Molecularly distinct sets of SNARE proteins localize to specific intracellular compartments and catalyze membrane fusion events. Although their central role in membrane fusion is appreciated, little is known about the mechanisms by which individual SNARE proteins are targeted to specific organelles. Here we investigated functional domains in Sec22p that direct this SNARE protein to the endoplasmic reticulum (ER), to Golgi membranes, and into SNARE complexes with Bet1p, Bos1p, and Sed5p. A series of Sec22p deletion mutants were monitored in COPII budding assays, subcellular fractionation gradients, and SNARE complex immunoprecipitations. We found that the N-terminal “profilin-like” domain of Sec22p was required but not sufficient for COPII-dependent export of Sec22p from the ER. Interestingly, versions of Sec22p that lacked the N-terminal domain were assembled into ER/Golgi SNARE complexes. Analyses of Sec22p SNARE domain mutants revealed a second signal within the SNARE motif (between layers −4 and −1) that was required for efficient ER export. Other SNARE domain mutants that contained this signal were efficiently packaged into COPII vesicles but failed to assemble into SNARE complexes. Together these results indicated that SNARE complex formation is neither required nor sufficient for Sec22p packaging into COPII transport vesicles and subsequent targeting to the Golgi complex. We propose that the COPII budding machinery has a preference for unassembled ER/Golgi SNARE proteins.

In eukaryotic cells, most intracellular membrane fusion processes are mediated by a related family of small proteins, called SNARE (soluble NSF attachment protein receptors) proteins (1, 2). SNAREs share a conserved heptad repeat coiled coil sequence, called the SNARE domain, that is typically adjacent to a C-terminal-membrane bound region (3). Specific sets of SNARE domains assemble into parallel four-helix bundles forming the stable core of a SNARE protein complex (4–6). Current membrane fusion models theorize that cognate sets of SNARE proteins provided from opposing membranes assemble into “trans” SNARE complexes and drive membrane bilayers together during fusion (2, 7).

The assembly of cognate SNAREs has also been proposed to define the compartmental specificity of membrane fusion based on experiments showing that only specific combinations of SNARE proteins catalyze in vitro fusion when reconstituted into synthetic proteoliposomes (8–10). However, this proposal remains debated given the promiscuity of SNARE interactions in vitro (11–13), functional redundancy in vivo (14, 15), and requirements for individual SNARE proteins in multiple intracellular membrane fusion steps (16). These and other observations suggest that additional spatial and temporal determinants in SNARE proteins contribute to the specificity of the membrane fusion (2).

The N-terminal domains of SNARE proteins display significant diversity and appear to govern SNARE protein activity and distribution. For example, the N-terminal domain of the syntaxin-like SNARE proteins, such as neuronal syntaxin1 and yeast Sso1p, fold into three-helix bundles and inhibit SNARE pairing when folded back against their SNARE domains (17–20). The structure of the N-terminal domain of a non-syntaxin yeast SNARE, Ykt6p, resembles the overall fold of the actin regulatory protein, profilin. In vitro studies (21) reveal that the Ykt6p N-terminal domain interacts with the Ykt6p SNARE domain and influences the specificity of SNARE pairing and the kinetics of SNARE complex formation. Further studies (22) of mammalian Ykt6 demonstrate that this N-terminal domain also acts in directing Ykt6 to a specialized subcellular location. The N terminus of mouse Sec22b (mSec22b) also contains a profilin-like domain that is conserved among different Sec22 species. Unlike the syntaxin-related SNARE proteins and Ykt6p, the mSec22b N-terminal domain does not appear to interact with its SNARE domain and has no effect on the rate of SNARE complex assembly in vitro (23).

In Saccharomyces cerevisiae, Sec22p cycles between the endoplasmic reticulum (ER) and the Golgi complex. This localization permits Sec22p to act in both anterograde- and retrograde-directed fusion events in transport between these compartments (14, 24, 25). The assembly of Sec22p into SNARE complexes with Bet1p, Bos1p, and Sed5p catalyzes anterograde transport from the ER to the Golgi (2). In this report, we explore the features in yeast Sec22p that influence its localization and assembly into SNARE complexes. Our studies demonstrate that the N-terminal domain of Sec22p is required for targeting Sec22p to the Golgi complex but has no apparent effect on ER/Golgi SNARE complex assembly. As expected, the SNARE domain of Sec22p is critical for Sec22p SNARE interactions; however, a smaller region of the SNARE domain unexpectedly contributes to Sec22p trafficking. Taken together, our data indicate that the ER exit of Sec22p is independent of assembly into SNARE complexes.

**EXPERIMENTAL PROCEDURES**

**Plasmid Construction**—To generate plasmid pRS313-SEC22, the SEC22 coding sequence was amplified by PCR from yeast genomic DNA. The PCR product was digested with BamHI and SacI to remove the yeast SEC22 coding sequence with SEC22 flanking upstream and downstream sequences were excised from YEp511-SLY2/SEC22.
Sec22p Transport and SNARE Pairing

Sec22p consists of an N-terminal profilin-like domain followed by a conserved SNARE domain and a C-terminal transmembrane segment (Fig. 1). The function of the Sec22p N-terminal domain remains unknown, although it has been proposed to function in subcellular localization (2) and/or to play a regulatory role in SNARE complex assembly (23). To explore the role of this domain in Sec22p trafficking and SNARE protein interactions, we constructed a series of Sec22p N-terminal mutants for study. Structure-based sequence alignments show that the Sec22p N-terminal domain resembles its mouse homolog mSec22b consisting of mixed α-helical and β-sheet folds (23). Based on this structure, we constructed two N-terminal truncations, SEC22ΔN1 and SEC22ΔN2, deleting beyond the first and second α-helical segments of the N-terminal domain, respectively. We also generated an MBP-SEC22 fusion protein by appending a 40-kDa MBP tag on the N-terminus of Sec22p (Fig. 1). These constructs were introduced into sec22Δ cells on CEN vectors (pRS313) for single copy expression or 2-μm vectors (pRS425) for multicopy expression. As shown in Fig. 2, control SEC22 on a CEN plasmid was expressed in sec22Δ cells at a level equal to the wild-type. The N-terminal Sec22p mutants were expressed at their expected sizes although at apparently reduced levels compared with the wild-type protein. These expression levels were increased when the mutants were expressed from 2-μm vectors.

Sec22p as well as other ER/Golgi SNARE proteins are efficiently packaged into COPII transport vesicles (25). We first examined the effects of the N-terminal mutations on the COPII packaging efficiency of Sec22p. For these studies, in vitro vesicle budding reactions were performed from semi-intact yeast cell membranes supplied with purified COPII subunits (Fig. 3). As expected, wild-type Sec22p was sorted efficiently into COPII vesicles as was the ER vesicle protein Erv25p, whereas Sec61p, an ER resident protein, was excluded (30). In contrast, none of the Sec22p N-terminal mutants for study. Structure-based sequence alignments show that the Sec22p N-terminal domain resembles its mouse homolog mSec22b consisting of mixed α-helical and β-sheet folds (23). Based on this structure, we constructed two N-terminal truncations, SEC22ΔN1 and SEC22ΔN2, deleting beyond the first and second α-helical segments of the N-terminal domain, respectively. We also generated an MBP-SEC22 fusion protein by appending a 40-kDa MBP tag on the N-terminus of Sec22p (Fig. 1). These constructs were introduced into sec22Δ cells on CEN vectors (pRS313) for single copy expression or 2-μm vectors (pRS425) for multicopy expression. As shown in Fig. 2, control SEC22 on a CEN plasmid was expressed in sec22Δ cells at a level equal to the wild-type. The N-terminal Sec22p mutants were expressed at their expected sizes although at apparently reduced levels compared with the wild-type protein. These expression levels were increased when the mutants were expressed from 2-μm vectors.

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Sec22p N-terminal Truncation Mutants Accumulate in the ER—In wild-type cells, Sec22p is incorporated into COPII-coated vesicles and localizes to ER and Golgi compartments (25). Because the Sec22p N-terminal mutants were not efficiently packaged into COPII transport vesicles, we speculated

RESULTS

The N-terminal Domain of Yeast Sec22p Is Required for Incorporation into COPII-coated Vesicles—Sec22p localizes to membranes of the ER and early Golgi compartments. Genetic and biochemical experiments indicate that Sec22p function is required directly in anterograde transport from the ER to the Golgi by forming a SNARE complex with Sed5p, Bos1p, and Bet1p (8, 13, 37–39). Unlike other ER/Golgi SNAREs required for anterograde transport, Sec22p is dispensable for growth, because another SNARE protein, Ykt6p, functionally substitutes for Sec22p in sec22Δ strains (14, 26). The sec22Δ allele results in slow growth and temperature sensitivity (26). Sec22p also functions in retrograde transport from the Golgi to the ER (24) forming a complex with the ER-localized SNARE proteins Ufe1p, Sec20p, and Use1p/Slt1p (41, 42). Accordingly, Sec22p is efficiently packaged into COPII- (25) and COPII-coated (24) transport vesicles.

Sec22p consists of an N-terminal profilin-like domain fol-
that their subcellular distribution would be different compared with wild-type Sec22p. To examine the localization of these mutant proteins, we employed a subcellular fractionation procedure to resolve ER and Golgi compartments on sucrose gradients (31, 33). The resolution of ER and Golgi compartments with this method was confirmed by monitoring the Golgi-localized marker Och1p, which peaked in fractions that were separated from the ER marker, Sec61p. Wild-type Sec22p displayed a distribution between ER and Golgi membranes. In contrast, the N-terminal mutants, Sec22pΔN1 and MBP-Sec22p, were shifted to Sec61p-containing fractions indicating that these N-terminal mutants had accumulated in the ER (Fig. 4). Based on the COPII-budding and subcellular fractionation data, we conclude that the N-terminal domain of Sec22p is required for efficient packaging into COPII-coated vesicles and for Golgi localization. Thus, one role for the N-terminal domain is to direct trafficking of Sec22p into ER-derived transport vesicles.

Sec22p N-terminal Domain Mutants Assemble into ER/Golgi SNARE Complexes in Vivo—We next tested whether these N-terminal truncations interfered with the assembly of Sec22p into ER/Golgi SNARE complexes that operate in anterograde transport. Previously, we showed (14) that a set of ER/Golgi SNARE proteins co-immunoprecipitated with Bos1p from detergent-solubilized membranes. Using this technique, Bos1p or Sec22p was immunoprecipitated from wild-type and SEC22

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**Fig. 2.** Expression levels of Sec22p N-terminal truncation mutants. An immunoblot to monitor the expression of Sec22p in the semi-intact cells from WT (CYB740), sec22Δ (CYB773), and sec22Δ strains expressing the indicated N-terminal mutants is shown. Semi-intact cells were diluted in sample buffer and resolved by 15% SDS-PAGE followed by immunoblot for MBP-Sec22p (loading control). The amounts of Sec22p-containing SNARE complexes when anti-Bos1p antibodies. Similarly, Bet1p, Bos1p, and Sed5p were immunoprecipitated from the N-terminal truncation strains when anti-Sec22p antibodies were used. These results indicate that the Sec22p N-terminal domain is not required for SNARE complex formation. Furthermore, because Sec22pΔN1 and Sec22pΔN2 were localized to the ER, a significant fraction of ER/Golgi SNARE complexes must also be present in the ER.

The amounts of Sec22pΔN1 and Sec22pΔN2 in these SNARE complex immunoprecipitations were lower than with wild-type Sec22p probably due to lower expression levels of the N-terminal truncation mutants (Figs. 2 and 5). In agreement with this possibility, increased expression of Sec22pΔN1 and Sec22pΔN2 from 2μm plasmids resulted in increased amounts of each N-terminal mutant protein associated with Bos1p. A corresponding increase in the levels of Bet1p, Bos1p, and Sed5p associated with these N-terminal deletion mutants was also observed when the overexpressed proteins were immunoprecipitated with anti-Sec22p antibodies (Fig. 5). Notably, the amount of Sec22p containing ER/Golgi SNARE complexes did not exceed the wild-type level even when N-terminal deletion mutants were overexpressed. Therefore it seems unlikely that the N-terminal domain of Sec22p negatively regulates SNARE domain interactions with other ER/Golgi SNARE proteins, because the N-terminal deletions would be expected to alleviate...
this negative effect and increase Sec22p assembly into SNARE complexes. Instead, these results suggest that the N-terminal domain of Sec22p does not regulate SNARE complex assembly and is not necessary for their formation.

Because the N-terminal mutants of Sec22p still formed ER/Golgi SNARE complexes, we speculated that these mutant proteins would at least partially fulfill the function of Sec22p in vivo. We tested this hypothesis by comparing the growth phenotypes of sec22Δ strains that expressed these individual mutants. Dilution series of these strains were plated and incubated at 30 and 37 °C to observe thermosensitive growth defects (Fig. 6). As expected, the sec22Δ strain grew at a rate comparable with the wild type at 30 °C but displayed a reduced growth rate at 37 °C. When Sec22Δ on a CEN plasmid was introduced into the sec22Δ strain, wild-type growth rates were observed at both temperatures. Expression of the N-terminal mutants, SEC22ΔN1, SEC22ΔN2, and MBP-SEC22, from CEN plasmids partially complemented the growth defect of sec22Δ at 37 °C. Increased complementation was observed when SEC22ΔN1 and SEC22ΔN2 were overexpressed from 2 μm plasmids. These findings indicate that the Sec22Δ N-terminal truncation mutants are functional and assemble into SNARE complexes in vivo, although their steady state distribution is altered. We speculate that a small fraction of the N-terminal Sec22p mutants continue to cycle between ER and Golgi compartments in a “bulk flow” manner and function in membrane fusion events. Overexpression of the mutants would be expected to increase their concentration on ER–Golgi membranes and probably explains the increased level of complementation and SNARE pairing we observe when 2-μm versions are used.

Residues within the SNARE Motif Contribute to Sec22p Trafficking—Having established a role for the N-terminal domain of Sec22p in trafficking, we next investigated the function of the Sec22p SNARE domain in localization and activity. We initially constructed three different SNARE domain truncation mutants. Sec22pΔS removes most of the SNARE domain from the −4 to +8 layers. Sec22pΔS1 and Sec22pΔS2 partially delete the SNARE domain from layers −2 to +2 and +2 to +8, respectively (Fig. 1). These mutant proteins were expressed in wild type and sec22Δ backgrounds from CEN plasmids, and each was readily detected with anti-Sec22p antibodies (Fig. 7). An increased expression level of the SNARE domain mutant proteins was observed in the wild-type background compared with the sec22Δ background, indicating that wild-type Sec22p somehow influences expression of these mutants. In vitro budding reactions were performed to monitor the packaging efficiency of each of the SNARE domain mutants into COPII vesicles. Sec22pΔS incorporation into COPII-coated vesicles was not detected when expressed in either the wild-type or sec22Δ background (Fig. 8A). However, Sec22pΔS1 and Sec22pΔS2 were both sorted into COPII vesicles, although the packaging efficiency of Sec22pΔS1 was greatly diminished while Sec22pΔS2 was packaged near wild-type levels (Fig. 8, B and C). Similar results were observed when the COPII packaging of Sec22pΔS1 and Sec22pΔS2 was monitored in a sec22Δ background (Fig. 8, B and C). These results indicate that the N-terminal domain, though required, is not sufficient for optimal packaging of Sec22p into COPII vesicles. Additional determinants within the SNARE domain appear to be required for efficient COPII-dependent export of Sec22p from the ER.

Subcellular localization studies on the Sec22p SNARE do-

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main mutants are consistent with the in vitro budding results. The more extensive Sec22pS2 mutant, which was not packaged into COPII vesicles, localized to ER membranes in both wild-type and sec22Δ backgrounds (Fig. 9, A and B). In contrast, the Sec22pΔS1 and Sec22pΔS2 proteins, which were packaged into COPII vesicles at varying levels, were found distributed between the ER and the Golgi compartments (Fig. 9, C and D).

**ER Export of Sec22p Is Independent of SNARE Complex Assembly**—To determine whether these Sec22p SNARE deletions interfered with the assembly into SNARE complexes, we monitored the levels of Sec22pΔS1 and Sec22pΔS2 associated with the ER/Golgi SNARE proteins in anti-Bos1p immunoprecipitation experiments (Fig. 10). Whereas wild-type Sec22p associated with Bos1p, Sed5p, and Bet1p, neither Sec22pΔS1 nor Sec22pΔS2 was detected in Bos1p immunoprecipitates when isolated from a wild-type or sec22Δ background. These results demonstrate that the deletion of residues between the −2 and +2 layers (Sec22ΔS1) or the +2 and +8 layers (Sec22ΔS2) prevent assembly of Sec22p into the ER/Golgi SNARE complexes, at least under the conditions that normally preserve such complexes in vitro. Because Sec22pΔS2 is efficiently packaged into COPII vesicles but does not assemble into SNARE complexes, we concluded that Sec22p packaging is independent of SNARE pairing.

**A Short Segment within the Sec22p SNARE Domain Is Required for COPII-dependent Export**—To further refine a region within the Sec22p SNARE domain that is required for efficient COPII-dependent export, we constructed a deletion mutant (Sec22pΔS3) that removes 10 amino acid residues between the −4 and −1 layers (Fig. 1). Characterization of this mutant revealed properties that were virtually identical to the

**Fig. 8.** Packaging of Sec22p SNARE domain mutants into COPII-coated vesicles. In vitro budding reactions from sec22Δ + SEC22ΔS and WT + SEC22ΔS (A), sec22Δ + SEC22ΔS1 and WT + SEC22ΔS1 (B), and sec22Δ + SEC22ΔS2 and WT + SEC22ΔS2 strains (C). Lanes contain one-tenth of a total reaction (+), or a mock reaction without COPII proteins (−). Samples were resolved by SDS-PAGE and immunoblotted for Erv25p, Sec61p, and Sec22p.

**Fig. 9.** Subcellular distribution of Sec22p SNARE domain mutants. Cell lysates from SEC22Δ (WT + SEC22ΔS) (A), SEC22Δ (sec22Δ + SEC22ΔS) (B), SEC22ΔS1 (WT + SEC22ΔS1) (C), and SEC22ΔS2 (WT + SEC22ΔS2) (D) were resolved on 18–60% sucrose gradients as described in the legend to Fig. 4.

We next tested the functional properties of these Sec22p SNARE domain deletion mutants in vivo. As shown in Fig. 11, none of the SNARE domain mutants complemented the growth defect of sec22Δ strains at 37 °C. Instead, when CEN versions of the SNARE domain mutants were expressed in sec22Δ cells, negative effects on growth rate were observed. SEC22ΔS2 displayed a severe negative effect, even when examined at 30 °C. These results hint that the N-terminal region in these SNARE domain mutants may non-productively interact with a limiting component in sec22Δ cells. However, it should be noted that no significant effects on growth rate were observed when the SNARE domain mutants were introduced into a wild-type SEC22 background (Fig. 11). Further experimentation will be required to determine the mechanism by which the SNARE domain mutants inhibit growth. Regardless, these data are in accord with the co-immunoprecipitation experiments and show that Sec22p SNARE domain truncation mutants cannot function in membrane fusion, because they fail to assemble into ER/Golgi SNARE complexes.
invariant amino acid sequence between the are required for efficient ER export. Interestingly, there is an result indicates that residues within this 10-amino-acid stretch SNARE domain deletion mutants (data not shown). These reduced negative effects on growth rates as noted for the other /H9004 (N-I-E) that was deleted in both Sec22p /H9004 /H9004 (CBY740) (sec22/H9004), a darker exposure of the anti-Sec22p blot.

Sec22p SNARE domain mutants display growth defects in sec22Δ. A dilution series of wild type (WT) (CBY740), sec22Δ (CBY773), or sec22Δ (CBY773) plus (+) the indicated plasmid is shown. All proteins were expressed from CEN plasmids. Strains were grown in selective medium, adjusted to A600 of 1.0, and spotted onto YPD plates. The plates were incubated at 30 or 37 °C for 3 days.

Sec22pΔS1 protein. As observed for Sec22pΔS1, Sec22pΔS3 was inefficiently packaged into COPII vesicles (Fig. 12A) and displayed an ER and Golgi distribution pattern (Fig. 12B). In vivo, SEC22ΔS3 did not complement sec22Δ strains and produced negative effects on growth rates as noted for the other SNARE domain deletion mutants (data not shown). These results indicate that residues within this 10-amino-acid stretch are required for efficient ER export. Interestingly, there is an invariant amino acid sequence between the -2 and -1 layers (N-I-E) that was deleted in both Sec22pΔS1 and Sec22pΔS3 and may be critical for Sec22p trafficking.

DISCUSSION

In this report, we examined regions of the Sec22p profilin-like domain and the SNARE domain for their influence on Sec22p trafficking and assembly into SNARE protein complexes. Our results are summarized in Table I. The N-terminal domain mutants were not efficiently packaged into COPII vesicles and displayed a steady state distribution to the ER. Surprisingly, these mutant proteins assembled into ER/Golgi SNARE complexes and provided partial function in vitro when expressed at low levels and full complementation when gene dosage was elevated. These findings indicate that the N-terminal domain of Sec22p is required for recognition by the COPII budding machinery but apparently not required for SNARE complex assembly. Furthermore, because the N-terminal deletion mutants can assemble into SNARE complexes but are not efficiently exported from the ER, we conclude that SNARE complex assembly is not sufficient for export of Sec22p.

Our deletion analysis of the Sec22p SNARE motif revealed dual functions for this domain. First, this domain is essential for SNARE pairing as predicted from previous studies (4, 43). We observed that the Sec22p SNARE domain deletion mutants failed to assemble into SNARE complexes and were not functional in vivo. Second, residues within the Sec22p SNARE domain are required for optimal export of Sec22p from the ER. We observed that both Sec22pΔS1 and Sec22pΔS3 were inefficiently packaged into COPII vesicles, whereas Sec22pΔS2 was efficiently packaged. Because none of these mutants assemble into SNARE complexes, the COPII packaging of Sec22pΔS2 clearly does not depend on SNARE complex assembly. The Sec22pΔS mutant displayed even stronger COPII packaging defects than the Sec22pΔS1 and Sec22pΔS3 mutants. The cause of this difference is not known, but we speculate that the extensive size of this deletion may also interfere with the function or positioning of the Sec22p N-terminal domain, because Sec22pΔS exhibits a localization pattern similar to Sec22ΔN1 and Sec22ΔN2. Regardless, our results indicate that a 10 amino acid segment in the Sec22p SNARE domain contains a targeting signal required for recognition by the COPII budding machinery in addition to the role this domain serves in SNARE complex assembly. Indeed, assembly of Sec22p into SNARE complexes may mask this signal and influence packaging into COPII vesicles.

Recognition of Sec22p by the COPII Coat—In a recent study, Mossessova et al. (44) reported that a recombinant Sec22p fusion protein lacking the transmembrane domain bound di-
rectly to purified Sec23/24p, a subunit of the COPII coat. Moreover, versions of the Sec22p fusion protein that contained both the N-terminal and the SNARE domain were required to recapitulate the Sec23/24p interaction. Miller et al. (45) recently demonstrated that at least two separate sites within the Sec24p protein were necessary for the efficient export of Sec22p from the ER. These results are in good agreement with our observations that the Sec22p N-terminal domain and the 10 amino acid residues between the −4 and −1 layers of the SNARE motif are required for optimal packaging of Sec22p into COPII vesicles. Mossessova et al. (44) tentatively concluded that Sec22p bound to a single site in the Sec23/24p complex through the formation of a folded epitope in a “closed” arrangement that places the N-terminal domain near critical SNARE motif residues. Given our data that the Sec22p N-terminal domain does not influence SNARE complex assembly in vitro and other studies showing that the isolated N-terminal domain protein does not inhibit assembly of mSec22b into SNARE complexes in vitro (23), we can envisage an alternate model in which an “open” conformation of Sec22p is recognized by the Sec23/24p complex. In this model, coordinated contacts of both the N-terminal domain and the SNARE domain in Sec22p with two distinct binding sites in the Sec23/24p complex may promote optimal binding. Further structural analyses of Sec22p binding site(s) in the Sec23/24p complex will be required to fully understand the molecular detail of these interactions.

ER Export of Other ER/Golgi SNARE Proteins—Is the situation with Sec22p transport from the ER as an uncomplexed SNARE protein in yeast a specific case or is it a general transport mechanism for other SNARE proteins? Previously, we reported (14) that depleting Sec22p from ER-derived vesicles by adding affinity-purified anti-Sec22p antibodies to a COPII-budding reaction did not affect the packaging of other ER/Golgi SNARE proteins into COPII vesicles. Our observation raised the possibility that this set of SNARE proteins (i.e. Bos1p, Sed5p, and Bet1p) were transported individually, although this result does not rule out the possibility that Bos1p, Sed5p, and Bet1p exit the ER in a ternary SNARE complex. Studies on Sed5p show that it also binds efficiently to Sec24p in the absence of any other ER/Golgi SNARE proteins (44, 46). However, competition-binding experiments with purified components do suggest that binding affinities between Sed5p and Sec24p may be increased when Sed5p is assembled into SNARE complexes (44). In contrast, our preliminary findings indicate that depletion of Sed5p during COPII vesicle budding did not affect the uptake of Bos1p and Sec22p into COPII vesicles.2 Bet1p also binds directly to Sec24p, and its COPII binding motif is occluded when assembled into SNARE complexes, suggesting that Bet1p is also packaged into COPII vesicles in a free form (44). As for mammalian ER/Golgi SNARE proteins, heteromeric SNARE interactions are not required at any step in bet1 targeting or transport dynamics (47). Moreover, antibodies to the mammalian ER/Golgi SNARE proteins (i.e. syntaxin5, membrin, mSec22b, and rbet1) blocked the recruitment of these individual SNARE proteins into COPII vesicles without any effect on the packaging of other SNARE proteins (48, 49). Therefore, we propose that the trafficking of ER/Golgi SNARE proteins from the ER in unassembled states represents a general mechanism from yeast to mammals. It may be informative to test this model in a reconstituted proteoliposome budding assay (50) where the composition and assembly state of SNARE complexes could be controlled.

Regulatory Role of N-terminal Domains in SNARE Proteins—Despite the conserved structure and function of the SNARE domain, the N-terminal domain of distinct SNARE proteins appears to be divergent in both structure and function. Our experiments demonstrated that the N-terminal domain of Sec22p was required for selective packaging of Sec22p into COPII-coated vesicles and for its Golgi localization. Mutants lacking the entire N-terminal domain formed ER/Golgi SNARE complexes with Bos1p, Sed5p, and Bet1p in vitro, indicating the N-terminal domain has no apparent influence on SNARE protein pairing. These findings are consistent with other in vitro studies. For example, binding assays with purified recombiant proteins showed that a portion of Sec22p (amino acids 40–194) containing the SNARE motif but only part of the N-terminal domain bound to a Sed5p-GST fusion protein. In contrast, a C-terminal truncation protein (amino acids 1–154) that deletes the SNARE motif fails to associate with Sed5p-GST (43). Using CD spectroscopy to follow SNARE assembly over time, it was observed that the rates of SNARE complex assembly with the syntaxin1A SNARE domain, SNAP-25, and mSec22b were identical in the presence or absence of the N-terminal domain of mSec22b (23). These investigators concluded that the N-terminal domain of mSec22b had no effect on the rate of SNARE assembly in vitro.

Because N-terminal regions in Sec22p and other SNARE proteins are required for their proper intracellular targeting (17–20), it seems probable that signals within these regions will interact with distinct coat protein complexes in addition to COPII. Such differential interactions are likely to govern intracellular location and therefore contribute to the fidelity of membrane fusion reactions. Indeed, SNARE protein localization may play a significant role in specifying membrane fusion partners. For example, when the level of a Golgi/endosomal SNARE protein, Ykt6p, is elevated on COPII vesicles in the absence of Sec22p, it assembles into an ER/Golgi SNARE complex and can function with non-cognate SNAREs in the fusion of ER-derived vesicles with the Golgi complex (14). Once a more complete set of specific targeting signals in SNARE proteins has been identified, it will be instructive to test whether other

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2. Y. Liu, J. J. Flanagan, and C. Barlowe, unpublished data.
examples of SNARE cross-complementation can occur when a given SNARE protein is redirected.

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