Sec12p-dependent Membrane Binding of the Small GTP-binding Protein Sarlp Promotes Formation of Transport Vesicles from the ER

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Abstract. Sec12p is an integral membrane protein required in vivo and in vitro for the formation of transport vesicles generated from the ER. Vesicle budding and protein transport from ER membranes containing normal levels of Sec12p is inhibited in vitro by addition of microsomes isolated from a Sec12p-overproducing strain. Inhibition is attributable to titration of a limiting cytosolic protein. This limitation is overcome by addition of a highly enriched fraction of soluble Sarlp, a small GTP-binding protein, shown previously to be essential for protein transport from the ER and whose gene has been shown to interact genetically with sec12. Furthermore, Sarlp binding to isolated membranes is enhanced at elevated levels of Sec12p. Sarlp–Sec12p interaction may regulate the initiation of vesicle budding from the ER.

Genetic analysis has identified four yeast genes that are required for the formation of transport vesicles derived from the ER: SEC12, SEC13, SEC16, and SEC23 (Kaiser and Schekman, 1990). In vitro reconstitution of ER-Golgi transport vesicle formation also showed a requirement for Sec12p and Sec23p (Rexach and Schekman, 1991). Cell fractionation and DNA sequence analysis have shown that Sec12p is an integral membrane protein, and Sec23p, Sec16p, and Sec13p are either cytosolic or peripheral membrane proteins (Nakano et al., 1988; Hicke and Schekman, 1989; Kaiser, C., and N. Pryer, unpublished data). Interactions observed among the genes encoding these proteins suggest that they could act in concert or form a multisubunit complex possibly associating with the ER membrane via Sec12p (Kaiser and Schekman, 1990).

Sec12p is a type II membrane glycoprotein with a 40 kD NH2-terminal cytosolic domain that is essential for transport, and a COOH-terminal luminal domain that is dispensable (Nakano et al., 1988; d'Enfert, C., C. Barlowe, S. Nishikawa, A. Nakano, and R. Schekman, manuscript submitted for publication). The thermosensitive lethality associated with a mutation in the NH2-terminal domain of Sec12p is suppressed by overproduction of Sarlp, a 21-kD GTP-binding protein (Nakano and Muramatsu, 1989). Sarlp is itself required for protein transport from the ER (Nakano and Muramatsu, 1989). Cell fractionation experiments show Sarlp both in the cytosol and in tight association with a membrane fraction, however overproduction increases the fraction of Sarlp soluble in the cytosol (Nishikawa and Nakano, 1991). Membrane association of Sarlp is enhanced in vivo by overproduction of Sec12p (d'Enfert, C., C. Barlowe, S. Nishikawa, A. Nakano, and R. Schekman, manuscript submitted for publication). These results suggest a structural and functional interaction of Sarlp and Sec12p.

In the course of identifying cytosolic factors that stimulate protein transport from the ER in vitro, we observed that addition of a membrane fraction with elevated levels of Sec12p was inhibitory, and that a limiting cytosolic factor restored transport when provided in an enriched form. This report describes the identification of this limiting factor as Sarlp. Extensive purification of a functional, apparently monomeric soluble form of Sarlp is achieved.

Materials and Methods

Strains, Plasmids, Materials, and General Methods

The yeast strains used in this study were RSY607 (leu2-3,112 ura3-52 pep4::URA3 MATα) and YPH500 (ura3-52 lys2-801 ade2-101 trp1-063 his3-A200 leu2-A MAT4 ; Sikorski and Hieter, 1989). Plasmids pANY1-9 (2μ URA3 SEC12), pCEY5 (2μ URA3 GALI-SEC12), pANY2-7 (2μ URA3 SARD), pANY18 (CEN4-ARS1 TRP1 GALI-SARD), and pSEY8 have been previously described (Nakano et al., 1988; d'Enfert, C., C. Barlowe, S. Nishikawa, A. Nakano, and R. Schekman, manuscript submitted for publication; Nakano and Muramatsu, 1989; Emr et al., 1986). pCGS109 (2μ URA3 GALI-α) is a gift of D. Moir (Collaborative Research, Lexington, MA). pSEC113 (2μ URA3 SEC13) and pSEC1614 (2μ URA3 SEC16) were kindly provided by C. Kaiser (Division of Biochemistry and Molecular Biology, and Howard Hughes Medical Institute, University of California, Berkeley, CA).

Yeast cells were grown in YP (2% Bacto-peptone 1% Yeast extract broth both from Difco Laboratories Inc., Detroit, MI) containing 2% glucose or in MV (0.67% yeast nitrogen base without amino acid; Difco Laboratories Inc.) containing 2% glucose and supplemented with the appropriate amino acids and/or 0.5% vitamin assay Casamino acids (Difco Laboratories Inc.).
For induction of GALI-regulated genes, cells were first grown in MV containing 2% lactate, pH 5.5, 0.1% glucose, and then induced by transfer to either 2% galactose for 20 h or MV 2% lactate, pH 5.5, 2% galactose for 5 h.

Anti-Sec23p, anti–Sarlp, anti–Sec23p, anti–Kar2p, and anti–α-1,6-mannose antibodies have been described (Nakano et al., 1988; Nishikawa and Nakano, 1991; Hicke and Schekman, 1989; Rose et al., 1989; Baker et al., 1988). Anti-Sec33p, anti-Yptlp, and anti-phosphoglycerokinase were kindly provided by C. Kaiser, D. Baker, and J. Thormer, respectively (Division of Biochemistry and Molecular Biology, and Howard Hughes Medical Institute, University of California, Berkeley, CA). Transfer of proteins from SDS-PAGE gels to nitrocellulose filters (Schleicher & Schuell, Inc., Keene, NH) was performed as described (Towbin et al., 1979). Filters were blocked and all incubations were conducted in 20 mM Tris-Cl, pH 7.4, 150 mM NaCl, 0.1% NP-40 with or without 2% non-fat dry milk. Detection of filter-bound antibodies with horseradish peroxidase-conjugated goat anti-rabbit IgG and with ECL immunoblotting reagents were performed according to the supplier’s instructions (Amersham Corp., Arlington Heights, IL). Protein concentrations were determined by the procedure of Lowry et al. (1951) in the presence of 1% sodium dodecyl sulfate or with the Bio-Rad protein assay (Bio-Rad Laboratories, Richmond, CA) using BSA as a standard.

Preparation of Subcellular Fractions for In Vitro Assays

Subcellular fractions were prepared from wild-type cells grown in 2% glucose or from transformed cells grown in 2% galactose (cytosol) or MV 2% lactate, 2% galactose (cytosol, microsome, and high speed pellet fractions). Cytosol was prepared from bead-lysed cells as described (Baker et al., 1988) and concentrated (12–15 mg protein/ml) by centrifugation in a 12,000-g supernatant from a bead-lysed cell extract (Baker et al., 1988). Alternatively, the high speed pellet fraction was obtained after a 100,000-g centrifugation of a 12,000-g supernatant from osmotically lysed cells. Activity but stimulated ER-Golgi transport as efficiently as an HSP fraction derived from a 12,000-g supernatant from a bead-lysed cell extract (Baker et al., 1988). In these conditions, transport activity was linearly dependent upon addition of cytosol and HSP fractions and the concentration of trypsin and trypsin inhibitor was adjusted accordingly. Alternatively, trypsin-treated membranes were washed by centrifugation (27,000 g, 10 min) in reaction buffer to remove soluble trypsin before isolation. After a 10 min incubation in ice, microsomes containing translocated [35S]-pro-α-factor were added and the second stage of the reaction was initiated. An 80% inhibition was reproducibly obtained with 4–5 μg of Sec12p-enriched membranes.

To test the rescue activity of various fractions, cytosol and HSP fractions were mixed with the appropriate amount of trypsin-treated Sec12p-enriched microsomes such that ~80% inhibition was obtained in the absence of any additional component. This mixture was kept on ice for 10 min, distributed into tubes containing the fractions to be tested, and incubated an additional 10 min on ice. Microsomes containing translocated [35S]-pro-α-factor then were added and the second stage of the reaction was conducted.

Results

Sec12p-enriched Membranes Inhibit Transport

Protein transport from the ER to the Golgi apparatus has been reconstituted with yeast membranes and cytosol (Baker et al., 1988, 1990; Ruohola et al., 1988). In the first stage of transport, [35S]methionine-labeled α-factor precursor is translocated into a crude ER membrane fraction to form core-glycosylated precursor. Transport to the Golgi complex is achieved in a second stage which measures the coupled addition of outer chain carbohydrate to the core-glycosylated precursor. In the assay developed by Baker et al. (1990), transport is stimulated by a cytosol fraction (100,000-g supernatant), a particulate fraction (HSP, 100,000-g pellet), and ATP. The particulate fraction appears to provide an enriched source of factors that are present in limited supply in the cytosol, rather than an essential membrane compartment. Addition of a salt wash soluble fraction, obtained by extraction of permeabilized spheroplasts, replaced the requirement
for HSP material (Baker, D. and L. Wuestehube, unpublished results).

To identify cytosolic factors that interact with Sec12p, we designed an assay in which these components become limiting through titration by excess Sec12p. This was achieved by mixing wild-type ER membranes containing 35S-core-glycosylated pro-α-factor with nonradioactive membranes isolated from cells transformed with a multicopy vector containing the SEC12 gene. An outline of this approach is depicted in Fig. 1. If the inhibitory membranes consume a specific limiting component, inhibition should be overcome by supplementing the reaction with an enriched source of this limiting protein.

Membranes were isolated from two strains varying only in their content of Sec12p. Wild-type (YPH500/pCGS109; YEp::GAL) and a Sec12p-overproducing strain (YPH500/pCEY5; YEp::Gal-SEC12) were grown on galactose to induce maximal synthesis of Sec12p in the latter. Membranes were isolated and treated with trypsin under conditions where Sec12p remains intact, but ER-Golgi transport activity is inactivated (Nakano et al., 1988; d'Enfert, C., unpublished data). Membranes from the transformant contained at least 60-fold more Sec12p than preparations from wild-type cells. Equivalent amounts of membrane protein from transformed and untransformed cells were mixed with cytosol and HSP fractions for 10 min at 0°C, and aliquots then added to a stage II incubation containing wild-type ER membranes loaded with [35S]α-factor precursor. Transport inhibition related to the amount of Sec12p in the overproducer membrane fraction was observed (Fig. 2). Competitor membranes isolated from cells transformed with a multicopy plasmid containing SEC12 under control of its own promoter (six- to eightfold less Sec12p than the GAL-regulated SEC12) were threefold more potent than control membranes in inhibiting transport (not shown). Inhibition was proportional to but not linearly related to the Sec12p level. Not all of the Sec12p may be in a form capable of competing for a transport-limiting component.

**Figure 1.** Inhibition of ER-Golgi transport by Sec12p-enriched membranes. In vitro-reconstituted ER–Golgi transport monitors the transfer of core-glycosylated 35S-labeled pro-α-factor (dotted circle) from the endoplasmic reticulum to the Golgi apparatus where it acquires outer-chain glycosylation (solid circle). Interaction between Sec12p (membrane protein) and an unknown cytoplasmic component (solid triangle) is thought to be necessary for the production of a transport vesicle. Addition of Sec12p-enriched membranes to a standard transport reaction should result in a titration of this cytoplasmic component by these membranes and thus in an inhibition of transport.

**Figure 2.** Sec12p-enriched membranes specifically inhibit transport. Standard ER-Golgi transport reactions were carried out with 10 μg wild-type cytosol, and 20 μg wild-type HSP prepared from osmotically lysed cells. Increasing amounts of trypsin-treated microsomes derived from a control strain (YPH500/pCGS109; YEp::GAL) or a strain overproducing Sec12p (YPH500/pCEY5; YEp::Gal-SEC12), both of which were grown in galactose-containing medium, were preincubated with the cytosol and the HSP fractions for 10 min at 0°C before mixing with the membranes that contained pro-α-factor. The efficiency of transport for each reaction was compared to the maximum transport efficiency obtained when competitor membranes were omitted (25%).

**Sarlp Is Limiting in the Inhibited Reaction**

The model presented in Fig. 1 implies that Sec12p titrates a limiting cytosolic component, however, Sec12p could equally well limit a factor present in the membrane or HSP fraction. Trypsin treatment of the Sec12p-rich membrane should reduce the contribution of a peripheral membrane protein that could partition between the cytosol and membrane fraction. Indeed, trypsin treatment enhanced (1.5-fold) the inhibitory potency of the competitor membrane fraction.
Figure 3. Cytosol-dependent reversal of Sec12p-mediated inhibition. Cytosols prepared from YPH500(pSEY8) (YEp), YPH500(pANY2-17) (YCp::GAL-SARI) grown in MV2% lactate 2% galactose were compared for their Sarlp content and their effect on the Sec12p-dependent transport inhibition. (A) 25 µg of each cytosol was resolved by SDS-PAGE and immunoblotted with anti-Sarlp antibodies. (B) Standard transport reactions were carried out with wild-type membranes containing translocated pro-α-factor, 150 µg wild-type cytosol, 40 µg wild-type HSP prepared from bead-lysed cells, 5 µg trypsin-treated Sec12p-enriched microsomes, and increasing amounts of cytosol from each strain. The efficiency of transport for each reaction was compared to the maximum transport efficiency obtained when Sec12p-rich microsomes and additional cytosol were omitted (23%). Addition of Sec12p-rich microsomes alone resulted in an 81% inhibition of transport.

Given the known genetic interaction between sec12 and SARI (Nakano and Muramatsu, 1989), we examined the effect of cytosols prepared from strains transformed either with a 2µ multicopy vector carrying SARI under control of its own promoter, with a single-copy plasmid carrying SARI under control of the more active GAL-10 promoter, or with a control 2µ plasmid. The relative content of Sarlp in these cytosol fractions was compared by immunoblotting with a polyclonal antiserum (Fig. 3 A). The 2µ SARI produced ~20-fold more, and the CEN GAL::SARI ~45-fold more Sarlp than the untransformed and control transformed strains, as determined by densitometry of the SDS-PAGE immunoblot. Significantly greater rescue of transport was observed with cytosols that contained more Sarlp (Fig. 3 B). Although the antidote effect was proportional but not linearly related to the level of Sarlp overproduction, other factors may become limiting or not all of the Sarlp may be functional. Other genes (SEC13, SEC16) have been associated with SEC12 by genetic analysis (Kaiser and Schekman, 1990). Cytosol fractions prepared from cells transformed with multicopy vectors carrying these genes were no more potent than control cytosol in ameliorating inhibition by Sec12p-rich membranes, although they contained increased amounts of these SEC proteins (not shown). Thus the inhibition by excess Sec12p is overcome by Sarlp or by some factor that is coordinately induced by overproduction of Sarlp.

To examine the specificity of rescue by Sarlp, cytosol from the GAL::SARI transformant was fractionated by gel filtration on Sephacryl S-100HR. Sarlp filtered in the included volume with a peak of immunoreactive material in fractions 30-34 (Fig. 4 A). Rescue assays were performed by mixing trypsin-treated Sec12p-rich membranes with a normal dose of the cytosol and HSP fractions and aliquots of the Sephacryl column fractions. After 10 min at 0°C the mixture was added to wild-type membranes containing pro-α-factor followed by incubation in a stage II transport reaction. Rescue activity fractionated coincidentally with Sarlp and was recovered in 18% yield with respect to the starting cytosol fraction. This level of recovery is consistent with the possibility that another cytosolic factor became limiting in the presence of competitor membranes. Although Sarlp is a 21-kD protein (Nakano and Muramatsu, 1989), the polypeptide eluted from the Sephacryl column at the position expected of a 7.5-kD protein. This may account for the striking separation of Sarlp (Fig. 4 B, closed circles) from the total protein (open circles) on this column. Indeed, SDS-PAGE of Sarlp peak fractions of the Sephacryl column showed the protein highly enriched in relation to other polypeptides (Fig. 5). Sarlp overproduction was insufficient to detect a difference in SDS-PAGE comparison of cytosol fractions prepared from untransformed and transformed cells (not shown). Parallel Sephacryl fractionation of cytosol from a control strain grown on galactose showed little rescue activity (not shown) and Sarlp was not detected in equivalent column fractions (Fig. 5). Sarlp appears to be directly responsible for reversal of the inhibitory effect of excess Sec12p.

Sarlp Stimulates Vesicle Budding

Although Sarlp is required for protein transport from the ER (Nakano and Muramatsu, 1989), a role in vesicle budding or fusion has not been distinguished. We used a vesicle budding assay that detects the release of transport vesicles from the ER to monitor the effect of Sec12p-rich membranes and
rescue by Sarlp. In this assay, vesicle budding from an enriched ER fraction is monitored by the appearance of protease-protected core-glycosylated \[^{[35S]}\text{pro-}\alpha\text{-factor}\] in a slowly sedimenting compartment that is resolved from ER by differential centrifugation (Wuestehube, L., and R. S. Schekman, manuscript in preparation). Vesicle release is cytosol- and energy-dependent and is retarded by GTP\(_Y\)S. Released vesicles are competent for transfer of core glycosylated \[^{[35S]}\text{pro-}\alpha\text{-factor}\] to a fresh supply of membranes.

Sec12p-rich membranes inhibited vesicle budding (Fig. 6). Membranes isolated from the GAL-regulated SEC12 transformant decreased budding by 69\%, whereas membranes isolated from an untransformed cell decreased budding by 4\%. Budding inhibition was overcome by addition of cytosol from a Sarlp overproducing strain (restored to 75\% of normal), while the same amount of cytosol from an untransformed strain offered no rescue. Sephacryl S-100 enriched fractions of Sarlp also were active as an antidote to inhibition of budding by Sec12p-rich membranes (not shown). These results suggest that Sarlp interacts with Sec12p to facilitate the formation of transport vesicles from the ER.

**Membrane Binding of Sarlp Is Stimulated by Sec12p**

Sec12p may act to sequester Sarlp by recruitment to the membrane fraction or by inactivation of a form that remains cytosolic. Membranes containing normal or overproduced levels of Sec12p were used to examine recruitment of Sarlp from a crude cytosol or of Sarlp purified on Sephacryl S-100. Membrane fractions were treated with trypsin to remove endogenous Sarlp and mixed with crude or purified Sarlp fractions at 4\°C. The content of Sec12p and the redistribution of Sarlp to membranes was evaluated by immunoblotting particulate (medium speed pellet [MSP]) and soluble (medium speed supernatant [MSS]) fractions resolved by SDS-PAGE (Fig. 7). Sec12p was detected by immunoblot only in membranes derived from cells transformed with a multicopy vector containing SEC12 (Fig. 7, S, lanes 2, 4, 7, and 10); Sec12p was only partially sensitive to trypsin cleavage (compare lane 2 with lanes 4, 7, and 10). Untreated membranes from the SEC12 transformant contained more endogenous Sarlp than was found in untransformed control membranes (Fig. 7, compare lanes 1 and 2). This species was quantitatively converted by trypsin to a Sarlp fragment (Fig. 7, Sarl\(\Delta\)) that sedimented in the MSP fraction. Exogenous Sarlp became sedimentable only when mixed with a membrane fraction (Fig. 7, compare lanes 5 and 8 with lanes 6 and 7 and 9 and 10), and the proportion of Sarlp recruited to membranes was enhanced by elevated levels of Sec12p (three- to fivefold, compare Fig. 7, lanes 6 and 7, and lanes 9 and 10). Recruitment to the membrane occurred at 4\°C and was independent of ATP or GTP (not shown). Although membrane association of Sarlp occurred even with membranes that contained the normal low level of Sec12p, this could reflect some non-specific binding. Purified Sarlp binds...
Discussion

Sarlp is a small GTP-binding protein required for efficient protein transport from the endoplasmic reticulum to the Golgi apparatus (Nakano and Muramatsu, 1989; Nishikawa and Nakano, 1991). The SARI gene was isolated as a multicopy suppressor of a thermosensitive mutation in the SEC12 gene which encodes a membrane glycoprotein involved in the formation of ER-to-Golgi transport vesicles (Nakano and Muramatsu, 1989; Kaiser and Schekman, 1990; Rexach and Schekman, 1991). These results suggested that Sarlp could interact with the cytosolic domain of Sec12p to promote vesicle assembly. This assumption is now confirmed by our observation that Sarlp becomes limiting in a vesicle budding reaction when the level of Sec12p is elevated in a competitor membrane fraction. In addition, Sec12p-rich membranes bind Sarlp more abundantly than do normal membranes which have a low level of Sec12p. Independently, Oka et al. (1991) have shown that Sarlp isolated from transformed yeast or Escherichia coli suppresses temperature sensitive transport in the ER-Golgi transport reaction reconstituted with sec12 mutant membranes. Although all these results point to an interaction between Sarlp and Sec12p, the contact may not be direct or stable, and additional factors that may not be rate-limiting could mediate the interaction.
Sarlp Requirement for the Formation of ER to Golgi Transport Vesicles

Apart from Sarl, genetic analysis had identified three other genes, SEC4, YPT1, and ARF, that encode small GTP-binding proteins required for protein transport at different steps along the secretory pathway (Salminen and Novick, 1987; Segev et al., 1988; Stearns et al., 1990). In the case of Sec4p, localization of the protein as well as mutational analysis suggest that it is required for proper targeting and fusion of secretory vesicles to the plasma membrane (Goud et al., 1988; Walworth et al., 1989). Biochemical studies have shown that ER-to-Golgi transport and cis-to-medial Golgi transport is inhibited by GTPyS (Malençon et al., 1987; Baker et al., 1988; Ruohola et al., 1988; Beckers and Balch, 1989) and by synthetic peptides corresponding to the effector domain of members of the rab family of mammalian Sec4p/Yptlp-related proteins (Plüttner et al., 1990; Zaharoui et al., 1989). In mammalian cells, GTPyS inhibition results in the accumulation of intra-Golgi non-clathrin-coated-vesicles docked to their acceptor compartment (Melançon et al., 1987; Orci et al., 1989; Malhotra et al., 1989). In yeast, Yptlp is required for efficient in vitro ER to Golgi transport (Baker et al., 1990; Bacon et al., 1989) and its immobilization by Yptlp antibody Fab fragments results in the accumulation of transport vesicles en route to the Golgi (Rexach and Schekman, 1991). Bourne (1988) has proposed a unifying model which suggests that each GTP-binding protein is required at a specific step to ensure an efficient targeting of vesicular carriers to their acceptor compartment.

It appears that GTPyS also inhibits vesicle budding or release from the trans-Golgi network (Tooze et al., 1990) and from the ER (Rexach and Schekman, 1991; Wuestehube, L., and R. Schekman, manuscript in preparation). We have shown that Sarlp is required for vesicle assembly during ER-Golgi transport and is thus the likely target of GTPyS inhibition in this process. A model for the function of Sarlp in vesicle formation is proposed in Fig. 8. Together with the involvement of Yptlp in ER-Golgi transport (Segev et al., 1988; Baker et al., 1990; Rexach and Schekman, 1991), our results suggest that Sarlp and Yptlp may function in successive steps using GTP binding and hydrolysis to facilitate protein transport from the ER.

Structural differences between Sarlp and Yptlp support the view that they act at different stages during a single round of vesicular traffic. Whereas Yptlp is closely related to the mammalian oncogene ras (Gallwitz et al., 1982), the Sarlp nucleotide binding site (G-1 and G-3 regions; Bourne et al., 1991) is more closely related to the family of ADP-riboseylation factors (ARF; Kahn, 1990; Nakano and Muramatsu, 1989; Bourne et al., 1991). ARF is associated with intra-Golgi transport vesicles in mammalian cells (Serafini et al., 1990) and its yeast homologue, Arflp, is required for protein transport and also is localized to the Golgi apparatus (Seewell and Kahn, 1988; Stearns et al., 1990). ARF could play a role similar to that of Sarlp but at a later stage in secretion.

Sec12p-dependent Membrane Attachment of Sarlp

A functional difference between Yptlp and Sarlp is also suggested by different possible mechanisms of membrane attachment. Yptlp, Sec4p, and members of the rab family of small GTP-binding proteins (Haubrück et al., 1987; Touchot et al., 1987; Zahraoui et al., 1989; Chavrier et al., 1990) have at least one cysteine that is located near the COOH terminus of the protein. By analogy with the mammalian ras oncogene (Hancock et al., 1989), this residue is thought to be modified by a lipid, possibly a farnesyl analogue, which mediates membrane attachment (Molenaar et al., 1988; Walworth et al., 1990). Sarlp does not have a carboxy-terminal cysteine nor any site of putative lipid modification that could explain its strong affinity for membranes (Nishikawa and Nakano, 1991). In this study, we have established an in vitro assay that measures the binding of Sarlp to ER membranes. Results presented in Fig. 7 show that Sarlp membrane attachment is stimulated by elevated levels of Sec12p. Thus, Sarlp may become membrane bound through the intervention of Sec12p.

Sec12p could act as a membrane anchor for Sarlp or alter the conformation of Sarlp so as to expose a hydrophobic surface enabling direct contact with the lipid bilayer. In both cases, Sec12p-specific membrane attachment of Sarlp could result in the regulated formation of an ER-Golgi transport vesicle at specific sites on the ER membrane. Studies with purified forms of both proteins will distinguish these possibilities.

We thank A. Nakano for Sarlp antibody.

This work was supported by grants from the National Institutes of Health (National Institute of General Medical Sciences) and the Howard Hughes Research Institute. C. d'Enfert was supported by the French INRA and L. Wuestehube by a fellowship from the American Cancer Society, California Division.

Received for publication 3 April 1991 and in revised form 17 May 1991.

References