Abstract. Uso1p, a *Saccharomyces cerevisiae* protein required for ER to Golgi transport, is homologous to the mammalian intra-Golgi transport factor p115. We have used genetic and biochemical approaches to examine the function of Uso1p. The temperature-sensitive phenotype of the *uso1-1* mutant can be suppressed by overexpression of each of the known ER to Golgi v-SNAREs (Bet1p, Bos1p, Sec22p, and Ykt6p). Overexpression of two of them, Bet1p and Sec22p, can also suppress the lethality of *Δusol*, indicating that the SNAREs function downstream of Uso1p. In addition, overexpression of the small GTP-binding protein Ypt1p, or of a gain of function mutant (SLY1-20) of the t-SNARE associated protein Sly1p, also confers temperature resistance. Uso1p and Ypt1p appear to function in the same process because they have a similar set of genetic interactions with the v-SNARE genes, they exhibit a synthetic lethal interaction, and they are able to suppress temperature sensitive mutants of one another when overexpressed. Uso1p acts upstream of, or in conjunction with, Ypt1p because overexpression of Ypt1p allows a *Δusol* strain to grow, whereas overexpression of Uso1p does not suppress a *Δypt1* strain. Finally, biochemical analysis indicates that Uso1p, like Ypt1p, is required for assembly of the v-SNARE/t-SNARE complex. The implications of these findings, with respect to the mechanism of vesicle docking, are discussed.

In eukaryotic cells, newly synthesized proteins destined for secretion, or residence in organelles of the secretory pathway, must enter and transit the secretory pathway for their proper processing and targeting. This pathway consists of a number of membrane-bounded organelles, including the ER and the Golgi apparatus. The proteins are transported between these organelles via small membrane-bounded secretory vesicles (Palade, 1975), which bud from the donor membrane and are targeted to the acceptor membrane where they fuse (Orci et al., 1989). Since proper functioning of the secretory pathway is crucial for normal cell function, transport between the various compartments must be tightly regulated to ensure that vesicles fuse only with the appropriate membrane.

Both biochemical and genetic approaches have led to the identification of numerous components of the molecular machinery that mediate transport. Utilization of an in vitro intra-Golgi transport assay, which reconstitutes *cis* to *medial* Golgi transport, has allowed the purification of several cytosolic transport factors. These include N-ethylmaleimide sensitive factor (NSF) (Block et al., 1988) and α, β, and γ soluble NSF attachment protein (SNAP) (Clary and Rothman, 1990). The yeast homologues of NSF and α-SNAP, namely Sec18p and Sec17p, were identified genetically (Novick et al., 1980; Eakle et al., 1988; Wilson et al., 1989; Griff et al., 1992). Together, NSF, and SNAPs, which are required for the binding of NSF to the Golgi membrane (Clary et al., 1990; Weidman et al., 1989), are considered part of the general fusion apparatus (Wilson et al., 1992), which functions at several transport steps, including endosome–endosome fusion (Diaz et al., 1989), vacuolar sorting (Graham and Emr, 1991), transcytosis (Sztul et al., 1993), and synaptic vesicle fusion (Söllner et al., 1993).

Recently the molecular basis of vesicle targeting and fusion has been further illuminated. A set of integral membrane proteins, known to reside in the neuronal presynaptic terminal, was shown to function as a receptor for α-SNAP (Söllner et al., 1993b). This result led to the formulation of the SNAP receptor (SNARE) hypothesis, which posits that the fidelity of vesicular transport is dependent on the presence of specific molecules on the vesicle (the v-SNARE) and on the target membrane (the t-SNARE). According to this model, the SNARE molecules physically interact with one another in a specific manner, thereby assuring that a given vesicle can only fuse with the appropriate target membrane. The binding of the SNAPs and NSF to the complexed v- and t-SNAREs results in a fusion-competent complex, called the SNARE complex (Aalto et al., 1993; Söllner et al., 1993a,b; Rothman and Warren, 1994).
SNAREs also function in the yeast secretory pathway (Dascher et al., 1991; Hardwick and Pelham, 1992; Aalto et al., 1993; Protopenov et al., 1993), and recently have been shown to enter into complexes analogous to those in neurons (Søgaard et al., 1994). In the ER to Golgi leg of the yeast secretory pathway, this complex includes the putative v-SNAREs Bet1p, Bos1p, Sec22p, and Ykt6p, the t-SNARE–Sec5p, the t-SNARE–associated protein Sly1p, and several other proteins (Søgaard et al., 1994). Although the small GTP-binding protein Ypt1p appears to impact on v-SNARE/t-SNARE complex formation (Lian et al., 1994; Søgaard et al., 1994), it has not been found in the SNARE complex (Søgaard et al., 1994).

An in vitro intra-Golgi transport assay that was designed to be dependent on high molecular weight proteins was used to identify and purify an additional protein, called p115, that is involved in vesicular transport (Waters et al., 1992). p115 is a peripheral membrane protein localized predominantly to the Golgi apparatus (Waters et al., 1992). p115 has also been identified as a component of transcytotic vesicles and thus was termed transcytosis-associated protein (TAP) (Szul et al., 1993). Recently, it was demonstrated that p115 is required in vitro, along with NSF and SNAPs, for the reassembly of post-mitotic Golgi fragments into Golgi cisternae (Rabouille et al., 1995).

Biochemical characterization and electron microscopy indicated that p115 exists as a parallel homo-dimer, with two globular “heads” and an extended rod-like “tail” domain (Waters et al., 1992; Sapperstein et al., 1995). Analysis of the p115 cDNA (Barroso et al., 1995; Sapperstein et al., 1995), which encodes a 108-kD protein, revealed that the “head” comprises approximately the amino-terminal two-thirds of the molecule. The “tail” comprises an approximately 250 residue coiled-coil domain followed by a small highly acidic domain at the extreme COOH terminus.

p115 is homologous to Uso1p (Barroso et al., 1995; Sapperstein et al., 1995), a yeast protein required for ER to Golgi transport (Nakajima et al., 1991). The two proteins share an overall “head-tail-acid” structural organization. Interestingly, the 206-kD Uso1p is significantly larger than p115, with a much longer coiled-coil dimerization domain accounting for most of its additional mass. In addition to their structural similarity, the two proteins share three regions of significant homology at the amino acid level: two regions in the head domain are more than 60% identical, and a third region just after the end of the predicted coiled-coil domain is 25% identical.

Several lines of evidence suggest that p115 and Uso1p function in the docking step of vesicular transport. First, the cell-free intra-Golgi transport assay used to purify p115 (Waters et al., 1992) measures only the docking and fusion stages of the vesicular transport cycle, not the formation of vesicles (Elazar et al., 1994). Second, p115 was shown to be required for the binding of transcytotic vesicles to the plasma membrane at a step before the ATP-dependent step of the transport cycle (Barroso et al., 1995). Finally, Uso1p is required in vitro for the targeting and fusion of ER-derived vesicles with purified Golgi membranes, and like p115, also functions at a step before ATP hydrolysis (Lupashin et al., 1996).

We have used genetic and biochemical approaches to further dissect the function of Uso1p in yeast vesicular transport. Multiply-suppressing suppressor analysis has revealed that Uso1p is similar to Ypt1p with respect to its genetic interactions with components of the vesicular targeting apparatus. Furthermore, we found that Uso1p, like Ypt1p, is required for assembly of the ER to Golgi v-SNARE/t-SNARE complex in vivo.

**Materials and Methods**

**Reagents**

Oligonucleotide primers were synthesized at the Princeton University Synthesis/Sequencing facility. Zymolyase 20T was obtained from Seikagaku Kogyo Co. (Tokyo, Japan), DNA modifying enzymes and restriction endonucleases were obtained from New England Biolabs (Beverly, MA), Tran35S-Label was from ICN Radiochemicals (Irvine, CA), protein A-Sepharose was obtained from Pharmacia LKB Nuclear (Piscataway, NJ), and 0.45-mm glass beads were from Thomas Scientific (Swedesboro, NJ).

Antiserum against Sed5p and Boslp were affinity purified as previously described (Søgaard et al., 1994) using anti-Sed5p and anti-Boslp sera and the corresponding recombinant proteins (generous gifts from M. Søgaard and J. Rothman, Sloan Kettering Cancer Center), except that antibodies were coupled to protein A-Sepharose instead of Protein G beads. Antiserum against Bet1p and Sec22p were obtained from R. Schekman, and the anti-

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**Table 1. Yeast Strains Used in This Study**

| Strain | Genotype | Source |
|--------|----------|--------|
| MY2788 | MAT a leu2Δ1 trp1-Δ63 ura3-52 | M. Rose |
| MY2789 | MAT a leu2Δ1 trp1-Δ63 ura3-52 | M. Rose |
| GGY30  | MAT a leu2Δ1 trp1-Δ63 ura3-52 | This study |
| GGY32  | MAT a leu2Δ1 trp1-Δ63 ura3-52 | This study |
| GGY33  | MAT a leu2Δ1 trp1-Δ63 ura3-52 | This study |
| GGY76  | MAT a leu2Δ1 trp1-Δ63 ura3-52 | This study |
| GFU16D | MAT a GAL10-YPT1::HIS3 leu3-112 trp1-Δ63 ura3-52 | H.D. Schmitt |
| RSY255 | MAT a sec32-1 his3-112 trp1-Δ63 ura3-52 | R. Schekman |
| RSY271 | MAT a sec32-1 his3-112 trp1-Δ63 ura3-52 | R. Schekman |
| RSY942 | MAT a sec32-1 his3-112 trp1-Δ63 ura3-52 | R. Schekman |
| RSY944 | MAT a sec32-1 his3-112 trp1-Δ63 ura3-52 | R. Schekman |
| RSY954 | MAT a sec32-1 his3-112 trp1-Δ63 ura3-52 | R. Schekman |
| RSY976 | MAT a sec32-1 his3-112 trp1-Δ63 ura3-52 | R. Schekman |
| GGY67  | MAT a sec32-1 his3-112 trp1-Δ63 ura3-52 | This study |
| GGY71  | MAT a sec32-1 his3-112 trp1-Δ63 ura3-52 | This study |

*usol-l* is a version of the original usol-l* mutation (Nakajima et al., 1991; Seog et al., 1994) that contains three consecutive termination codons (amber, ochre, and opal) rather than the single amber mutation. *The SEC32 gene is likely to be identical to BOS1* (Wuestebebe et al., 1995).
carboxypeptidase Y (CPY) antisera were obtained from S. Emr. HRP-conjugated secondary antibodies were obtained from Bio-Rad Labs (Her- 
cules, CA).

**Strains, Plasmids, and Media**

*S. cerevisiae* strains used in this study are listed in Table I. Yeast media were prepared as described (Sherman, 1991). The *Escherichia coli* strain XL1-Blue [supE44 thi-1 recA1 gyrA96 hsdR17 relA1 (F'proAB lacZAM15 Tn10) (Strategene Inc., La Jolla, CA)] was used for all routine manipulations; BMB171-18 [dua supE3 lac-proAB (hns:-Tn10) (F' proAB lacZAM15)] (Kramer et al., 1984) was used in site-directed mutagenesis procedures where required, and MC1066 [pCP8030 leu8 ara- 
pyrF74-Tn5 (Karplus)] (Casadaban et al., 1983) was used to select ligation products bearing the *LEU2* gene. Bacterial strains were grown on stan-
ard media (Miller, 1972).

The plasmids used in this study were as follows: pSN2d (2 µm BETI 
URA3), pMG18 (2 µm SEC22A), pAN109 (2 µm SEC7 URA3), 
pNB167 (2 µm YPT1 URA3; Bacon et al., 1989) (from S. Ferro-Novic 
Yale University School of Medicine); pANY2-7 (2 µm SARI URA3; 
(d’Enfert et al., 1991]), pSEC7 (2 µm SEC7 URA3), pARFI (2 µm AF 
ARFI URA3), and pSEC21 (2 µm SEC21 URA3) (from R. Schekman, Un 
cerity of California, Berkeley, CA); pYEpSN1 (2 µm SN1 URA2) 
and pYEpSN2 (2 µm SN2 URA2) and pYEpSC2 (2 µm SNC2 LEU2) 
(from J. Gerst, Weizmann Institute of Science, Rehovot, Israel); 
pSEC7 (2 µm SEC7 URA3) and pYEpSFT1 (2 µm SFT1 URA3) (from 
H. Petham, MRC, Cambridge, England); pSEC62 (2 µm SEC62 URA3) 
(from S. Emr, University of California, San Diego, CA); 
pSEC17 (2 µm SEC17 URA3) (from C. Kaiser, MIT); and pYCP50-SLY1- 
20 (CEN SLY1-20 URA3), pSLY1 (2 µm SLY1 URA3), pSLY4 (2 µm 
SLY4 URA3). All pSK plasmids used in this study are described below.

**Microbial Techniques**

Genetic techniques were essentially as described by Rose et al. (1990). The recovery of plasmids from yeast employed an additional chloroform 
 extraction with a subsequent ethanol precipitation. Yeast transforma 
tions were performed by the method of Eithile (1992), except that 20 µl 1 M 
DTT was added to each transformation to increase efficiency. Each trans 
formation reaction contained 500 µl log-phase cells, 1 µg plasmid DNA, 
and 3 µl 10 mg/ml sheared denatured carrier DNA. Salmon sperm DNA 
was prepared as previously described (Schiessl and Gietz, 1989), ex 
cept that the phenol chloroform extraction and subsequent ethanol pre 
 precipitation were omitted. Cells were plated on synthetic complete (SC) me 
dium was called pSK21. The 4.9-kb NsiI fragment of pSK21, which con 

Primers flanking the site of the disruption (Saiki et al., 1988), and by 
sporulation and dissection of the resulting *Leu* diploid. All 11 of the tet 
rice disrupted segregated viable *Leu* spores in a 2:2 pattern.

The plasmid pSK47 (2 µm USO1 URA3) was generated from pSK19 
as follows: the 5.6-kb SacI-KpnI fragment from pSK19 was isolated and sub 
cloned into pRS426 digested with KpnI and SacI. To generate a plasmid 
containing the isolated YKT76 gene, the 850-bp EcoRV-ClaI fragment from 
the multicopy suppressor isolate SQU197, which contains the YKT76 
gene, was subcloned into pRS426 (Christianson et al., 1992) digested with 
the same restriction enzymes. The resulting plasmid was selected.

The *uso1-*17 mutant allele utilized throughout these studies bears three 
consecutive nonsense mutations (amber, ochre, and opal) in the *USO1* 
gene instead of the single amber mutation present in the *uso1-*1 allele 
(Seog et al., 1994); the mutant proteins encoded by *uso1-*1 and *uso1-*17 are identical. *uso1-*17 was generated in pSK19 by site-directed mutagenesis 
using the unique site elimination method (Deng and Nickeloff, 1992) and 
defined by DNA sequence analysis with the dideoxy method (Sanger et 
al., 1977). To introduce the mutation into the genome, *uso1-*17 was sub 
cloned into the YEp-URA vector PRS306 (Sikorski and Hieter, 1989) on 
a KpnI-Sacl fragment. The resulting plasmid, pSK20, was linearized with 
BspEI, which cuts in *uso1-*1, and transformed into both MY2789 (wt), 
RSY255 (wt), RSY271 (sec18-1). Transformants were selected on SC-Ura 
media, purified once on the same media, and then patched onto a YPD 
plate. After growth at room temperature for 2 d, the YPD patch plates 
were then replica plated onto 5-fluoroorotic acid (5-FOA) media (Rose et 
al., 1990) to force the loss of the *URA3* gene and either the wild-type 
*USO1* allele or the *uso1-*1 allele. After 2 d at room temperature, cells 
from the patches on 5-FOA media were streaked for single colonies on 
5-FOA plates. The presence of the *uso1-*1 mutation in individual colo 
nies was determined by a directed double mutant (GWY71) also causes temperature 
sensitivity, to detect the presence of the introduced mutation in the sec18 
*uso1-*1 double mutant (GWY71) it was necessary to first transform 
the strains with a *SEC18* plasmid (provided by T. Graham, Vanderbilt 
University). In this way the presence of the *uso1-*1 mutation was detectable.

**Multicopy Suppressor Screen**

For the multicopy suppressor screen of *uso1-*1, we used a modified version 
of the mutation, called *uso1-*1*, that contains three consecutive stop 
codons (see above), to eliminate a potential background of nonsense sup 
pressors. For the screen, 29,000 transformants of a yeast genomic YEp24 
library (Carlson and Bottstein, 1982) were grown on SC-Ura plates. Since 
the average insert size for this library is >10 kb, we screened >19 genome equivalents. Each of these plates was serially replica plated to two 
YPD plates. The replica YPD plates were then incubated for 3 d at 37°C. Serial 
replica plating was employed because we have found that when the *uso1-*1 
strain is incubated at the restrictive temperature revertant colonies arise 
at a frequency of ~10^-5. Under the plating conditions used for this screen 
it is sometimes difficult to distinguish between plasmid-dependent tem 
perature-resistant colonies and colonies that have revertants growing in 
them. Therefore, from these plates we chose those transformants that 
were able to grow on both plates; 355 transformants met this criterion.

Cells from each temperature-resistant transformant were patched onto 
SC-Ura media to check that each of the isolates bore plasmid DNA. All but one were able to grow on the selective media. Cells from each isolate were then retested for temperature-resistance on YPD at 37°C. Of the 355 isolates tested, 305 still exhibited a temperature-resistant phenotype. These were divided into two groups, strong and weak, based on the degree 
to which each was able to confer temperature resistance. Plasmids from 
the 173 strains in the strong suppressor group were isolated and retrans 
formed into GWY33. Of the resulting strains, 132 exhibited plasmid 
dependent suppression. To identify known genes in the suppressors we employed Southern blotting (Sambrook et al., 1989). 1 µg of the plasmid 
DNA isolated from each candidate was applied to BA35 nitrocellu 
lose (Schleicher & Schuell, Keene, NH) using a Schleicher & Schuell 
Manifold II. Eight identical filters were generated, one for each probe to be tested. Radiolabeled probes were made by the random-primer labeling 
method (Feinberg and Vogelstein, 1984) with DNA fragments generated 
by PCR with the following oligonucleotide primers: USO1: USK2 
5'CTTGGTTCACGAGTCGAGTGG3' and USK8 5'GTGAATCGTTCA 
TACGTCGAGTGG3'.
immunoprecipitation of Sed5p protein complexes

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Except for minor modifications. In brief, ice between each burst. 180 μl of bead buffer were added to each tube, the samples were vortexed and centrifuged for 10 min. The supernatants were transferred to fresh tubes and 0.6 ml of 50 mM EDTA, 1.25% Triton X-100 was added. Protein extract from a S.

Experiments were performed essentially as described (Segaard et al., 1994), except for minor modifications. In brief, S. cerevisiae RSY255 (wild-type), RSY271 (sec18-1), GYW67 (uso1-1*), GYW71 (sec18-1 upUSO1) and GYW85 (sec18-1 upUSO1) were grown to mid-logarithmic phase in either SC or SC-Ura media supplemented with 1% casamino acids (Difco, Detroit, MI) at 25°C. Cells were harvested, spheroplasted, and incubated in YPD-sorbitol for 1 h at either the permissive (24°C) or restrictive temperature (37°C). The isolated spheroplasts were lysed in buffer D (Søgaard et al., 1994) and debris was removed by centrifugation. The extracts were collected and frozen in aliquots in liquid nitrogen. Spheroplast detergent extracts (60 μg of protein) were diluted in buffer E (Søgaard et al., 1994) and rotated overnight with 20 μl of affinity-purified anti-Sed5p antibodies coupled to protein A-Sepharose with dimethylpimelimidate (Harlow and Lane, 1988). Beads were washed four times with buffer E, and eluted twice with 0.15 ml of 0.1 M glycine (pH 2.4). The eluates were pooled, precipitated with 10% (wt/wt) trichloroacetic acid, and resuspended in Laemmli sample buffer. Membranes were probed with 1,000-fold diluted, affinity-purified anti-Sec22p and anti-Bet1p antisera. Immunoblots were developed using chemiluminescent detection (Renaud; DuPont-NEN, Boston, MA).

Results

Ypt1p and the ER to Golgi v-SNAREs Can Suppress a Uso1p Defect

We undertook a genetic analysis of USO1 to determine how Uso1p fits into the molecular framework of proteins already known to function in the yeast secretory pathway, and to potentially identify new genes involved in secretion. We began by searching for genes that could suppress the temperature-sensitive phenotype of the yeast uso1-1 mutant when overexpressed. This strain harbors a nonsense mutation in the USO1 gene that causes the Uso1p protein to be truncated after approximately one-fourth of its coiled-coil domain (Seog et al., 1994). To identify multicopy suppressors of uso1-1, a total of 29,000 transformants were replica-plated to YPD at the restrictive temperature of 37°C (see Materials and Methods for details). From these, 355 temperature-resistant colonies were isolated. After restaging the temperature-sensitive phenotype, 305 of the isolates were still resistant. Plasmids from the 173 transformants exhibiting the strongest suppression were isolated and retransformed into the uso1-1 strain. Upon retransformation, 132 plasmids were able to confer temperature resistance in a plasmid dependent manner.

Since it had been previously demonstrated that Uso1p functions in ER to Golgi transport (Nakajima et al., 1991), we investigated whether any of the plasmid-linked suppressors contained genes known to function in this portion of the secretory pathway. To this end, all suppressor plasmids were hybridized with probes corresponding to a panel of genes known to function in ER to Golgi or intra-Golgi transport. The probes were BET1, BOS1, SEC7, SEC17, SEC22, SED5, SLY1, SLY41, and USO1. This hybridization procedure revealed that six of the plasmids contained the USO1 gene itself (data not shown). In addition, the collection contained six plasmids bearing BET1, five bearing SEC22, and one bearing BOS1. Interestingly, BET1, BOS1, and SEC22 encode proteins that are localized to ER-derived vesicles and are putative v-SNARE molecules (Lian and Ferro-Novick, 1993; Rexach et al., 1994). Furthermore, all three proteins appear to be components of the yeast ER to Golgi v-SNARE/t-SNARE complex (Søgaard et al., 1994). The other 114 plasmids did not hybridize with any of the probes (data not shown).

The remaining collection of 114 suppressor plasmids

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was subjected to restriction mapping to identify duplicates; 27 plasmids were eliminated in this manner. Of the remaining 88 plasmid-linked suppressors, we sequenced the ends of the inserts from the 48 strongest suppressors. In this manner we hoped to potentially identify the genomic regions borne by these plasmids and thus, the candidate open reading frames responsible for the suppression phenotype. Sequence analysis revealed that several regions of the genome were represented multiple times among this group. One of these regions, a segment from chromosome XI, contains the Ykt6p gene. Ykt6p is also a member of the yeast ER to Golgi SNARE complex (Søgaard et al., 1994), and based on its homology to Sec22p, Ykt6p is considered to be a v-SNARE.

Therefore, overexpression of each of four different plasmids, each encoding a putative ER to Golgi v-SNARE (Bet1p, Bos1p, Sec22p, and Ykt6p), was able to suppress the temperature-sensitive phenotype of the usol-1 mutant (Fig. 1 A). Multicopy BET1 and BOS1 are able to suppress the temperature-sensitive growth phenotype as well as the USO1 gene itself (Fig. 1 A). In comparison to these genes, SEC22 and YKT6 appear to suppress this phenotype somewhat more weakly (Fig. 1 A).

The remaining group of suppressor plasmids have been found to contain 13 distinct chromosomal regions. Two of the inserts, however, cannot be localized to a sequenced chromosomal region since they do not align with any sequences currently in the yeast sequence database. We are currently focusing our attention on the only two suppressors which have demonstrated an ability to suppress the usol-1 transport defect.

To confirm the results of the multicopy suppression screen and to extend our analysis, we examined whether a number of genes previously shown to function in the yeast secretory pathway could suppress the usol-1 mutation. For this analysis, isolated genes, rather than genomic fragments, were employed. In agreement with the results of the multicopy suppressor screen, we found that BET1, BOS1, SEC22, and YKT6 were each able to suppress the temperature-sensitive phenotype of the usol-1 mutant (Fig. 1 B). Again, BOS1 and BET1 are somewhat better suppressors of usol-1 than either YKT6 or SEC22.

Although YPT1, which encodes the small rab-like GTP-binding protein Ypt1p, was not recovered in the multicopy suppressor screen, it was found to be a good suppressor of usol-1 (Fig. 1 B). Similarly, SLY1-20, which contains an activating mutation in SLY1 (Dascher et al., 1991), was able to suppress the usol-1 mutation. For all experiments involving suppression by SLY1-20 we used a CEN plasmid; suppression by this construct was identical to that observed for a 2 μm SLY1-20 plasmid (data not shown). Interestingly, SLY1-20 was originally identified as a mutation that enables the SLY1 gene to compensate for the reduced levels of Ypt1p found in cells after galactose shut-off of a GAL10-YPT1 expression plasmid (Dascher et al., 1991). Wild-type SLY1, however, which encodes a t-SNARE--associated protein (Søgaard et al., 1994), has no effect on the temperature sensitivity of either the usol-1 or the ypt1-3 mutant (data not shown). We also found that multicopy ARF1, SARI, SEC7, SEC17, SEC18, SEC21, SED5, SFT1, SNC1, and SNC2 were unable to suppress the usol-1 strain.

The suppression data are summarized in Table II. The temperature-sensitive phenotype of the usol-1 mutant was not suppressed by overexpression of "fusion components" (Sec17p or Sec18p), by overexpression of Golgi to plasma membrane v-SNAREs (Snc1p or Snc2p), by overexpression of a putative intra-Golgi v-SNARE (Sft1p), or by the

| Gene | Function of gene product |
|------|--------------------------|
| USO1 | ER-Golgi docking/fusion  |
| BOS1 | ER-Golgi v-SNARE         |
| BET1 | ER-Golgi v-SNARE         |
| SEC22| ER-Golgi v-SNARE         |
| YKT6 | Putative ER-Golgi v-SNARE|
| YPT1 | ER-Golgi docking/fusion regulator |
| SLY1-20| Gain of function mutation in SLY1 |

| Gene | Function of gene product |
|------|--------------------------|
| SED5 | ER-Golgi t-SNARE         |
| SLY1 | ER-Golgi t-SNARE--associated protein |
| SEC17| SNAP                     |
| SEC18| NSF                      |
| SNC1 | Golgi-PM v-SNARE         |
| SNC2 | Golgi-PM v-SNARE         |
| SFT1 | Putative intra-Golgi v-SNARE |
| SEC7 | ER through Golgi factor  |
| ARF1 | Low MW GTPase involved in COPI budding |
| SEC21| COPI subunit             |
| SARI | COPIII-associated GTP binding protein |

**Figure 1.** Suppression of the temperature-sensitive growth phenotype of the usol-1 mutant. (A) Suppression of usol-1 by plasmids isolated in the multicopy suppressor screen. (B) Suppression of usol-1 by the individual suppressor genes, each isolated from their genomic background. All plasmids were 2 μm except for pSLY1-20 which was a CEN plasmid. Plates were incubated for 3 d at 37°C.

| Table II. Genes Tested for Their Ability to Suppress the Temperature-sensitive Growth Defect of usol-1 Cells |
|-----------------------------------------------------------------------------------------------------------------|
overexpression of several vesicle budding components (Arflp, Sarlp, Sec2lp). Therefore, all of the factors able to suppress usol-1 appear to be specific for the targeting/docking step of ER to Golgi transport.

**Restoration of ER to Golgi Transport in the usol-1 Mutant**

Since overexpression of the ER to Golgi v-SNAREs, Ypt1lp, and Sly1-20p suppressed the growth defect of usol-1 at the restrictive temperature, we investigated whether this ability correlated with an ability to suppress the secretory defect of this mutant strain. To do so we examined the processing of the well-characterized vacuolar protease CPY. In wild-type cells the earliest glycosylated form of CPY, called p1 CPY, is associated with the ER (Stevens et al., 1982). p1 CPY is then further glycosylated in the Golgi to generate the p2 form, which is subsequently proteolytically processed in the vacuole to generate mature CPY.

To monitor the ability of each of these strains to process CPY to its mature form a pulse-chase analysis was performed using a wild-type strain, the usol-1 strain, and usol-1 strains bearing plasmids containing the different suppressor genes. Cells were grown at the permissive temperature (24°C), then shifted to the restrictive temperature (38°C) and immediately pulse-labeled for 5 min with Tran35S-label. After a 20-min chase at the restrictive temperature, the cells were lysed, and CPY was immunoprecipitated and analyzed by SDS-PAGE and fluorography (Fig. 2). Whereas wild-type cells had processed 85% of the radiolabeled CPY to the mature form, usol-1 cells had matured only 30% of the CPY; the balance of the CPY was in the p1 form indicating that it had not exited the ER (Fig. 2). This observation is consistent with the previously reported accumulation of the ER form of invertase in the usol-1 mutant (Nakajima et al., 1991). Compared to the usol-1 strain, the overexpression of each of the v-SNAREs or Ypt1lp resulted in generation of twice the amount of

**Figure 2.** Suppression of the usol-1 transport defect. (A) Autoradiograph of carboxypeptidase Y (CPY) immunoprecipitations from wild-type cells, usol-1 cells, or usol-1 cells transformed with each of the suppressor plasmids. All plasmids were 2 μm except for pSLY1-20 which was a CEN plasmid. Cells were shifted to the restrictive temperature of 38°C, pulse labeled for five minutes, and allowed to chase for 20 min at the same temperature. CPY was isolated from cell extracts by immunoprecipitation and resolved by SDS-PAGE. The different forms of CPY are indicated on the left. (p1) The core-glycosylated form, ER form; (p2) the outer-chain glycosylated Golgi form, and m: the mature, vacuolar form. (B) Quantitation of the ability of the suppressor genes to restore maturation of CPY in usol-1 cells. All three species of CPY (p1, p2, and mature) were quantitated by Phosphorimager analysis with background subtraction. The percentage of mature CPY was then calculated for each lane as follows: % conversion to mature = mature CPY/(p1 CPY + p2 CPY + mature CPY) × 100. In three independent experiments, the relative ability of each of the suppressors to restore transport was similar. *The YKT6 gene in this strain is in the genomic background in which it was isolated from the multicopy suppressor screen.
mature CPY, ~60%. Notably, expression of Slyl-20p was able to suppress the transport defect almost completely (Fig. 2). These results suggest that the ability of the BETI, BOS1, SEC22, YKT6, and SLY1-20 to suppress the usol-1 growth defect stems directly from their ability to restore, at least partially, ER to Golgi transport.

**Suppression of a Deletion of US01**

To determine whether the proteins encoded by the usol-1 suppressors were able to bypass the requirement for Usol1p, we tested whether any of them suppress the lethality of a US01 deletion. The ability of one gene to suppress a complete deletion of another suggests that the function of the suppressor is downstream of the missing gene product. To test this, a heterozygous US01/Δusol diploid, in which the disrupted Δusol allele is marked with the LEU2 gene (Δusol::LEU2), was transformed with each of the usol-1 multicopy suppressor plasmids. The resulting strains were sporulated and dissected. Representative tetrads are shown in Fig. 3.

The US01/Δusol::LEU2 strains containing BOS1 or YKT6 on 2-μm plasmids yielded two viable Leu− spores and two inviable spores (Fig. 3), indicating that overexpression of these genes was unable to suppress the Δusol null allele. Microscopic examination of the inviable spores revealed the presence of microcolonies containing two or more cells, indicating that these spores were able to germinate and divide at least once. Therefore, their inability to grow into visible colonies was due to a vegetative growth defect. In contrast, for tetrads resulting from the dissection of diploids containing SEC22 or BET1 on 2-μm plasmids we observed two normal sized Leu+ colonies and two slowly growing Leu− colonies (Fig. 3). Therefore, two of the v-SNAREs weakly suppress the Δusol allele, indicating that they function are downstream of Usol1p.

In addition to two of the v-SNAREs, the Δusol allele could also be suppressed by overexpression of Yptlp. However, this suppression was somewhat weaker than that observed for BET1 or SEC22. Whereas small Leu− colonies were detected in the BET1 and SEC22 containing strains after 5 d of growth at room temperature, colonies were not observed for the YPT1 bearing strain until after one week (Fig. 3). Furthermore, the frequency of these small Ura+ Leu− colonies was low; in 23 tetrads only five such colonies were observed after 9 d at room temperature.

Finally, tetrad dissection of US01/Δusol::LEU2 strain containing pSLY1-20 produced normal-sized Leu+ colonies (Fig. 3). Therefore, SLY1-20, which is the best suppressor of the usol-1 temperature sensitivity and the usol-1 transport defect, is also the best suppressor of a complete deletion of US01. Taken together, these results indicate the v-SNAREs Sec22p and Bet1p, the t-SNARE associated protein Sly1p, and the rab-like GTP-binding protein Yptlp function downstream of Usol1p.

Interestingly, the multicopy suppression of the Δusol allele follows a pattern similar to that observed for the Δyptl allele. Specifically, BET1, SEC22, and SLY1-20, are also able to suppress the functional loss of Yptlp (Dascher et al., 1991). This led to their designation as SLY genes (for suppressor of loss of Yptlp function); SEC22 is allelic to SLY2, and BET1 is allelic to SLY12 (Dascher et al., 1991).

**The usol-1 Suppressors Are yptl-3 Suppressors**

All but one of the multicopy suppressors of usol-1 have previously been demonstrated to interact genetically with YPT1 (Dascher et al., 1991; Newman et al., 1990). To examine this observation in a systematic fashion we determined the ability of each of the usol-1 suppressors to allow growth of the temperature-sensitive yptl-3 strain (Rexach et al., 1994) at the restrictive temperature. As shown in Fig. 4, all of the usol-1 suppressors can also suppress the temperature-sensitive phenotype of the yptl-3 strain. SLY1-20 is the strongest yptl-3 suppressor, followed closely by SEC22 and BOS1. BET1 and YKT6 are somewhat weaker suppressors, and US01 is the weakest. There are two notable differences between the patterns observed for suppression of the yptl-3 and usol-1 temperature-sensitive alleles. First, SEC22 is a better suppressor of yptl-3 than of usol-1. Second, whereas YPT1 is a strong suppressor of usol-1, US01 is a very weak suppressor of yptl-3.

**Genetic Interactions of US01 and YPT1**

The finding that the same set of genes can suppress either yptl-3 or usol-1 temperature sensitivity, as well as the observation that the same subset of genes can suppress a deletion of either US01 or YPT1, suggests that Usol1p and Yptlp may function in the same process. This is supported by the ability of YPT1 and US01 to suppress temperature sensitive alleles of each other. Based on these findings we analyzed the genetic interaction between US01 and YPT1 in more detail.

Although US01 had not been identified as a suppressor of Δyptl in the original SLY screen (Dascher et al., 1991), we tested whether US01 is able to suppress a loss of Yptlp. We examined this in two ways. First, we assessed the ability of multicopy US01 to suppress reduced levels of Yptlp, which was the method employed in the original SLY screen. For this purpose, we used a haploid strain that contains YPT1 under the control of the GAL10 promoter. Due to the essential nature of the YPT1 gene, this

**Cloned genes:**

![Figure 4. Suppression of yptl-3 by the same panel of suppressor genes shown in Fig. 1A. Cells were plated on YPD and permitted to grow for 3 d at 37°C. All plasmids were 2 μm except for pSLY1-20 which was a CEN plasmid.](image-url)
strain is viable only on galactose media, which induces the expression of *YPT1*. In contrast, cells grown on glucose media express low, but detectable, levels of Ypt1p (Ossig et al., 1995). We transformed this strain with either 2 μm pYPT1, 2 μm pUSO1, or the 2 μm vector alone and tested the ability of each strain to grow on galactose media and glucose media. As expected, all three strains were able to grow equally well on media containing galactose, when Ypt1p is expressed (Fig. 5, left). On glucose media, however, the strain containing the vector alone was unable to grow, whereas the strains containing the YPT1 plasmid or the USO1 plasmid were able to grow (Fig. 5, right). This result indicates that overexpression of *USO1* is able to compensate for reduced levels of wild-type Ypt1p, a result consistent with suppression of ypl-3 by *USO1*.

To test the ability of *USO1* to suppress the complete loss of Ypt1p, a heterozygous *YPT1*/Δypt1::LEU2 diploid was transformed with 2 μm *USO1*, 2 μm *YPT1*, or the 2-μm vector alone, and then sporulated and dissected. Upon dissection, all tetrads from the strains containing either the vector alone or the *USO1* plasmid contained only two viable Leu- spores (data not shown) indicating that *USO1* cannot suppress a Δypt1 allele. Thus, although high levels of Usolp can compensate for low levels of Ypt1p activity, some Ypt1p function is required for this suppression by Usolp to occur. This suggests that Usolp exerts its function through, or in conjunction with, Ypt1p.

We also determined whether mutant alleles of the *USO1* and *YPT1* exhibit a synthetic lethal interaction with one another. Both *usol-1* and *yptl-3* are able to grow well at 24 and 30°C, but exhibit a temperature-sensitive growth phenotype at 34°C or higher. To test the phenotype of the double mutant, we crossed the strains bearing the individual *usol-1* and *yptl-3* mutant alleles. Diploids resulting from the *usol-1*/Δypt1::LEU2 cross were sporulated, dissected, and then tested for temperature sensitivity and for the presence of the *usol-1* and *yptl-3* alleles by complementation. Colonies derived from this dissection are shown in Fig. 6 (left). In cases where four spores grew at 24°C, all were inviable at 37°C, indicating that each spore contained a single mutant allele (parental diatype); this was confirmed by complementation. In several cases, three spores were able to grow at 24°C, yet only one of the three spores was viable at 37°C (tetraplotype). The three viable spores always consisted of one wild-type, one *usol-1*, and one *yptl-3*; by inference, the inviable spore was the *usol-1 yptl-3* double mutant. Finally, some tetrads yielded a 2 viable:2 inviable segregation pattern at both 24 and 37°C (non-parental diotypes); complementation indicated that the viable segregants were wild-type, and by inference, the inviable spores were the *usol-1 yptl-3* double mutants.

To be certain that the synthetic lethal interaction was due exclusively to the interaction of *usol-1* and *yptl-3* we transformed the *usol-1 yptl-3* diploid with either *USO1* or *YPT1* 2 μm plasmids and performed tetrad analysis of the resulting strains (Fig. 6, right). As expected, all spores from the transformed strain were able to grow at 24°C since all of the double mutant segregants were covered by either *YPT1* or *USO1*. However, when these colonies were replica plated to 5-FOA media, which forces cells to

![Figure 5. Overexpression of Usolp can suppress reduced levels of Ypt1p. All sectors contain GFU1-6D cells, which contains GAL10-YPT1::HIS3 at the YPT1 locus. These cells also contain either the 2 μm vector (pRS426; Christianson et al., 1992), pSK47 (2 μm USO1 URA3), or pNB167 (2 μm YPT1 URA3). (left) On galactose media, which permits expression of Ypt1p. (right) On glucose media, which represses expression of Ypt1p.](image)

![Figure 6. usol-1 and yptl-3 exhibit a synthetic lethal interaction. (left) The two mutant strains were crossed and the resulting diploid was sporulated and dissected. The tetrads were permitted to grow at room temperature, and then were replica-plated to YPD at 37°C to determine which colonies contained temperature-sensitive alleles. (right) To verify that the synthetic lethal phenotype observed was due to mutations in the *USO1* and *YPT1* genes, the *USO1/usol-1 yptl-3/YPT1* diploid was transformed with either pUSO1 URA3 (pSK47) or pYPT1 URA3 (pNB167). The resulting strains were sporulated and dissected. The dissected tetrads were permitted to grow on selective media (control). They were then replica plated to 5-FOA plates to force the loss of the plasmids. Plates were incubated at 24°C for 3 d. In a separate experiment the genotype of all temperature-sensitive segregants was confirmed by complementation. In all cases the genotype of these spores was either *usol-1* or *yptl-3.*](image)
lose the plasmids, the synthetic phenotype was uncovered (Fig. 6, right). This synthetic lethal interaction underscores the existence of a strong genetic interaction between USO1 and YPT1 and suggests that Usolp and Yptlp may function in the same step of ER to Golgi transport.

**Uso1p Is Required for Assembly of the SNARE Complex**

Taken together, our genetic data suggest that Usolp and Yptlp function at the same step in vesicular transport upstream of the v-SNAREs, and that Usolp may act through, or in concert with Yptlp. Previously, it has been suggested that Yptlp is required for assembly of the ER to Golgi SNARE complex because v-SNARE/t-SNARE complexes were not demonstrable in yptl-3 cells (Søgaard et al., 1994). Furthermore, it has been proposed that Yptlp mediates SNARE complex assembly through specific activation of the v-SNAREs Boslp and Sec22p (Lian et al., 1994). Based on the similar suppression profiles of the USO1 and YPT1 genes, we were interested to determine whether Usolp is also required for assembly of the ER to Golgi SNARE complex. To address this question, wild-type, usol-1, sec18-1, and usol-1 sec18-1 cells (with or without a 2 μm USO1 plasmid) were grown to mid-logarithmic phase and spheroplasted. The cells were then incubated for 1 h at either the permissive (24°C) or restrictive (38°C) temperature. After incubation, the cells were lysed and the t-SNARE Sed5p was immunoprecipitated with affinity-purified antibodies. A control reaction without anti-Sed5p antibodies was performed as well. The immunoprecipitates were then analyzed for their Betlp and Sec22p content (v-SNAREs) by immunoblotting with specific antibodies (Fig. 7). In wild-type cells neither Betlp nor Sec22p was associated with Sed5p at either temperature (top), although these proteins were detected in the supernatant of the immunoprecipitation (bottom) indicating that they were present. The fact that the v-SNAREs are not associated with Sed5p in wild-type cells is expected since the v-SNARE/t-SNARE complex should exist only transiently in wild-type cells. Likewise, no complex accumulated in sec18-1 cells grown at the permissive temperature. However, in agreement with previously published data (Søgaard et al., 1994), sec18-1 cells accumulated the Sed5p/Sec22p/Betlp complex at the restrictive temperature. Presumably, complex accumulation occurs at the restrictive temperature because the mutant version of Sec18p, which is the yeast equivalent of NSF, cannot disassemble the complex as a prelude to membrane fusion (Söllner et al., 1993a,b). The usol-1 strain incubated at either the permissive or restrictive temperature did not accumulate the complex. However, since the usol-1 strain contains wild-type Sec18p, even if the complex could assemble in these cells, it would not accumulate. Therefore, to determine whether Usolp was required for assembly of the complex, we tested a usol-1 sec18-1 double mutant. In this strain, if the v-SNARE/t-SNARE complex formed it would accumulate at the restrictive temperature, due to the absence of Sec18p activity. The absence of complex formation in the double mutant would indicate a defect in its formation. Indeed, we found that usol-1 sec18-1 double mutant cells failed to accumulate the Sed5p/Sec22p/Betlp complex at the restrictive temperature. Identical results were obtained when the immunoprecipitations were performed with anti-Boslp antibodies (data not shown). As a final control to confirm that the inability to form a v-SNARE/t-SNARE complex was due to the usol-1 mutation we tested a usol-1 sec18-1 double mutant strain containing a

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**Figure 7.** Usolp is required for assembly of the ER to Golgi SNARE complex. Wild-type (RSY255), sec18-1 (RSY271), usol-1 (GWY67), usol-1 sec18-1 (GWY71), or usol-1 sec18-1/pUSO1 (GWY71 + pSK47 (2 μm USO1 URA3)) spheroplasts were incubated at 24 or 38°C for 1 h, and then lysed with Triton X-100. 60 μg of each detergent extract was immunoprecipitated with anti-Sed5p antibodies covalently coupled to protein A-Sepharose beads (20 μl). Immunoprecipitated proteins were loaded on a SDS-12% PAGE gel. The separated polypeptides were transferred to nitrocellulose and analyzed by Western blotting using affinity-purified antibodies against Sed5p, Sec22p and Betlp (top). One-half of the supernatant from each immunoprecipitation reaction was also subjected to PAGE and immunoblotted (bottom). Bound antibodies were visualized using chemiluminescence.
USO1 plasmid. In this strain, the SNARE complex once again accumulated at the restrictive temperature. Therefore, functional Usolp is required for formation of the ER to Golgi v-SNARE/t-SNARE complex.

Discussion

Usolp was identified in a novel screen for secretion mutants and has been shown to be required for ER to Golgi transport (Nakajima et al., 1991). It is homologous to the mammalian transport factor p115 (or TAP), which is required for the docking or fusion step of intra-Golgi transport (Elazar et al., 1994; Waters et al., 1992), for binding of transcytotic vesicles to the plasma membrane (Barroso et al., 1995), and for the reassembly of the Golgi apparatus from dispersed Golgi fragments after mitosis (Rabouille et al., 1995). The precise biochemical function of neither p115 nor Usolp has been determined. To further elucidate how Usolp, and possibly p115, function in membrane trafficking, we undertook an analysis of Usolp function in yeast.

We performed a biochemical analysis to determine whether assembly of the ER to Golgi v-SNARE/t-SNARE complex is affected in usol-1 mutant cells. To do this we used a yeast strain that has a temperature-sensitive mutation in the SEC18 gene, which encodes the yeast homologue of NSF (Wilson et al., 1989). The Sec18p ATPase acts to disassemble the v-SNARE/t-SNARE complex (Söllner et al., 1993a,b) thereby allowing membrane fusion to ensue. By immunoprecipitation of the t-SNARE Sed5p, and examination of whether the v-SNAREs Bet1p and Sec22p are associated with it, we have found, in agreement with the results of Søgaard et al. (1994), that Bet1p and Sec22p are not associated with Sed5p in wild-type cells; this is most likely due to the transient nature of the targeting complex in the presence of active Sec18p. In contrast, when the same experiment is performed in a sec18-1 mutant strain at the restrictive temperature, where Sec18p is inactive, Bet1p and Sec22p coimmunoprecipitate with Sed5p. Preparation of a double mutant strain containing both the sec18-1 and usol-1 mutations allowed us to perform an epistasis experiment to determine whether functional Usolp is required for assembly of the v-SNARE/t-SNARE complex. Indeed, we found that the presence of the usol-1 allele prevented the accumulation of the SNARE complex that is normally evident in a sec18-1 strain at the restrictive temperature. SNARE complex assembly was restored by complementing the usol-1 mutation with USO1 on a plasmid. From these results we conclude that functional Usolp, like Ypt1 (Søgaard et al., 1994), is required for assembly of the ER to Golgi v-SNARE/t-SNARE complex.

Although the v-SNARE/t-SNARE complex is unable to form in the absence of Usolp function, Usolp does not appear to be a member of the SNARE complex. Using a version of the sec18-1 usol-1 strain that contained a 2-μm plasmid encoding epitope-tagged Usolp, we were unable to detect Usolp in the complex which accumulates at the restrictive temperature (data not shown). This is consistent with the absence of Usolp in the yeast ER to Golgi SNARE complex (Søgaard et al., 1994), as well as with the absence of p115 in the mammalian SNARE complex (Söllner et al., 1993b).

Genetic analysis of USO1 and its relationship to other genes involved in ER to Golgi transport (summarized in Table III) has provided insight into how Usolp may facilitate SNARE complex assembly. We have found that overexpression of each of the known yeast ER to Golgi v-SNAREs (Bet1p, Bos1p, Sec22p, and Ykt6p) confers temperature resistance, as well as partial restoration of ER to Golgi transport, to usol-1 mutant cells. The simplest explanation for this multicopy suppression is that high levels of v-SNARE molecules can increase v-SNARE/t-SNARE complex assembly by virtue of mass action (see Fig. 8). Such enhanced assembly may be able to compensate, at least partially, for the loss of Usolp function. This interpretation is supported by the finding that two of the v-SNAREs, Bet1p and Sec22p, can suppress a complete deletion of Usolp. Overexpression of these v-SNAREs was also found to suppress the loss of Ypt1p (Dascher et al., 1991), albeit to a lesser degree (Dascher et al., 1991). The other two v-SNAREs, Bos1p and Ykt6p were able to suppress the temperature-sensitive alleles of USO1 and YPT1, but were unable to suppress either the usol1 null or the ypt1 null. Although both of these proteins, along with Sec22p and Bet1p, are known to be members of the ER to Golgi SNARE complex (Søgaard et al., 1994), our results suggest that Bos1p and Ykt6p are functionally different from the other two v-SNAREs, Bet1p and Sec22p. Furthermore, since overexpression of Bet1p and Sec22p is able to suppress the usol1 and ypt1 null alleles, it appears that the v-SNAREs do not operate through Usolp, but rather, that they function downstream of Usolp. Consistent with this is the fact that overexpression of USO1 is not able to suppress the temperature sensitivity of the v-SNARE mutants sec22-3, bet1-1, or bos1-1 (data not shown).

In contrast to the v-SNAREs, overexpression of the t-SNARE Sed5p did not suppress a usol-1 mutation. This might be construed as conflicting with the idea that SNARE assembly can be driven by mass action. However, we believe this paradox can be resolved by considering the two activities of Sly1p. First, since SLY1 is an essential gene (Dascher et al., 1991), and mutations in Sly1p disrupt ER to Golgi transport (Ossig et al., 1991), it must have a positive role in promoting this vesicular transport event.

| Table III. Summary of Multicopy Suppression Data |
|-----------------------------------|
| 2 μm plasmid | usol-1*/ | ypt1-3* | Δusol1* |
|------------------|-----------|-----------|-----------|
| YEp24             | −         | −         | −         |
| USO1              | + + +     | +         | + + +     |
| BET1              | + +       | ++        | ++        |
| SEC22             | +         | ++ +      | + +       |
| BOS1              | +         | + +       | −         |
| YKT6             | +         | +         | −         |
| SLY1-20            | + + +     | + + +     | + + +     |
| YPT1              | + + +     | +         | +         |

* Suppression of the temperature-sensitive phenotype.
1 Suppression of lethality.
2 The YKT6 gene in this plasmid is contained in the genomic fragment as it was isolated from the multicopy suppressor screen.
3 For suppression of the null allele, a CEN plasmid was used. For suppression of temperature sensitivity both CEN and 2 μm plasmids were tested; identical results were obtained.
A.

\[ \text{v-SNAREs} \xrightarrow{\text{Yptlp}} \text{Activated v-SNAREs} \xrightarrow{\text{Usolp}} \text{v-SNARE/t-SNARE complex} \]

B.

\[ \text{v-SNAREs} \xrightarrow{\text{Slylp}} \text{t-SNARE} \xrightarrow{\text{Usolp}} \text{v-SNARE/t-SNARE complex} \]

**Figure 8.** Potential sites of action for Usolp and Yptlp in assembly of the ER to Golgi SNARE complex. (A) Usolp may facilitate the action of Yptlp in v-SNARE activation. (B) Usolp and Yptlp may act by relieving the inhibitory effect that Slylp imparts on v-SNARE/t-SNARE complex formation (see discussion for further explanation).

Secondly, Slylp appears to act as a negative regulator of ER to Golgi transport because a dominant gain of function allele, \textit{SLY1-20}, can abrogate the requirement for both Yptlp (Ossig et al., 1991), and Usolp in vesicle docking. A plausible model (Dascher et al., 1991; Søgaard et al., 1994) to explain these observations is that Slylp, which is found in association with the t-SNARE Sed5p (Søgaard et al., 1994), impacts on Sed5p activity in a regulated fashion. Thus, the association of Slylp with Sed5p may prevent illicit interactions of Sed5p with v-SNAREs until Slylp is converted to another conformation, perhaps one similar to that of Sly1-20p, that permits Sed5p to interact with the v-SNAREs. Similarly, n-secl (the neuronal homologue of Slylp) appears to regulate the ability of syntaxin (the neuronal homologue of Sed5p) to interact with VAMP (the neuronal homologue of the v-SNAREs) (Pevsner et al., 1994). In support of this model is our finding that overexpression of wild-type Slylp (the inhibitory conformation) was unable to suppress the \textit{usol-1} mutation, while expression of even moderate levels of Sly1-20p (the stimulatory conformation) can completely restore ER to Golgi transport in the \textit{usol-1} mutant. Therefore, it appears that the lack of v-SNARE/t-SNARE complex assembly in \textit{usol-1} cells can be compensated for by either increasing the concentration of any of the v-SNAREs, or by increasing the effective concentration of the "active" t-SNARE.

Interestingly, all of the \textit{usol-1} suppressors (with the exception of \textit{YKT6}) had been previously described as having some genetic interaction with \textit{YPT1} (Dascher et al., 1991). In this study we have shown that all of the \textit{usol-1} suppressors can suppress the \textit{ypt1-3} temperature-sensitive phenotype and that \textit{usol-1} and \textit{ypt1-3} mutations display a synthetic lethal interaction (Fig. 6). These data, together with our observation that Usolp, like Yptlp (Lian et al., 1994; Søgaard et al., 1994), is required for assembly of the ER to Golgi v-SNARE/t-SNARE complex, suggest that Usolp and Yptlp function in the same process. We have also observed that overexpression of Usolp can suppress reduced levels of Yptlp, but it cannot compensate for the complete loss of Yptlp. In contrast, the overexpression of \textit{YPT1} is capable of weakly suppressing the complete loss of Usolp. Thus, it appears that Usolp requires some minimal amount of Yptlp to impact on SNARE assembly, but that Yptlp, when present at high levels, can function in the complete absence of Usolp. Therefore, Usolp most likely functions upstream of Yptlp. An alternative possibility is that Usolp functions in conjunction with Yptlp, and that the high levels of Yptlp, even in the absence of Usolp, impart sufficient function to allow slow growth.

What is the nature of the interaction between Usolp and Yptlp? Since Yptlp is a member of the rab family of small GTP-binding proteins, its function should be regulated by other proteins. These would include a GTPase activating protein (GAP), a GTP dissociation inhibitor (GDI), and a GDP dissociation stimulator (GDS) (Nuoffer and Balch, 1994). Usolp is unlikely to be the GDI for Yptlp since it is known that Sec19p can function as a Yptlp GDI (Garrett et al., 1994). Potential GAP or GDS activities of Usolp however remain to be examined. Another possibility is that Usolp impacts on Yptlp indirectly, perhaps through an as of yet undiscovered factor.

Since Usolp functions in ER to Golgi transport, and its mammalian homolog \textit{pl15} functions in both intra-Golgi transport and transcytosis, we, and others, had proposed that \textit{pl15} is a general transport factor (Barroso et al., 1995; Sapperstein et al., 1995). By analogy, if Usolp is a general transport factor, one would predict that overexpression of ER to Golgi SNAREs might facilitate movement of proteins from the ER to the Golgi, but that later intra-Golgi transport steps would still be defective. Surprisingly, we have not observed a defect in intra-Golgi transport when the \textit{usol-1} secretory defect was suppressed by overexpression of the ER to Golgi v-SNAREs (see Fig. 2). Therefore, either Usolp is specifically required for the ER to Golgi transport step, or it is required for multiple steps with the ER to Golgi step being the most sensitive to perturbations in Usolp function.

Fig. 8 presents two models for how Yptlp and Usolp could function to regulate v-SNARE/t-SNARE complex assembly. The first model (Fig. 8 A), based on the proposal of Lian et al. (Lian et al., 1994), and consistent with the experiments presented here, posits that Usolp and Yptlp activate the v-SNAREs (perhaps by facilitating their association) rendering them competent for interaction with the t-SNARE. Alternatively (Fig. 8 B), Usolp and Yptlp may impact on Slylp, perhaps to relieve an inhibitory effect of Slylp on SNARE complex assembly. In this regard, it is noteworthy that \textit{SLY1-20}, the dominant gain of function allele of \textit{SLY1}, is the best suppressor of \textit{ypt1-3} and \textit{usol-1} temperature sensitivity, and of suppression of the \textit{usol-1} transport defect. The models depicted in Fig. 8 are, however, not mutually exclusive: Usolp and Yptlp could impact on both v-SNARE activation and the relief of t-SNARE inhibition, perhaps in concert, to promote assembly of the v-SNARE/t-SNARE complex. In addition, Usolp could also function as a docking checkpoint by monitoring whether the correct v-SNAREs are
present on the vesicle, or whether the v-SNAREs have been sufficiently activated.

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