Abstract. In Saccharomyces cerevisiae, vesicles that carry proteins from the ER to the Golgi compartment are encapsulated by COPII coat proteins. We identified mutations in ten genes, designated sec-thirteen, that were lethal in combination with the COPII mutation sec13-1. ST1 showed synthetic-lethal interactions with the complete set of COPII genes, indicating that ST1 encodes a new COPII function. ST1 codes for a protein similar in sequence to the COPII subunit Sec24p. Like Sec24p, ST1 is a peripheral ER membrane protein that binds to the COPII subunit Sec23p. Chromosomal deletion of ST1 is not lethal, but inhibits transport of the plasma membrane proton-ATPase (Pma1p) to the cell surface, causing poor growth on media of low pH. Localization by both immunofluorescence microscopy and cell fractionation shows that the export of Pma1p from the ER is impaired in ST1Δ mutants. Transport of other proteins from the ER was not affected by ST1Δ, nor was Pma1p transport found to be particularly sensitive to other COPII defects. Together, these findings suggest that a specialized form of the COPII coat subunit, with ST1 in place of Sec24p, is used for the efficient packaging of Pma1p into vesicles derived from the ER.

Key words: endoplasmic reticulum • transport vesicle • COPII • proton-ATPase • Saccharomyces cerevisiae

The plasma membrane proton-ATPase (Pma1p) is an essential integral membrane protein that couples ATP hydrolysis to the translocation of protons across the plasma membrane (Serrano et al., 1986). The proton gradient generated by Pma1p then drives the uptake of nutrients, such as amino acids, from the extracellular medium (Vallejo and Serrano, 1989). A second physiological function of Pma1p is to maintain the cytosol at a neutral pH. In medium of low pH, the growth rate is limited by the amount of cellular Pma1p (McCusker et al., 1987; Portillo and Serrano, 1989). Pma1p transport to the cell surface depends upon the secretory pathway defined by the sec genes (Brada and Schekman, 1988; Chang and Slayman, 1991). Pma1p is one of the most abundant cargo molecules of the secretory pathway, constituting 25–50% of the total plasma membrane protein (Serrano, 1991). Because of its abundance and physiological importance, one might expect that yeast cells would have specialized mechanisms to ensure efficient transport of Pma1p through the secretory pathway. Such a function has been suggested for two proteins, Ast1p and Ast2p, in the transport of Pma1p from the Golgi compartment to the plasma membrane (Chang and Fink, 1995). For early steps in the secretory pathway, proteins that are specifically required for the transport of Pma1p have not yet been identified.

Proteins destined for the plasma membrane are transported from the ER to the Golgi compartment by vesicles coated with a set of proteins known as COPII (Barlowe et al., 1994). These COPII coats are thought to cause the deformation of the membrane into a vesicle and to recruit cargo molecules into vesicle buds (reviewed by Schekman and Orci, 1996). The stepwise recruitment and assembly of the COPII coat onto the membrane is thought to occur as follows. Action of the ER resident membrane protein Sec12p, a guanine nucleotide exchange factor for Sar1p, causes Sar1p to bind to the ER membrane (Barlowe and Schekman, 1993). Membrane-associated Sar1p, in turn, recruits the soluble Sec23p/Sec24p and Sec13p/Sec31p complexes (Matsuoka et al., 1998). Sec16p resides on the ER membrane and binds to both the Sec23p/Sec24p and Sec13p/Sec31p complexes, likely organizing their assembly onto the membrane (Espenshade et al., 1995; Gimeno et al., 1996; Shaywitz et al., 1997). To examine the role of different COPII coat subunits in recruitment of cargo molecules to vesicles, partially assembled COPII complexes have been tested for their ability to associate with cargo proteins. A association of a membrane-bound complex of Sar1p and...
Sec23p/Sec24p with integral membrane proteins indicates that cargo proteins may laterally partition into the vesicle membrane by virtue of their affinity for the Sec23p/Sec24p protein complex (A ridor et al., 1998; K uhn et al., 1998).

An early indication that the COPII coat subunits would physically interact came from specific genetic interactions between mutations in COPII genes. When temperature-sensitive mutations in COPII genes are combined, the resulting double mutants are almost always more growth-restrictive than the component single mutations, and are usually inviable at 24°C. These synthetic-lethal interactions are restricted to genes involved in COPII vesicle formation and do not occur when mutations in genes required for vesicle formation are combined with genes required for vesicle fusion (Kaiser and Schekman, 1990). The specificity of this type of genetic interaction suggested that synthetic lethality with known COPII mutations would be a useful criterion to identify new mutations involved in the assembly of the COPII coat.

We screened for mutations that were lethal with the COPII mutation sec13-1 and identified ten LST genes (lethal with sec-thirteen). As we describe elsewhere, most of the LST genes are related to an unanticipated role for SEC13 in the regulated delivery of specific amino acid precursors to the cell surface (Roberg et al., 1997a,b). Accordingly, these LST genes display synthetic-lethal interactions with SEC13, but not with the other COPII genes. On the other hand, mutations in LST1 were lethal with the full set of mutations defective in COPII vesicle budding, but not with mutations defective in vesicle budding, indicating that LST1 does participate in vesicle budding at the ER. Here we show that LST1 encodes a homologue of the COPII subunit, Sec24p, and that LST1p is a peripheral membrane protein localized to the ER that can form complexes with Sec23p. The LST1 gene is not essential, but by examination of the phenotypes of lstΔ mutants we show that LST1 is required for the efficient export of Pma1p from the ER to the Golgi compartment. These results suggest a specialized form of the vesicle coat that is responsible for recruitment of Pma1p into COPII-coated vesicles.

Materials and Methods

Media, Strains, and Plasmids

The Saccharomyces cerevisiae strains used in this study are listed in Table I. Rich medium (YPD) and supplemented minimal medium (SM M) were prepared according to K aiser et al. (1994). To evaluate growth at low pH, YPD was adjusted to pH 3.8 with HCl (this medium remained at pH 3.8 throughout the growth of a yeast culture). For some experiments, SMM rich medium (YPD) and supplemented minimal medium (SMM) were prepared according to Kaiser et al. (1994). To evaluate growth at low pH, I. Rich medium (YPD) and supplemented minimal medium (SMM) were adjusted to pH 3.8 with HCl (this medium remained at pH 3.8 throughout the growth of a yeast culture). For some experiments, SMM was adjusted to pH 3.8 with HCl (this medium remained at pH 3.8 throughout the growth of a yeast culture).

Synthetic-lethal Screen

The following plasmids and strains were constructed for use in the sec13-1 synthetic-lethal screen. The plasmid pKR1 carries SEC13 on a 1.8-kb SalI/BamHI fragment excised from pCK1313 (P ryer et al., 1993), inserted into pRS316 (Sikorski and H eiter, 1989). pKR4 carries the same 1.8-kb SalI/BamHI fragment and a 3.8-kb NheI/BamHI fragment containing AD E3 from pD K255, both inserted into the vector pRS315 (Sikorski and H eiter, 1989). CYU563 and CKY54 were crossed to produce a MATa ade2 ade3 leu2 ura3 sec13-1 segregant, which was transformed with pKR4 to give CKY423. The mating type of CKY423 was switched by ectopic expression of the H O gene (H erskovitz and J ensen, 1991) to give CKY424.

 Cultures of CKY423 and CKY424 were mutagenized by irradiation with a germicidal UV lamp at a dose resulting in 10% cell survival. Mutagenized cells were plated on YPD at a density of 150 colonies per plate. After 5 d of growth at 24°C, colonies with a solid red color and no white sectors were selected for further analysis. The dependence of the nonsecreting phenotype on the sec13-1 mutation was tested by transforming candidate mutants with either pKR1 or pRS316. Strains that sectored after transformation with pKR1, but not after transformation with pRS316, were scored as sec13-1–dependent.

Complementation tests were performed by mating mutants isolated from CKY423 with those isolated from CKY424. Zygotes isolated by micromanipulation were scored for their ability to form sectored colonies on YPD plates. The genes defined by these complementation groups were designated LST. All lst mutant strains were backcrossed to a parental strain twice. The lst sec13-1 double mutants were converted to lst single mutants by integration of a wild-type copy of SEC13 at the sec13-1 locus, using the integrating plasmid p1312 (S ikerski and H eiter, 1989). The integrants were grown on YPD and cells from white mutants (indicating loss of plasmid) were used as an additional test to establish that the inviable spores always carried the sec mutation, and therefore were not the result of random spore death.

To test for synthetic-lethal interactions between lst mutations and mutations in sec genes, lst mutants CKY435 (lst1Δ-1), CKY436 (lst2-1), CKY437 (lst3-1), CKY438 (lst4-1), CKY439 (lst5-1), CKY440 (lst6-1), CKY441 (lst7-1), and CKY442 (lst8-1) were crossed to the sec mutants CKY45 (sec13-1), CKY50 (sec16-2), CKY78 (sec23-1), and CKY450 (sec31-2). Viability of a given lst sec double mutant was inferred from crosses where lethality segregated as a two-gene trait (most tetrad giving a segregation pattern of 1:3 for lethality), an outcome that was easily detectable since crosses to wild-type typically gave >95% spore viability. The segregation pattern of the sec mutation in the surviving sister spores was used as an additional test to establish that the inviable spores always carried the sec mutation, and therefore were not the result of random spore death.

Construction of lstΔ Mutants

Replacement of the chromosomal LST1 gene with an allele disrupted with the LEU2 gene was constructed as follows. pKR18 carries the 5′ half of LST1 on a 2.0-kb XhoI/indlI fragment inserted into pRS316. A 2.0-kb HindIII/BamHI fragment containing the LEU2 gene from plasmid pG252 (J ones and P rakas, 1990) and a 250-bp BclI/SacI fragment from the 3′ noncoding region of LST1 were inserted into pKR18 to construct pKR28. The NH2-terminal coding region of LST1 (except for codons 1–13) was removed by deleting a 1.7-kb Eco47II/HindIII fragment from pKR28 to gen-
erate pKR 28A. The lst1Δ::LEU2 construct, liberated from pKR 28A by digestion with Xho I, was transformed into the wild-type diploid strain CKY 348 (MATα leu2-3,112/leu2-3,112 ura3-52/ura3-52). On sporulation and dissection, this diploid gave four viable spore clones, and haploid segments carrying lst1Δ::LEU2 were confirmed by Southern blotting. One such segregant was further backcrossed to our S288C genetic background to give strains CKY 536 and CKY 542.

**Proton Efflux from Intact Yeast Cells**

Pma1p activity was assayed by proton efflux from intact cells into the external medium. Cells were grown to exponential phase in YPD at 37°C, washed, and then stored in deionized water at 4°C overnight. Cell number was measured by light scattering, and a total of 25 A600 units (~5 x 10^8 cells) was suspended in 5 ml of 100 mM KCl, 10 mM sodium phosphate buffer, pH 4.0. The pH of the cell suspension was measured using a combination electrode at 25°C with constant stirring. Once the pH had stabilized (~10 min), glucose was added to a final concentration of 40 mM and the ensuing drop in pH was recorded at 30-s intervals over 15 min. In comparison of wild-type (CKY 443) and lst1Δ (CKY 536) strains, both suspensions had identical cell concentration as measured by light scattering, and showed the same response to calibration pulses with HCl.

**Immunofluorescence Microscopy**

The intracellular location of Pma1p in wild-type (CKY 443) and lst1Δ (CKY 536) cells was examined by indirect immunofluorescence microscopy using techniques described previously (Pringle et al., 1991; Espenshade et al., 1995). Strains were grown exponentially in 50 mM medium, pH 7.2, at 30°C. Cells were fixed in 3.7% formaldehyde and then converted to spheroplasts by digestion with lyticase. Both primary and secondary antibody incubations were for 1 h at 25°C. Afinity-purified anti-Pma1p antibody was prepared as follows. A crude preparation of yeast membranes was resolved by preparative SD-S-PAGE, and after transfer of proteins to a nitrocellulose membrane by electrophoresis, the strip of membrane that contained Pma1p was excised. A rabbit antiserum to Pma1p was applied to the nitrocellulose strip, and after the strip was washed with 20 mM Tris, pH 7.5, 150 mM NaCl, 0.5% Tween 20, the bound antibody was eluted with 100 mM glycine, pH 2.8, 500 mM NaCl, 0.5% Tween 20. A affinity-purified Pma1p was used at a 1:100 dilution and FITC-conjugated anti-rabbit IgG was used at a 1:200 dilution. Munting medium was supplemented with 4,6-diamidino-2-phenylindole (DAPI). Micrographs were taken with a Nikon Eclipse TE300 microscope with a Hamamatsu Orca C4742-95 CCD camera.

For the localization of Lst1p-HA, CKY 535 was grown on 5 M NaCl to exponential phase and prepared as described above. For visualization of Lst1p-HA, the 12CA 5 antibody (Berkely A nitrobl, Co., Inc.) was used at a 1:5,000 dilution and FITC-conjugated goat anti-mouse IgG was used at a 1:50 dilution. Rabbit anti-Kar2p polyclonal serum (a gift of M. Rose, Princeton University, Princeton, NJ) was used at a 1:1,000 dilution and rhodamine-conjugated goat anti-rabbit IgG was used at a 1:200 dilution. Samples were viewed and imaged using a Nikon OPTIPHOT 2 microscope and a Photometric ImagePoint CCD camera. Images were recorded using IP Lab software (Molecular Dynamics, Inc.).

**Cell Fractionation**

Cell organelles were fractionated on equilibrium density gradients as previously described (Roberg et al., 1997a). Cultures were grown exponentially at 24°C and then shifted to 37°C for 3 h. 1.6 x 10^8 cells were collected by centrifugation and suspended in 0.5 ml ST E10 (10% [wt/wt] sucrose, 10 mM Tris-HCl, pH 7.6, 10 mM EDTA) with a protease inhibitor cocktail (1 mM PM SF, 0.5 μg/ml leupeptin, 0.7 μg/ml pepstatin, 0.7 μg/ml aprotinin) and lysed by vortexing with glass beads. A 2 ml of ST E10 was added, and the lysate was cleared of unbroken cells and large cell debris by centrifugation and suspended in 0.5 ml ST E10 (10% [wt/wt] sucrose, 10 mM Tris-HCl, pH 7.6, 10 mM EDTA) prepared for an SW50.1 rotor (Beckman Instruments, Inc.). Samples were centrifuged 100,000 g for 18 h at 4°C and fractions of 300 μl were collected from the top of the gradient. Protein was precipitated from each fraction by the addition of 100 μl of 0.15% deoxycholate and 100 μl of 72% trichloroacetic acid. Protein pellets were collected by centrifugation at 13,000 g, washed with cold acetone, and.

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**Table I. S. cerevisiae Strains**

| Strain       | Genotype                                      | Source or reference                  |
|--------------|-----------------------------------------------|--------------------------------------|
| CUY563       | MATα ade2-101 ade3-24 leu2-3,112 ura3-52      | T. Huffaker (Cornell University)      |
| CUY564       | MATα ade2-101 ade3-2 leu2-3,112 ura3-52       | T. Huffaker (Cornell University)      |
| EGY40        | MATα ura3-52 leu2 his3 tp1                     | Golemis and Brent, 1992              |
| CKY45        | MATα sec13-1 his4-619 ura3-52                 | Kaiser Lab Collection                |
| CKY50        | MATα sec16-2 his4-619 ura3-52                 | Kaiser Lab Collection                |
| CKY54        | MATα sec17-1 his4-619 ura3-52                 | Kaiser Lab Collection                |
| CKY58        | MATα sec18-1 his4-619 ura3-52                 | Kaiser Lab Collection                |
| CKY78        | MATα sec23-1 his4-619 ura3-52                 | Kaiser Lab Collection                |
| CKY348       | MATα leu2-3/leu2-3 ura3-52/ura3-52             | Kaiser Lab Collection                |
| CKY423       | MATα sec13-1 ade2-101 ade3-24 leu2-3,112 ura3-52 | [pKR4]                              |
| CKY424       | MATα sec13-1 ade2-101 ade3-24 leu2-3,112 ura3-52 | [pKR4]                              |
| CKY426       | MATα lst1::sec3-1 ade2-101 ade3-24 leu2-3,112 ura3-52 | [pKR4]                              |
| CKY435       | MATα lst1::sec13-1::SEC13, URA3 ade2-101 ade3-24 leu2-3,112 ura3-52 | [pKR4]                              |
| CKY436       | MATα lst2::sec13-1::SEC13, URA3 ade2-101 ade3-24 leu2-3,112 ura3-52 | [pKR4]                              |
| CKY437       | MATα lst3::sec13-1::SEC13, URA3 ade2-101 ade3-24 leu2-3,112 ura3-52 | [pKR4]                              |
| CKY438       | MATα lst4::sec13-1::SEC13, URA3 ade2-101 ade3-24 leu2-3,112 ura3-52 | [pKR4]                              |
| CKY439       | MATα lst5::sec13-1::SEC13, URA3 ade2-101 ade3-24 leu2-3,112 ura3-52 | [pKR4]                              |
| CKY440       | MATα lst6::sec13-1::SEC13, URA3 ade2-101 ade3-24 leu2-3,112 ura3-52 | [pKR4]                              |
| CKY441       | MATα lst7::sec13-1::SEC13, URA3 ade2-101 ade3-24 leu2-3,112 ura3-52 | [pKR4]                              |
| CKY442       | MATα lst8::sec13-1::SEC13, URA3 ade2-101 ade3-24 leu2-3,112 ura3-52 | [pKR4]                              |
| CKY443       | MATα prototroph                               | Kaiser Lab Collection                |
| CKY473       | MATα leu2-3,112 ura3-52 Gal°                   | Kaiser Lab Collection                |
| CKY534       | MATα lst1::LEU2 leu2-3,112 ura3-52             | [pKR17HA]                           |
| CKY535       | MATα lst1::LEU2 leu2-3,112 ura3-52             | [pKR17HA]                           |
| CKY536       | MATα lst1::LEU2 ura3-52 leu2-3,112             | [pNV31]                             |
| CKY540       | MATα leu2-3,112 ura3-52                        | [pNV31]                             |
| CKY541       | MATα sec12-4 ura3-52                          | [pNV31]                             |
| CKY542       | MATα lst1::LEU2 leu2-3,112 ura3-52             | [pNV31]                             |
| CKY552       | MATα lst1::LEU2 leu2-3,112 ura3-52             | [pNV31]                             |

All strains are from this study unless otherwise indicated.

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then solubilized in ESB (60 mM Tris-HCl, pH 6.8, 100 mM DTT, 2% SDS, 10% glycerol, 0.02% bromophenol blue). Pma1p, Gas1p, and Sec61p were resolved by SDS-PAGE and were detected by immunoblotting. The relative amount of each protein in cell fractions was determined by densitometry using an Ultroscan 2002 (LKB Instruments, Inc.). The Golgi GDPase activity was assayed in gradient fractions before protein precipitation using standard methods (A beijon et al., 1989).

The subcellular distribution of Lst1p-HA was examined using techniques described previously (Espenshade et al., 1995). CYK 535 carrying pKR17HA, which expresses Lst1p-HA, was grown to exponential phase in SM M without uracil. 2 × 10^6 cells were harvested, converted to spheroplasts, and then gently lysed by glass beads in 500 μl of cell lysis buffer (20 mM MES, pH 6.5, 100 mM NaCl, 5 mM MgCl2) including the protease inhibitor cocktail. The cell extract was sequentially centrifuged at 500 g for 20 min, 10,000 g for 20 min, and 150,000 g for 60 min, to give one soluble and three particulate fractions.

Release of Lst1p-HA from the particulate fraction was examined by treating cell extracts with 500 mM NaCl, 100 mM sodium carbonate, pH 11.5, 2.5 M urea, or 1% Triton X-100. A 1 ng of 1-h incubation at 4°C, samples were centrifuged at 50,000 g for 30 min to separate soluble and particulate fractions. Fractions from both experiments were solubilized in sample buffer and analyzed by immunoblotting.

**Immunoblotting**

Samples of 10–30 μl in ESB were resolved by SDS-PAGE and immunoblotting was conducted according to standard protocols (Harlow and Lane, 1988). For transfer of Lst1p to nitrocellulose membranes, 0.1% SDS was included in the transfer buffer. The following antibodies were used: mouse monoclonal 12CA 5 anti-HA at 1:1,000 dilution; rabbit anti-Pma1p (a gift of A. Chang, A. Libert E. College of Medicine, B. Ronx, NY) at 1:500 dilution; rabbit anti-Gas1p (a gift of H. Riezman, University of Basel, Basel, Switzerland) at 1:10,000 dilution; rabbit anti-Sec61p (a gift of R. Scheckman, University of California, Berkeley, CA) at 1:13,000 dilution; rabbit anti-Gdh2p (a gift of B. Magasanik, Massachusetts Institute of Technology, Cambridge, MA) at 1:1,000 dilution; HRP-coupled sheep anti–mouse Ig and HRP-coupled sheep anti–rabbit Ig (Nycomed A mersham Corp.) at 1:1,000 dilution. Blots were developed using chemiluminescence detection system (Nycomed A mersham Corp.).

**Pulse–Chase Kinetics of Invertase Maturation**

The strains used for radiolabeling all carried the plasmid pNV31, which carries the SUC2 gene under the constitutive TPI1 promoter (a gift of M. Lewis, Medical Research Council Laboratories of Molecular Biology, Cambridge, UK). Wild-type (CYK 540) and rlsΔ1 (CYK 542) strains were grown in SMM without methionine (buffered with 50 mM MES and 50 mM NaCl) for 5 min before the addition of 2% galactose as described above. 2 h after induction, 2 × 10^6 cells were collected by centrifugation and resuspended in 20 μl of lysis buffer. 20 μl of cell lysate buffer (20 mM MES, pH 6.5, 100 mM NaCl, 5 mM MgCl2) was added to 200 μl of 2 × ESB, and incubation was continued for 2 h at 30°C to allow for complete lysis. The lysate was cleared of unlysed cells and large cell debris by centrifugation at 300 g for 3 min. 50 μl of the supernatant was reserved for a total extract sample and the remainder was centrifuged to pellet ER membranes at 10,000 g for 30 min at 4°C in a microcentrifuge. A equal number of cell equivalents of total extract, membrane-pellet, and supernatant fractions was solubilized in ESB and analyzed by immunoblotting. The cytosolic protein Gdh2p was found only in the soluble fractions, demonstrating cell lysis was complete (data not shown).

**Results**

**Mutations Synthetically Lethal with sec13-1**

To find new genes required for the budding of COP11 vesicles, we screened for mutations that displayed synthetic lethality with the COP11 mutation sec13-1 using a plasmid sectoring assay (Roberg et al., 1997b). Strain CYK 423 has the chromosomal mutations ade2 ade3 sec13-1 and harbors the plasmid pKR4, which carries wild-type copies of SEC13 and ADE3. This strain accumulates a red pigment because of the ade2 mutation, but the spontaneous loss of pKR4 during the growth of a colony gives white sectors of ade2 ade3 segregants. In this strain, a mutation that is lethal with sec13-1 will produce a nonsectoring colony. Mutation of CYK 423 and the isogenic strain of opposite mating type, CYK 424, yielded 139 nonsectoring mutants (Fig. 1). These strains were then tested for restored ability to sector after transformation with pKR1, which carries wild-type SEC13, but lacks the ADE3 gene. By this test, 57 of the mutants had synthetic-lethal mutations that could be rescued by wild-type SEC13. In backcrosses, 52 mutants gave a segregation pattern indicating that the trait was due to a single nuclear mutation (Fig. 1).
Table II. Mutations Lethal with sec13-1

| Gene  | Number of alleles |
|-------|-------------------|
| LST1  | 11                |
| LST2  | 6                 |
| LST3  | 4                 |
| LST4  | 5                 |
| LST5  | 5                 |
| LST6  | 1                 |
| LST7  | 1                 |
| LST8  | 1                 |
| LST9  | 1                 |
| LST10 (SEC16) | 2       |

Table III. Growth of lst sec Double Mutants at 24°C

| lst sec | sec13-1 | sec16-2 | sec23-1 | sec31-1 | sec17-1 | sec18-1 |
|---------|---------|---------|---------|---------|---------|---------|
| lst1-1  | -       | -       | -       | -       | +       | +       |
| lst2-1  | -       | +       | +       | +       | ND      | ND      |
| lst3-1  | +       | -       | +       | +       | ND      | ND      |
| lst4-1  | +       | +       | -       | +       | ND      | ND      |
| lst5-1  | -       | +       | -       | +       | ND      | ND      |
| lst6-1  | -       | +/-     | -       | +       | -       | +       |
| lst7-1  | -       | +       | +       | +       | ND      | ND      |
| lst8-1  | -       | +       | +       | +       | ND      | ND      |

Growth is represented in decreasing order by: + > +/- > +/− > −. ND, not determined.
fragment into pRS306 to produce pKR7. pKR 7 was inte-
gerated at its chromosomal locus after linearization with
MscI and was then checked for linkage to
lst1-1. Tetrad analysis showed that pKR7 was not linked to
lst1-1 and we concluded that pKR7 carries an unlinked suppressor gene.

The 3.5-kb insert of p77-2 was inserted into the XhoI site of the centromeric vector pRS316 to construct pKR17. The base sequence of this insert was determined and found to contain a single open reading frame encoding a protein of 929 amino acids. This sequence corresponds to the open reading frame YHR098c located on chromosome VIII (Saccharomyces Genome Database, Cherry et al., 1997). The predicted amino acid sequence of LST1 shows significant similarity to SEC24 (YIL109C). The two proteins share 23% sequence identity that extends over most of their length (Fig. 2), suggesting that Lst1p may have a function similar to that of Sec24p as a subunit of the COPII vesicle coat.

**Phenotypes of lst1Δ**

One copy of the LST1 gene in the wild-type diploid strain CY 348 was disrupted to generate a lst1Δ::LEU2/LST1 heterozygote. Sporulation and dissection of this diploid gave >95% spore viability on YPD medium and the L E U 2 marker segregated 2:2, showing that LST1 is not essential for growth. A lst1Δ::LEU2 mutant spore clone was crossed to sec mutants to test for synthetic lethality. In crosses of lst1Δ to sec12, sec13, sec16, sec23, sec24, or sec31 mutants, inviability segregated as a two-gene trait (segregation patterns for dead/viable spore clones were 2:2, 1:3, and 0:4). Tests of the genotype of the surviving sister spore clones showed that the inviable spores in these crosses were always lst1Δ sec double mutants. Crosses between lst1Δ and sec17 or sec18 produced viable double mutants. These findings confirmed and extended our earlier tests for synthetic lethality with lst-1, and demonstrated that lst1Δ was synthetically lethal with all the known genes required for COPII vesicle formation, but not with genes required for vesicle fusion.

We evaluated the growth of lst1Δ::LEU2 mutants under a variety of conditions. On Y PD , the lst1Δ::LEU2 strain grew, as well as an isogenic wild-type strain at temperatures ranging from 14 to 37°C. However, on SM M the lst1Δ::LEU2 strain grew poorly at temperatures above 30°C. Since YPD (pH 6.5) and SM M (pH 3.8) differed markedly in pH, we suspected that lstΔ mutants may be particularly sensitive to an acidic environment, and we tested the effect of pH on the growth of lstΔ mutants. A lthough lstΔ mutants grew as well as wild-type on YPD at all temperatures, when YPD was brought to pH 3.8, lstΔ mutants grew much more slowly than wild-type at 37°C.
Having identified conditions where \( LST1 \) was needed for growth, we investigated whether overexpression of \( SEC24 \) could supply the function lost in \( lst1\Delta \). Some restoration of function was indicated by the ability of an \( lst1\Delta \) mutant to grow on acidic medium when provided with extra copies of \( SEC24 \) on either centromeric or \( 2\mu \) plasmids (Fig. 3 B). These findings imply some functional overlap between \( LST1 \) and \( SEC24 \). In parallel tests for suppression, we found that the genes \( SEC23, SEC13, SEC31, \) or \( SEC23 \), when expressed from \( 2\mu \) plasmids, could not restore the ability of an \( lst1\Delta \) mutant to grow on acidic medium. We found that the \( lst1\Delta \) mutation caused a selective defect in the trafficking of Pma1p from the ER, and we also examined the ability of overexpressed \( SEC24 \) to suppress this phenotype caused by the \( lst1\Delta \) mutation. By immunofluorescence microscopy, the proper localization of Pma1p to the cell surface was restored in an \( lst1\Delta \) strain that also carried \( SEC24 \) on a \( 2\mu \) plasmid (see Fig. 5).

In an attempt to test the effect of overexpression of \( LST1 \), we found that \( LST1 \) on a \( 2\mu \) plasmid severely impaired growth of wild-type yeast cells. To examine the response of cells to different doses of \( Lst1p \), we designed a way to express different levels of \( Lst1p \) according to the amount of galactose in the growth medium. A wild-type strain (CKY473) carrying a plasmid that expressed \( LST1 \) from \( pGAL1 \) (pKR35) was spread on an SM M plate with 2% raffinose, a carbon source that allows yeast growth without repression of the \( G A L1 \) promoter. When these cells are exposed to a gradient of galactose concentrations, from 3 mg of galactose in a filter disk on top of the lawn, growth was inhibited in a halo 1.5 cm beyond the edge of the filter (Fig. 3 C). A strain that did not contain \( pKR35 \) grew uniformly up to the edge of the filter, showing that the galactose itself was not inhibitory. Given the similarity of \( Lst1p \) to \( Sec24p \), we asked whether the overexpression of \( SEC24 \) could compensate for overexpression of \( LST1 \). Cells carrying both the \( pGAL1-LST1 \) plasmid (\( pKR35 \)) and the \( SEC24 \) gene on a \( 2\mu \) plasmid (\( pKR41 \)) were tested in an identical halo assay, and were found to be resistant to the effect of galactose (Fig. 3 C). Suppression by \( SEC24 \) appeared to be specific, as parallel tests of \( 2\mu \) plasmids carrying \( SEC12, SEC13, SEC31, \) or \( SEC23 \) failed to show suppression. It is worth noting that \( SEC23 \) expressed from a \( 2\mu \) plasmid significantly slows the growth of our yeast strains. Any suppression afforded by overexpression of \( SEC23 \) might be counteracted by this inherent toxicity of \( SEC23 \). A simple conclusion that can be drawn from these overexpression studies is that too great of a stoichiometric excess of \( Lst1p \) over \( Sec24p \) is lethal. This observation can be explained if \( Lst1p \) and \( Sec24p \) compete with one another in the assembly of vesicle coat complexes and that excess \( Lst1p \) causes sequestration of vesicle components into complexes that fail to satisfy some essential function of COPII.

**Lst1Δ Diminishes the Activity of the Plasma Membrane Proton-ATPase**

The sensitivity of \( lst1\Delta \) mutants to low pH suggested the involvement of Pma1p, which has been shown to be the limiting cell component for growth on acidic medium (M-Cusker et al., 1987; Portillo and Serrano, 1989). The dependence of Pma1p activity on \( LST1 \) was supported by the observation that \( lst1\Delta \) mutants exhibited an unusual morphology characteristic of pma1 mutants. When \( lst1\Δ \) mutants were grown in low pH (SM M or Y PD brought to pH...
3.8) at 37°C, ~10% of the cells formed multibudded rosettes; in some cases, as many as 15 daughters radiated from a single large mother cell (Fig. 4 A). The unseparated daughter cells contained nuclei that could be stained with DAPI and the daughter cells could be separated from their mothers by micromanipulation, indicating they had completed cytokinesis. Cells depleted of Pma1p produce similar multibudded cells with attached daughters that had completed cytokinesis. In this case, multibudded rosettes are thought to form because a mother cell formed with sufficient Pma1p in the plasma membrane will continue to bud, whereas daughter cells formed after Pma1p transport is compromised will have insufficient Pma1p to form buds themselves (Cid et al., 1987). The morphology of lst1Δ cells grown at relatively high pH (YPD or medium buffered to pH 6.5) at 37°C appeared normal, with few cells having more than one attached daughter.

As a more direct test of the effect of lst1Δ on the activity of Pma1p, we measured the capacity of mutant cells to pump protons into the external medium. Wild-type and lst1Δ strains were cultured in YPD at 37°C, conditions under which both strains grow equally well. After starvation by prolonged incubation in water, the cells were placed in a weakly buffered medium and proton efflux on addition of glucose was measured as a drop in extracellular pH. For both wild-type and lst1Δ strains, addition of glucose produced a sharp decline in pH (after a 30-s lag), which began to level off after ~5 min (Fig. 4 B). Although the responses of wild-type and lst1Δ cells were qualitatively similar, proton efflux from lst1Δ cells was compromised: in the first 5 min after addition of glucose the rate of change in pH produced by the lst1Δ mutant was 65% of that of wild-type. These findings indicate that the lst1Δ mutant grown at 37°C has about half of the Pma1p activity as wild-type cells.

**Figure 4. Pma1p defects caused by lst1Δ.** (A) lst1Δ cells (CKY 534) were photographed using differential interference contrast microscopy after growth at 37°C on YPD, pH 3.8. A montage of multibudded cells is shown. Cells of this type comprise ~10% of a lst1Δ culture, but are never seen in wild-type grown under the same conditions. Bar, 10 μm. (B) Reduced capacity for proton pumping by lst1Δ cells. Wild-type (CKY 443) and lst1Δ (CKY 536) were grown to exponential phase in YPD medium, pH 6.8, at 37°C. Cells were incubated in water overnight and then suspended in 10 mM glycine buffer at pH 4.0. Proton efflux from the cells after addition of glucose was recorded as a decrease in the pH of the external medium. Based on the average rate of change in pH over the first 5 min after glucose addition, lst1Δ cells exhibited 65% the rate of proton efflux as wild-type.

**LST1 Is Required for Efficient Transport of Pma1p Out of the ER**

To determine whether the reduced Pma1p activity in lst1Δ mutants was due to a defect in the transport of Pma1p to the cell surface, we compared the localization of Pma1p in wild-type and lst1Δ mutant cells by immunofluorescence microscopy. Cells were grown at 30°C in YPD medium to avoid possible secondary effects due to the pH sensitivity of lst1Δ mutants. In lst1Δ cells, Pma1p was located primarily at the nuclear periphery and at the cellular rim, indicating that a large proportion of Pma1p remains in the ER (Fig. 5). This pattern of localization differed markedly from the surface localization of Pma1p in wild-type cells incubated at 30°C (Fig. 5) or in lst1Δ cells incubated at 24°C (data not shown).

We also examined the subcellular distribution of Pma1p in lst1Δ cells by cell fractionation. Lysates from cells grown at 37°C for 3 h were fractionated on sucrose density gradients under conditions where the ER and plasma membrane are well separated on the basis of their buoyant density. Pma1p from wild-type cells was located in dense fractions of the gradient in a peak that was coincident with that of Gas1p, a GPI-linked plasma membrane protein (Nuoffer et al., 1991). In contrast, ~35% of the total Pma1p from lst1Δ cells coincided with the plasma membrane marked by Gas1p protein and the majority of Pma1p was located in fractions containing the ER (Fig. 6). Interestingly, the ER from lst1Δ mutants (marked by Sec61p) reproducibly resolved into two peaks of different density, suggesting that accumulation of Pma1p segregates ER membranes into subdomains of relatively high and low density. Given that most of the Pma1p was located in the ER peak of higher density, it is possible that the density of the ER had been increased because of the accumulation of Pma1p. A similar increase in density of a portion of the ER is caused when folding mutants of PMAL1 are retained within the ER (Harris et al., 1994).

The fact that transport of Pma1p, but not of Gas1p, was affected by deletion of LST1 suggested that LST1 may be specifically required for the export of Pma1p from the ER. The absence of a general protein secretion defect in lst1Δ mutants was implied by the normal growth of lst1Δ mutants at 37°C in medium of pH 6.5 (the doubling time of both lst1Δ and wild-type was 1.75 h in YPD), indicating a normal rate of expansion of the plasma membrane. A specific test for the rate of ER to Golgi transport, pulse-chase experiments were performed to follow the rate of maturation of invertase from its core glycosylated ER form to the Golgi and secreted forms. No delay in invertase transport was observed in lst1Δ mutants that had been grown at 37°C for 3 h, conditions that caused the accumulation of Pma1p (Fig. 7). Similarly, no defect in the maturation of carboxypeptidase Y from the ER form to the Golgi and vacuolar forms of the enzyme could be detected (data not shown).

We also considered the possibility that transport of Pma1p may be partially sensitive to any subtle defect in vesicle formation. We addressed this possibility by examining the localization of Pma1p in sec24-1 and sec31-2 mutants cells at the semipermissive temperature of 28°C. Although the growth rate of both mutants was compromised...
at this temperature (doubling time on YPD: 2.9 h for sec24 and 2.4 h for sec31, as compared with 1.7 h for wild-type), no accumulation of Pma1p was detected in the perinuclear region of either mutant by immunofluorescence microscopy (data not shown). Thus, partial defects in COPII functions did not lead to the extensive accumulation of Pma1p in the ER that was observed for lst1D mutants. Taken together, comparisons between the lst1D mutation and COPII gene mutations indicate that the lst1D mutation is unusual in its ability to inhibit Pma1p exit from the ER without interfering with the transport of other cargo proteins.

Localization of Lst1p

To examine the intracellular distribution of Lst1p, an epitope-tagged derivative was constructed by inserting six copies of the 10–amino acid HA near the NH2 terminus of Lst1p. The HA-tagged LST1 was functional, as demonstrated by its ability to complement lst1Δ in a sectoring assay, and to restore the ability of a lst1Δ mutant to grow on acidic medium at 37°C (not shown). In cells expressing Lst1p-HA that were fixed for immunofluorescence microscopy, staining was found primarily at the nuclear periphery (Fig. 8). No signal was seen in cells expressing untagged Lst1p, verifying that the origin of the staining pattern was due to Lst1p. Although Lst1p-HA staining largely coincided with the ER marker Kar2p, there were subtle differences in their patterns of localization: Kar2p appeared uniformly distributed around the nuclear periphery, whereas Lst1p-HA staining had a more punctate appearance indicating that Lst1p might be concentrated in particular regions of the ER. In addition, weak punctate staining was observed throughout the cell body, some of which may correspond to ER membranes near the cell periphery.

The intracellular distribution of Lst1p was also examined by subcellular fractionation. Cells expressing Lst1p-HA were converted to spheroplasts and then gently lysed. This cell lysate was subjected to differential centrifugation and most of Lst1p-HA was found to pellet at either 500 g or 10,000 g (Fig. 9 A). A II of the soluble marker protein Gdh2p (Miller and Magasanik, 1990) was found in the 150,000 g supernatant fraction, indicating complete cell lysis (data not shown). The association of the Lst1p protein with the sedimenting fraction was analyzed by chemical treatment of cell lysates before centrifugation at 50,000 g. Incubation of cell extracts in 1% Triton X-100, 2.5 M urea,
100 mM sodium carbonate, pH 11.5, or 500 mM NaCl resulted in the release of a portion of the Lst1p-HA into the soluble fraction (Fig. 9B). The partial dissociation of Lst1p-HA from the sedimenting fraction by these agents suggested that Lst1p is a peripheral membrane protein that adheres tightly to the membrane.

**Lst1p Binds Sec23p**

Sec24p was first identified as a protein that formed a 400-kD complex with Sec23p (Hicke et al., 1992). Because of the similarity of Lst1p to Sec24p, we investigated whether Lst1p could also bind to Sec23p. To assay potential interactions by the yeast two-hybrid assay, LST1 was fused to the lexA DNA-binding domain (pKR37) and SEC23 was fused to an acidic activation domain (pPE81). Interaction between the two fusion proteins was tested by assaying for activation of a lacZ reporter gene. Induction of β-galactosidase was observed when the LST1 and SEC23 fusions were coexpressed, but not when expressed alone (Table IV). The level of induction caused by interaction of LST1 and SEC23 was similar to that seen for interaction of SEC24 and SEC23 (Gimeno et al., 1996).

To confirm the interaction between Lst1p and Sec23p, association of these proteins was examined in yeast cell extracts. The coding sequence of LST-1-HA (codons 14–927) was fused to GST and expressed in yeast from the pGAL1 promoter. SEC23 was also expressed from pGAL1. Since activation of a lacZ reporter gene. Induction of β-galactosidase was observed when the LST1 and SE C23 fusions were coexpressed, but not when expressed alone (Table IV). The level of induction caused by interaction of LST1 and SEC23 was similar to that seen for interaction of SEC24 and SEC23 (Gimeno et al., 1996).

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Table IV. Two-Hybrid Interaction between LST1 and SEC23

| LST1 | SEC24 | No fusion |
|------|-------|-----------|
| SEC23 | 395 ± 8 | 629 ± 1 | 24.4 ± 0.1 |
| No fusion | 30.4 ± 4.0 | 25.3 ± 1.4 | 44.8 ± 5.3 |

Fusions to the LexA DNA-binding domain and to a transcriptional activation domain were induced by growth in galactose for 10 h. Activities shown are the mean from five independent transformants. Units of β-galactosidase activity are nmol/mg × min.

Both proteins are largely associated with intracellular membranes (Fig. 10 B), membranes prepared from cells overexpressing both Sec23p and GST–Lst1p-HA were first extracted with 600 mM NaCl to release protein complexes from the membrane, the salt extracts were clarified by centrifugation at 90,000 g, and diluted to give a final concentration of 200 mM NaCl. GST–Lst1p-HA was isolated from the extracts by affinity to glutathione Sepharose beads. Sec23p was found in association with GST–Lst1p-Ha, but not in control extracts prepared from cells expressing Sec23p and GST alone (Fig. 10 A). Together, these experiments show that Lst1p, like Sec24p, can form a complex with Sec23p.

Sec23p and Sec24p have been shown to assemble onto the ER membrane as a complex (Matsuoka et al., 1998). While working out conditions to optimize recovery of Sec23p bound to GST–Lst1p-HA, we discovered that assembly of an Lst1p/sec23p complex appears to enhance the association of both proteins with the ER membrane. When both GST–Lst1p-HA and Sec23p were overexpressed in the same cell, >60% of the Sec23p, and 70% of the GST–Lst1p-HA were found in a fraction that pelleted at 10,000 g (Fig. 10 B). This pellet contains most of the ER, as marked by the ER membrane protein Sec61p (data not shown). When material that pelleted at 10,000 g was suspended in 60% sucrose and applied to the bottom of a sucrose density gradient, >90% of the GST–Lst1p-HA and Sec23p cofractionated with the ER resident membrane protein, Sec61p, at a density corresponding to 45% sucrose, showing that GST–Lst1p-HA and Sec23p were associated with membranes (data not shown). In contrast to the case when Sec23p and GST–Lst1p-HA were expressed together, <10% of the Sec23p pelleted at 10,000 g in lysates from a strain overexpressing Sec23p alone. Similarly, <20% of the GST–Lst1p-HA pelleted at 10,000 g in lysates from a strain expressing GST–Lst1p-HA alone (Fig. 10 B). Thus, when either Sec23p or GST–Lst1p-HA was overexpressed alone, most of the overexpressed protein was soluble, but when the proteins were expressed together, most of the proteins were associated with the ER membranes. These data support the observation that Lst1p can form a complex with Sec23p, and that the Lst1p/Sec23p complex has affinity for ER membranes.

**Discussion**

By screening for mutants that exhibited synthetic-lethal genetic interactions with the COPII mutation sec13-1, we identified the LST1 gene. Subsequent genetic tests showed that lst1Δ is lethal when combined with mutations in genes required for COPII vesicle budding from the ER (SEC12, SEC13, SEC16, SEC23, SEC24, and SEC31), but lst1Δ is not lethal when combined with mutations in genes that are required for vesicle fusion with the Golgi compartment (SEC17 and SEC18). This pattern of genetic interactions indicated that LST1 participates in the process of vesicle budding from the ER, an expectation that was born out by the examination of the LST1 gene and its product. The following observations indicate a role for Lst1p as part of a COPII-like vesicle coat: (I) LST1 encodes a 90-kD protein that is homologous to the COPII-coat subunit Sec24p. The two proteins share 23% amino acid identity over their entire lengths. (II) Lst1p is a peripheral ER membrane protein as shown by immunofluorescence microscopy and cell fractionation. (III) Lst1p, like Sec24p, can bind to Sec23p as shown by tests for two-hybrid interaction and affinity purification of a complex of GST–Lst1p and Sec23p. (IV) A assembly of the Sec23p–Lst1p complex appears to enhance the membrane association of both Lst1p and Sec23p: when both proteins are overexpressed together, most associate with membranes, whereas either protein overexpressed alone is mostly cytosolic. (V) Although strains with chromosomal deletion of LST1 are viable and appear normal for secretion of marker proteins, these mutants show a pronounced accumulation of Pma1p in the ER, indicating a selective defect in ER to Golgi traffic. Based on these findings, we propose that Lst1p takes the place of Sec24p in a specialized COPII coat complex that is used for the recruitment of Pma1p into vesicles.

Strains carrying lst1Δ have the phenotypic hallmarks of a deficiency in Pma1p activity, including sensitivity to growth in an acidic environment, the formation of multibudded cells, and a decreased rate of proton influx from intact cells. All three traits are expressed only at temperatures of 30°C and above, indicating that LST1 is only re-
required for Pma1p activity at high temperature. Localization of Pma1p in lst1Δ cells by immunofluorescence and sucrose density cell fractionation demonstrate that the transport of Pma1p from the ER is compromised in lst1Δ at 37°C.

Export of Pma1p from the ER cannot be completely dependent on Lst1p, since Pma1p transport appears normal in lst1Δ mutants at 24°C. Even at 37°C, the block in Pma1p transport may not be complete since ~35% of the total Pma1p fractionates with the plasma membrane, although some of the Pma1p detected in the plasma membrane in this experiment was probably synthesized before the shift to restrictive temperature. Therefore, it seems likely that Lst1p and Sec24p share the burden of transporting Pma1p from the ER. At 24°C, it appears that Sec24p (or some other protein) can compensate for the absence of Lst1p, but at temperatures of 30°C or higher, compensation is no longer possible unless extra copies of Sec24p are provided by expression from a multicopy plasmid.

The transport defect caused by deletion of LST1 appears to be specific for Pma1p. Under conditions where a defect in Pma1p transport was observed in lst1Δ mutants, transport of Gas1p, carboxypeptidase Y, and invertase was unaffected. Using growth as a more general assay for trafficking defects, we found that lst1Δ mutants grew at an identical rate to wild-type at 37°C, when we compensated for the defect in Pma1p transport by using media at pH 6.5. This indicates that rate of expansion of the plasma membrane, including the transport of all essential plasma membrane proteins, is not significantly affected by the absence of LST1.

We also considered the possibility that there may be differences among cargo molecules in their response to general defects in the protein transport machinery. Of particular concern was the possibility that Pma1p transport might be particularly sensitive to slowed ER to Golgi transport, such that a defect in transport too subtle to have an effect on our standard marker proteins might have a significant effect on the rate of transport of Pma1p. If this were the case, partial defects in other COPII components should also interfere with Pma1p transport. Therefore, we examined sec24 and sec31 mutants, but could find no evidence for a defect in Pma1p transport, even at semipermissive temperatures where the rate of growth was inhibited. Although Pma1p was the only essential protein for which we could detect a transport defect in lst1Δ mutants, a defect in the transport of any nonessential protein could have been overlooked by our analysis.

Factors required for the transport of specific membrane proteins have been documented in a number of other cases. The SHR3 gene encodes an ER resident protein that is required for the transport of amino acid permeases out of the ER, but is not required for the transport of a variety of other proteins (Ljungdahl et al., 1992; Kuehn et al., 1996). A set of ER proteins, Vma12p, Vma21p, and Vma22p, are required for transport from the ER of the integral membrane subunit of the vacuolar ATPase (Hill and Stevens, 1994; Portillo, 1997). Similarly, mutational studies have shown that the small ER membrane protein Erv14p is specifically required for transport of the plasma membrane protein Axl2p out of the ER (Powers and Barlowe, 1998).

Finally, A st1p has been suggested to be a factor specifically needed for the transport of Pma1p from the Golgi compartment to the plasma membrane (Chang and Fink, 1995). In all of these cases, the question remains whether Shr3p, the Vma proteins, Erv14p, or A st1p act directly in vesicular transport of their respective cargo molecules, or whether they are primarily involved in protein folding and influence protein sorting indirectly through quality control mechanisms. Because Lst1p appears to be a component of a vesicle coat, Lst1p seems more likely to have a direct role in the sorting of Pma1p rather than in its folding.

Expression of a variety of dominant PMA1 mutations can cause accumulation of both mutant and wild-type Pma1p in proliferated ER (Harris et al., 1994; Portillo, 1997). Similarly, the transport of wild-type Pma1p from the ER is blocked when PMA2 (an isoform of PMA1) or plant plasma membrane proton-A TPases are overexpressed in yeast (Villalba et al., 1992; Supply et al., 1994; de Kerchove d’Eaerde et al., 1995). One proposal was that a special factor may be required for the transport of Pma1p from the ER in a manner analogous to the requirement for Shr3p in the transport of amino acid permeases (Supply et al., 1994). The specific role of Lst1p in the transport of Pma1p suggests that it may be the factor depleted by the expression of dominant forms of Pma1p. In the future, it may be possible to test this idea by evaluating the ability of L st1p overexpression to reverse the effects of dominant PMA1 mutations.

The mechanism by which Lst1p acts in the transport of Pma1p may be inferred from recent studies examining the recruitment of cargo molecules into COPII vesicles. Using ER-derived microsomes and purified COPII components, Kuehn et al. (1998) have shown that the Sec23p/Sec24p complex, along with Sar1p, associate with amino acid permeases and other integral membrane protein that are destined for the plasma membrane. In parallel experiments using mammalian microsomes, mammalian Sec23p/Sec24p and Sar1p were found to bind to microsomal membranes and form a complex that contains the cargo protein VSV-G (Aridor et al., 1998). The conclusion from both experimental systems is that the Sec23p/Sec24p complex contains specific binding sites for the capture of membrane cargo proteins within the plane of the ER membrane. Based on the data presented here, Lst1p appears to be an isoform of Sec24p that is adapted for selection of Pma1p. This provides the first evidence that Sec24 family members carry information specifying the type of cargo molecules that are accepted by ER-derived vesicles.

We have looked for association of Lst1p with ER-derived vesicles, but under the conditions of an in vitro budding reaction, a large quantity of Lst1p-HA is released from the membrane in soluble form. Soluble Lst1p-HA gives a high background in vesicle fractions preventing us from reliably determining whether there is a specific association of Lst1p with vesicles. In future experiments, it may be possible to isolate vesicles coated with Lst1p by performing an in vitro budding reaction using purified cytosolic components, including a purified complex of Lst1p and Sec23p. It may also be possible to determine whether vesicles that are formed using a Sec23p/Lst1p complex more efficiently incorporate Pma1p than vesicles formed using the Sec23p/Sec24p complex. Finally, it will be of in
terest to determine if there is direct binding of Lst1p to Pma1p.

The identification of a Sec24p homologue that also acts in transport from the ER raises the possibility that the coats of ER-derived vesicles may be heterogeneous. It is possible that Sec23p/Lst1p complexes act to form a class of vesicle that is distinct from those formed by Sec32p/Sec42p complexes. Alternatively, it is possible that the two complexes assemble together forming vesicles with coats of mixed composition. The identification of additional homologues of Sec23p and Sec24p suggest the existence of coats with even greater combinatorial complexity. We have identified a third Sec24p family member, which we call Iss1p, as a protein that binds to Sec16p. Iss1p (YNL049c) also binds Sec23p and appears to be associated with the ER membrane (Gimeno, 1996). In addition, the Saccharomyces genome contains an uncharacterized open reading frame (YHR035w) that is 21% identical to Sec23p (Saccharomyces Genome Database, Cherry et al., 1997). If each of the Sec23p and Sec24p homologues carry different determinants for cargo selection, and if mixed coats can form, the possible combinations of Sec23p and Sec24p homologues should allow the formation of a wide variety of COPII-like vesicles with different capacities to carry different cargo molecules.

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