Abstract. Localization of yeast Kex2 protease to the TGN requires a signal (TLS1) in its cytosolic tail (C-tail). Mutation of TLS1 results in rapid transit of Kex2p to the vacuole. Isolation of suppressors of the Tyr713Ala mutation in TLS1 previously identified three SOI genes. SOII, cloned by complementation of a sporation defect, encodes a novel, hydrophilic 3,144-residue protein with homologues in Caenorhabditis elegans, Drosophila melanogaster, and humans. Epitope-tagged Soi1p existed in a detergent-insensitive, sedimentable form. Deletion of SOII impaired TGN localization of wild-type Kex2p and a fusion protein containing the C-tail of Ste13p, and also caused misrouting of carboxypeptidase Y and accelerated vacuolar degradation of the Vps10p sorting receptor. Deletion of SOII improved retention of Tyr713Ala Kex2p in the pro-α-factor processing compartment but, unlike the original soi alleles, did not increase the half-life of Tyr713Ala Kex2p. These results suggested that Soi1p functions at two steps in the cycling of Kex2p and other proteins between the TGN and prevacuolar compartment (PVC). This hypothesis was confirmed in several ways. Soi1p was shown to be required for optimal function of TLS1. Suppression of the Tyr713Ala mutation by mutation of SOII was shown to be caused by activation of a second signal (TLS2) in the Kex2p C-tail. TLS2 delayed exit of Kex2p from the TGN, whereas TLS1 did not affect this step. We propose that Soi1p promotes cycling of TGN membrane proteins between the TGN and PVC by antagonizing a TGN retention signal (TLS2) and facilitating the function of a retrieval signal (TLS1) that acts at the PVC.

The yeast (Saccharomyces cerevisiae) Kex2 protease is required in MATα1 haploid cells for the production of the mating pheromone α-factor (19). A type 1 transmembrane protein, Kex2p, is localized to a late Golgi compartment that is analogous to the TGN in mammalian cells (15, 35, 55). Localization of Kex2p to the TGN requires a TGN localization signal (TLS) in the COOH-terminal, cytosolic tail (C-tail) of the protein. This signal includes important aromatic residues Tyr713 and Phe715 (36, 55). Substitution of alanine for either Tyr713 or Phe715 results in rapid transport of Kex2p to the vacuole without passing through the plasma membrane (34, 55). Truncation of Kex2p after Ile718 results in a form of the protein, I718tail Kex2p (called D718Amb in reference 34), which possesses only the first 19 residues of the 115-residue C-tail (34). I718tail Kex2p is localized efficiently to the TGN, suggesting that the TLS is sufficient for localization (34). A similar aromatic residue–containing TLS has been identified in the NH2-terminal, cytosolic tail of Ste13p (dipeptidyl aminopeptidase A), a type 2 transmembrane protein that is also required for α-factor biosynthesis (29, 30). Kex1p, a carboxypeptidase involved in α-factor maturation, and Vps10p, a vacuolar protein sorting receptor, also require sequences in their COOH-terminal, cytosolic tails for proper TGN localization (6, 8, 9). In each case, mutation or deletion of TLS sequences in these proteins results in mislocalization to the vacuole (6, 8, 9).

Several studies suggest that steady-state localization of membrane proteins to the TGN is mediated by a cycling pathway between the TGN and the prevacuolar compartment (PVC), the intersection between vacuolar biosynthetic traffic and endocytic traffic that corresponds to the mammalian late endosome (31, 32, 37, 51). In class E vps mutants, which accumulate active vacuolar proteases in an
ing retrieval from the TGN to the PVC. This function is mediated through the modulation of the function of two TGN localization signals.

Materials and Methods

Strains, Antibodies, Reagents, and Media

Yeast strains used appear in Table I. SPB227-5C was crossed to JBY11 from which JBY11-r1 was a segregant (Table I). JBY96 was produced by a series of crosses between JBY11-r1, SEY6210, and YJM82. Synthetic minimal (SD) and synthetic complete containing glucose (SDC) or galactose (SCG), or rich medium (YPD: glucose) were as described (39), except that isoleucine, glutamate, aspartate, valine, and serine were omitted from SDC, and 50 μg/ml adenine sulfate was included in YPD. Dropout media are indicated as SDC-Ura, etc. Anti–alkaline phosphatase (ALP) serum was provided by Steve Nothwehr (University of Oregon, Eugene, OR); anti-CPY and anti-Vps10p sera were furnished by Scott Emr (University of California, San Diego, CA). Monoclonal anti–carboxypeptidase Y (CPY) was from Molecular Probes, Inc. (Eugene, OR). Anti–hemagglutinin (HA) mAb 12CA5 was obtained from Boehringer Mannheim (Indianapolis, IN). Rabbit anti–mouse IgG and gold-conjugated IgG (Jackson ImmunoResearch Laboratories, West Grove, PA). Unless otherwise noted, chemicals were from Sigma Chemical Co. (St. Louis, MO).

SOI1 Cloning, Sequencing, Mapping, and Disruption

To create a selection for Spo" transformants, we used a soil-2/soil-2 strain that was heterozygous at two recessive drug resistance loci, can1 and cyh2 (JBY96). After sporulation, one quarter of the haploid progeny of such a strain should be resistant to both drugs. Library (YPHI; LEU2 CEN yeast genomic library, ATCC No. 77162) transformants that were complemented for the Spo" phenotype were identified by selecting for haploid progeny on SD-Arg containing 40 μg/ml canavine sulfate and 10 μg/ml cycloheximide after incubation on sorbitol medium. Transformants producing drug-resistant progeny were picked from the sorbitol medium and examined microscopically for the production of ascii. Plasmids were retrieved into Escherichia coli as described (53).

Oligonucleotide primers were synthesized and sequencing reactions were performed by the University of Michigan core facility. Both strands of SOI1 were sequenced, and sequence analysis was performed using software from Intelligenetics (Palo Alto, CA).

To determine whether the insert in pSOI1.1 was linked to the SOI1 locus, a 3.3-kb ClaI fragment from pSOI1.1 was cloned into pRS315 (HIS3 integrating vector) to create pSOI1.3. pSOI1.3, digested at a unique Nco site in the insert, was used to transform CRY2. Four independent transformants were crossed to SPB227-5C, and 10 tetrads were dissected from the resulting diploids. All 39 complete tetrads displayed parental diploidal segregation of these phenotypes (2 His", Vps": 2 His", Vps"), indicating that the DNA insert from pSOI1.1 was tightly linked to the SOI1 locus.

SOI1 was replaced by the E. coli kan" gene by PCR amplification of the kan" gene using primers whose 5' sequences were derived from SOI1 (52). Transformants were selected on YPAD + 400 mg/liter Geneticin (GIBCO BRL, Gaithersburg, MD). Two different disruptions were created: soil-1 was a partial deletion of SOI1 with kan" in place of bases 2,178–9,078 in the coding sequence of SOI1. Deletion soil-1 was removed the entire SOI1 structural gene. No differences were observed between soil-2 and soil-1 strains (our unpublished data). The primers used to amplify the kan" gene from pFAkanMX2 were (5' to 3'): soil-2 upstream, (upstream), CCCCTGTT-GCCATCATTGAGCCGTACCAGTTATGCTATGACTTGATTTAAC-AGCTTGATACCCTGAG; soil-1 downstream, (downstream), TCACGGTCTT-GAGGACCATGATTGCTCCTGGGCTCCTAGTAGTACATGAAGGGG-GGACCATAGTGATCTCA; soil-2 downstream, (downstream), TGACTAACAAA-AGGGAAAAAGGCGAGAAAAAAATGTTAAAGCTGATTTAA-AAGCTCTTGACCAGCTGAG; soil-1 downstream, (downstream), GAATATTAGCTATGTAGTAAAGAAAGAGGGGTTATATACGATACCAGTGG-AGGGCAGTATTGGATCTA; soil-2 downstream, (downstream), GAATATTAGCTATGTAGTAAAGAAAGAGGGGTTATATACGATACCAGTGG-AGGGCAGTATTGGATCTA; PCR products were introduced into diploid strains, and disruption was confirmed by three-primer PCR using genomic DNA from G418" transformants. The primers used to confirm disruptions were (5' to 3'): kan" forward, GACTGACGTTTCGGAGCCCG; soil-1 mid (antisense), ACATTGACGGCGGCTTTCATT; soil-2 mid (antisense), GAATGGGGTACGTTAACCGGAG; soil-1 upstream (sense), GACTGACGTTTCGGAGCCCG; soil-2 upstream (sense), GACTGACGTTTCGGAGCCCG.
Table I. Yeast Strains

<table>
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*Only those loci differing from the parental strain are described.
‡Provided by Dr. Scott Emr.
*Provided by Dr. John McCusker (Duke University School of Medicine, Durham, NC).

TCAAATTGCCCAACCACGC TCC, soi1Δ-2 upstream (sense), ATTTAATTTGATGCGGATAGAA. Tetrads from diploid transformants were dissected, and the progeny were analyzed for the Vps phenotype and G418 resistance.

**Indirect Immunofluorescence**

Indirect immunofluorescence localization of Kex2p was performed as described (35), visualized using an Axioskop (Carl Zeiss, Inc., Thornwood, NY) microscope and photographed using Hypered Kodak Techpan 2415 film ASA 800 (Lumicon, Livermore, CA).

**Phenotypic Assays and Complementation Analysis**

The onset of impotence assay was performed as described (34). The Vps phenotype was analyzed essentially as described (40), using a monoclonal anti-CYp antibody followed by rabbit anti-mouse IgG and then donkey anti-rabbit IgG conjugated to HRP. Filters were processed for enhanced chemiluminescence according to the manufacturer’s instructions (Amer sham, Arlington Heights, IL). The Spo phenotype was scored microscopically as the production of asc. Resistance to G418 was scored as growth on YPAD + 400 mg/liter Geneticin.

The soi1 mutants were previously characterized as class A vps mutants (34), which display no alterations in vacuolar morphology (32). Complementation analysis with a complete collection of vps and pep mutants, provided by Dr. Bruce Horazdovsky and Dr. Scott Emr (University of California, San Diego, CA), indicated that all (1561-2, 0534-2, 1564-2) but one of the mutants designated as vps13 failed to complement the Vps and Spo* phenotypes caused by the soi1-2 mutation (data not shown). The allele that did complement soi1 (0594-2) was not allelic with the other soi1-2 mutants (data not shown).

**Subcellular Fractionation**

Cells were grown and labeled with [35S]H2SO4 (54) for 30 min. 2 × 10^6 cells were harvested by centrifugation, washed twice in 100 mM Tris, pH 9.4, 10 mM DTT, resuspended in spheroplasting buffer (10 mM Tris, pH 7.4, 1 mM DTT, 0.7 M sorbitol, 625 μg/ml zymolase 100T), and incubated at 30°C for 30 min. Spheroplasts were washed twice with 0.7 M sorbitol, 50 mM Hepes, pH 7, and resuspended in lysis buffer (0.3 M sorbitol, 10 mM Hepes, pH 7). DEAE dextran was then added slowly to a final concentration of 15 mg/ml (10), and the spheroplasts were incubated for 2 min on ice, at 37°C for 5 min, and then back on ice.

The supernatant from a 1,000 g centrifugation was spun at 13,000 g for 10 min to produce a pellet (P13) and supernatant fraction (S13). The S13 was then added to either lysis buffer or Triton X-100 (0.1% final concentration), and was spun at 50,000 rpm for 90 min in a TL155 rotor (Beckman Instruments, Palo Alto, CA). SDS was added to 1% to supernatant (S150) and the S150 pellets were resuspended in 8 M urea + 1% SDS. All samples were heated to 95°C for 3 min, diluted into 1 ml immunobuffer, and processed for immunoprecipitation (34).
Radioactive band intensities were quantified using a Molecular Dynamics PharosImager (Sunnyvale, CA) and IPLabGel software (Signal Analytics, Vienna, VA), and t12 values were determined by linear regression using data from five or more time points.

Site-directed Mutagenesis

DNA manipulations were performed as described (42). A NotI site was created at the 3’ end of SOI1 by mutagenesis of single-stranded, uracil-containing DNA (24) using the following primer: AGTACTGTGAAAGCGGGCCGCTGATCACATATG. After subcloning this mutation into pUC19 (pUC19-NotI,1), the triple-HA tag from pGTEP1 (provided by Dennis Thiele, University of Michigan, Ann Arbor, MI) was introduced as a NotI fragment to create pUC19-HA1 (50). A 3.2-kb PstI fragment containing DNA (24) using the following primer: AGTACTGTGAAGTGACCCT, 2) AGGGTCACTTCATGAATTTTCGTT; 3) CGAAAATCC, 2) GGATTTTCGTTTGTTCATACACTATC. After subcloning this mutation into pUC19 (pUC19-NotI,1), the triple-HA tag from pGTEP1 (provided by Dennis Thiele, University of Michigan, Ann Arbor, MI) was introduced as a NotI fragment to create pUC19-HA1 (50). A 3.2-kb PstI fragment containing DNA (24) using the following primer: AGTACTGTGAAGTGACCCT, 2) AGGGTCACTTCATGAATTTTCGTT; 3) CGAAAATCC, 2) GGATTTTCGTTTGTTCATACACTATC.

Results

Cloning of SOI1

In addition to Soi1-2 and Vps-2 phenotypes, soil mutations also resulted in a severe sporulation defect. As measured by the production of viable spores (38), the sporulation efficiency was reduced ~2,500-fold in soil homoygous diploid strains (data not shown). However, asci were not identifiable by microscopic inspection of cultures of soil homoygous diploid strains. Because of the stringent nature of the sporulation phenotype, we exploited it to clone SOI1 by complementation, as described in Materials and Methods. Screening a single-copy yeast genomic library, only 2 of 20,000 transformants were Spor-1. Plasmid loss and retransformation experiments confirmed that complementation of this phenotype was plasmid dependent (data not shown). Two Spor-1 transformants contained the same plasmid, hereafter called pSOI1.1, that had a 12-kb insert. Transformation of a MATα soil-2 kex2Δ strain with pSOI1.1 complemented both the Vps- and Soi1-2 phenotypes caused by the soil-2 mutation (Fig. 2 A).

Genetic linkage between soil-2 and the pSOI1.1 insert confirmed that pSOI1.1 contained the SOI1 gene (see Materials and Methods). Efforts to isolate smaller complementing fragments from pSOI1.1 were unsuccessful, suggesting that the length of the complementing gene was between 6 and 10 kb (Fig. 2 B). Sequencing of the insert from pSOI1.1 revealed a single long open reading frame of 9,432 bp (GenBank accession number AF001317; Fig. 2 B) predicted to encode a 3,144-residue polypeptide of 358 kDa (GenBank/EMBL/DDBJ accession number Z73145) revealed no significant differences. BLAST and FASTA similarity searches of the GenBank/EMBL/DDBJ database using the deduced amino acid sequence of Soil1 identified an open reading frame from Caenorhabditis elegans with significant similarity to SOI1 (T08G11.1; GenBank/EMBL/DDBJ accession number Z73145).

Figure 1. Schematic of mutations in the Kex2p cytosolic tail (C-tail). The positions of mutations in various forms of Kex2p that were used in this study are indicated. The Y713A (55) and I718tail (34) mutations have been described. TMD, transmembrane domain.

Figure 2. Isolation of SOI1. (A) KRY18-1A (MATα kex2Δ) and JBY11-1r1 (MATα kex2Δ soil-2), expressing Y713A Kex2p under the control of the GAL1 promoter from plasmid pCWXX1 (55), were transformed with either pBS32 (vector control) or pSOI1.1, and were analyzed for their Soi1 and Vps phenotypes, as described in Materials and Methods. For the mating assay, strains were shifted to glucose for 6 h before testing mating competence. (B) Restriction map of pSOI1.1 and analysis of subcloned fragments. At the top is shown a restriction map of the insert from pSOI1.1. Sequencing of the insert from pSOI1.1 revealed a single long open reading frame of 9,432 bp (GenBank accession number AF001317; Fig. 2 B) predicted to encode a 3,144-residue polypeptide of 358 kDa (GenBank/EMBL/DDBJ accession number Z73145) revealed no significant differences. BLAST and FASTA similarity searches of the GenBank/EMBL/DDBJ database using the deduced amino acid sequence of Soil1 identified an open reading frame from Caenorhabditis elegans with significant similarity to SOI1 (T08G11.1; GenBank/EMBL/DDBJ accession number Z73145).
Bank/EMBL/DDBJ accession number 1546759). This open reading frame would encode a protein of 3,212 amino acids that is 22% identical and 42% similar to Soi1p over its entire length, with the highest degree of similarity in the NH2 and COOH termini (Fig. 3B). Given the strong conservation of NH2- and COOH-terminal domains along with nearly identical overall length and similar sequence composition, T08G11.1 represents a likely Soi1p homologue in C. elegans, suggesting conservation of the protein and its function between yeast and metazoans.

The sequence composition of Soi1p, as assessed by analysis of overlapping 100 residue segments, was surprisingly homogeneous throughout the length of the sequence. The sequence contained 25% charged residues (Glu, Asp, Arg, and Lys) with a predicted pI of 5.23. The sequence was rich in residues found in α-helical coiled coils (Leu1Ile1Val1Met526%). Levels of Ala (4.5%) and Gly (4.2%) in Soi1p were low. These features are suggestive of a protein with an extensive structural motif; however, no heptad repeats longer than two to three turns were predicted (using the program Coils; references 25, 26). Although the program TMpred (17) predicted three potential transmembrane domains in Soi1p, the interpretation of this result was uncertain because each potential transmembrane domain contained several strongly hydrophilic or charged residues. No other motifs, domains, or identifiable structural features were predicted through analysis of the Soi1p sequence with various search programs (data not shown).

SOI1 Encodes a High Molecular Weight Protein That Exists in a Sedimentable Complex

To identify the protein encoded by SOI1, we introduced three copies of the HA epitope at the carboxy terminus of Soi1p (see Materials and Methods). This allele of SOI1 (encoding Soi1p-HA) was integrated at the SOI1 locus and complemented both the Vps2 and Spo2 phenotypes of the soi1D strain (data not shown). Monoclonal anti-HA antibodies selectively immunoprecipitated a 300-kD polypeptide from strains expressing Soi1p-HA (data not shown). To assess the possible association of Soi1p with membranes, cells labeled with [35S]SO4 were converted to spheroplasts, and osmotic lysates were fractionated by differential centrifugation (51). In this fractionation, the low-speed pellet (P13) should contain ER and vacuolar membranes. The high-speed pellet (P150) should contain Golgi membranes, transport vesicles, and secretory vesicles, and the high speed supernatant fraction (S150) should contain soluble proteins (see Materials and Methods). Because Soi1p-HA was very sensitive to proteolytic degradation in lysates (data not shown), the experiments were performed in a strain devoid of vacuolar proteases. Similar to Kex2p, 90% of Soi1p-HA sedimented at 150,000g, whereas very little (5%) sedimented at 13,000g (Fig. 4). In contrast, the vacuolar ALP, as expected (51), was found in both the P13 and P150 fractions (data not shown). However, addition of 0.1% Triton X-100 to the 13,000-g supernatant fraction before high speed centrifugation efficiently solubilized Kex2p, sedimentation of Soi1p-HA in the P150 fraction was unchanged (Fig. 4). Likewise, neither 1% deoxycholate nor 0.1 M Na2CO3, pH 11, had any effect on the sedimentation of Soi1p (data not shown). Thus, Soi1p-HA was associated with a sedimentable complex that was insensitive to detergent. Fractionation of lysates by discontinuous sucrose equilibrium density gradients revealed that >50% of Soi1p-HA cofractionated with one
a subpopulation of Kex2p-containing membranes (Sipos, G., J.H. Brickner, and R.S. Fuller, unpublished results). In Triton X-114 phase separation experiments, during which integral membrane proteins typically partition into the detergent phase (2), Soi1p-HA partitioned into the aqueous phase (data not shown). Therefore, Soi1p appears to exist in a high molecular weight heterooligomeric or homooligomeric complex that is peripherally associated with membranes.

**Disruption of SOI1**

Chromosomal deletions of *SOI1* were created by gene replacement using the bacterial *kanr* gene (52), which confers resistance to G418. Diploid strains heterozygous for either of two *SOI1* deletions (see Materials and Methods) were sporulated, and the tetrads were dissected. Nearly all tetrads produced four viable spores, indicating that *SOI1* was not essential. In all complete tetrads, the Vps- and G418' phenotypes segregated as single loci (2:2) and displayed tight linkage. All G418' progeny were Vps- and all G418' progeny were Vps+ (data not shown). Thus, like the original soi1 mutant isolates (34), soi1Δ strains were Vps+.

**The soi1 Null Mutation Affects Kex2p Localization, as Judged by Indirect Immunofluorescence**

By indirect immunofluorescence, Kex2p displays a punctate cytoplasmic distribution (35) that is also observed for other Golgi proteins (12, 29, 31). We examined localization of both WT and Y713A Kex2p in *SOI1* and soi1Δ strains by indirect immunofluorescence (Fig. 5). In the *SOI1* strain (i.e., the soi1Δ strain transformed with pSOI1.1), WT Kex2p localized as expected to punctate structures throughout the cytoplasm (Fig. 5 a). WT Kex2p was also localized in a similar punctate distribution in the soi1Δ strain (Fig. 5 b); however, the Kex2p-containing structures were often larger and/or more elongated (see wide arrows in Fig. 5 b). Such structures were also observed in the *SOI1* strain, but much less frequently (Fig. 5 a). Y713A Kex2p localized to faint punctate spots and occasionally to the vacuolar membrane in both the *SOI1* and soi1Δ strains (Fig. 4, c and d). In the soi1Δ strain, Y713A Kex2p was also found associated with larger structures similar to those observed in the case of WT Kex2p in the soi1Δ strain (Fig. 5 d). Thus, the soi1Δ mutant exhibited a qualitative effect on the localization of both WT Kex2p and Y713A Kex2p that may reflect a change in either the structure of Kex2p-containing compartments or the organelar distribution of Kex2p.

**TGN Localization of WT Kex2p, Vps10p, and Ste13p (A-ALP) Is Impaired in a soi1Δ Mutant**

The original soi1 mutant alleles accelerated the degradation of WT Kex2p (34). To examine the effect of the soi1Δ mutation on the vacuolar degradation of WT Kex2p, we measured the t1/2 of the protein in the soi1Δ strain. As in the case of the original soi1 alleles, WT Kex2p was degraded more rapidly in the soi1Δ strain (Fig. 6 A; t1/2 = 50 min ± 4 min [SEM], n = 3) than in the wild-type strain (t1/2 = 112 min ± 10 min, n = 3). Therefore, localization of WT Kex2p was perturbed by the soi1Δ mutation in the same way as by the original mutant alleles.

The Vps- phenotype of the original soi1 mutant was evaluated qualitatively by a colony immunoblotting assay for CPY secretion (Fig. 2 A). To analyze the missorting of vacuolar proteins in greater detail, we examined the sorting and processing of CPY by pulse chase/immunoprecipitation. Transport of CPY through the secretory pathway can be followed by the modifications that occur during its transport to the vacuole. Core-glycosylated proCPY, p1CPY, exits the ER and is further glycosylated in the Golgi complex to produce p2CPY, which is proteolytically processed upon delivery to the vacuole to produce mature enzyme (mCPY; 47). Spheroplasts were labeled with [35S]H2SO4 for 10 min and then chased for 60 min. After 60 min of chase, all of the CPY in the *SOI1* strain was found as intracellular mCPY (Fig. 6 B). In contrast, the soi1Δ strain was found to be impaired in the processing of CPY to the cell surface after 60 min of chase (Fig. 6 B). A smaller fraction of proPrA, another vacuolar proenzyme (20), was also missorted to the cell surface in the soi1Δ mutant (data not shown).

In the late Golgi, p2CPY is actively sorted to the vacuole by the Vps10p sorting receptor (16, 27). Vps10p is lo-
Deletion of SOI1 Suppresses the Effect of the Y713A Substitution on Mating but Does Not Alter the Overall Rate of Delivery of Y713A Kex2p to the Vacuole

Mutations in SOI1 were originally isolated as suppressors of the rapid loss of mating competence exhibited by a MATa strain after shutting off expression of Y713A Kex2p (34). Deletion of SOI1 also resulted in suppression (Fig. 7A). All three original soil alleles also decreased the rate of delivery of Y713A Kex2p to the vacuole (34). In contrast, deletion of SOI1 did not affect on the rate of vacuolar delivery of Y713A Kex2p. The rate of vacuolar degradation of Y713A Kex2p was the same in SOI1 and soilΔ strains (Fig. 6A; \( t_{1/2} = 21 \text{ min} \pm 2 \text{ min} \) [SEM] in soilΔ strain, \( n = 4 \); \( t_{1/2} = 23 \text{ min} \pm 3 \text{ min} \) in SOI1 strain, \( n = 4 \)). The onset of impotence assay reflects the level of Kex2p activity in the pro-α-factor processing compartment, presumably the TGN, whereas the \( t_{1/2} \) of Kex2p may also reflect downstream events, e.g., the rate of Kex2p delivery from the PVC to the vacuole. Therefore, complete loss of Soilp function apparently increased the concentration of Y713A Kex2p in the pro-α-factor processing compartment without measurably affecting the net rate of delivery of the protein to the vacuole. These results are consistent with a model in which Soilp functions in two distinct steps in a pathway or cycle involved in Kex2p localization, one step governing the rate of transport of Kex2p from the PVC to the vacuole and the other affecting the concentration of the protein in the TGN.

These data also demonstrate that the original three soil alleles, by their effects on the rate of transport of Y713A
Y<sub>713</sub>A Kex2p. Suppression by the original soi<sup>1</sup> allele and the deletion allele was identical in each case (data not shown). Therefore, suppression in this assay results from loss of Soi1p function, and the degree of suppression by the soi<sup>1</sup> mutations does not correlate with the <i>t</i><sub>1/2</sub> of the Y<sub>713</sub>A Kex2p.

**Analysis of the soi<sup>1</sup> Mutant Reveals the Existence of a Second TGN Localization Signal in the Kex2p C-tail, TLS2**

Consistent with the decreased <i>t</i><sub>1/2</sub> of WT Kex2p in soi<sup>1</sup> mutant strains, the original soi<sup>1</sup> alleles also exhibited a measurable effect on WT Kex2p in the onset of impotence assay (34). We therefore assessed the effect of the soi<sup>1</sup> mutation on WT Kex2p using this assay. As can be seen in Fig. 7A, 16 h after shutting off expression of WT Kex2p, the soi<sup>1</sup> strain mated slightly less well than the SOI1 strain, though much better than the soi<sup>1</sup> strain expressing Y<sub>713</sub>A Kex2p.

Also examined by the onset of impotence assay was I718tail Kex2p, which has only the first 19 amino acids of the tail. I718tail Kex2p behaved like WT Kex2p in both soi<sup>1</sup> and in the onset of impotence assay, suggesting that it contains all sequences within the C-tail necessary for localization (34). Despite the presence of the Tyr<sub>713</sub>-based signal in I718tail Kex2p, loss of Soi1p function had a much more profound effect on I718tail Kex2p than on WT Kex2p. I718tail Kex2p was lost more quickly from the TGN (Fig. 7B) and was delivered more rapidly to the vacuole (Fig. 7B) in the soi<sup>1</sup> strain than in the SOI1 strain. These results lead to two conclusions. First, Soi1p is required for the function of the TLS in I718tail Kex2p, presumably the Tyr<sub>713</sub>-based signal (Fig. 7A, compare I718tail Kex2p in SOI1 and soi<sup>1</sup> strains after 16 h on glucose). Second, to account for the superior TGN localization of WT Kex2p in the soi<sup>1</sup> strain (Fig. 7A, compare WT Kex2p to I718tail Kex2p in the soi<sup>1</sup> strain after 12 or 16 h on glucose), a second localization signal must exist in the WT Kex2p C-tail that is not present in I718tail Kex2p. We have designated this second signal TLS2 and the Tyr<sub>713</sub>-based signal TLS1.

The rates of degradation of both the WT and I718tail forms of Kex2p in the soi<sup>1</sup> strain represent the rates of transport to the vacuole because these proteins were stable in strains lacking vacuolar proteases (Fig. 7C).

**Suppression of Y<sub>713</sub>A Kex2p by the soi<sup>1</sup> Mutation Requires TLS2: Soi1p Antagonizes TLS2 Function**

The suppression of the mating defect of Y<sub>713</sub>A Kex2p in soi<sup>1</sup> strains could be explained in two ways. It might have resulted from loss of active discrimination against Ala at position 713, which might also explain the defect in the function of the WT TLS1. Alternatively, suppression might have resulted from the activation of TLS2 function by removal of Soi1p. The first model predicts that suppression of Tyr<sub>713</sub>Ala should be identical in the context of either the full-length tail or the I718tail, whereas the second model predicts that suppression should depend on TLS2. To distinguish between these models, we substituted Ala for Tyr<sub>713</sub> in I718tail Kex2p and assessed the TGN localization of this protein in SOI1 and soi<sup>1</sup> strains using the onset of impotence assay. Y<sub>713</sub>A I718tail Kex2p behaved...
identically in **SOII** and **soi1Δ** strains in the onset of impotence assay (Fig. 8, A and B). Suppression of Y713A was only observed when TLS2 was present, i.e., only in the context of the full-length tail. Therefore, suppression of Y713A Kex2p in **soi1** mutants resulted from activation of TLS2, implying that TLS2 is normally antagonized by Soi1p. Y713A-I718tail Kex2p, lacking both TLS1 and TLS2, behaved like C-tailΔ Kex2p in this assay (Fig. 8 A). **soi1** mutations were classified as allele-specific suppressors because they suppressed Ala and other substitutions at Tyr713, but not complete deletion of the C-tail in Kex2p (34). Examination of C-tailΔ Kex2p by both half-life determination (Fig. 7 D) and onset of impotence (Fig. 8 A) indicated that deletion of **SOII** had no effect on either the concentration of this protein in the TGN or its rate of delivery to the vacuole, consistent with results obtained with **soi1-1**, -2, and -3 strains (34). The basis for the allele-specific suppression of **soi1** mutations, therefore, is the activation of TLS2, a signal that is absent from both Y713A-I718tail Kex2p and C-tailΔ Kex2p.

An obvious question was whether TLS2 functioned at all in the presence of Soi1p. If so, the TGN localization of Y713A Kex2p in the **SOII** strain would be measurably better than that of Y713A I718tail Kex2p in the same strain. Y713A I718tail Kex2p was delivered to the vacuole and degraded with a t1/2 of 15 min in the wild-type **SOII** strain (Table II). The t1/2 of Y713A Kex2p was 50% longer (t1/2 = 23 ± 3 min; Table II) in the **SOII** strain. Thus, TLS2 appears to function to some degree even in a wild-type **SOII** strain.

### Optimal TLS1 Function Requires Soi1p, but TLS1 Exhibits Residual Function in the Absence of Soi1p

As discussed previously, I718tail contains a TGN localization signal that requires Soi1p for optimal function (Fig. 7 A; see also Fig. 8 B). Substitution of Ala for Tyr713 in the I718tail Kex2p completely abrogated localization in both **SOII** and **soi1Δ** strains (Fig. 8 B). Therefore, the Soi1p-dependent signal responsible for TGN localization of I718tail Kex2p is TLS1. However, comparison of I718tail Kex2p with Y713A I718tail Kex2p in the **soi1Δ** strain at both the 7- and 11-h time points clearly indicated that TLS1 exhibited significant residual function in the absence of Soi1p (Fig. 8 B).

### Soi1p Antagonizes a TGN Localization Signal in the Ste13p C-Tail That Is Distinct from the F-X-F87 Signal

Previously, we found that the original **soi1** mutations suppressed the effects of the F87A substitution in the Ste13p C-tail, as measured by the t1/2 of maturation of the A-ALP fusion protein (34). To determine conclusively whether suppression in this case resulted from loss of Soi1p function, we examined the t1/2 for maturation of F87A A-ALP in the **soi1Δ** strain (Fig. 9 A). In the **SOII** strain, F87A A-ALP

<table>
<thead>
<tr>
<th>Protein</th>
<th><strong>SOII</strong></th>
<th><strong>soi1Δ</strong></th>
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<tbody>
<tr>
<td>WT Kex2p</td>
<td>112 ± 10</td>
<td>50 ± 4</td>
</tr>
<tr>
<td>I718tail Kex2p</td>
<td>80</td>
<td>35</td>
</tr>
<tr>
<td>Y713A Kex2p</td>
<td>23 ± 3</td>
<td>21 ± 2</td>
</tr>
<tr>
<td>Y713A I718tail Kex2p</td>
<td>15</td>
<td>12</td>
</tr>
<tr>
<td>C-tailΔ Kex2p</td>
<td>8*</td>
<td>11</td>
</tr>
<tr>
<td>Vps10p</td>
<td>122 ± 9</td>
<td>70 ± 6</td>
</tr>
<tr>
<td>A-ALP</td>
<td>&gt;300</td>
<td>158 ± 7</td>
</tr>
<tr>
<td>F87A A-ALP</td>
<td>60</td>
<td>125</td>
</tr>
</tbody>
</table>

*The t1/2 for C-tailΔ Kex2p in the WT (**SOII**) background was previously measured as 11 min (55).
matured with a $t_{1/2}$ of $\sim 60$ min (Fig. 9; references 29, 36). In the soiI$\Delta$ strain, maturation of F$_{87}$A A-ALP was delayed, occurring with a $t_{1/2}$ of 125 min (Fig. 9 and Table II). Thus, deletion of SOII resulted in a slower rate of delivery of F$_{87}$A A-ALP to the vacuole, suggesting that loss of Soi1p resulted in activation of a second TGN localization signal in the Ste13p C-tail.

Deletion of SOII Does Not Affect Early Transport Steps in the Secretory Pathway

It was conceivable that the reduced rate of transport of F$_{87}$A A-ALP to the vacuole and the suppression of Y$_{713}$A Kex2p in the soiI$\Delta$ mutant might have resulted from slower transport steps proximal to the late Golgi. To address this possibility, we analyzed the rate of transport from the ER to the Golgi by following the rate of post-translational modification of Kex2p. If transport from the ER to the Golgi were slowed in the soiI$\Delta$ mutant, then conversion of core-glycosylated Kex2p (I$_2$; reference 54) to Golgi-glycosylated Kex2p (J) would be expected to be slowed. However, rapid pulse-chase immunoprecipitation indicated that the conversion from core-glycosylated pro-Kex2p (I$_1$) to I$_2$ and from I$_2$ to J were identical in SOII and soiI$\Delta$ strains (Fig. 9 B). This result also shows that TLS2 does not express its function during the early steps of the secretory pathway.

We also analyzed the rate of transport of proALP through the Golgi and to the vacuole. If the rate of ER-to-Golgi and/or intra-Golgi transport steps were slowed in the soiI$\Delta$ strain, then the rate of delivery of ALP to the vacuole should have been slowed. Analysis of the proteolytic maturation of pro-ALP, which occurs upon delivery of this protein to the vacuole (21), indicated that deletion of SOII had no effect on the rate of delivery of ALP to the vacuole. Therefore, mutation of SOII had no effect on the transport of proteins from the ER to the Golgi or through the Golgi.

TLS2 Regulates the Rate of Delivery of Kex2p to the PVC

The fact that activation of TLS2 can suppress mutation of TLS1 (i.e., in Y$_{713}$A Kex2p) suggested that TLS2 functions before TLS1. Specifically, the fact that deletion of SOII increased the amount of Y$_{713}$A Kex2p in the pro-a-factor processing compartment suggested that TLS2 delays the exit of Kex2p from the TGN. Conversely, TLS1 might function to promote retrieval of Kex2p from the PVC. To test this hypothesis, we measured the rate of transport of Kex2p to the PVC directly using a class E Vps mutant strain. We reasoned that, if TLS2 functioned to improve TGN retention of Kex2p, then it should slow the rate of degradation of Kex2p in class E PVC compartment. Furthermore, if TLS1 functioned in the retrieval of membrane proteins from the PVC to the TGN, then its integrity should have no effect on the rate of degradation of Kex2p in the class E vps mutant strains.

We analyzed the rate of turnover of WT Kex2p coexpressed with either I718tail Kex2p or Y$_{713}$A I718tail Kex2p in a vps28 strain (Fig. 10). All three forms of Kex2p were degraded rapidly in the vps28 strain at 30°C (Fig. 10 A), with I718tail Kex2p and Y$_{713}$A I718tail Kex2p exhibiting nearly identical $t_{1/2}$ values of 24 and 23 min, respectively (Fig. 10). This indicated that TLS1 has no effect on the rate of delivery to the PVC and likely functions to promote retrieval to the TGN. There was only a small difference between the rates of delivery of I718tail Kex2p and of WT Kex2p (i.e., full-length; $t_{1/2} = 27$ min $\pm$ 1 min) to the PVC. To amplify the possible difference between WT Kex2p and I718tail Kex2p in this assay, we compared the $t_{1/2}$ values of the two proteins in the vps28 mutant at 23°C (see Materials and Methods). Under these conditions, WT Kex2p was degraded with a $t_{1/2}$ of 37 min $\pm$ 2 min, while I718tail

Figure 9. (A) Improved TGN localization of F$_{87}$A A-ALP in a soiI$\Delta$ mutant strain. Strains JBY135-1A (soiI$\Delta$-1) and JBY135-2D (SOII), transformed with pSN98 (expressing F$_{87}$A A-ALP; reference 29), were labeled for 10 min with $[^{35}$S]H$_2$SO$_4$ and chased for 120 min. Samples were collected after 10, 20, 30, 60, and 120 min of chase and processed for immunoprecipitation using anti-ALP antisum. Band intensity of pro-A-ALP was quantified after SDS-PAGE (see Materials and Methods) and plotted relative to initial band intensity. (B) Deletion of SOII does not affect transport through early secretory compartments. Strains JBY154-1D (KEX2 soiI$\Delta$-2) and JBY154-8B (KEX2 SOII) were labeled with EXPRE$^{[35]$S}S label for 2 min before addition of chase. Cells were collected at the indicated times after addition of chase. Indicated beside the top panel are precursor forms of Kex2p, I$_1$ (pro-Kex2p possessing core glycosyl modifications) and I$_2$ (mature, core glycosylated Kex2p), and Golgi-modified, mature Kex2p (J; reference 54). Indicated beside the bottom panel are proALP and mature ALP (21).
of TLS2 leads to an increase in the concentration of Y713A to the PVC. Together with the observation that activation
strain. However, deletion of length and I718tail Kex2p to the PVC in the
regulates the rate of exit of Kex2p from the TGN.

SDS PAGE, WT Kex2p, I718tail Kex2p, or Y713A I718tail Kex2p
tated using antisera against the Kex2p lumenal domain. After
dition of chase, cells were collected, lysed, and immunoprecipi-
min (see Materials and Methods). At the indicated times after ad-
labeled for 10 min at 30
8
Fig. 10
arrows
Figure 10. TLS2 slows delivery of Kex2p to the PVC. (A) Mutation of TLS1 does not affect the rate of delivery to the PVC. Strain 0472-28 (vps28 KEX2) expressing either I718tail Kex2p (pCWXX10-17I8tail; top panel) or Y713A 17I8tail Kex2p (pCWXX11-17I8tail; bottom panel) on a CEN plasmid was pulse-
labeled for 10 min at 30°C in SDC-Met-Ura and chased for 80
min (see Materials and Methods). At the indicated times after addition of chase, cells were collected, lysed, and immunoprecipitated using antisera against the Kex2p lumenal domain. After SDS PAGE, WT Kex2p, I718tail Kex2p, or Y713A 17I8tail Kex2p were quantified, and t_{1/2} values were obtained by linear regression. Indicated are the positions of WT Kex2p (Full length; filled arrows), 17I8tail Kex2p, or Y713A 17I8tail Kex2p (open arrows). (B) Strain 0472-28 containing pCWXX10-17I8tail was grown at 23°C in SD + Ade, His, Leu, Lys, and Trp, labeled, chased, and processed for immunoprecipitation as described in A. Note that under otherwise identical conditions, the rate of degradation of Kex2p in the vps28 mutant is slower in SDC-Met-Ura than in SD + Ade, His, Leu, Lys, and Trp (data not shown). This may be caused by increased proteolytic activity in the PVC caused by amino acid limitation.

Kex2p was degraded with a t_{1/2} of 27 min ± 3 min (n = 2; Fig. 10 B). Thus, TLS2 slows the rate of delivery of Kex2p to the PVC. Together with the observation that activation of TLS2 leads to an increase in the concentration of Y713A Kex2p in the TGN (Fig. 8 A), these data suggest that TLS2 regulates the rate of exit of Kex2p from the TGN.

We attempted to analyze the rates of transport of full-length and I718tail Kex2p to the PVC in the soi1 vps28 strain. However, deletion of SOI1 in the context of the vps28 mutation stabilized I718tail Kex2p as well as full-length Kex2p, suggesting that the combination of the two mutations reduced proteolytic activity in the PVC (data not shown).

Mapping of TLS2

To map the Kex2p TLS2, we examined a series of truncated forms of Kex2p in the soi1 Δ strain using the onset of impotence assay. These truncation mutants were indistinguishable from full-length Kex2p in the SOI1 strain (Fig. 11). In the soi1 Δ strain, however, mating behavior indicated that whereas 778tail Kex2p possessed TLS2, its function was lost by deletion of six additional residues (see 772tail Kex2p in Fig. 11). Thus, the COOH-terminal endpoint of TLS2 appears to be discrete.

**Discussion**

Analysis of the soi1 Δ strain led to the identification of a second signal (TLS2) in the Kex2p C-tail whose function is antagonized by Soi1p. This signal appears to function at the TGN because it delays transport from the TGN to the PVC in the class E vps28 mutant, and because it increases the concentration of Kex2p in the pro-α-factor processing compartment. TLS1 does not appear to function at the TGN. Its presence does not affect the rate of transport from the TGN to the PVC in the class E vps28 mutant. This is consistent with the idea that transport of Kex2p from the TGN to the PVC does not depend on sequences in the C-tail (36). However, TLS1 function is important for proper TGN localization. Therefore, it must function in an event downstream from TGN to PVC transport. The most likely role of TLS1 is in promoting retrieval of Kex2p from the PVC. A similar conclusion has been reached concerning the role of the aromatic residue-containing signal, FXFXD, in the Ste13p C-tail (5). In contrast to TLS2, which is activated in the absence of Soi1p, TLS1 requires the presence of Soi1p for full function. Thus, Soi1p appears to function both at the TGN and the PVC.

Figure 11. Mapping TLS2. A series of Kex2p truncation mutants (shown schematically in Fig. 1) was expressed under the control of the GAL1 promoter in JBY-154-1A (MATα kex2Δ soi1Δ) and JBY154-2A (MATα kex2Δ SOI1), and was tested for mating competence in the soi1 Δ strain led to the identification of a second mutation at the carboxy terminus (P778S). This second mutation was inconsequential in that other isolates of this truncation that lacked this second mutation behaved identically to the one shown (data not shown).
A model for Soi1p function and the localization of TGN transmembrane proteins such as Kex2p and Ste13p is presented in Fig. 12. In this model, Soi1p promotes cycling of TGN transmembrane proteins between the TGN and PVC. Soi1p antagonizes the function of "retention" signals (TLS2 in Kex2p) in the TGN, promoting entry of proteins into transport vesicles targeted to the PVC. At the PVC, Soi1p promotes entry of proteins containing aromatic residue–containing retrieval signals (TLS1 in Kex2p) into retrograde transport vesicles. The proposed function of Soi1p at two organelles and at two distinct steps in this cycling pathway is an unusual and important feature of this model.

A second important feature is that Soi1p function is not required for transport of proteins between the TGN and PVC per se. Rather, Soi1p regulates the function of TLS1 and TLS2 which, in turn, regulate the entry of proteins into the cycling pathway. The localization of TLS2 that lack both signals (e.g., Y713A I718tail Kex2p or CtailΔ Kex2p) is unaffected by the loss of Soi1p. In other words, the function of Soi1p in promoting cycling is achieved through regulating the function of localization information and not through the efficiency of the transport steps that constitute this pathway. The role proposed for Soi1p is different from that of other proteins shown to be involved in TGN-to-PVC transport, including clathrin heavy chain and Vps1p. These proteins appear to be required for transport of all proteins from the TGN to the PVC. Loss of their function blocks transport of proteins regardless of the presence of signals in their tails (28, 36).

Although we have not characterized the role of Soi1p in localization of Ste13p in as much detail, it is likely to be similar to the role of Soi1p in Kex2p localization. Loss of Soi1p impaired the localization of WT A-ALP, but it improved the localization of F87A A-ALP, which lacks the aromatic residue–containing retrieval signal. These effects are consistent with a positive role for Soi1p function in the retrieval of Ste13p, which depends on the aromatic residue containing signal, and with Soi1p-dependent antagonism of a second signal in the Ste13p C-tail. Analysis of the Ste13p C-tail recently revealed the presence of a second signal, analogous to TLS2 in Kex2p, that is distinct from the aromatic residue–containing TLS, and that appears to prevent exit of Ste13p from the TGN (5). It seems likely that this signal is the one that is activated in the soi1Δ mutant, although this has not been tested. Analysis of residues 758–778 in Kex2p revealed two sequence blocks (E761-G763 and T768-D770) that had potential counterparts in the Ste13p C-tail. Moreover, truncation of the Kex2p C-tail between or downstream of these blocks disrupted TLS2 function (Fig. 11, 763tail and 772tail). However, mutant forms of Kex2p in which three alanines were substituted for either of these blocks behaved like full-length Kex2p in both SOI1 and soi1Δ strains, indicating that neither of these blocks was required for TLS2 function (data not shown). Conversely, deletions that inactivated the retention signal in the Ste13p C-tail did not remove the counterparts of the E761-G763 and T768-D770 sequences in the Ste13p C-tail (5). Thus, although they may fulfill analogous roles, the TGN retention signals in Kex2p and in Ste13p lack obvious sequence similarity.

Several observations suggest that this second TGN localization signal in Ste13p is a stronger signal than TLS2 in Kex2p. In SOI1 strains, the difference in the rate of delivery to the PVC between full-length Kex2p and I718tail Kex2p was small, indicating that TLS2 is ordinarily a weak signal (Fig. 10). In an analogous experiment using a class E vps27 strain, there was a substantial difference in the rate of delivery to the PVC between forms of A-ALP having the retention signal (t1/2 = 60 min) and forms lacking it (t1/2 = 15 min; reference 5). Deletion of SOI1, and, hence activation of TLS2, had no effect on the net rate of delivery of Y713A Kex2p to the vacuole. In contrast, deletion of SOI1 resulted in a significant decrease in the t1/2 of delivery of F87A A-ALP to the vacuole. Moreover, mutation of the aromatic residue–containing retrieval signal of Ste13p in F87A A-ALP resulted in a relatively modest defect in TGN localization (29). Therefore, it seems likely that the retention signal of Ste13p is more effective than the Kex2p TLS2 and, conversely, that the aromatic residue–based TLS1 is more important in the localization of Kex2p.

The difference between the Kex2p TLS2 and the Ste13p retention signal provides a reasonable explanation for the large difference in t1/2 between the two proteins (Figs. 5 and 6; references 29, 55). Why might different proteins

Figure 12. Model for role of Soi1p, TLS1, and TLS2 in the cycling of Kex2p between the TGN and PVC. TLS2 inhibits/delays entrance of Kex2p into the PVC transport vesicle at the TGN. Soi1p inhibits this function of TLS2. TLS1 and Soi1p together promote entry of Kex2p into newly forming TGN transport vesicle at PVC.
have localization signals of different strengths? Variation in the strength of a TGN retention signal offers a mechanism to adjust both the distribution of membrane proteins between the TGN and the PVC and the relative rates of turnover of TGN membrane proteins. The weak TLS2 in Kex2p might result in a more balanced distribution of the protein between the TGN and the PVC. This could have physiological significance if, for example, Kex2p processing activity were required in both the TGN and in the PVC. Some proteins may use one or the other kind of localization signal exclusively. Kex1p carboxypeptidase, for example, depends on sequences in its C-tail for proper TGN localization, but lacks an obvious aromatic residue–containing TLS (8). It is possible that the Kex1p C-tail contains only a TGN retention signal. Conversely, because the role of Vps10p in vacuolar protein sorting requires that it cycle efficiently between the TGN and PVC, Vps10p might not be expected to have a TGN “retention” signal. Localization of Vps10p might be mediated exclusively by signal- and Soi1p-dependent retrieval from the PVC.

Although the deduced amino acid sequence of Soi1p is unlike that of any proteins of known function, it does appear to be conserved between yeast and C. elegans (Fig. 3B). We have also identified human (cDNA clone 727106, GenBank/EMBL/DDBJ accession numbers AA292831 and AA398770) and Drosophila melanogaster (cDNA clone CK01879, GenBank/EMBL/DDBJ accession number AA141511) expressed sequence tags that represent other likely homologues. The existence of these probable homologues argues that mechanisms of TGN membrane protein localization are conserved between yeast and higher eukaryotes, including mammals. The cycling of Kex2p, Ste13p and Vps10p between the TGN and PVC is similar to that of mannose-6 phosphate receptors between the TGN and the late endosome in mammalian cells (22). In addition, net TGN localization of the mammalian Kex2p homologue furin is achieved by cycling between the TGN, the cell surface, and endosomal compartments (3, 7, 48).

The biochemical role of Soi1p is still largely unknown. Even though extensive heptad repeat regions are not found, the highly conserved domains at the NH2 and COOH termini, combined with a monotonous amino acid composition rich in aliphatic and charged residues in between, are reminiscent of intermediate filament proteins (13). Models of Soi1p function must accommodate roles at both the TGN and PVC. Soi1p might be associated with the TGN and PVC independently or might itself cycle between the two organelles in association with transport vesicles. An interesting possibility is that Soi1p forms a cytoskeletal element that physically links the TGN and PVC. Unfortunately, efforts to visualize Soi1p tagged with the HA epitope or with green fluorescent protein have been unsuccessful thus far, most likely due to the fact that Soi1p appears to be present at very low levels in cells (data not shown). On the basis of comparing the efficiency of labeling with [35S]methionine, we estimate Soi1p to be 5–10-fold less abundant than Kex2p, which is present at only a few hundred molecules per cell (reference 14 and data not shown).

It remains to be determined whether Soi1p physically interacts with TLS1 or TLS2. The sorting of TLS1-containing proteins back to the TGN is impaired in soi1Δ strains, which is consistent with a requirement for Soi1p in effect-tive TLS1-dependent retrieval. Soi1p is not absolutely required for the recognition of TLS1 in that substitution of Ala for Tyr134 in the Kex2p C-tail has a small but measurable effect in a soi1Δ strain (Fig. 8B). The defect in retrieval exhibited by the soi1Δ strain may result from either a sorting defect or a subtle defect in transport from the PVC to the TGN. A unifying role for Soi1p that is consistent with all the current data is in the recruitment of TGN membrane proteins into transport vesicles leaving both the TGN and the PVC. Such a function would necessarily antagonize a TGN retention signal and would also facilitate the use of a retrieval signal. This “recruitment factor” role for Soi1p is analogous to roles proposed for proteins such as Emp24p (43), Bst2p (11), and Shr3p (23), which are thought to promote sorting of cargo molecules into COPII-coated vesicles at the ER. Given the apparent disposition of Soi1p as a cytosolic peripheral membrane protein, we think Soi1p must function through interactions with other cytosolic factors or with cytosolic domains of transmembrane proteins. Identification of the interacting partners of Soi1p should help in dissecting the signal-mediated events in the localization of TGN membrane proteins.

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Note Added in Proof. While this paper was in press, a paper was published that described the identification of an insertion mutation in VPS13 (SOI1) as a suppressor of mutation in plasma membrane ATPase that diverts Pma1p to the vacuole (Luo, W., and Chang, A. 1997. Novel genes involved in endosomal traffic in yeast revealed by suppression of a target-
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