ER export: **More than one way out**

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Protein export from the ER is mediated by COPII vesicles. Glycosylphosphatidylinositol-linked proteins seem to be segregated from other cargo proteins during ER export, suggesting that ER membranes produce more than one type of vesicle.

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Transport between compartments of the endomembrane system involves three known vesicle coat complexes [1]: clathrin, coat protein I (COPI) and coat protein II (COPII). Clathrin functions in endocytosis and in biosynthetic transport from the trans-Golgi network to endosomes. COPI vesicles carry material between Golgi cisternae and from the Golgi to the endoplasmic reticulum (ER), whereas COPII vesicles carry newly synthesized proteins from the ER to the Golgi. It is well established that cells employ at least two types of clathrin-coated vesicle, and the same is probably true for COPI vesicles. A new paper by Muñiz and coworkers [2] suggests that COPII vesicles may also come in multiple varieties.

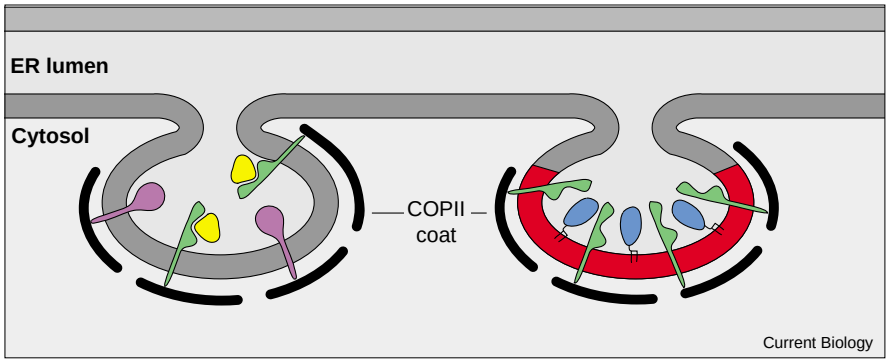
Why do cells need so many different kinds of vesicle? Part of the answer is the diverse nature of the cargoes that are transported. For example, clathrin-coated vesicles at the trans-Golgi network package lysosomal enzymes with the aid of the AP-1 adaptor, whereas other clathrin-coated vesicles at the plasma membrane package receptor proteins with the aid of the AP-2 adaptor. Similarly, COPI vesicles transport various cargo proteins between multiple compartments of the ER–Golgi system, and immunocytochemical studies indicate that different cargo proteins are segregated into distinct populations of COPI vesicles [3]. The molecular basis for this heterogeneity in COPI vesicles is not known, but the paradigm that has emerged from the analysis of clathrin and COPI is that vesicle coats contribute to the specificity of cargo packaging [1].

COPII vesicles also mediate selective cargo transport. Although some proteins seem to exit the ER by a 'bulk flow' pathway [4], most of the proteins that are packaged into COPII vesicles interact specifically with coat subunits and/or cargo receptors [5]. This packaging step must involve several different mechanisms, because the cargo proteins include those that are soluble in the ER lumen, those that span the membrane once, and those that span the membrane multiple times. How are all of these proteins recognized by the COPII machinery? One recognition system employs accessory proteins in the ER membrane. For example, in the yeast *Saccharomyces cerevisiae*, Shr3p recognizes newly synthesized amino acid permeases and delivers them to nascent COPII vesicles [6]. Shr3p itself remains behind in the ER. A variation on this theme is Erv14p, which escorts newly synthesized molecules of the membrane protein Axl2p into COPII vesicles and then travels together with Axl2p to the Golgi [7]. Erv14p is subsequently recycled to the ER.

Another possible way to export diverse cargoes from the ER would be to make multiple classes of COPII vesicles. This idea gained credence with the discovery of Lst1p, a homolog of the Sec24p subunit of COPII [8]. Lst1p recognizes newly synthesized molecules of the proton pump Pma1p, which is a major component of the yeast plasma membrane. An initial speculation was that one class of COPII vesicles might incorporate only Sec24p into the coat, while another class would incorporate only Lst1p. Subsequent work, however, indicated that individual COPII vesicles contain a mixture of Sec24p and Lst1p [9]. Furthermore, when COPII vesicles are generated *in vitro*, different cargo proteins are present in the same vesicles [10]. These data suggested that COPII vesicles are uniform with respect to both their coat composition and their cargo.

A new twist on this story involves proteins that are anchored to the membrane by a glycosylphosphatidylinositol (GPI) group. GPI is added to newly synthesized proteins in the ER, yielding hybrid molecules in which the polypeptide faces the ER lumen while the fatty acids of GPI reside in the lumenal leaflet of the membrane. In yeast, the most extensively studied GPI-linked protein is Gas1p, a cell-surface glycoprotein that functions in cell wall dynamics [11]. The ER-to-Golgi transport of Gas1p shows several unusual features. First, attachment of the GPI anchor is essential for the packaging of Gas1p into COPII vesicles [12]. Second, in yeast cells that carry either a mutation in the α subunit of COPI or a deletion of the putative cargo receptor Emp24p, the export of Gas1p from the ER is strongly inhibited, whereas the export of other cargoes is largely unaffected [13,14]. Third, blocking ceramide synthesis inhibits the ER-to-Golgi transport of Gas1p but not of other cargoes [14]. Similar behavior is seen with Yps1p, another GPI-linked protein. These findings suggest that GPI-linked proteins may exit the ER by a unique pathway.

Figure 1



Heterogeneity in the ER membrane may allow a single type of COPII coat to generate more than one class of transport vesicle. In the model depicted, the vesicle on the left is incorporating a patch of non-raft membrane (gray) while the vesicle on the right is incorporating a lipid raft (red). Some transmembrane proteins (purple) will be largely excluded from rafts, and will enter vesicles by interacting with the COPII coat. A subset of the soluble secretory proteins (yellow) will associate with cargo receptors such as Emp24p (green), which spans the membrane and probably binds to the COPII coat. GPI-linked proteins (blue) will be concentrated in rafts. Because GPI-linked proteins do not span the membrane, each raft must also contain transmembrane proteins such as Emp24p that will bind to the COPII coat and help package the entire raft into a vesicle.

Muñiz *et al.* [2] now present evidence that the transport carriers for GPI-linked proteins are distinct from the standard COPII vesicles. The experimental strategy was to reconstitute protein export from the ER of permeabilized yeast cells, and then assess the contents of the vesicles released into the supernatant. Immunoprecipitation and density-gradient fractionation revealed that Gas1p and Yps1p were largely excluded from the vesicles that contained conventional secretory proteins. The authors conclude that GPI-linked proteins are segregated from other secretory proteins during export from the ER.

What is the nature of the ER-derived vesicles that contain GPI-linked proteins? COPII function is needed for the ER-to-Golgi transport of GPI-linked proteins [13–15], and Gas1p can be packaged into COPII vesicles *in vitro* [12], suggesting that GPI-linked proteins probably exit the ER in COPII vesicles. It remains possible, however, that GPI-linked proteins exit the ER in COPI vesicles [16]. The data of Muñiz *et al.* [2] do not address this issue. Moreover, only indirect evidence is presented that the vesicles containing GPI-linked proteins are actual transport intermediates. Despite these cautions, the new work presents the best indication to date that the ER generates multiple types of transport carrier.

Perhaps the most interesting aspect of this story is that ongoing ceramide synthesis is needed for the ER export of GPI-linked proteins [14]. Ceramide is made in the ER and serves as a precursor for sphingolipid synthesis, which occurs in the Golgi [17]. GPI-linked proteins are thought to partition into sphingolipid-containing 'raft' subdomains in the Golgi and plasma membrane [18]. An intriguing possibility is that rafts also exist in the yeast ER, and that GPI-linked proteins must partition into these rafts to be packaged into COPII vesicles [19]. Recent size estimates suggest that a typical raft would be small enough to fit inside a COPII vesicle [20]. The formation of ER-localized rafts may require the COPI-mediated transport of sphingolipids from the Golgi to the ER; this idea would explain why the ER export of GPI-linked proteins depends upon both ceramide synthesis and COPI function. Figure 1 shows a model in which a given COPII vesicle incorporates either a patch of non-raft ER membrane containing standard cargo proteins, or a raft subdomain containing GPI-linked proteins. Such a mechanism would allow a single COPII machinery to produce biochemically distinct vesicle populations.

This discussion highlights the potential importance of membrane subdomains in ER export. Our understanding of these subdomains is still in its infancy. Many cell types contain discrete ER regions, known as 'transitional ER sites' or 'ER exit sites', which are specialized for COPII vesicle production [21]. Discrete transitional ER sites exist in the budding yeast *Pichia pastoris*, but have not been detected in *S. cerevisiae* [22]. Nevertheless, the ER membrane of *S. cerevisiae* may contain heterogeneities that facilitate protein export (Figure 1). This particular model is very speculative, but the general picture is coming into focus: ER export apparently involves a generic COPII machinery, with individual cargo proteins using a host of specific strategies to make their way into COPII vesicles.

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