Proc. Natl. Acad. Sci. USA
Vol. 94, pp. 837–842, February 1997
Biochemistry

Selective packaging of cargo molecules into endoplasmic reticulum-derived COPII vesicles

(Sar1p/Sec16p/secretory proteins/SNARE proteins)

JOSEPH L. CAMPBELL AND RANDY SCHEKMAN

Department of Molecular and Cell Biology, and Howard Hughes Medical Institute, Barker Hall, University of California, Berkeley, CA 94720-3202

Contributed by Randy Schekman, November 27, 1996

ABSTRACT **Coated vesicles transport proteins from the endoplasmic reticulum (ER) to the Golgi apparatus. The formation of transport vesicles *in vitro* requires the incubation of an ER-membrane fraction with three protein fractions collectively known as *co*at *p*rotein II (COPII; Sar1p, Sec23p/Sec24p, and Sec13p/Sec31p). We used this assay to investigate how targeting [v-SNARE, *v*esicle-*s*oluble *N*SF (*N*-ethylmaleimide-*s*ensitive *f*actor) *a*ttachment protein *re*ceptor], putative adapter (e.g., Emp24p), and cargo molecules are captured into ER-derived COPII vesicles. Analysis of fusion proteins strongly suggests that the cytoplasmic domain of the v-SNARE protein Sec22p is required for its packaging into ER-derived COPII vesicles. We examined the packaging requirements for various molecules by individually titrating each of the COPII components. More Sar1p (the GTP-binding protein that initiates vesicles budding) is needed to package the membrane-associated v-SNAREs and Emp24p than is needed to package the soluble secretory protein glycosylated pro-α-factor (gpαF). Microsomes prepared from a strain overproducing Sec12p (the nucleotide exchange protein that recruits Sar1p to the ER) produce vesicles containing gpαF without the addition of exogenous Sar1p, whereas the v-SNAREs and Emp24p are not efficiently packaged under these conditions. Addition of Sar1p to these microsomes leads to increased packaging of v-SNAREs and Emp24p with no increase in the packaging of gpαF. Finally, we show that membranes prepared from strains with mutations in the *SEC16* gene are more defective for the packaging of v-SNARE molecules and Emp24p than they are for the packaging of gpαF. These results point to the possibility that diverse signals or adapters participate in the capture of secretory and membrane cargo molecules into COPII transport vesicles.**

Palade (1) originally proposed that vesicles carry secretory proteins between organelles. Subsequent genetic and biochemical experiments have identified many of the proteins required for producing these vesicles and fusing them with the appropriate target membrane. These studies have also shown that homologous molecules perform the same functions in yeast and mammalian cells (for review, see ref. 2). A set of soluble proteins (the Sec13p/Sec31p complex, the Sec23p/Sec24p complex, and Sar1p) that can substitute for cytosol in the formation of these vesicles were identified in a cell-free assay (3). This set of soluble proteins forms a coat, COPII, around the vesicles during the budding process (4). The first COP (*co*at *p*rotein) complex to be identified, COPI, was discovered in mammalian cells and mediates the formation of a different class of vesicles (for review, see ref. 2).

Despite our knowledge of the cytosolic factors involved in vesicle formation, little is known about how cargo is recognized and packaged into these vesicles. One class of proteins that must be packaged into vesicles is known collectively as v-SNARE [*v*esicle-*s*oluble *N*SF (*N*-ethylmaleimide-*s*ensitive *f*actor) *a*ttachment protein *re*ceptor] proteins (for review, see ref. 5). v-SNARE proteins are membrane-bound proteins that interact with their cognate t-SNARE (target SNARE) protein (t-SNARE proteins are located in the membrane of the acceptor organelle) to ensure that vesicles only fuse with the membrane of the appropriate organelle. To examine v-SNARE packaging signals, we employed fusion proteins between Sec22p, a v-SNARE protein found in COPII vesicles, and Sec12p, an endoplasmic reticulum (ER) membrane protein required for the formation of COPII vesicles but not found in these vesicles. The results of these and other experiments suggest that there is a signal for the packaging of Sec22p on the cytoplasmic domain of Sec22p.

We also examined the packaging characteristics of glycosylated pro-α-factor (gpαF), Sec22p, Bos1p, Bet1p, and Emp24p. Bos1p and Bet1p are two additional v-SNARE proteins found in COPII vesicles, and Emp24p is a transmembrane protein believed to be involved in the packaging of specific cargo molecules (6). We find differential sensitivity to COPII component levels and to the presence of functional Sec16p, suggesting that all packaging is not handled identically.

Preliminary data on the fusion proteins appeared previously in the proceedings of a meeting (7).

MATERIALS AND METHODS

Strains. RSY255, *MATα ura3-52, leu2-3,112*; RSY267, *MATα sec16-2 ura3–52 his 4-619*; RSY317, *MATα sec16-1, leu 2-3,112*; RSY658 W303-1B, *sec12*::*LEU2* (pANY1-9); and RSY772, *MATα sec16-3 ura3-52, leu2-3,112*. pANY1-9 is a 2-μm plasmid from which the expression of Sec12p is driven by its own promoter (8).

Reagents. Restriction enzymes, T4 DNA polymerase, and T4 DNA ligase were purchased from New England BioLabs and Boehringer Mannheim. PFU DNA polymerase was purchased from Stratagene. Antibodies for Sec22p (9), Bos1p (10), Bet1p (10), Emp24p (6), and Sec12p (C. Barlowe, Dartmouth Medical School) were used in Western blots. ^{125}I-labeled protein A was purchased from ICN. Chemicals were purchased from Sigma, and media were purchased from Difco.

Construction of Fusion Proteins. The fusion proteins were constructed with a two-step PCR-based method described by Horton *et al.* (11). For Sec22-12p (a hybrid protein bearing the

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

0027-8424/97/94837-6$2.00/0
PNAS is available online at **http://www.pnas.org**.

Abbreviations: v-SNARE, *v*esicle-*s*oluble *N*SF (*N*-ethylmaleimide-*s*ensitive *f*actor) *a*ttachment protein *re*ceptor; COP, *co*at *p*rotein; Sec22-12p, hybrid protein bearing the N terminus of Sec22p and the C terminus of Sec12p; Sec12-22p, hybrid protein bearing the N terminus of Sec12p and the C terminus of Sec22p; gpαF, glycosylated pro-α-factor; ER, endoplasmic reticulum.

N terminus of Sec22p and the C terminus of Sec12p), the following oligonucleotides were used: 5′-GCT TCG AGA TCT AAA CCC TGA CAG TGA CAC-3′, 5′-CGC AAA AGA TCA ACT TCG ATT TTT TCA CCA ACT TCA TCC T-3′, 5′-AGG ATG AAG TTG GTG AAA AAA TCG AAG TTG ATC TTT TGC G-3′, and 5′-GCC GAT TAT CTA GAC GTC ACT CTC CTT TTC GC-3′. For Sec12-22p (a hybrid protein bearing the N terminus of Sec12p and the C terminus of Sec22p), the following oligonucleotides were used: 5′-GCC GAT TAA GAT CTG ATA CGT GAT AGA AAT AC-3′, 5′-TGA AAC AAA AAA TCT CTA AAC TCT TGA TCA GTC AAT ATG C-3′, 5′-GCA TAT TGA CTG ATC AAG AGT TTA GAG ATT TTT TGT TTC A-3′, and 5′-GCT TCG TCT AGA CTT GGA CCA AAT TGA TCG-3′. The desired fragments were gel-purified, and the ends were made blunt with T4 DNA polymerase. Blunt fragments were ligated into the *Eco*RV site of Bluescript II KS$^+$ (Stratagene). The resulting plasmids were then cut with *Bgl*II and *Xba*I and the fragment coding for the fusion protein was cloned into pRH98-1 cut with *Bam*HI and *Xba*I. pRH98-1, a gift of Randy Hampton (University of California, Berkeley, CA), was derived as described for pRH98-2 (12) except the parent vector was YCplac33 (*ARSCEN*, *URA3*) instead of YIp lac211 (integrating, *URA3*) (13). When genes were cloned into the *Bam*HI site of pRH98-1, their expression was driven by the glyceraldehyde-3-phosphate dehydrogenase promoter (14). The plasmid bearing Sec22-12p was called pJCY26, and the plasmid bearing Sec12-22p was called pJCY30.

Construction of Sec22p Overproducer. PCR was used to generate a fragment containing the entire coding sequence of Sec22p. The following oligonucleotides were used in these reactions: 5′-GCT TCG AGA TCT AAA CCC TGA CAG TGA CAC-3′ and 5′-GCC GAT TAT CTA GAG CCA TAC TAT ACT AAT AC-3′. The resulting fragment was gel-purified, and the ends were made blunt with T4 DNA polymerase. This blunt fragment was ligated into the *Eco*RV site of Bluescript II KS$^+$. The resulting plasmid was cut with *Bgl*II and *Xba*I, and the fragment coding for Sec22p was cloned into pRH98-3 cut with *Bam*HI and *Xba*I. pRH98-3, a gift of Randy Hampton, is a 2-μm version of pRH98-1, whose construction was identical to that of pRH98-2 (12) except the parent vector was YEplac195 (2 μm, *URA3*) instead of YIp lac211 (integrating, *URA3*) (13). This plasmid is called pJCY40.

Preparation of Microsomes. Microsomes were prepared as described by Wuestehube and Schekman (15) with minor modifications. To prepare microsomes containing Sec22-12p or Sec12-22p, RSY255 was transformed with the appropriate vector (see above). The Sec22p overproducer microsomes were made from RSY255 transformed with pJCY40. For the experiments shown in Table 1 and Fig. 2, wild-type microsomes were made from RSY255 transformed with pRH98-3. The Sec12p overproducer microsomes were made from RSY658. For all these microsomes, cells were grown at 30°C in 2% peptone, 2% glucose, 0.67% yeast nitrogen base, plus amino acids as needed. For the microsomes used in the experiments described in Fig. 4, strains (RSY255, RSY267, RSY317, and RSY772 all untransformed) were grown at 26°C in yeast extract/peptone/dextrose.

Preparation of COPII Components. The preparation of COPII components has been described (3, 4).

Vesicle Budding Reactions. [^{35}S]Pre-pro-α-factor was post-translationally translocated into microsomes as described by Wuestehube and Schekman (15). The microsomes containing [^{35}S]gpαF were then incubated at 20°C (except for those shown in Fig. 4, for which the incubations were at 30°C) in buffer 88 (20 mM Hepes, pH 7/150 mM KOAc/250 mM sorbitol/5 mM $MgOAc_2$) with the following additions; 0.1 mM GTP, 2 mM ATP, and the COPII components. The reactions were performed in a volume of 50 μl, and the concentration of membrane proteins contained in the microsomes was 500 μg/ml. For all reactions except the titrations shown in Fig. 2, the COPII components were used at the following concentrations: Sar1p, 20 μg/ml; Sec23p/Sec24p, 24 μg/ml; and Sec31p/Sec13p, 44 μg/ml. In the titrations shown in Fig. 2, two components were added at the concentrations indicated above, and the concentration of the third component was varied. The Sar1p concentration ranged from 0 to 80 μg/ml, the Sec23p/Sec24p concentration ranged from 0 to 96 μg/ml, and the Sec13p/Sec31p concentration ranged from 0 to 176 μg/ml. For all of the vesicle budding reactions, after a 30-min incubation at the appropriate temperature, the reactions were placed on ice for 5 min, and the vesicles were separated from donor membranes by centrifugation at 14,000 $\times$ *g* for 3 min in a refrigerated microcentrifuge. The release of gpαF into the vesicle-containing supernatant was quantified as described by Rexach and Schekman (16), and release of the other assayed molecules into the supernatant was quantified by ^{125}I-labeled protein A immunoblots and a Molecular Dynamics PhosphorImager. Immunoblots were performed as described by Towbin *et al.* (17).

RESULTS

Sec22p May Possess a Positive Transport Signal. To gain information about how v-SNARE proteins are packaged into COPII vesicles, we constructed fusions between two type II ER membrane proteins, Sec22p and Sec12p. A schematic view of these proteins is shown in Fig. 1. Sec22p is a v-SNARE protein that is packaged efficiently into ER-derived vesicles, whereas Sec12p is required for the formation of COPII vesicles but does not efficiently enter COPII vesicles (4, 9, 16, 18). *In vivo*, a fusion of the cytoplasmic domain of Sec22p and the transmembrane and lumenal domains of Sec12p (Sec22-12p; Fig. 1) behaves like Sec22p. A plasmid encoding Sec22-12p complemented a temperature-sensitive allele of *sec22*, and the fusion protein rapidly acquired Golgi-specific glycosyl modifications, indicating a rapid exit from the ER (data not shown). Similarly, the reverse construct (Sec12-22p; Fig. 1) complemented temperature-sensitive and null alleles of *sec12* (data not shown).

To test how these fusion proteins behaved in our *in vitro* vesicle budding assay, we prepared ER membranes (microsomes) from strains transformed with plasmids encoding the fusions. We showed previously that COPII components efficiently package Sec22p and gpαF into ER-derived vesicles, whereas ER resident proteins such as Sec12p are not packaged into vesicles under these conditions (4, 10). As expected, we found that when COPII components were added to microsomes containing either fusion protein, Sec22p and gpαF were packaged into vesicles and Sec12p remained in the ER membrane (Table 1). We also found that Sec22-12p was packaged into vesicles, whereas Sec12-22p was not (Table 1), consistent with the *in vivo* data. Because Sec12-22p provided Sec12p function *in vivo*, we believe it is not excluded from vesicles due

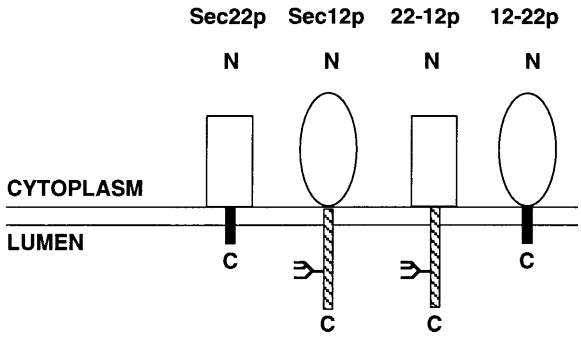

FIG. 1. A schematic view of Sec22p, Sec12p, and the fusions proteins used in this study. A glycosylation site on the lumenal domain of Sec12p is indicated.

Table 1. Packaging of wild-type and fusion proteins into ER-derived COPII vesicles

Membrane source	% release of				
	Alpha factor	Sec22p	Sec12p	Sec22-12p	Sec12-22p
Sec22-12p	38.5	23	<3	14	ND
Sec12-22p	37.5	17	<3	ND	<3
Wild type	37	29	<3	ND	ND
SEC22 OP	40	13.5	<3	ND	ND

The membrane source is the type of microsome used in COPII vesicle budding reactions. ND, not detected.

to misfolding. These data suggested either a positive signal for the packaging of Sec22p on its cytoplasmic domain or a retention signal for Sec12p on its cytoplasmic domain. We reasoned that if Sec22p contained a positive signal, we might be able to saturate its receptor by overproducing the ligand. Therefore, we measured the release of Sec22p from microsomes made from a strain overproducing the protein (microsomes prepared from this strain contain 15-fold more Sec22p, as determined by quantitative immunoblotting) and found that these microsomes, when compared with wild-type microsomes, packaged a 2.5-fold lower percentage of the Sec22p into the vesicles (13% versus 29%), even though they packaged the same percentage of gpαF (Table 1).

We considered the possibility that the reduced efficiency of Sec22p packaging may be attributable to mislocalization of the overproduced protein such that a reduced fraction was contained in ER membranes in the microsomal preparation. For this to be so, over half of the overproduced Sec22p must be mislocalized to other membranes, such as Golgi cisternae, that are contained in the microsomes, even though in untransformed cells Sec22p appears to be localized to the ER (19). Furthermore, microsomes are enriched for ER membranes: less than 20% of the Golgi marker, Och1p, and over half of ER marker, Wbp1p, are found in microsomes (20). The gene fusion and overproduction data together argue for a positive signal on the cytoplasmic domain of Sec22p and suggest that the receptor for this signal can be saturated.

Sar1p Requirement Is Different for Soluble and Membrane Proteins. If cargo and v-SNARE proteins possess distinct and saturable transport signals, the efficiency of their packaging may vary according to the concentration of an interacting COPII subunit. To assess this, we titrated each of the COPII components individually and measured the packaging of the soluble protein gpαF and the membrane proteins Emp24p, Bos1p, Bet1p, and Sec22p into vesicles from wild-type microsomes. When Sar1p was titrated, we observed a significant difference in the dose–response curves for these proteins (Fig. 2*A*). Specifically, the gpαF curve plateaued at a lower concentration of Sar1p than did the curves for Bos1p and Emp24p. The curves for Sec22p and Bet1p plateaued at an intermediate concentration of Sar1p. The titration curves for Sec23p/Sec24p and Sec13p/Sec31p showed no such striking effect (Fig. 2 *B* and *C*).

Budding from Microsomes Prepared from Strains Overproducing Sec12p. We next explored an independent means of delivering varied levels of Sar1p to the ER membrane. The cytoplasmic domain of Sec12p interacts with Sar1p to facilitate nucleotide exchange and recruitment of Sar1p-GTP (21–24). Microsomes were prepared from strains overproducing Sec12p (microsomes prepared from this strain contain 40-fold more Sec12p, as determined by quantitative immunoblotting). As previously reported (22), these microsomes were enriched in Sar1p. Indeed, on addition of Sec23p/Sec24p and Sec13p/Sec31p, no additional Sar1p was needed to package gpαF into COPII vesicles (Fig. 3). Under the same conditions, the packaging of Bos1p and Emp24p was inefficient (Fig. 3). The difference between the amount of gpαF packaged and the amount of Bos1p and Emp24p packaged was greater in the Sec12p-overproducing membranes than the difference observed at any concentration when Sar1p was titrated in incubations containing wild-type membranes. Sec12p overproduction appeared to exaggerate the distinct Sar1p dependencies observed in Fig. 2*A*. In the presence of exogenous Sar1p, the packaging of Bos1p and Emp24p improved dramatically (greater than 4-fold), even though no additional gpαF was packaged (Fig. 3). Sec22p and Bet1p, again, show an intermediate phenotype in this assay (Fig. 3).

GTP is required for the formation of COPII vesicles (16). Sar1p is a GTPase, and one potential role for GTP in this reaction is in the recruitment of Sar1p to the membrane. We found that formation of vesicles containing gpαF still required GTP even when the reactions were performed with the Sec12p-overproducer membranes in the absence of exogenous Sar1p (release of gpαF dropped to 2.5% when GTP was omitted). Thus, GTP may serve an additional role independent of its role in the recruitment of Sar1p to membranes.

Reduction of Sec16p Function also Reveals a Distinction Between Soluble and Membrane Proteins. Finally, we examined Sec16p, another membrane protein possibly involved in COPII cargo discrimination. Sec16p is a peripheral membrane protein of the ER that has been shown genetically to be required for the formation of ER-derived vesicles *in vivo* (18) and is recruited onto COPII vesicles *in vitro* (25). Given the cargo distinctions demonstrated by the COPII titration experiments, we considered the possibility that Sec16p may be similarly selective in its involvement. Microsomes were prepared from strains bearing temperature-sensitive alleles of *sec16* (*sec16-1*, *sec16-2*, and *sec16-3*) and from wild-type strains, and the COPII-dependent packaging of gpαF, Bos1p, Bet1p, Sec22p, and Emp24p from these microsomes was measured. As shown in Fig. 4, when the function of Sec16p was compromised, the packaging of gpαF was reduced 2- to 3-fold and the packaging of Emp24p and the v-SNAREs was reduced to a greater extent. This defect was most pronounced in the packaging of Bos1p and Emp24p into vesicles using microsomes from a *sec16-2* strain. Taken together, these data lead us to conclude that Sar1p, Sec12p, and Sec16p cooperate to distinguish various cargo components destined for packaging into COPII vesicles.

DISCUSSION

A Positive Sorting Signal for Packaging of Sec22p. We used fusions of Sec22p and Sec12p to assess which protein domains determine the different fates of these proteins during the formation of COPII vesicles. Our data suggest that there is either a positive packaging signal on the cytoplasmic domain of Sec22p or a retention signal on the cytoplasmic domain of Sec12p, because the presence of either one dictates the fate of the hybrid protein. Two lines of reasoning constitute evidence in support of the positive signal on Sec22p. First, Sec22p overproduction reduces the efficiency of Sec22p packaging without affecting the efficiency of gpαF packaging. Thus, passive transport without a transport signal seems quite unlikely. Second, Sec22p is present in coated vesicles that bud from the ER, and the concentration depends on the coat (9).

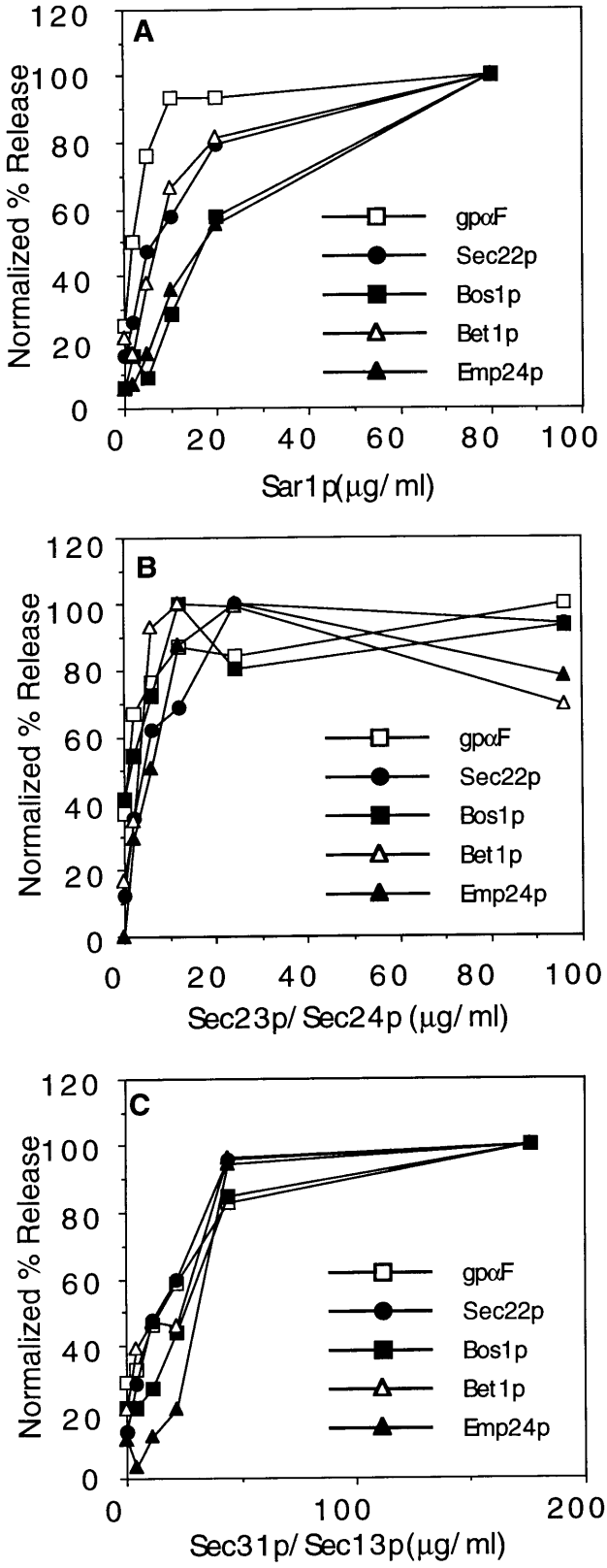

FIG. 2. Normalized % release of the assayed cargo molecules is shown for a titration of each of the COPII components. For the Sar1p titration in *A*, 100% equals the following values: 39.25% gpαF, 36.5% Sec22p, 28% Bos1p, 53.25% Bet1p, and 21.25% Emp24p. For the Sec23p/Sec24p titration in *B*, 100% equals the following values: 43% gpαF, 39.3% Sec22p, 19.3% Bos1p, 37.5% Bet1p, and 13.5% Emp24p. For the Sec13p/Sec31p titration in *C*, 100% equals the following values: 48.5% gpαF, 31.5% Sec22p, 18.3% Bos1p, 29.3% Bet1p, and 22% Emp24p.

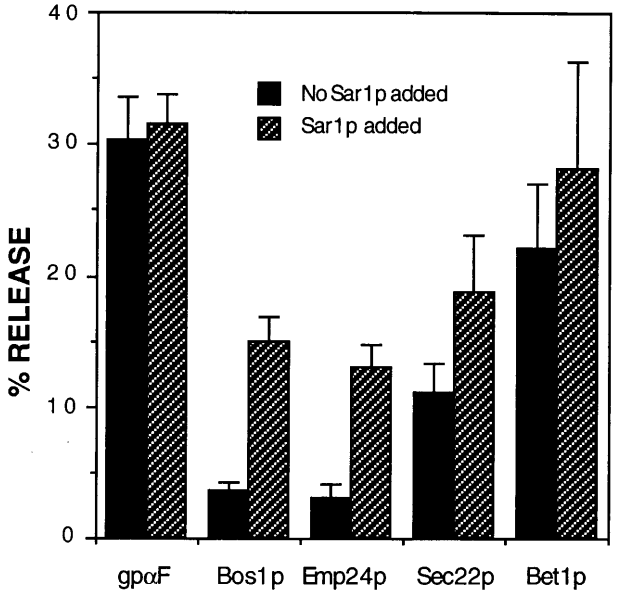

FIG. 3. The absolute % release from Sec12p overproducer microsomes is shown for the five assayed molecules when Sar1p was either omitted or added at a concentration of 20 μg/ml. Error bars are as indicated, based on four repetitions of the reactions.

COPII vesicles have a higher concentration of Sec22p than COPI vesicles (9). This strongly suggests that Sec22p is concentrated before budding (in the yeast equivalent of the mammalian transitional zone) or becomes clustered by coat proteins. In either case, we conclude that this enrichment depends upon some as yet undefined signal in the N-terminal domain of Sec22p. *In vivo* studies have shown that the transmembrane domain of Sec12p contains a signal that mediates its retrieval from the Golgi to the ER (26, 27). Our *in vitro*

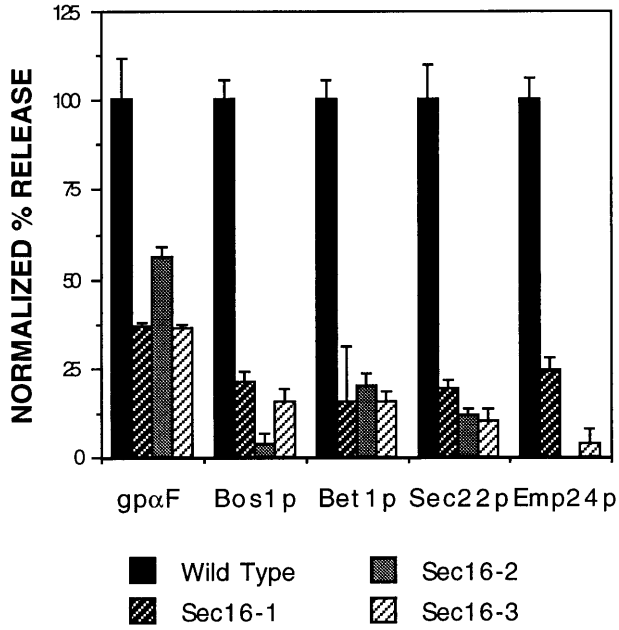

FIG. 4. Vesicle budding reactions were performed at 30°C with microsomes prepared from strains bearing *sec16* alleles. Release of the five assayed molecules was normalized to the wild-type values obtained under the same conditions. Error bars are indicated, based on two repetitions of the reactions. In these experiments, the wild-type values were as follows: 29% gpαF, 23.3% Sec22p, 8.5% Bos1p, 14.8% Bet1p, and 7.5% Emp24p. There was no detectable release of Emp24p from the *sec16-2* microsomes.

experiments only monitor the packaging of cargo into anterograde vesicles and thus do not detect retrieval signals. Based on *in vivo* experiments with chimeras between Dap2p and Sec12p, Nakano and coworkers have concluded that the cytoplasmic domain of Sec12p contains a retention signal (27). This conclusion is not incompatible with our evidence that Sec22p contains a positive transport signal. However, an alternative interpretation of Nakano and coworkers is that Dap2p has a positive signal on its cytoplasmic domain and Sec12p has no retention signal. In other work, Stevens and coworkers (28) developed evidence against Dap2p having a positive signal for forward transport from the late Golgi to the vacuole; however, the issue of how Dap2p is transported from the ER to the Golgi was not addressed. We believe that all the data can be most simply explained if the cytoplasmic domains of Sec22p and Dap2p contain positive signals that lead to their packaging into ER-derived vesicles.

Selective Cargo Capture. We have uncovered three instances that distinguish the packaging of gpαF from the packaging of v-SNAREs and Emp24p. First, when the three proteins that compose COPII are titrated individually and the packaging of gpαF, Bet1p, Sec22p, Bos1p, and Emp24p from wild-type membranes is measured, significantly lower concentrations of Sar1p are required to capture gpαF than are required to capture the other assayed proteins. Second, the packaging of gpαF is less impaired than that of the other assayed molecules, when microsomes were prepared from *sec16* mutant strains. Finally, when microsomes are made from a strain overproducing Sec12p, gpαF is efficiently packaged into vesicles in the absence of exogenous Sar1p, whereas the v-SNAREs and Emp24p are not. Addition of exogenous Sar1p to these membranes leads to efficient packaging of the v-SNAREs and Emp24p but to no increase in gpαF packaging. In all three of the above cases, Bos1p and Emp24p are more dramatically different from gpαF than Sec22p and Bet1p, suggesting a common cause. Perhaps correspondingly, we showed previously that the packaging of v-SNAREs can be uncoupled from the packaging of cargo (29).

We have provided evidence that Sec22p contains a positive signal for its packaging into vesicles. If one assumes that the other molecules we have assayed the release of also contain a positive packaging signal, the distinctions discussed above can be explained most simply if Sar1p recognizes the packaging signals of the assayed molecules either directly or indirectly by the recognition of an adapter molecule. In the case of a soluble molecule such as gpαF, the engagement by Sar1p must be indirect through the intervention of an adapter that spans the ER membrane and which may capture only a subset of cargo molecules. Thus, Sar1p may have a higher affinity for the adapter molecule responsible for gpαF than it does for the packaging signals or adapters of the other molecules we have assayed. The intermediate results obtained with Sec22p and Bet1p would be explained by intermediate affinities for these proteins or their adapters. Sar1p may engage the adapter or cargo molecules in collaboration with other proteins including other COPII subunits.

In the context of this model, the observation that microsomes made from strains bearing *sec16* alleles are more defective for the packaging of v-SNAREs and Emp24p than they are for the packaging of gpαF can be explained if Sec16p increases the effective concentration of Sar1p at the site of vesicle budding, as suggested by genetic interactions between Sar1p and the membrane-associated Sec16p (24, 30). It is possible that in a wild-type membrane, Sec12p and Sec16p combine to form a high affinity site that recruits Sar1p for vesicle formation. In a *sec16* mutant, Sar1p may only interact with Sec12p and thus have a lower affinity for the site of vesicle formation. Lowering the effective concentration of Sar1p at the site of vesicle formation (by mutating *SEC16*) should have less effect on the packaging of gpαF than it does on the packaging of the other molecules assayed, if Sar1p is more efficient at recruiting gpαF. In the extreme, it may be possible to suppress the sorting defect of *sec16* membranes by incubation with a higher concentration of Sar1p, though for technical reasons we have been unable to do so. In this context, one could explain the dramatic differences observed with *sec16-2* by invoking multiple roles for Sec16p in vesicle formation, one of which is the recruitment of Sar1p. Accordingly, *sec16-2* may be more defective in the recruitment of Sar1p (and less defective in the other roles of Sec16p) than the other alleles we tested. Sec16p may also serve as a subunit of COPII and aid in the recruitment of the Sec23/Sec24 complex, as suggested by genetic interactions (18, 25). Mutant alleles defective in these functions are likely to affect equally the recruitment of all cargo molecules.

Sec12p overproduction may also lower the concentration of Sar1p at the site of vesicle formation. It may be that Sec12p molecules are found in proximity to Sec16p, which together form a high affinity site for Sar1p. Thus, overproducing Sec12p would lead to an imbalance, creating potentially low affinity binding sites that serve as a sink for Sar1p, and consequently reducing the concentration of Sar1p at the site of vesicle formation.

These hypotheses may be distinguished when the sorting signals and adapter molecules that lead to the packaging of proteins are more rigorously defined. Another important step will be the isolation of a soluble, active form of Sec16p. This has been difficult to achieve because Sec16p is firmly bound to membranes (25). However, now that a clear defect in vesicle formation is reproduced with *sec16* mutant microsomes, it may be possible to develop a biochemical complementation assay such as was used to assay and purify the other components of COPII. Further efforts may require detergent solubilization and reconstitution of vesicle budding with synthetic proteoliposomes. These tools plus purified COPII proteins should allow the protein–protein interactions involved in the capture of cargo into vesicles to be defined.

We thank members of the Schekman laboratory past and present (especially Charlie Barlowe, Sebastian Bednarek, and Tom Yeung) for helpful discussions during the course of this work; Tom Yeung for invaluable practical advice on how to perform budding reactions; Bob Lesch for purification of COPII components; Howard Riezman, Susan Ferro-Novick, and Randy Hampton for the gift of reagents; Charlie Barlowe, Sebastian Bednarek, Ann Corsi, Tamara Doering, Rainer Duden, Chris Kaiser, and Meta Kuehn for careful reading of this manuscript; and Jon Bertsch and Ann Corsi for help with the preparation of figures. This work was funded by the Howard Hughes Medical Institute. At the beginning of this work, J.L.C. was supported by a fellowship from the Helen Hay Whitney Foundation.

1. Palade, G. (1975) *Science* **189,** 347–358.
2. Schekman, R. W. (1996) *Harvey Lect.* **90,** 41–57.
3. Salama, N. R., Yeung, T. & Schekman, R. W. (1993) *EMBO J.* **12,** 4073–4082.
4. Barlowe, C., Orci, L., Yeung, T., Hosobuchi, M., Hamamoto, S., Salama, N., Rexach, M. F., Ravazzola, M., Amherdt, M. & Schekman, R. (1994) *Cell* **77,** 895–907.
5. Rothman, J. E. & Warren, G. (1994) *Curr. Biol.* **4,** 220–233.
6. Schimmöller, F., Singer-Krüger, B., Schröder, S., Krüger, U., Barlowe, C. & Riezman, H. (1995) *EMBO J.* **14,** 1329–1339.
7. Campbell, J. L. & Schekman, R. (1996) in *Molecular Dynamics of Biomembranes*, ed. Op den Kamp, J. A. F. (Springer, Berlin), NATO ASI Series, Vol. H 96, pp. 209–217.
8. Nakano, A., Brada, D. & Schekman, R. (1988) *J. Cell Biol.* **107,** 851–863.
9. Bednarek, S. Y., Ravazzola, M., Hosobuchi, M., Amherdt, M., Perrelet, A., Schekman, R. & Orci, L. (1995) *Cell* **83,** 1183–1196.
10. Rexach, M., Latterich, M. & Schekman, R. (1994) *J. Cell Biol.* **126,** 1133–1148.
11. Horton, R. M., Hunt, H. D., Ho, S. N., Pullen, J. K. & Pease, L. R. (1989) *Gene* **77,** 61–68.
12. Hampton, R. Y. & Rine, J. (1994) *J. Cell Biol.* **125,** 299–312.

13. Gietz, R. D. & Sugino, A. (1988) *Gene* **74,** 527–534.
14. Schena, M., Picard, D. & Yamamoto, K. R. (1991) *Methods Enzymol.* **194,** 389–398.
15. Wuestehube, L. J. & Schekman, R. (1992) *Methods Enzymol.* **219,** 124–136.
16. Rexach, M. & Schekman, R. W. (1991) *J. Cell Biol.* **114,** 219–229.
17. Towbin, M. T., Staehlin, T. & Gordon, J. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 4350–4353.
18. Kaiser, C. A. & Schekman, R. (1990) *Cell* **61,** 723–733.
19. Jiang, Y., Sacher, M., Singer-Krüger, B., Lian, J., Stone, S. & Ferro-Novick, S. (1995) *Cold Spring Harbor Symp. Quant. Biol.* **60,** 119–126.
20. Gaynor, E. C., te Heesen, S., Graham, T. R., Aebi, M. & Emr, S. D. (1994) *J. Cell Biol.* **127,** 653–665.
21. Barlowe, C. & Schekman, R. (1993) *Nature (London)* **365,** 347–349.
22. d'Enfert, C., Barlowe, C., Nishikawa, S.-i., Nakano, A. & Schekman, R. (1991) *Mol. Cell. Biol.* **11,** 5727–5734.
23. d'Enfert, C., Wuestehube, L. J., Lila, T. & Schekman, R. (1991) *J. Cell Biol.* **114,** 663–670.
24. Nakano, A. & Muramastu, M. (1989) *J. Cell Biol.* **109,** 2677–2691.
25. Espenshade, P., Gimeno, R. E., Holzmacher, E., Teung, P. & Kaiser, C. A. (1995) *J. Cell Biol.* **131,** 311–324.
26. Boehm, J., Ulrich, H. D., Ossig, R. & Schmidt, H. D. (1994) *EMBO J.* **13,** 3696–3710.
27. Sato, M., Sato, K. & Nakano, A. (1996) *J. Cell Biol.* **134,** 279–293.
28. Roberts, C. J., Stevens, F. N. & Stevens, T. H. (1992) *J. Cell Biol.* **119,** 69–83.
29. Yeung, T., Barlowe, C. & Schekman, R. (1995) *J. Biol. Chem.* **270,** 30567–30570.
30. Gimeno, R. E., Espenshade, P. & Kaiser, C. A. (1995) *J. Cell Biol.* **131,** 325–338.