COPII and secretory cargo capture into transport vesicles

Meta J Kuehn* and Randy Schekman†

Yeast cytosolic coat proteins (COPII) direct the formation of vesicles from the endoplasmic reticulum. The vesicles selectively capture both cargo molecules and the secretory machinery that is necessary for the fusion of the vesicle with the recipient compartment, the Golgi apparatus. Recent efforts have aimed to understand how proteins are selected for inclusion into these vesicles. A variety of cargo adaptors may concentrate and sort secretory and membrane proteins by direct or indirect interaction with a subset of coat protein subunits.

Addresses
Department of Molecular and Cell Biology, Howard Hughes Medical Institute, 401 Barker Hall, University of California, Berkeley, CA 94720, USA
*e-mail: Kuehn@mendel.berkeley.edu
†e-mail: Schekman@mendel.berkeley.edu

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Abbreviations

COP	coat protein
ER	endoplasmic reticulum
NEM	*N*-ethylmaleimide
NSF	NEM-sensitive factor
SNAP	soluble NSF-attachment protein
SNARE	SNAP receptor
t-SNARE	SNARE on target membrane
v-SNARE	SNARE on vesicle

Introduction

Secreted proteins move between compartments of the cellular secretory pathway and become modified by glycosyltransferases, glycosidases, proteases and other enzymes as they travel through the cell. At each step, cargo is collected from one compartment into transport vesicles and delivered to the subsequent compartment by fusion of the vesicles with the target compartment. The proteins that accomplish the specific, sequential modifications remain behind or are retrieved to the station in which they function. A third group of proteins, involved in the mechanics of vesicle formation and fusion, may remain in the donor organelle or may travel with the cargo; in the latter case, these proteins must be recycled in retrograde vesicles together with escaped resident proteins of the early compartments.

Two types of transport vesicle coated by two distinct cytosolic protein complexes, termed coatomer (or coat protein [COP]I) and COPII, shuttle molecules between the endoplasmic reticulum (ER) and the Golgi compartment (for reviews, see [1,2]). Both COPI- and COPII-coated vesicles carry fusion proteins, called v-SNAREs (see abbreviations list), namely, Bet1p, Bos1p, and Sec22p in yeast [3]. These v-SNAREs are involved in fusion events, and act in concert with the putative Golgi t-SNARE (see abbreviations list), Sed5p [4,5], and the putative ER t-SNARE, Ufe1p [6]. Both COPI- and COPII-coated vesicles bud directly from the ER, but only COPII-coated vesicles have been shown to transport anterograde cargo from the ER [3]. Unlike COPII, COPI associates with cargo containing an ER retrieval motif [7,8]. Together, these results suggest that COPI-coated vesicles may shuttle retrieved proteins between the ER and the Golgi so that the compartments are continuously replenished with the necessary budding and fusion protein machinery. Several retrograde amino acid sequence signals have been elucidated (carboxy-terminal KKXX, KXKXX, and (K or H)DEL amino acid sequences, where X represents any amino acid). The effect of COPI mutations on anterograde traffic may be indirect [9–11] and may be explained by the role that COPI-coated vesicles play in the retrieval of recycled transport components to the ER [8,12].

Anterograde traffic from the ER has been reconstituted in a cell-free system in yeast and in mammalian cell extracts. COPII proteins mediate anterograde vesicle budding in both systems. At a critical step during vesicle emergence, cargo and constitutive secretory proteins must be included into vesicles, whereas resident ER proteins should be excluded. This could occur through an active selection method, an active retention system, or a combination thereof. The concentration of cargo in COPII-coated vesicles [3] favors the active selection model, which would predict the existence of specific cargo receptors. A variety of receptors may exist, although only one, Emp24p, and a family of related proteins have been ascribed this role [13–15]. Specific cargo receptors or coat adaptors may interact with one or more positive transport signals on cargo molecules. Unfortunately, no such signals have yet been documented.

In this review, we will discuss current data that address COPII-coated-vesicle formation and the selective sorting process. ER-derived-vesicle transport has been most extensively characterized in yeast and, therefore, yeast proteins will be the main focus of this review. The isolation and localization of homologous proteins in mammalian cells supports the generality of the yeast model.

COPII-coated-vesicle formation

As depicted in Figure 1, COPII components interact sequentially with the ER membrane. The process begins with recruitment of Sar1p, a small GTP-binding protein, to the ER membrane where it exchanges GDP for GTP under the influence of a specific guanine nucleotide dissociation factor, Sec12p [16]. Sec23p–Sec24p binds to

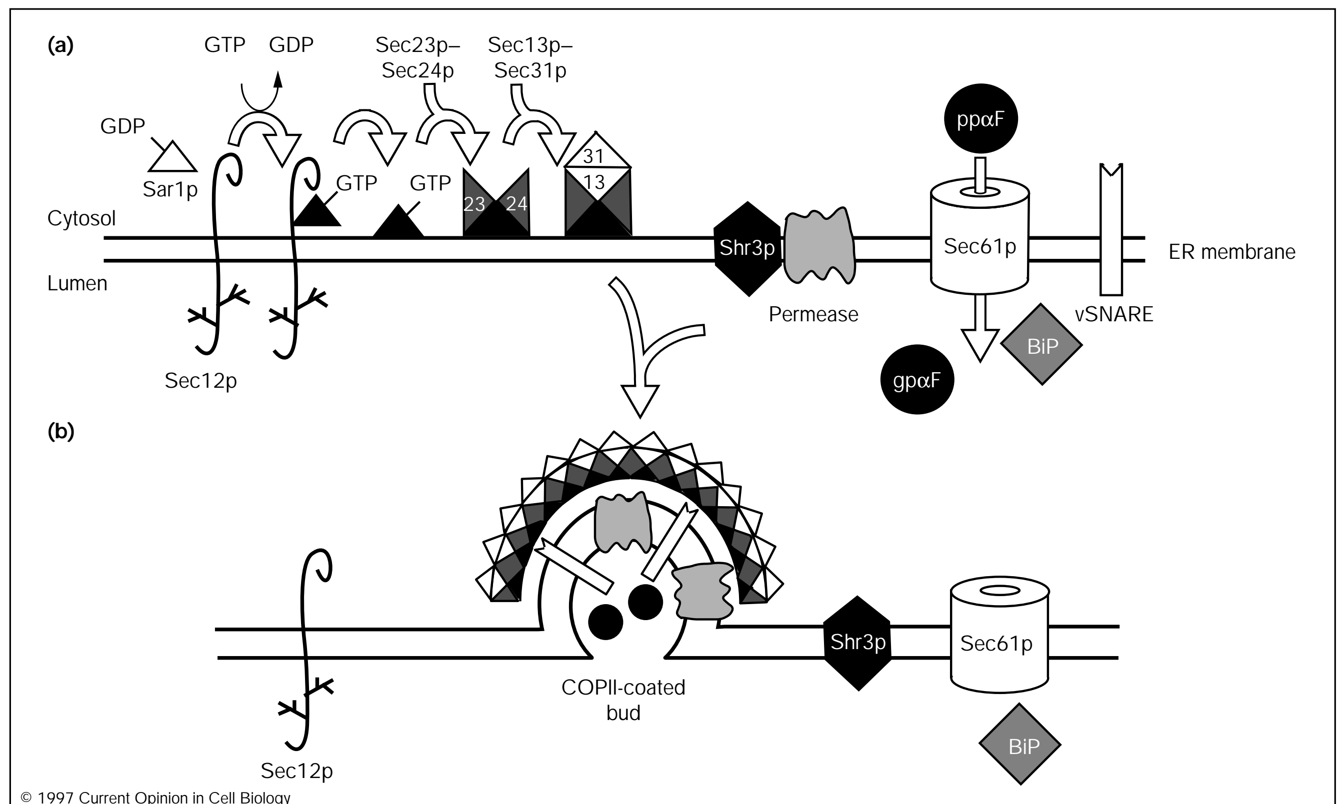
membrane-anchored Sar1p·GTP, at least partly because Sec23p is a GTPase-activating protein that interacts uniquely with Sar1p [17]. Finally, the Sec13p–Sec31p complex binds to initiate coat and vesicle formation [18,19]. Binding of GTP to Sar1p but not GTP hydrolysis is necessary to complete vesicle budding [20]. GTP hydrolysis allows Sar1p (in a GDP-bound form) to dissociate from the membrane, rendering the remaining COPII components labile and easily displaced from a completed vesicle. Although the details are not as well established, the formation of COPII-coated vesicles on mammalian ER membranes probably employs the same mechanism as in yeast [21,22].

An additional gene, *SEC16*, is essential for budding *in vivo*, but it may not contribute directly to vesicle morphogenesis. Sec16p is a large (240 kDa) peripheral membrane protein that associates with the ER and is probably part of the COPII coat on transport vesicles [23]. Two-hybrid analysis reveals a direct contact between Sec16p with Sec23p and Sec24p [24]. However, *sec16* temperature-sensitive mutants express a somewhat selective budding defect *in vitro*. v-SNARE protein packaging is more dramatically defective than is packaging of cargo, such as glyco-pro-α-factor, when budding assays are conducted with *sec16* mutant lysates [25]. Thus, the essential role of Sec16p may result more from a function in cargo sorting than from one in vesicle budding.

COPII homologs

Several homologs of COPII components exist in yeast and mammals. The homology between Sar1p and the *arf* family of proteins in yeast is almost certainly related to the role of each protein in initiating the formation of a non-clathrin coat [26,27]. Two mammalian homologs of yeast Sar1p were found by degenerate PCR [28] and functional homologs have been isolated from *Arabidopsis thaliana* and *Schizosaccharomyces pombe* [29]. A yeast database search revealed a gene with weak homology to *SEC23* and two with homology to *SEC24* (*Saccharomyces* Genome Database, Stanford University; URL http://genome-www.stanford.edu/). These yeast COPII

Figure 1



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Sequential interaction of COPII components with the ER membrane. **(a)** Sar1p·GDP is recruited to the ER membrane (left-hand side of the figure) and is converted to Sar1p·GTP by the guanine nucleotide exchange factor Sec12p. Subsequently, Sec23p–Sec24p and Sec13p–Sec31p are added to the membrane complex, leading to **(b)** formation of the coat. (a) Cargo (e.g. pre-pro-α-factor, ppαF) is translocated into the ER via the Sec61p channel and the chaperone BiP (right-hand side of the figure). As an example of cargo preparation, amino acid permeases are shown to interact with Shr3p, and ppαF becomes proteolytically processed and glycosylated to produce glyco-pro-α-factor (gpαF). (b) The COPII-coated bud includes cargo molecules (i.e. gpαF and permeases) and v-SNARES and excludes resident ER proteins such as BiP, Sec12p, Sec61p and Shr3p.

homologs may function in the formation of vesicles with different destinations or in the ER-to-Golgi transport of a distinct set of cargo molecules. Cross-reactive yeast antisera revealed that Sec23p exists in elements of the transitional mammalian ER [30]. Two conserved forms of rat *SEC23* were identified by homology searching; one of these forms functionally replaces a mutant form of *sec23* in yeast [31]. The human homolog of yeast *SEC13* (*mSEC13*) cannot completely complement a *sec13* defect in yeast; however, chimeras of human and yeast Sec13p do rescue the *sec13* secretion defect [32]. mSEC13 also appears to compete with Sec13p in yeast, indicating that it interacts with essential budding components and probably plays a role in COPII coats in mammalian cells [33]. Interestingly, Sec13p and a homolog of Sec13p , Seh13p, are associated with the yeast nuclear pore complex where they may function in a rather different assembly event [34]. Mammalian homologs of Sec22p and Bet1p have also been identified and appear to be expressed in all tissues [35]. Thus, at the structural and functional level, the conservation of COPII components (and of v-SNAREs) in different species is quite remarkable.

Cargo inclusion/sorting into COPII-coated vesicles – the signals required

For efficient export of secretory proteins and maintenance of organelle integrity, ER resident proteins must be distinguished from v-SNAREs and cargo proteins, and immature cargo must be distinguished from mature cargo. Retention of resident ER proteins could simply be due to a selective permeability barrier between hypothetical subcompartments of the ER, for example, between the rough ER and the transitional or smooth exit face of the ER. Alternatively, these proteins may not possess a signal directing forward movement. The lumenal Hsp70 ER chaperone BiP (or Kar2p) is transported slowly to the *cis* Golgi cisterna, at which point a retrieval signal returns it to the ER [36,37]. BiP or other chaperones, such as Hsp47 and cyclophilin B, may only leave the ER in complex with a cargo protein [38]; thus, their ‘escape’ may not be arbitrary and instead may serve a function beyond the ER for some secreted proteins.

Some proteins, particularly those involved in the mechanics of the secretory process, may be localized by means of signals for ER retention or for packaging into COPII-coated vesicles. A recent study dissected the localization signals contained in Sec12p by evaluating the fate of fusions of Sec12p with Dap2p, a protein secreted to the vacuole by default [39•]. These data showed that a cytosolic domain of Sec12p and of its homolog, Sed4p, is responsible for ER retention and that their transmembrane domains are essential for retrieval from the Golgi. v-SNAREs, such as Sec22p, Bos1p and Bet1p, are required in the membrane of both COPII- and COPI-coated vesicles for the fusion of anterograde and retrograde vesicles with target membranes. These proteins may directly interact with elements of both coat protein complexes. *In vitro* data support the suggestion of a signal for anterograde transport: the cytosolic domain of Sec22p possesses a saturable forward-directing signal; and the v-SNAREs and Emp24p, a putative component of the constitutive vesicle machinery, are more sensitive than is soluble cargo to a limiting amount of Sar1p in the budding assay [25]. Thus, secretory machinery proteins may have tracking devices that locate them to the appropriate compartment.

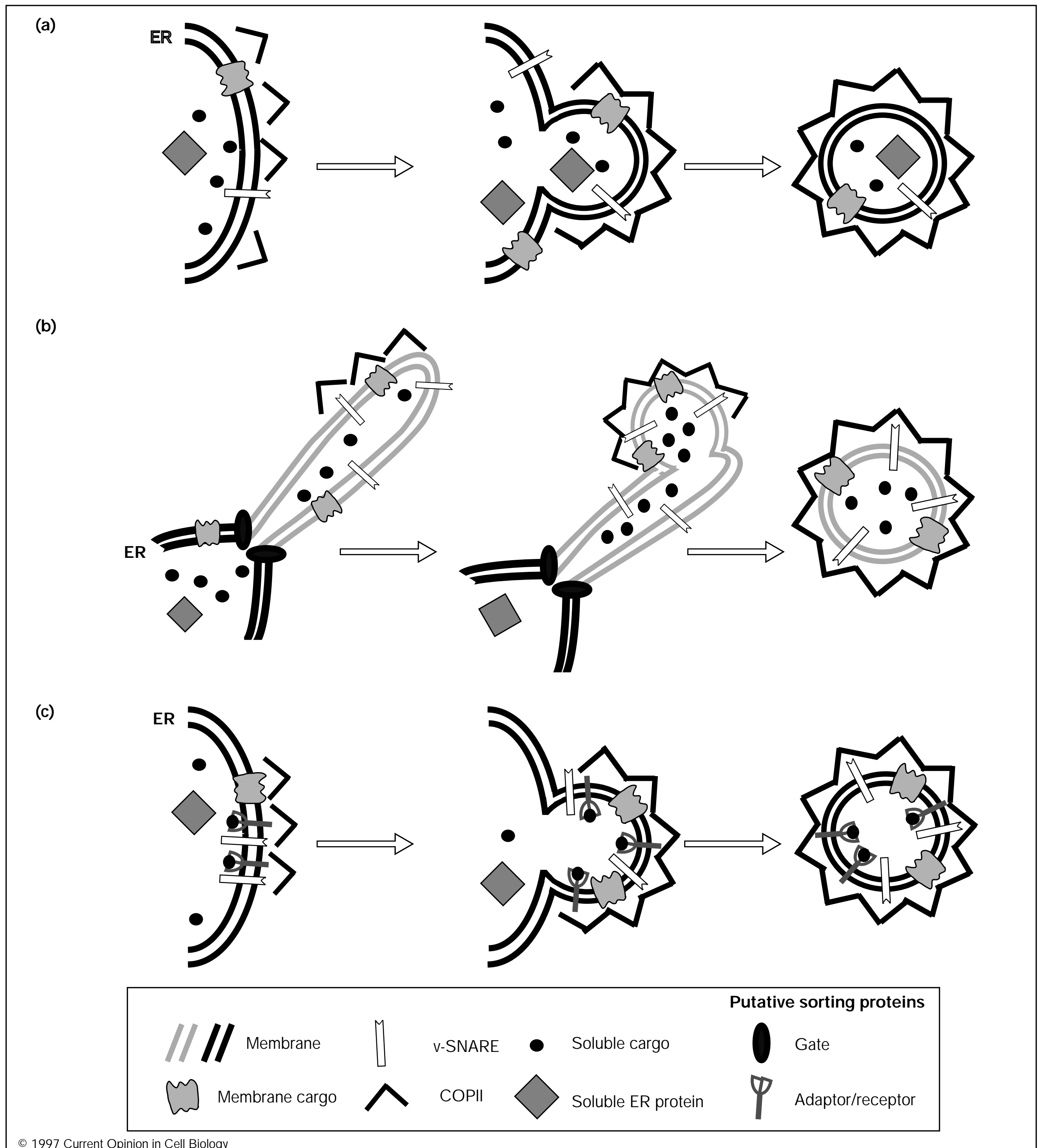
It is difficult to distinguish cargo selection from quality control. Once a protein is properly glycosylated, folded, and/or assembled, it may be incorporated immediately into a transport vesicle. For example, a glycosylphosphatidylinositol (GPI)-anchored protein, Gas1p, is transported via COPII-coated vesicles [40]. If the protein is not modified by GPI anchor addition, it lingers in the ER and is not incorporated into the vesicles. It is not yet known whether the GPI anchor machinery is in contact with the secretory apparatus. An ER resident protein, Shr3p, is required for packaging of amino acid permeases into ER-derived COPII-coated vesicles [41•]. Although in the absence of Shr3p the permeases are present in the ER, they are not recognized as cargo for incorporation into transport vesicles. Vma12p, Vma21p and Vma22p are implicated in vacuolar membrane ATPase assembly at the level of transport out of the ER [42–44]. These assembly proteins may be part of either the quality control machinery or the vesicle entry machinery. There appear to be a variety of protein-specific criteria by which cargo is judged to be ready for inclusion into vesicles.

How are assembled proteins selected for transport in COPII-coated vesicles?

Several experiments suggest that cargo is concentrated into transport vesicles [3,45,46] although it is difficult to calculate the amount of cargo per unit of membrane. Interestingly, cycloheximide treatment, which inhibits protein synthesis thus leading to a depletion of cargo in the ER, does not inhibit vesicle budding in yeast [47]; thus, the presence of cargo does not drive COPII-coated vesicle formation. It should be noted that these studies were done *in vitro* and potentially lack important physiological controls present *in vivo*. Components present in these cargoless vesicles may be important structural or recognition proteins required for the vesicle to form [47,48•].

Several possible modes of transport out of the ER are outlined in Figure 2. The first model, in which there is no ER cargo selection event, is called bulk flow (Figure 2a). Here, proteins not retained by specific signals move into transport vesicles without a concentration step; any resident proteins that escape are retrieved from a later compartment. This model does not, however, explain why cargo and membrane trafficking proteins are enriched and why resident ER proteins are depleted in the vesicle preparations [3,20], or why glyco-pro-α-factor is packaged

Figure 2



Models for cargo incorporation into ER-derived COPII-coated vesicles. At the left-hand side of each part of the figure is shown the recruitment of COPII components to the ER membrane; in the center, vesicle budding is shown; and fully formed vesicles are shown at the right. **(a)** Bulk flow. Cargo is not selected or concentrated for transport into COPII-coated vesicles. All proteins present in the ER (including resident soluble ER proteins such as BiP) may be included into the vesicles. **(b)** Privileged site budding. Concentration of cargo and v-SNARES occurs in a specific region of the ER membrane that may be formed by gating proteins. This subcompartment of the ER attracts COPII components, leading to vesicle formation. **(c)** Direct binding. COPII components interact directly with membrane cargo, v-SNARES, and soluble-cargo adaptors (or receptors). Accumulation of COPII–cargo complexes leads to formation of a vesicle. In all cases, ER resident proteins and targeting machinery (v-SNARES) may be returned from the Golgi via retrograde vesicles (not shown).

into COPII-coated vesicles and not COPI-coated vesicles that bud from the ER [3].

In the second model, transmembrane ER 'sieving' or 'gating' proteins act selectively to allow cargo molecules access to a site where COPII coat assembly and membrane budding occur (Figure 2b). These gating proteins may direct transmembrane cargo proteins laterally to a privileged site in the membrane. Lumenal cargo proteins may either be tethered to the membrane by an integral membrane cargo receptor, or be sieved through a pore that segregates a transitional zone from the rest of the ER. This model would predict active hot-spots for vesicle budding where cargo and coat proteins congregate. Such hot-spots have been identified in mammalian membranes [49], but evidence of this is lacking in *Saccharomyces cerevisiae*, which may have a less organized ER membrane structure. This model requires that secreted proteins be recognized by one or more signals or structures that have not yet been identified.

The third model of cargo selection is the most direct. Here, membrane proteins and membrane-bound secretory proteins are tethered directly or indirectly by COPII components which assemble spontaneously into a coated bud (Figure 2c). Soluble cargo requires a transmembrane adaptor to interact with the COPII proteins. The second and third models predict recognition motifs, structural or sequence, that allow selection by COPII components or intermediate adaptor proteins. Different adaptor proteins could be redundant in function and therefore would not be evident in genetic screens for *sec* mutants.

Possible cargo selection proteins

One possible candidate for the role of cargo adaptor is the p24 family, members of which appear to be cycled between the ER and the Golgi in COPII- and COPI-coated vesicles [14,15,50,51]. There is a reproducible but marginal secretion defect associated with a deletion of one or two of these proteins: the secretion of most proteins is wholly unaffected and the secretion of two proteins (Gas1p and periplasmic invertase) is merely delayed [13,48•]. These results indicate that the role of the p24 family members is either unessential or redundant. Although an association with cargo has not yet been demonstrated, several members of this family have been found to interact with coatomer via their carboxy-terminal sequences [15,51]. Two members of this family in yeast, Erv25p and Emp24p, have been shown to interact with each other biochemically and are interdependent for stability *in vivo* [48•]. Mutations in three genes, one identified as *EMP24*, suppress a *sec13* anterograde secretion block and cause a defect in resident ER protein retention [52•]. Emp24p and other proteins may restrict ER resident protein access into vesicles, and thus a mutation in Emp24p may reduce the fidelity of sorting by the COPII coat. It will be essential to assess the direct or indirect interaction of the p24 family of proteins with cargo and ER resident proteins.

Other candidates for selective cargo transporters are the yeast and mammalian p53/58 lectin-type molecules which recycle between the ER (and the ER–Golgi intermediate compartment, or ERGIC, in mammalian cells) and the Golgi ([53,54]; for review, see [55]). These type I integral membrane proteins use the carboxy-terminal recycling signal (KKXX; single-letter code for amino acids, where X represents any amino acid) as well as less well defined lumenal signals in order to recycle [53,56]. As lectins, p53/58 may bind to sugar moieties of glycosylated cargo proteins (mannose, in the case of ERGIC-53 [57]) in one compartment and release them in another in a manner that is dependent on a change in environment or local concentration of the ligand. However, the secretion of three glycosylated proteins is not affected by a mutation in Emp47p, a member of the p53/58 family of proteins in yeast [56]. Thus, either Emp47p has a narrow range of substrate specificity, or it is functionally redundant, or it has no role in cargo capture. Because a majority of secreted proteins become glycosylated, this family of proteins deserves further attention as potential chaperones or targeting proteins.

Conclusions

It is now generally agreed that COPII-coated vesicles represent the major and perhaps sole vehicle for anterograde protein traffic from the ER. However, the identification of Sec23p and Sec24p homologs in *S. cerevisiae* and in mammalian cells suggests that the COPII-related proteins may function to specify a particular cohort of cargo molecules. A growing body of evidence suggests that a cargo selection machinery exists that coordinates cargo with coats. The membrane proteins that mediate this interaction remain to be clearly documented. Likewise, the signal or signals that specify these interactions have yet to be uncovered.

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- •• of outstanding interest

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