complexes from the immunoprecipitates were resolved on polyacrylamide gels and analyzed by immunoblot. For immunoprecipitation of Yip1p, 0.5 μl of anti-Yip1p polyclonal antiserum was used in combination with 25 μl of 20% protein A-Sepharose beads in the manner described above.

In Vivo Labeling

Pulse-chase experiments were performed as described previously (Belden and Barlowe, 1996), with minor differences. Wild-type and *yos1-1* cells were grown at 25°C in minimal medium containing 2% glucose to an OD_{600} of 0.4. Cultures were harvested, washed, and resuspended at 1/10 the original volume in minimal medium lacking sulfate. After preculturing for 5 min at the permissive (25°C) and restrictive (37°C) temperatures, cells were pulsed for 7 min by the addition of [^{35}S]methionine (Amersham Biosciences, Pisca-

taway, NJ) and chased by the addition of excess unlabeled methionine. Cell samples were taken at the end of the pulse period and after 30 min of chase. Cell lysates were prepared by bead-beat lysis, and labeled species were precipitated from a common extract with specific antibodies for CPY.

In Vitro Vesicle Budding, Tethering, and Transport Assays

Yeast semi-intact cells from wild-type and mutant strains were prepared as described previously (Baker *et al.*, 1988). Vesicle budding, tethering, and fusion assays following [^{35}S]gpαf were published previously (Barlowe, 1997; Cao *et al.*, 1998). Experiments to assay packaging of proteins into vesicles by Western blot were performed as described previously (Otte *et al.*, 2001) from microsomes. For in vitro assays, data points are the average of duplicate determinations and the error bars represent the range.

Microscopy

For analysis of wild-type and *yos1–1* cells by electron microscopy (EM), cells were harvested on filters after shift to the restrictive temperature (1 h, 37°C). Cells were then washed once with buffer (0.1 M PIPES, 0.1 M sorbitol, 1 mM $CaCl_2$, and 1 mM $MgCl_2$, pH 6.8), fixed with 1.25% glutaraldehyde in buffer at RT for 1 h, and then overnight at 4°C. Permanganate fixation and staining was performed according to the procedure of Kaiser and Schekman (1990).

RESULTS

Identification of YER074W-A as a Novel Multicopy Suppressor of the yip1-4 Strain

Our recent biochemical, genetic, and morphological studies of Yip1p function in protein transport between the ER and the Golgi complex led to the finding that Yip1p plays a critical role in COPII vesicle biogenesis (Heidtman *et al.*, 2003). To extend these studies and gain further insight into the mechanism of Yip1p action, a genetic approach was undertaken. Specifically, a yeast genomic YEp24 library bearing the *URA3* marker (Carlson and Botstein, 1982) was used to select for genes that could rescue the growth defect of the *yip1-4* strain at the nonpermissive temperature of 34°C. Approximately 12,000 transformants were selected on minimal plates lacking uracil at the permissive temperature. Transformants were then replica plated to rich media and incubated at 34°C for 48 h. Fifty-five colonies were found to grow at the restrictive temperature. Plasmid DNA was recovered from these colonies and used to retransform the *yip1-4* mutant to test for plasmid-linked suppression. Twenty-two of the 55 plasmids were found to confer suppression of the growth defect in the *yip1-4* strain at the restrictive temperature upon retransformation. DNA sequencing identified the genomic insert present in each of the suppressing plasmids. Two of these plasmids contained a region of DNA harboring the *YIP1* open reading frame (ORF). The remaining 20 plasmids all contained a similar region of genomic DNA from chromosome V. Inspection of this region revealed three ORFs common to all 20 plasmids. These ORFs were *ALD5*, *RPS24*, and YER074W-A. Subcloning revealed that YER074W-A was solely responsible for the suppression of the growth defect in the *yip1-4* strain at 34°C. We have termed this gene *YOS1* based on the manner in which this gene was identified.

To more closely examine the high copy suppression activity of *YOS1* with regard to *yip1-4*, we performed dilution series experiments. Strains were grown overnight in selective medium to stationary phase, back diluted to an equivalent OD_{600}, and a 10-fold dilution series was spotted onto YPD plates. As shown in Figure 1A, the *yip1-4* strain transformed with empty vector displayed a severe growth defect after 48 h at 34°C. In contrast, multi-copy *YOS1* was able to significantly rescue this growth defect. We next examined whether multi-copy *YOS1* was able to rescue any of the secretion defects present in the *yip1-4* strain. We previously reported that membranes prepared from the *yip1-4* strain display a significant defect in a cell-free assay that measures

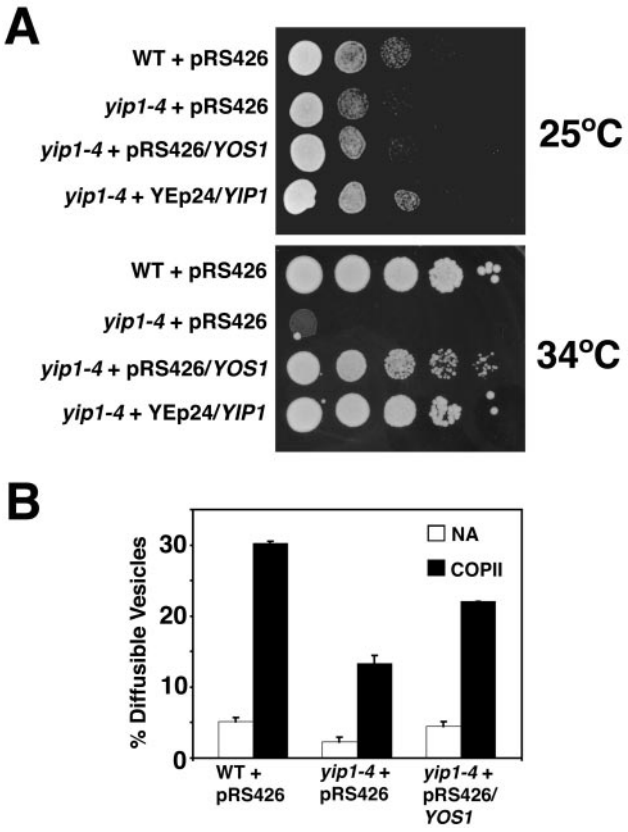

Figure 1. *YOS1* is a multicopy suppressor of *yip1-4*. (A) Strains were grown to saturation in minimal media lacking uracil to maintain selection of plasmids, adjusted to an OD_{600} of 1.0, and 5 μl of a 10-fold dilution series was spotted onto YPD plates. Plates were then incubated at 25 or 34°C for 48 h. (B) Multicopy *YOS1* partially rescues the budding defect in the *yip1-4* strain. Wash semi-intact cell membranes from different strains containing [^{35}S]gpαf were incubated with COPII proteins and an energy regeneration system to measure vesicle budding. After 30 min at 23°C, freely diffusible vesicles containing [^{35}S]gpαf were separated from semi-intact cell membranes by centrifugation at 18,000 $\times$ *g*, and [^{35}S]gpαf was quantified by concanavalin A precipitation.

the COPII-dependent budding of [^{35}S]glyco-pro-α factor (Heidtman *et al.*, 2003). Using this assay, we examined the budding efficiencies of washed semi-intact cell membranes prepared from *yip1-4* cells bearing empty vector or multicopy *YOS1*. As shown in Figure 1B, wild-type membranes displayed a budding efficiency of ~30%. In contrast, *yip1-4* membranes displayed a budding efficiency of only ~12%, consistent with our previously published results (Heidtman *et al.*, 2003). Interestingly, we observed that membranes prepared from the *yip1-4* strain containing multi-copy *YOS1* produced a budding efficiency of ~23%. This result indicates that multi-copy *YOS1* can partially rescue the budding defect displayed by the *yip1-4* membranes in vitro and suggests that the suppression activity of *YOS1* with regard to the *yip1-4* allele is direct.

YOS1 is an uncharacterized ORF identified by comparison of the *S. cerevisiae* genome to that of the related fungi *Ashbya gossypii* (Blandin *et al.*, 2000). *YOS1* contains two introns and is predicted to encode a protein of 87 amino acids with a molecular mass of 9.5 kDa. As shown in Figure 2, Yos1p is well conserved across species, possessing homologues in higher eukaryotic organisms including humans.

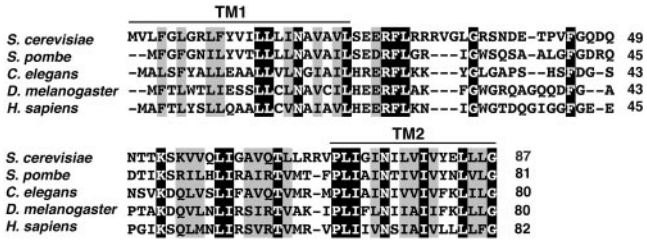

Figure 2. Sequence comparison of Yos1p from different species. The amino acid sequence of Yos1p from *S. cerevisiae* is aligned with those of related proteins from *Schizosaccharomyces pombe* SPAC19A8.09, *Caenorhabditis elegans* W09G3.8, *Drosophila melanogaster* CG32069, and *Homo sapiens* HSPC039. Alignments were generated using the ClustalW program. Invariant residues are shaded in black, whereas regions in which conserved substitutions have been observed are shaded in gray. Lines indicate predicted transmembrane segments.

Yos1p Is an Essential Protein Required for Protein Transport between the ER and the Golgi Complex

YOS1 has not been analyzed by previous large-scale studies that sought to classify yeast ORFs as either essential or nonessential for growth. Therefore, we next examined whether *YOS1* is essential for growth in yeast cells. A polymerase chain reaction (PCR)-based approach (Longtine *et al*., 1998) was used to direct the *S. pombe his5*$^+$ gene to the *YOS1* locus, replacing the entire *YOS1* ORF. Colony PCR was performed to confirm that the *his5*$^+$ gene had integrated correctly at the *YOS1* locus (our unpublished data). A heterozygous *YOS1*/*yos1*Δ diploid strain was then transformed with empty vector, or a centromere (CEN)-based plasmid containing *YOS1*. As shown in Figure 3A, dissection of asci from the heterozygous deletion strain containing empty vector resulted in only two viable spores in each case. Tetrad analysis of the surviving spores confirmed that none possessed the *his5*$^+$ marker, indicating that *YOS1* is essential for viability. As shown in the right panel of Figure 3A, inviability could be rescued when the heterozygous deletion strain was transformed with *YOS1* on a single-copy plasmid. In this case, three and sometimes four viable spores could be recovered. This observation indicates that the 2$^+$:2$^-$ segregation pattern observed in the presence of empty vector is a direct consequence of the deletion of *YOS1*.

Because *YOS1* is an essential gene, we next created a conditional allele of *YOS1*. *YOS1* was placed under transcriptional control of the *GAL1* promoter, allowing for the depletion of Yos1p from cells in glucose-containing medium (Longtine *et al*., 1998). Cells containing *GAL1-YOS1* grew normally in galactose-containing medium but displayed a severe growth defect ~6 h after shift to medium containing 2% glucose. Cells depleted of Yos1p, as monitored with a specific anti-Yos1p polyclonal antiserum, accumulated the proforms of secretory proteins en route to various cellular destinations (Figure 3B). Specifically, the core-glycosylated ER form (p1) of the vacuolar hydrolase CPY was observed to accumulate in the *GAL1-YOS1* strain ~5 h after shift to glucose. Similarly, the core-glycoslyated ER form (p) of the glycosylphosphatidylinositol-anchored plasma membrane protein Gas1p also was observed to accumulate in cells depleted of Yos1p. The ER proform of vacuolar alkaline phosphatase also was seen to accumulate under conditions of Yos1p depletion (our unpublished data). Together, these results indicate that Yos1p is an essential protein in yeast and is required for transport of secretory proteins from the ER to the Golgi complex.

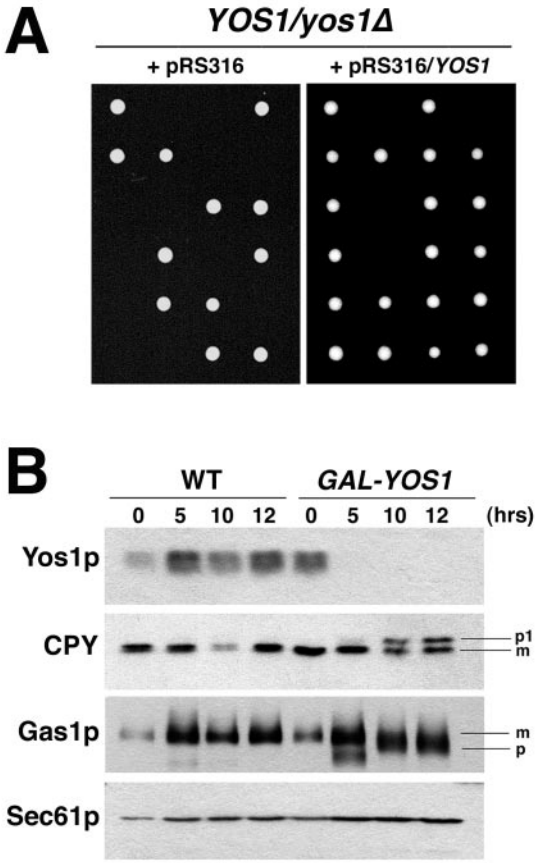

Figure 3. *YOS1* is an essential gene and is required for protein transport between the ER and the Golgi complex. (A) A *YOS1/yos1*Δ strain was transformed with empty vector or a centromere-based plasmid bearing wild-type *YOS1*. Transformants were sporulated, and asci were dissected on YPD plates at 25°C. The *YOS1/yos1*Δ strain transformed with empty vector (pRS316) displayed a 2$^+$:2$^-$ segregation pattern, indicating *YOS1* is an essential gene (left). Inviability could be rescued in certain cases by the presence of pRS316/*YOS1* (right), demonstrating that the 2$^+$:2$^-$ segregation pattern is a specific consequence of the disruption of *YOS1*. (B) Depletion of Yos1p causes a block in protein transport between the ER and the Golgi complex. A *GAL1-YOS1* strain and an isogenic wild-type strain were grown overnight in media containing 2% galactose/2% glucose. Cells were then washed and back diluted to an OD_{600} of 0.1 in media containing 2% glucose to induce transcriptional silencing of the *GAL1* promoter-controlled *YOS1* gene and allowed to grow at 30°C. Equivalent OD_{600} units were harvested from each strain at various time points after shift to glucose. Membrane fractions were prepared and analyzed by immunoblot for the secretory proteins CPY and Gas1p. Sec61p was immunoblotted as a loading control, whereas immunoblot of Yos1p is a control to show efficient depletion of Yos1p upon *GAL1* repression.

Yos1p Is an Integral Membrane Protein

Hydrophobicity plotting (Kyte and Doolittle, 1982) shows that Yos1p contains two potential transmembrane domains located at the NH_2 and COOH termini. These potential transmembrane segments are separated by a relatively hydrophilic stretch of residues (Figure 4A). We sought to confirm that Yos1p is an integral membrane protein by using Western blotting in combination with cellular fractionation under various conditions. As shown in Figure 4B, Yos1p pelleted with membranes when cells were treated with buffer, or with buffer at pH 11, which efficiently extracts the

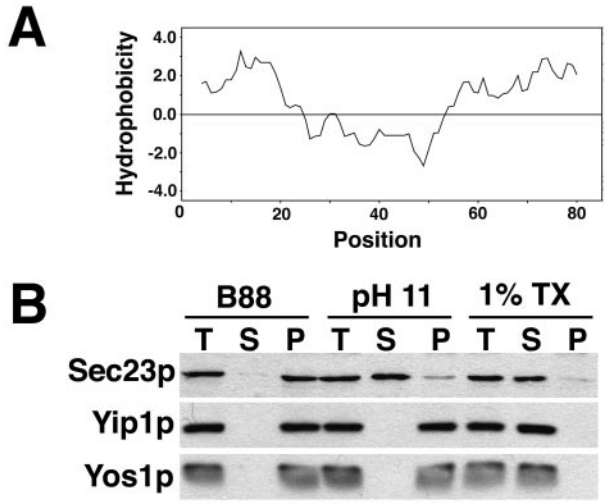

Figure 4. Yos1p is an integral membrane protein. (A) Hydrophobicity plot of the Yos1p amino acid sequence as described previously (Kyte and Doolittle, 1982). (B) Microsomes prepared from wild-type cells (FY834) were treated with buffer (B88), 0.1 M Na_2CO_3 (pH 11), or buffer containing 1% Triton X-100 (1% TX) and centrifuged at 100,000 × *g*. Totals before centrifugation (T), supernatant (S), and pellet fractions (P) were analyzed on a 15% polyacrylamide gel and immunoblotted for Sec23p (peripheral membrane protein), Yip1p (integral membrane protein), and Yos1p.

peripherally associated membrane protein Sec23p into the soluble fraction. Yos1p shifted to the soluble fraction when membranes were treated with 1% Triton X-100, in a manner similar to the known integral membrane protein Yip1p. These results confirm that Yos1p is an integral membrane protein. Further experimentation will be required to establish the membrane topology of Yos1p.

Yos1p Localizes to the Early Secretory Pathway

Because Yos1p was identified as a multicopy suppressor of the *yip1-4* strain, we hypothesized that Yos1p would exhibit a similar subcellular localization pattern as Yip1p. Previous studies have shown that Yip1p and Yif1p localize to ER and Golgi membranes as well as COPII transport vesicles (Yang *et al*., 1998; Matern *et al*., 2000; Heidtman *et al*., 2003). In addition, a thermosensitive allele of *sec12* that blocks ER export but allows retrograde trafficking causes the accumulation of both Yip1p and Yif1p in ER-enriched membrane fractions (Heidtman *et al*., 2003). Together, these observations indicate that Yip1p and Yif1p cycle between ER and Golgi membranes. Therefore, we examined the subcellular localization of Yos1p by resolution of membrane organelles on sucrose gradients. As shown in Figure 5A, Yos1p sedimented in one broad peak that overlapped with the Golgi marker Och1p and the ER marker Sec61p. This localization pattern was very similar to that of Yip1p, which also sedimented in one broad peak overlapping with both ER and Golgi markers.

The observation that Yos1p sediments with both Golgi and ER membranes suggests that Yos1p cycles between these membranes and is therefore packaged into COPII transport vesicles. To test this hypothesis, we prepared microsomes from wild-type cells for use in an in vitro budding reaction. Microsomes were incubated with an energy regeneration system in the presence or absence of COPII proteins, and the vesicles synthesized in each condition were then

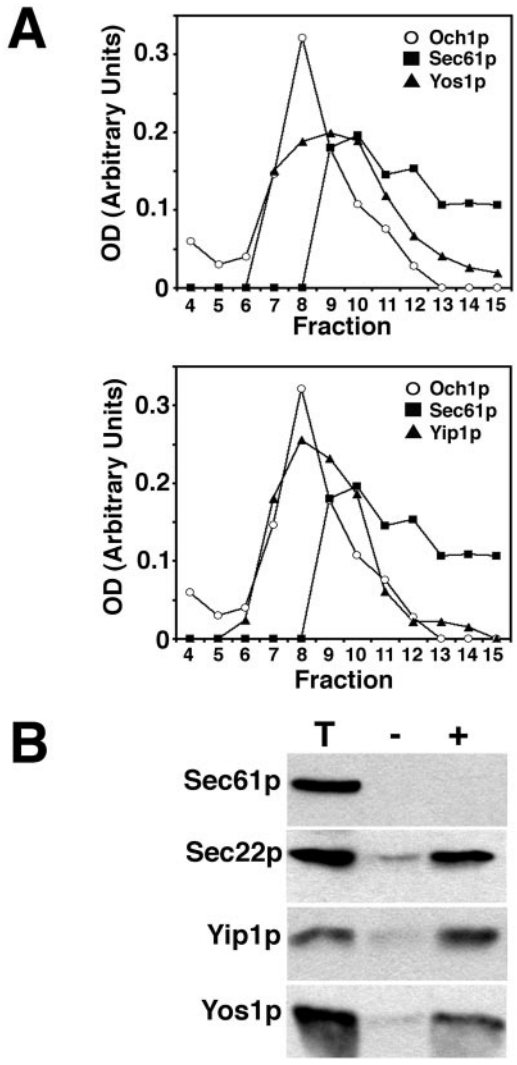

Figure 5. Yos1p localizes to the early secretory pathway. (A) Yos1p fractionates with ER and Golgi membranes. A whole cell lysate from wild-type cells was separated on a sucrose density gradient (20–60%), and fractions were collected from the top. Relative levels of Sec61p (ER marker), Och1p (Golgi marker), Yos1p, and Yip1p were quantified by densitometry of immunoblots. (B) Yos1p is packaged into COPII-coated vesicles. COPII-dependent budding reactions were performed from wild-type (FY834) microsomes. One-tenth of a total reaction (T) and budded vesicles isolated after a mock incubation (−) or after incubation with COPII proteins (+) were resolved on a 15% polyacrylamide gel. Sec61p (ER-resident protein), Sec22p (SNARE protein), Yip1p, and Yos1p were detected by immunoblot.

isolated and analyzed by immunoblotting. As shown in Figure 5B, Yos1p was packaged into vesicles in a COPII-dependent manner, at efficiencies similar to that of Yip1p and the SNARE protein Sec22p. The ER-resident protein Sec61p was not packaged into vesicles in the presence of COPII proteins, demonstrating the selective sorting in this budding assay. We conclude that Yos1p localizes to Golgi and ER membranes and is efficiently packaged into ER-derived COPII transport vesicles.

Yos1p Forms a Complex with Yip1p and Yif1p

Our localization studies indicate that Yos1p displays a similar subcellular distribution to that of Yip1p and Yif1p. In

addition, overproduction of Yos1p is able to rescue the growth defect of the *yip1-4* strain at the restrictive temperature. These observations suggest that Yos1p function is closely linked to that of Yip1p and therefore Yos1p may physically associate with the Yip1p–Yif1p complex. To test this hypothesis, we prepared microsomes from a strain expressing Yif1p-3×HA as the sole source of Yif1p in the cell. This COOH-terminally tagged version of Yif1p is under transcriptional control of the endogenous *YIF1* promoter and fully complements growth (Otte *et al*., 2001). Microsomes also were prepared from an untagged, isogenic wild-type strain to serve as a negative control. Microsomes from each strain were solubilized at room temperature with 0.5% digitonin. Solubilized extracts were harvested from each strain and subjected to immunoprecipitation with monoclonal anti-HA antibodies in the presence of protein A-Sepharose beads. As shown in Figure 6A, Western blotting revealed that Yif1p-3×HA could be efficiently immunoprecipitated from a solubilized extract generated from the tagged strain. Yip1p was enriched in the immunoprecipitated fraction, confirming the observation that Yip1p and Yif1p form a complex (Matern *et al*., 2000). Strikingly, Yos1p also was efficiently coimmunoprecipitated under these conditions, indicating that Yos1p was physically associated with the Yip1p–Yif1p complex. The integral membrane protein Sec61p and the SNARE protein Sec22p were not coimmunoprecipated with Yif1p-3×HA, demonstrating that the association of Yos1p with Yip1p–Yif1p was specific. We also analyzed the immunoprecipitates for the presence of Ypt1p but could not detect this protein in these fractions.

To further explore the observation that Yos1p physically interacts with Yip1p–Yif1p, we performed additional immunoprecipitation experiments. Specifically, a digitonin solubilized extract was generated from the *YIF1–3×HA* strain and subjected to immunoprecipitation with polyclonal antibodies directed against Yos1p. As shown in Figure 6B, Yos1p was efficiently immunoprecipitated. Both Yip1p and Yif1p-3×HA could be coimmunoprecipitated with Yos1p under these conditions, providing a second line of evidence for the association of Yos1p with Yip1p–Yif1p. Sec61p and Ypt1p were both absent from the immunoprecipitated fractions, confirming the specificity of these interactions. Densitometry analysis of the immunoblot in Figure 6A indicated that ~9% of the total Yif1p-3×HA was immunoprecipitated from the soluble extract, with ~5% of Yip1p and Yos1p coimmunoprecipitating. This observation suggests a stoichometric association of each subunit of the complex; however, further biochemical analysis of the complex will be required to prove this point. Together, these results demonstrate that Yos1p is physically associated with Yip1p–Yif1p and suggests that Yos1p is a novel subunit of the Yip1p–Yif1p complex. Furthermore, this observation is entirely consistent with the subcellular localization of Yos1p as well as the ability of Yos1p to function as a multicopy suppressor of the *yip1-4* strain.

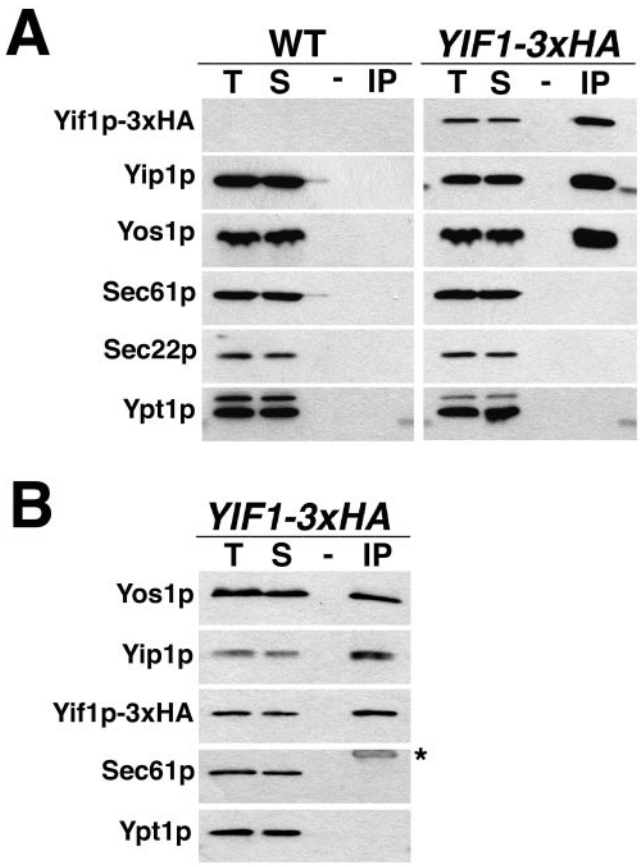

Figure 6. Yos1p forms a complex with Yip1p and Yif1p. Solubilized proteins were bound to anti-HA or anti-Yos1p antibodies coupled to protein A beads (IP) or beads alone (−) as described in *Materials and Methods*. (A) Immunoblots of native anti-HA immunoprecipitations from *YIF1–3×HA* and untagged (WT) solubilized microsomes. Total lanes (T) and solubilized (S) lanes represent 1.5% of the starting material. Note specific coimmunoprecipitation of Yip1p and Yos1p with Yif1p-3×HA. (B) Immunoblots of native anti-Yos1p immunoprecipitations from *YIF1-3×HA* microsomes. T and S lanes represent 1.5% of the starting material. Note the specific coimmunoprecipitation of Yip1p and Yif1p-3×HA with Yos1p. Asterisk indicates antibody heavy chain cross-reactivity with secondary antibodies.

Yos1p Interaction with Yip1p Is Disrupted in the yip1-4 Strain

We next analyzed the nature of complex formation between Yos1p and Yip1p in the *yip1-4* strain. We immunoprecipitated Yos1p from either wild-type or *yip1-4* digitonin solubilized membranes and monitored the amount of Yip1p and other proteins that coimmunoprecipitated. As seen in Figure 7, Yip1p efficiently coimmunoprecipitated with Yos1p from wild-type–soluble extracts. Interestingly, Yip1p was not coimmunoprecipated with Yos1p from *yip1-4* extracts. This observation suggests that complex formation between Yos1p and Yip1p in the *yip1-4* strain is disrupted or weakened.

Mutant yos1-1 Cells Display Transport Defects In Vivo and In Vitro

To further characterize Yos1p function in the early secretory pathway, we undertook a directed mutagenesis approach to generate thermosensitive alleles of *YOS1*. Conserved residues were mutated to either an alanine or to a residue of opposite property. These mutant versions of Yos1p were then expressed as the sole source of Yos1p by using a plasmid-shuffling approach. The results of the mutagenesis are shown in Table 2. Interestingly, mutation of the invariant asparagine at position 18 to alanine in the first transmembrane domain of Yos1p resulted in a strain that was thermosensitive for growth. We have termed this mutant allele *yos1-1*. The *yos1-1* cells grew near wild-type rates at 30°C but displayed a severe growth defect when shifted to 37°C. We examined the transport kinetics of secretory proteins in this strain compared with wild-type cells. A pulse-chase exper-

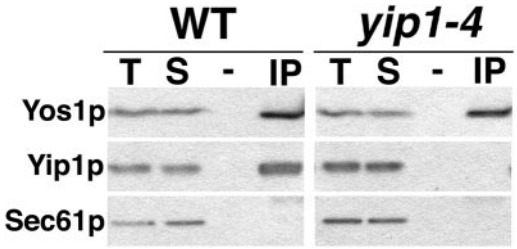

Figure 7. Yos1p interaction with Yip1p is disrupted in the *yip1-4* strain. Solubilized proteins were bound to anti-Yos1p antibodies coupled to protein A beads (IP) or beads alone (−) as described in *Materials and Methods*. Total lanes (T) and Solubilized (S) lanes represent 3% of the starting material. Note the specific coimmunoprecipitation of Yip1p with Yos1p from wild-type membranes, whereas this interaction is significantly reduced in the *yip1-4* strain.

iment was performed in which cells were grown in minimal media and pulsed for 7 min with [^{35}S]methionine to label newly synthesized proteins. Excess unlabeled methionine was added for the chase phase, and maturation of vacuolar CPY was monitored by immunoprecipitation with an anti-CPY antibody. CPY first occurs in the ER as the P1 precursor form of 67 kDa and is then modified in the Golgi to produce the P2 form of 69 kDa. Finally, CPY is processed in the vacuole to yield a mature form of 61 kDa (Stevens *et al.*, 1984). As seen in Figure 8A, the *yos1-1* strain exhibited a significant delay in the transport of CPY compared with wild-type cells, even at the permissive temperature of 25°C. Strikingly, the *yos1-1* strain exhibited a complete block in the transport of CPY at the restrictive temperature of 37°C. This observation further supports the argument that Yos1p is required for protein transport between the ER and the Golgi complex and is consistent with the phenotypes observed by *GAL1*-regulated depletion of Yos1p from cells.

We next characterized the *yos1-1* strain by using a reconstituted cell-free assay that measures protein transport to the Golgi complex. For this assay, washed semi-intact cell membranes containing [^{35}S]gpαf in the ER are incubated with purified factors (COPII, Uso1p, and LMA) to drive transport of [^{35}S]gpαf to the Golgi complex (Barlowe, 1997). On delivery to the Golgi complex, gpαf receives outer-chain α1,6-mannose residues that can be immunoprecipitated with α1,6-mannose–specific serum to quantify [^{35}S]gpαf transport (Baker *et al.*, 1988). As shown in Figure 8B, *yos1-1* membranes displayed a modest transport defect of [^{35}S]gpαf to the Golgi complex at 25°C. Specifically, the *yos1-1* membranes displayed a 30% reduction in [^{35}S]gpαf transport compared with wild-type membranes. A similar result was observed when these strains were compared at 29°C; there-

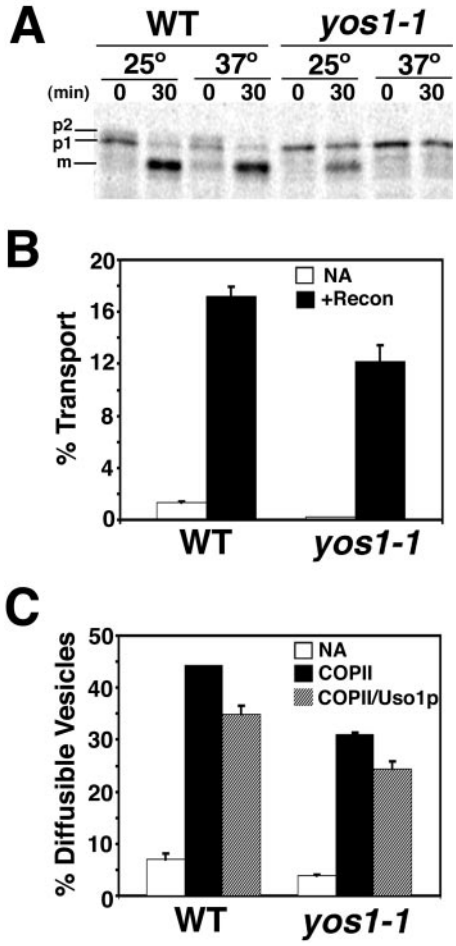

Figure 8. Mutant *yos1-1* cells display transport defects in vivo and in vitro. (A) Pulse-chase analysis of CPY maturation in wild-type and *yos1-1* strains. Wild-type (CBY1766) and *yos1-1* (CBY1822) strains were pulsed for 7 min with ^{35}S-labeled methionine and then chased for 30 min. Labeled CPY was immunoprecipitated from cell extracts, resolved on an 8% acrylamide gel, and visualized by autoradiography. (B) Washed semi-intact cells containing [^{35}S]gpαf were prepared from wild-type (CBY1766) and *yos1-1* (CBY1822) strains. Semi-intact cells were incubated with COPII proteins, Uso1p, LMA1, and an ATP regeneration system. After 80 min at 25°C, the amount of Golgi-modified [^{35}S]gpαf was measured to determine transport efficiency. (C) Semi-intact cells from wild-type or mutant strains were prepared as in A and incubated with COPII or COPII plus Uso1p to measure vesicle budding and tethering. After 30 min at 25°C, freely diffusible vesicles containing [^{35}S]gpαf were separated from semi-intact cell membranes by centrifugation at 18,000 × *g* and [^{35}S]gpαf quantified by concanavalin A precipitation.

Table 2. *YOS1* mutagenesis

Mutation	Growth (YPD)[a]
N18A	Conditional
E25A	Wild type
E26A	Wild type
R27A	Wild type
F28A	Wild type
F28S	Wild type
L29E	Wild type
R30A	Wild type
K53A	Wild type
L59S	Wild type
T65A	Wild type
P71A	Lethal
L72S	Wild type
I73N	Wild type
N76A	Wild type

[a] Growth phenotype of haploid cells. CBY1781 was transformed with different pRS314-*YOS1* alleles. Transformants were passaged on YMD-Trp + 5-FOA to eliminate the wild-type *YOS1* plasmid, and surviving cells were tested for growth at various temperatures.

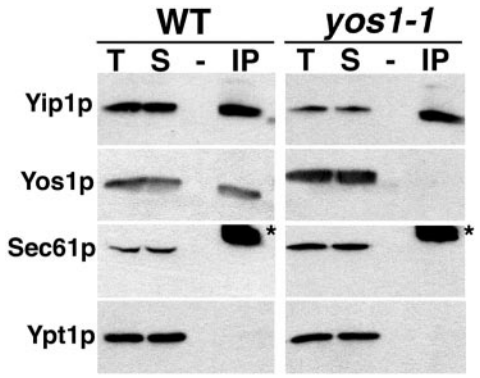

Figure 9. Yip1p interaction with Yos1p is disrupted in the *yos1-1* strain. Solubilized proteins were bound to anti-Yip1p antibodies coupled to protein A beads (IP) or beads alone (−) as described in *Materials and Methods*. Total lanes (T) and Solubilized (S) lanes represent 3% of the starting material. Note the specific coimmunoprecipitation of Yos1p with Yip1p from wild-type membranes, whereas this interaction is absent in the *yos1-1* strain. Asterisks indicate antibody heavy chain cross-reactivity with secondary antibodies.

fore, we were unable to replicate the thermosensitivity of the *yos1-1* allele in vitro (our unpublished data).

Subreactions in cell-free transport can be monitored by following the sedimentation properties of membranes containing [^{35}S]gpαf (Barlowe, 1997). Incubation of washed semi-intact cell membranes with purified COPII proteins catalyzes the formation of diffusible vesicles that can be separated from larger membranes by centrifugation. When purified Uso1p is included in this reaction, a fraction of the diffusible vesicles now pellet with heavier membranes, allowing a measurement of vesicle tethering. We examined the ability of the *yos1-1* strain to bud and tether COPII vesicles to identify the stage at which transport was compromised (Figure 8C). On the addition of COPII proteins, wild-type membranes budded vesicles at an efficiency of ~44%, whereas the *yos1-1* membranes budded vesicles at an efficiency of only ~31%. When the tethering factor Uso1p was included in these reactions, both wild-type and *yos1-1* membranes tethered ~23% of the diffusible vesicles. These results indicate that the *yos1-1* membranes can effectively tether COPII vesicles to the Golgi complex but are compromised in their ability to bud COPII vesicles from ER membranes.

Yip1p Interaction with Yos1p Is Disrupted in the yos1-1 Strain

We next sought to characterize the interaction of Yip1p with Yos1p in the *yos1-1* strain. Yip1p was immunoprecipitated from either wild-type or *yos1-1* digitonin-solubilized membranes. The amount of Yos1p and other proteins that was coimmunoprecipitated was then monitored by immunoblotting. As shown in Figure 9, Yos1p efficiently coimmunoprecipitated with Yip1p from wild-type soluble extracts. In contrast, Yos1p was not efficiently coimmunoprecipitated with Yip1p from *yos1-1* extracts. This result indicates that the interaction between Yip1p and Yos1p in the *yos1-1* strain is destabilized or disrupted. This result is consistent with the observation that the interaction between Yip1p and Yos1p also seems to be disrupted in the *yip1-4* strain (Figure 7).

Mutant yos1-1 Cells Display Morphological Phenotypes Characteristic of a Block in the Early Secretory Pathway

We further analyzed the *yos1-1* strain by thin-section EM to characterize the morphology of any transport intermediates that may accumulate at the restrictive temperature. As shown in Figure 10, *yos1-1* cells displayed morphological phenotypes consistent with a block in the early secretory pathway upon shift to 37°C for 60 min. Specifically, the *yos1-1* cells displayed tubulation and/or dislocation of ER membranes from the cell periphery (Figure 10, B and C, white arrows). In addition, clusters of vesicular or tubular elements were sometimes observed (Figure 10D, white arrow). Similar alterations in membrane organization were observed in the *yos1-1* strain after a 30-min shift to 37°C. These morphological phenotypes are consistent with a requirement for Yos1p function in the early secretory pathway.

DISCUSSION

Yip1p was originally identified as a protein factor that interacts with the Rab GTPases Ypt1p and Ypt31p by using yeast two-hybrid screens (Yang *et al*., 1998). Additional studies showed Yip1p forms a complex with a related integral membrane protein termed Yif1p (Matern *et al*., 2000). Like Yip1p, Yif1p also displays the ability to bind Rab GTPases in the two-hybrid system. These observations coupled with the localization of Yip1p–Yif1p to the early secretory pathway led to the suggestion that this complex may function as an integral membrane receptor for Rab GTPases (Yang *et al*., 1998; Matern *et al*., 2000). More recent studies using both two-hybrid and biochemical approaches have suggested Yip1p and Yif1p are able to bind Rab proteins in a more promiscuous manner (Calero *et al*., 2002; Calero *et al*., 2003). The interaction of Yip1p with Rabs in the two-hybrid system is dependent on an intact double prenylation motif contained at the COOH termini of Rabs (Calero *et al*., 2003). Our study of Yip1p function revealed a critical role for Yip1p in the biogenesis of COPII transport vesicles from ER membranes (Heidtman *et al*., 2003). Because Ypt1p does not seem to be required for COPII vesicle budding in vivo and in vitro (Becker *et al*., 1991; Segev, 1991; Rexach and Schekman, 1991; Cao and Barlowe, 2000), these data suggest that Yip1p function may be independent of Ypt1p function. It is also possible that Yip1p has dual functions in both COPII vesicle formation and in modulation of Rab activity during membrane fusion (Chen *et al*., 2004).

In this report, we used a multicopy suppressor screen to identify genes that could rescue the thermosensitive growth defect of the *yip1-4* strain. The *yip1-4* allele results from an E70K point mutation in the hydrophilic NH_2 terminus of Yip1p (Calero *et al*., 2003). This strain exhibits a severe growth defect at the restrictive temperature of 34°C on rich media with few revertants, making this strain ideal for genetic studies. Our screen identified the uncharacterized ORF YER074W-A as a novel multicopy suppressor of the *yip1-4* allele. We have termed this gene *YOS1*. Interestingly, multicopy *YOS1* is unable to suppress the previously described thermosensitive *yip1-1*, *yip1-2*, *yif1-2*, and *yif1-4* strains (Yang *et al*., 1998; Matern *et al*., 2000).

YOS1 encodes a conserved protein of 87 amino acids with a predicted molecular mass of 9.5 kDa. We show that *YOS1* is an essential gene in yeast, and Yos1p depletion results in the accumulation of the proforms of secretory proteins. In addition, we show that Yos1p localizes to ER and Golgi membranes as well as ER-derived COPII transport vesicles.

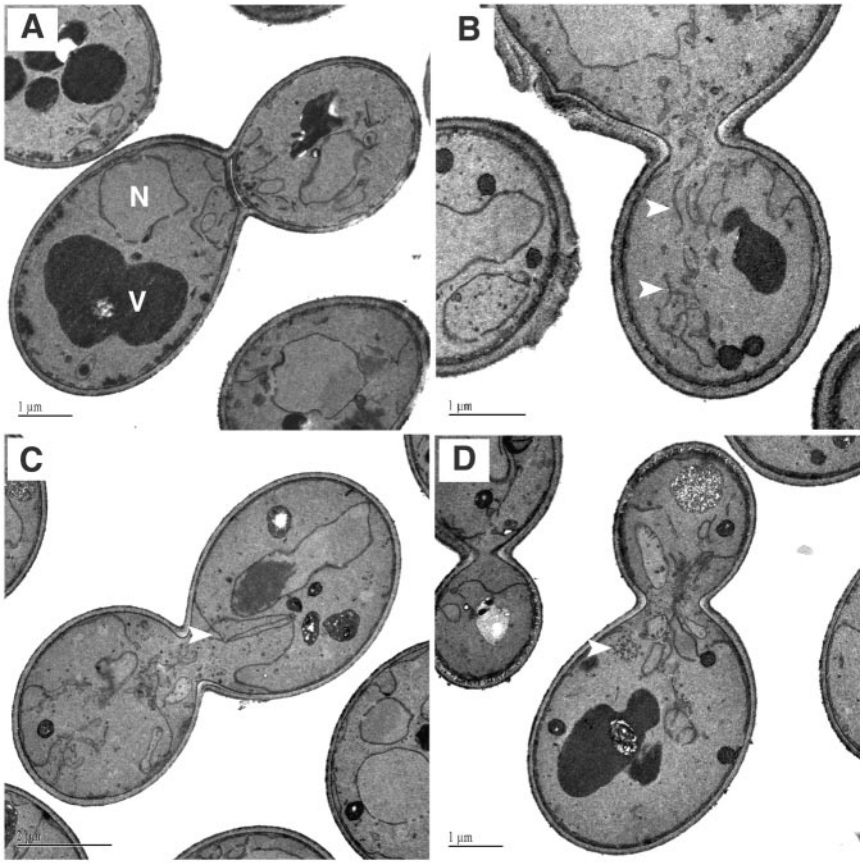

Figure 10. Mutant *yos1-1* cells display morphological phenotypes characteristic of a block in the early secretory pathway. Wild-type (A) and mutant cells (B–D) were shifted to 37°C for 60 min and then fixed and prepared for EM as described in *Materials and Methods*. Representative thin sections are shown for each condition. The white lettering in A denotes the nucleus (N) and vacuole (V). The white arrows in B and C highlight dislocated tubular ER elements, whereas the white arrow in D highlights a cluster of vesicular structures. Bars, 1 μm (A, B, and D) and 2 μm (C).

This pattern of localization is very similar to that of Yip1p and indicates that Yos1p cycles between ER and Golgi compartments. Importantly, we also demonstrate that Yos1p physically associates with Yip1p and Yif1p, indicating that Yos1p is a novel subunit of the Yip1p–Yif1p complex. This interaction was disrupted in the *yip1-4* strain, suggesting the NH_2 terminus of Yip1p may in some way regulate the interaction of Yip1p with Yos1p.

Our previous studies indicated that inhibition of Yip1p function blocks the formation of COPII vesicles from ER membranes. Consistent with these observations, pulse-chase analysis of the thermosensitive *yos1-1* strain showed a severe block in the transport of CPY at the restrictive temperature. Furthermore, membranes prepared from the *yos1-1* strain exhibited transport defects most specific to the COPII budding stage in a cell-free transport assay. EM analysis of the *yos1-1* strain at the restrictive temperature revealed tubulation and dislocation of ER membranes from the cell periphery. In addition, accumulation of vesicular structures or tubular elements was sometimes observed. These structures may represent transport vesicles or fragmented ER membranes. These phenotypes are similar to those reported for the *yif1-1* thermosensitive strain (Matern *et al*., 2000). Interestingly, these morphological phenotypes are somewhat distinct from that of the *yip1-4* strain at the restrictive temperature, which displays massive proliferation of ER membranes but no accumulation of vesicles (Heidtman *et al*., 2003). These observations may indicate that Yif1p and Yos1p function downstream of Yip1p to fulfill a critical function at the Golgi complex. Alternatively, the moderate accumulation of vesicular structures seen in the *yif1-1* and *yos1-1* strains may indicate an additional role for these proteins in retrograde transport. Thermosensitive mutations in genes involved in retrograde trafficking have been reported to result in an intermediate vesicle accumulation phenotype (Kaiser and Schekman, 1990; Sweet and Pelham, 1993).

The thermosensitivity of the *yos1-1* strain was caused by an N18A point mutation in the first predicted transmembrane domain of Yos1p. Western blot analysis of this strain revealed that Yos1-1p was stably expressed at both the permissive and restrictive temperatures (our unpublished data). These observations suggest that this mutant form of Yos1p is stably integrated into membranes but is defective in some other aspect of protein function. One possibility is that the N18A point mutation prevents efficient interaction of Yos1p with Yip1p–Yif1p via their respective transmembrane domains. In support of this idea, we observed that Yip1p interaction with Yos1p was disrupted in the *yos1-1* strain.

What is the nature of the Yos1p–Yip1p–Yif1p complex in terms of assembly and stability? To address this question, it may be useful to consider other characterized heteromeric protein complexes localized to the early secretory pathway. For example, yeast oligosaccharyltransferase (OST) is a conserved heteromeric complex that catalyzes the initial stages of N-linked protein glycosylation in the lumen of the ER (Dempski and Imperiali, 2002). Mutation in certain subunits of OST results in decreased stability of other associated subunits, indicating that at least some of the OST subunits depend on each other for stability (Silberstein *et al*., 1995). It is unclear whether individual subunits of the Yos1p–Yip1p–Yif1p complex are directly required for stability of other subunits. A

recent study showed that *GAL1* regulated depletion of Yip1p from cells resulted in decreased levels of Yif1p (Barrowman *et al*., 2003); however, this may be due to a general secretory block. We have observed that a reduction of Yip1p or Yif1p by using the conditional *yip1-1*, *yip1-2*, *yif1-2*, or *yif1-4* alleles (Yang *et al*., 1998; Matern *et al*., 2000) did not produce a decreased level of the reciprocal subunit (our unpublished data). This result suggests that the expression levels of Yos1p, Yip1p, and Yif1p are not interdependent. Moreover, we find that Yos1p, Yip1p and Yif1p can be individually overexpressed severalfold (our unpublished data), supporting the idea that subunits of the complex do not depend on each other for stability. These expression behaviors are more reminiscent of SNARE protein complexes in which subunits of the SNARE complex undergo cycles of assembly and disassembly in catalysis of membrane fusion (Bonifacino and Glick, 2004). Therefore, the Yos1p–Yip1p–Yif1p complex may cycle through assembly/disassembly states in temporal and spatial alignment with biochemical reactions required for ER/Golgi transport.

Interestingly, Yos1p interaction with Yip1p is disrupted in the *yip1-4* strain. The thermosensitivity of the *yip1-4* strain is the result of an E70K point mutation in the cytosolic NH_2 terminus of Yip1p. Therefore, the NH_2 terminus of Yip1p may regulate the association of Yip1p with Yos1p, perhaps in response to a signal or factors present in the cytosol. This idea may provide an explanation for the finding that Yos1p is a multicopy suppressor of the *yip1-4* strain. Specifically, overexpression of Yos1p may stimulate complex assembly in the absence of a proper signal, thus providing a minimal level of complex to allow growth at the restrictive temperature. Biochemical purification of the Yos1p–Yip1p–Yif1p complex will be required to determine composition, stoichiometry, and size of the complex.

What is the function of the Yos1p–Yip1p–Yif1p complex in the cell? The Yip1p–Yif1p complex was originally proposed to function as a receptor for the recruitment of Ypt1p to Golgi membranes (Yang *et al*., 1998; Matern *et al*., 2000). A recent study provided evidence of this type of activity for the human protein PRA-1, the human homologue of the yeast protein Yip3p (Sivars *et al*., 2003). In this study, the authors showed that PRA-1/Yip3 acts catalytically to dissociate complexes of endosomal Rab9 bound to GDI for Rab9 recruitment onto membranes. Although Yip3p shares a similar predicted membrane topology with Yip1p, Yip3p has a limited primary sequence relationship to Yip1p or Yif1p and therefore may be a member of a separate family of factors that act in intracellular trafficking. Depletion of Yip1p from yeast cells did not seem to effect the membrane binding or localization of Ypt1p (Barrowman *et al*., 2003), although the subcellular distribution of GFP-Ypt1p was altered in *yip1-4* strains at a restrictive temperature (Calero *et al*., 2003). In light of these observations, it is possible that the action of the Yos1p–Yip1p–Yif1p complex may be restricted to the budding stage of transport. A cycling heteromeric complex of Yos1p–Yip1p–Yif1p could act at both ER and Golgi membranes to promote COPII and COPI vesicle biogenesis, perhaps in promoting vesicle scission. Alternatively, Yip1p and associated proteins may interact with Ypt1p and other Rab GTPases to coordinate the budding and tethering stages of intracellular transport. Identification of Yos1p as a new member of the Yip1p–Yif1p complex will allow us to perform more defined biochemical and morphological experiments to understand the precise function of the Yos1p–Yip1p–Yif1p complex in eukaryotic cells.

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REFERENCES

Ausubel, R. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A., and Struhl, K. (1987). Current Protocols in Molecular Biology, New York, Greene Publishing Associates and Wiley-Interscience.

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

Barlowe, C. (1997). Coupled ER to Golgi transport reconstituted with purified cytosolic proteins. J. Cell Biol. *139*, 1097–1108.

Barrowman, J., Wang, W., Zhang, Y., and Ferro-Novick, S. (2003). The Yip1p·Yif1p complex is required for the fusion competence of endoplasmic reticulum-derived vesicles. J. Biol. Chem. *278*, 19878–19884.

Becker, J., Tan, T. J., Trepte, H. H., and Gallwitz, D. (1991). Mutational analysis of the putative effector domain of the GTP-binding protein Ypt1p in yeast suggests specific regulation by a novel GAP activity. EMBO J. *10*, 785–792.

Belden, W. J., and Barlowe, C. (1996). Erv25p, a component of COPII-coated vesicles, forms a complex with Emp24p that is required for efficient endoplasmic reticulum to Golgi transport. J. Biol. Chem. *271*, 26939–26946.

Blandin, G., *et al*. (2000). Genomic exploration of the hemiascomycetous yeasts: 4. The genome of *Saccharomyces cerevisiae* revisited. FEBS Lett. *487*, 31–36.

Bonifacino, J. S., and Glick, B. S. (2004). The mechanisms of vesicle budding and fusion. Cell *116*, 153–166.

Calero, M., Chen, C. Z., Zhu, W., Winand, N., Havas, K. A., Gilbert, P. M., Burd, C. G., and Collins, R. N. (2003). Dual prenylation is required for Rab protein localization and function. Mol. Biol. Cell *14*, 1852–1867.

Calero, M., Winand, N. J., and Collins, R. N. (2002). Identification of the novel proteins Yip4p and Yip5p as Rab GTPase interacting factors. FEBS Lett. *515*, 89–98.

Cao, X., Ballew, N., and Barlowe, C. (1998). Initial docking of ER-derived vesicles requires Uso1p and Ypt1p but is independent of SNARE proteins. EMBO J. *17*, 2156–2165.

Cao, X., and Barlowe, C. (2000). Asymmetric requirements for a Rab GTPase and SNARE proteins in the fusion of COPII vesicles with acceptor membranes. J. Cell Biol. *149*, 55–65.

Carlson, M., and Botstein, D. (1982). Two differentially regulated mRNAs with different 5′ ends encode secreted and intracellular forms of yeast invertase. Cell *28*, 145–154.

Chen, C. Z., Calero, M., DeRegis, C. J., Heidtman, M., Barlowe, C., and Collins, R. N. (2004). Genetic analysis of Yip1p function reveals a requirement for Golgi-localized Rab proteins and Rab-GDI. Genetics *168*, 1827–1841.

Dempski, R. E., and Imperiali, B. (2002). Oligosaccharyltransferase: gatekeeper to the secretory pathway. Curr. Opin. Chem. Biol. *6*, 844–850.

Heidtman, M., Chen, C. Z., Collins, R. N., and Barlowe, C. (2003). A role for Yip1p in COPII vesicle biogenesis. J. Cell Biol. *163*, 57–69.

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

Kuehn, M. J., Herrmann, J. M., and Schekman, R. (1998). COPII-cargo interactions direct protein sorting into ER-derived transport vesicles. Nature *391*, 187–190.

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

Liu, Y., and Barlowe, C. (2002). Analysis of Sec22p in endoplasmic reticulum/Golgi transport reveals cellular redundancy in SNARE protein function. Mol. Biol. Cell *13*, 3314–3324.

Longtine, M. S., McKenzie III, A., Demarini, D. J., Shah, N. G., Wach, A., Brachat, A., Phillipsen, P., and Pringle, J. R. (1998). Additional modules for versatile and economical PCR-based gene deletion and modification in *Saccharomyces cerevisiae*. Yeast *14*, 953–961.

Matern, H., Yang, X., Andrulis, E., Sternglanz, R., Trepte, H. H., and Gallwitz, D. (2000). A novel Golgi membrane protein is part of a GTPase-binding protein complex involved in vesicle targeting. EMBO J. *19*, 4485–4492.

Otte, S., and Barlowe, C. (2002). The Erv41p-Erv46p complex: multiple export signals are required *in trans* for COPII-dependent transport from the ER. EMBO J. *21*, 6095–6104.

Otte, S., Belden, W. J., Heidtman, M., Liu, J., Jensen, O. N., and Barlowe, C. (2001). Erv41p and Erv46p: new components of COPII vesicles involved in transport between the ER and the Golgi complex. J. Cell Biol. *152*, 503–517.

Powers, J., and Barlowe, C. (1998). Transport of Axl2p depends on Erv14p, an ER-vesicle protein related to the *Drosophila cornichon* gene product. J. Cell Biol. *142*, 1209–1222.

Rexach, M., and Schekman, R. (1991). Distinct biochemical requirements for the budding, targeting, and fusion of ER-derived transport vesicles. J. Cell Biol. *114*, 219–229.

Segev, N. (1991). Mediation of the attachment or fusion step in vesicular transport by the GTP-binding Ypt1 protein. Science *252*, 1553–1556.

Shakoori, A., Fujii, G., Yoshimura, S., Kitamura, M., Nakayama, K., Ito, T., Ohno, H., and Nakamura, N. (2003). Identification of a five-pass transmembrane protein family localizing in the Golgi apparatus and the ER. Biochem. Biophys. Res. Commun. *312*, 850–857.

Sherman, F. (1991). Getting started with yeast. Methods Enzymol. *194*, 3–20.

Silberstein, S., Collins, P. G., Kelleher, D. J., and Gilmore, R. (1995). The essential OST2 gene encodes the 16-kD subunit of the yeast oligosaccharyltransferase, a highly conserved protein expressed in diverse eukaryotic organisms. J. Cell Biol. *131*, 371–383.

Sivars, U., Aivazian, D., and Pfeffer, S. R. (2003). Yip3 catalyses the dissociation of endosomal Rab-Gdi complexes. Nature *423*, 856–859.

Stevens, T., Esmon, B., and Schekman, R. (1984). Early stages in the yeast secretory pathway are required for transport of carboxypeptidase Y to the vacuole. Cell *30*, 439–448.

Sweet, D. J., and Pelham, H. R. (1993). The TIP1 gene of Saccharomyces cerevisiae encodes and 80 kDa cytoplasmic protein that interacts with the cytoplasmic domain of Sec20p. EMBO J. *12*, 2831–2840.

Tang, B. L., Ong, Y. S., Huang, B., Wei, S., Wong, E. S., Qi, R., Horstmann, H., and Hong, W. (2001). A membrane protein enriched in endoplasmic reticulum exit sites interacts with COPII. J. Biol. Chem. *276*, 40008–40017.

Yang, X., Matern, H. T., and Gallwitz, D. (1998). Specific binding to a novel and essential Golgi membrane protein (Yip1p) functionally links the transport GTPases Ypt1p and Ypt31p. EMBO J. *17*, 4954–4963.