ER export: public transportation by the COPII coach

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The COPII coat produces ER-derived transport vesicles. Recent findings suggest that the COPII coat is a highly dynamic polymer and that efficient capture of cargo molecules into COPII vesicles depends on several parameters, including export signals, membrane environment, metabolic control and the presence of a repertoire of COPII subunit homologues.

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Abbreviations
ER endoplasmic reticulum
SCAP SREBP cleavage-activating protein
SREBP sterol regulatory element binding protein

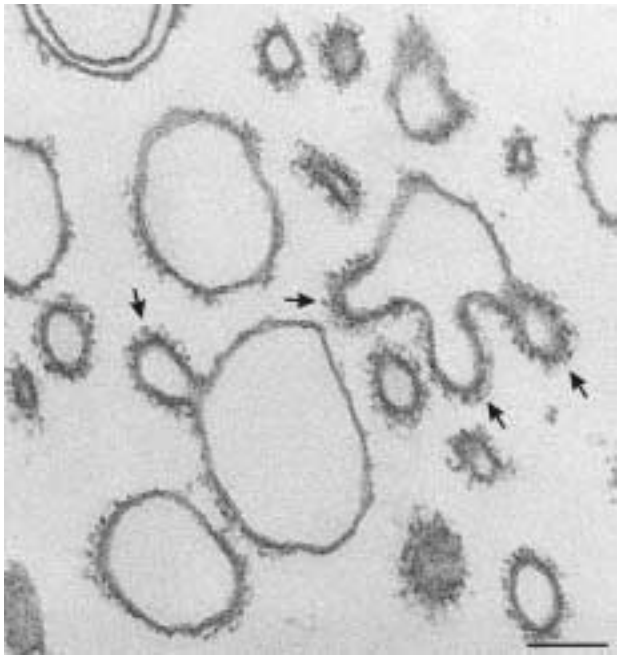
Introduction

Public transport systems have been used as a metaphor for vesicular traffic within eukaryotic cells. Transport vehicles operate along fixed pathways carrying varying numbers of passengers, each of whom selects a conveyance based on his destination. Protein passengers deposited in the endoplasmic reticulum (ER) may remain there or be shuttled to the Golgi apparatus to be presented with further transportation options. In this review, we discuss how COPII vesicles, the ER-specific shuttle coach, capture diverse passengers. Recent studies raise the following questions: are there different subtypes of COPII vesicles? Are there privileged passengers and do they have a choice in the sorting decision, and do they exert some control on vesicle formation?

COPII is number one

Like its distant cousin COPI, the COPII coat is a polymer formed by the ordered assembly of cytosolic proteins, which shape lipid membranes to produce transport vesicles [1]. Vesicles coated by the COPII coat mediate the export of newly synthesized proteins from the ER, whereas COPI-coated vesicles are involved at later stages of intracellular transport [2–4]. In mammalian cells, a relay between the two coats takes place at the level of ERGIC (ER–Golgi intermediate compartment), a dynamic compartment at the crossroads between the ER and the Golgi [4,5•]. This relay has been visualized by time-lapse microscopy in living cells [5•]. COPII-labeled areas remain close to the ER, whereas COPI-labeled areas, after an initial overlap with COPII, travel on a microtubule network toward the Golgi [4,5•].

Figure 1



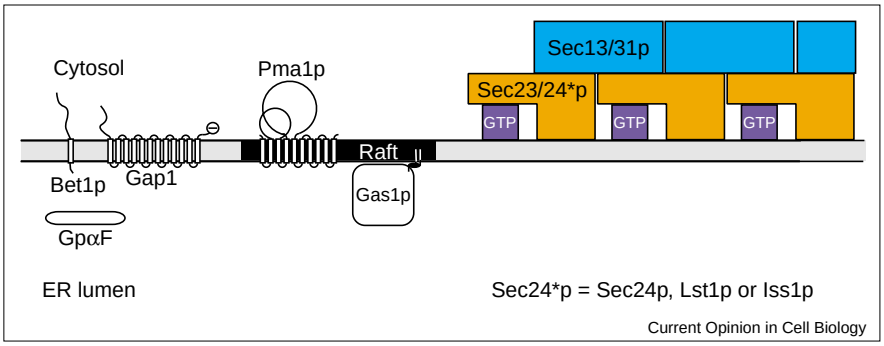
COPII budding profiles on anionic liposomes incubated with purified COPII proteins and GMP-PNP at room temperature. The arrows point to budding regions decorated by the coat. The bar indicates 100 nm. Micrograph courtesy of Lelio Orci.

A novel inhibitor of COPI movement is the viral glycoprotein NSP4, which is smuggled in COPII vesicles to the ERGIC, where it binds to microtubules through its cytosolic domain and impedes the movement of ERGIC [6•]. The spatial organization of the ER to Golgi pathway and the importance of the ERGIC and of the cytoskeleton vary between organisms [4].

A blind coat?

The base of the COPII coat is formed by the small G-protein Sar1p-GTP, which binds directly to the lipid surface. Two large complexes, Sec23/24p and Sec13/31p, then bind sequentially [1]. This ordered assembly has been observed on liposomes [7]. Remarkably, this assembly leads to the budding of coated vesicles, which is a similar process to that observed with the ER membrane ([7]; Figure 1). Thus, the coat has the intrinsic ability to deform a lipid bilayer. The absence of a membrane protein requirement in this reaction raises the possibility that cargo packaging and vesicle budding may not be coordinated. Indeed, biosynthetic cargos can be purged from ER membranes with no effect on COPII vesicle formation [8]. However, proteins that cycle between the ER and the Golgi may facilitate the budding reaction. COPI budding from

Figure 2



Very different cargo proteins are packaged into COPII vesicles. The COPII coat is formed by the small G protein Sar1p in the GTP-bound conformation and by two large complexes, Sec23/24p and Sec13/31p. The L shape of Sec23/24p symbolizes the ability of this complex to interact not only with Sar1pGTP but also with anionic lipids and cytosolic motifs from membrane cargo or receptor proteins. Some cargo molecules studied for ER export in yeast are schematized on the left of the figure. This includes a SNARE protein (Bet1p), a mating factor precursor (GpαF), an amino-acid permease (Gap1), the plasma membrane ATPase (Pma1p) and a GPI-anchored protein (Gas1p). The cartoon highlights the difference in size and membrane topology between these cargo proteins. The repertoire of Sec24 homologues may facilitate the packaging of some of these cargo proteins into COPII vesicles.

synthetic liposomes is stimulated by the cytoplasmic tail of one such recycled passenger, the mammalian p23 protein [9]. In addition, SNARE proteins may serve as primers to organize and potentiate the polymerization of the COPII coat at sites of cargo capture on the ER membrane [10]. Here, the interactions between the shell and its content reinforce one another.

Different passengers

Figure 2 schematizes some proteins that have been used as models for ER export in yeast. These cargo proteins are anything but similar. The COPII coat must accommodate cargo molecules of diverse size and membrane topology. Nevertheless, *in vitro*, the addition of purified COPII components and GTP to yeast microsomes promotes the incorporation of these proteins into COPII vesicles. In a few cases, the presence of different cargos in the same vesicle has been demonstrated [11]. However, the apparent extent of packaging varies from a few % for the GPI-anchored protease Gas1p to almost 30–50% for the α-factor precursor (gpαF). Differences in the rate of protein folding in the ER could explain this range, because only properly folded precursors are substrates for packaging. Additionally, protein substrates that are packaged at their prevailing concentration in the ER are likely to be less efficiently incorporated into COPII vesicles than proteins that are highly concentrated by direct or indirect interaction with coat subunits [11–14,15•].

Privileged customers

A large number of proteins found in COPII vesicles are not in transit to other destinations but are recycling continuously between the ER and the Golgi. This includes proteins that operate in tethering and fusion mechanisms but also many proteins whose functions are unknown [16–19]. As frequent flyers of COPII vesicles, they seem to have some privileges. Thus, the SNARE protein Bet1p interacts directly through its amino-terminal cytosolic domain with Sar1p and Sec23/24p [16]. Such proteins, called primers, are selected by the coat and organize coat polymerization at the ER [10]. Although mammalian p23/p24 has been proposed to nucleate coat polymerization [9], the full set of equivalent molecules has been deleted in yeast with no effect on cell growth and secretion [20]. Thus, the cell may employ redundant primers, any one of which could orient COPII coat polymerization to the ER.

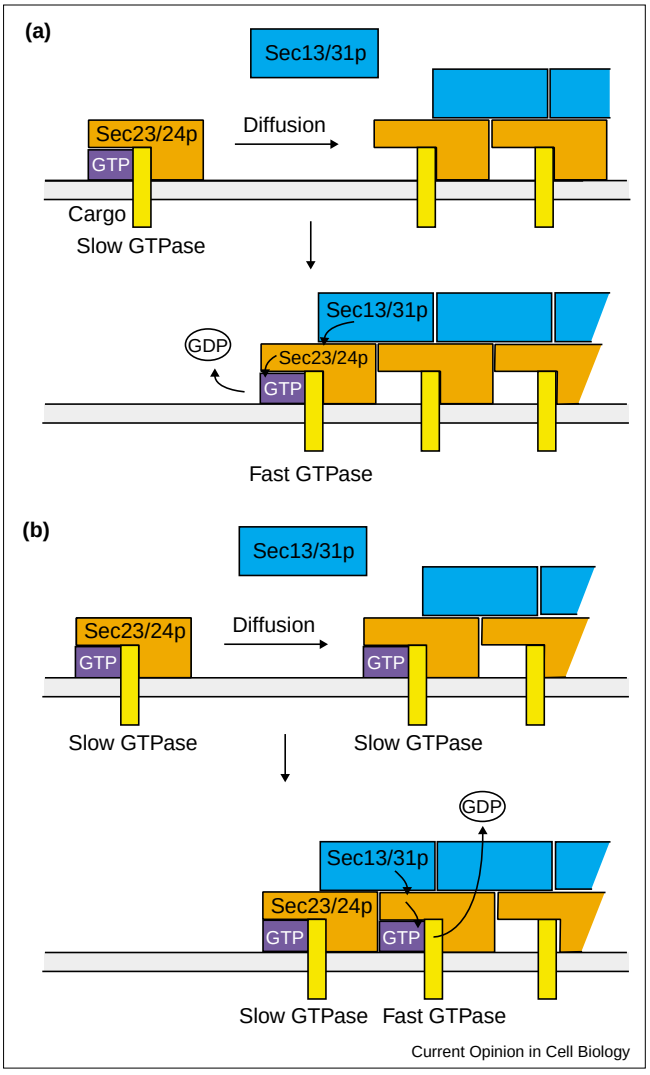
A direct interaction between COPII and a newly synthesized membrane protein, viral glycoprotein VSV-G, has been demonstrated. This protein is very efficiently sorted into COPII vesicles and interacts through an EXD signal in its carboxy-terminal cytosolic terminus with Sar1p and Sec23/24p [21,22,23•]. The importance of this sequence was recently highlighted by an extensive study of the transport of inward rectifying potassium channels [24•]. Ma and coworkers [24•] identified two alternative motifs within the carboxyl terminus of some of these channels that are necessary and sufficient to promote efficient ER export. Both motifs contain EXD or EXE sequences.

Some lumenal proteins are very efficiently exported from the ER, suggesting a specific interaction with the coat through membrane receptors. So far, the only example of a receptor for soluble cargos is ERGIC-53, whose lumenal domain interacts with some glycoproteins [25]. Members of the p24 family could be involved in the sorting of GPI-anchored protein [26].

Special COPII vesicles for special parcels?

Pma1p, the plasma membrane ATPase, is a much more cumbersome vesicle passenger than Bet1p. Pma1p displays a large cytoplasmic domain and can form oligomers. The accommodation of such a structure may require novel coat subunits. Incorporation of Pma1p into COPII vesicles is favored by the concomitant action of Sec23/24p and Sec23/Lst1p, a complex

Figure 3



A model for spatial and temporal control of GTP hydrolysis in the COPII coat. A prebudding complex comprising a cargo molecule, Sar1GTP and Sec23/24p, may form and remain stable long enough to diffuse within the plane of a membrane and be captured by the coat network including Sec13/31p. Once incorporated, the action of Sec13/31p on the GAP activity of Sec23p promotes rapid GTP hydrolysis and loss of Sar1GDP. In model (a), Sec13/31p acts on the most recently incorporated prebudding complex, whereas in (b), Sec13/31p acts on the penultimate prebudding complex. The models are distinguished by the presence in (b) of a stabilizing annulus of Sar1GTP at the perimeter of a laterally polymerizing coat. Whatever the exact mechanism, the effect of Sec13/31p on the GTPase reaction is consistent with the observation that the 'GTP' coat contains much less Sar1p than the 'GMP-PNP' coat [1].

including one of the two Sec24p homologues found in yeast [27,28•]. Vesicles formed with this mixed coat are larger than standard COPII vesicles, perhaps reflecting the greater surface area occupied by an ATPase oligomer [28•].

Membrane domains may influence the incorporation of some cargos into COPII vesicles. Lipid rafts are characterized by an ordered fluid phase in which cholesterol and saturated lipids are tightly packed [29]. In mammalian cells, lipid rafts form in the Golgi. However, detergent-resistant membranes (DRMs), the experimental hallmark of lipid rafts, have been detected in the ER of yeast cells [30•]. Notably, Pma1p and GPI-anchored proteins (e.g. Gas1p) partition in DRMs. Because of their peculiar physical properties, lipid rafts (or their ER precursors) may be challenging areas to incorporate into vesicles, and it raises many questions. For example, are they small enough? Can they adopt extreme curvatures? Is their cytosolic leaflet adapted to the COPII proteins? On liposomes, the binding of Sar1p is favored by unsaturated phospholipids [7]. At later stages of the secretory pathway, enrichment or exclusion of raft lipids in transport vesicles has been observed [31•,32].

Recently, Muñiz *et al.* [33•] showed by immunoisolation and density analysis that GPI-anchored proteins, such as Gas1p, are transported in different vesicles than Gap1p and gpαF. Although the coats associated with these vesicles were not characterized, a reasonable hypothesis is that there are various subtypes of COPII-coated vesicles adapted, thanks to the repertoire of Sec24 proteins, to cargos and membranes displaying particular features [27,28•,34–36].

Protein assistance and metabolic control

In addition to the intervention of protein specific 'outfitter' gene products that guide membrane proteins into COPII vesicles [37,38], recent evidence suggests an important role for metabolic regulation of the packaging of certain cargo proteins [39••]. In mammalian cells, two membrane proteins, SCAP (SREBP cleavage-activating protein) and SREBP, act in tandem to trigger the response to a low level of cholesterol. SCAP contains a sterol-sensing domain, whereas SREBP contains a cytosolic domain, which, after cleavage in the Golgi, acts as a transcription factor. The exit of SREBP from the ER requires the escort of SCAP, which itself is exported only upon sterol starvation. Sterols have no effect on the transport of other cargos, suggesting that SCAP may mediate the sterol deprivation-dependent access of SREBP to the COPII coat. Whether the converse adaptation (coat adaptation to cargos) can also occur is not known, but, if so, this may change the general spectrum of exported proteins.

Spatial and temporal control of COPII coat assembly and disassembly

At first glance, the connection between the GTPase cycle of Sar1p and the assembly of the COPII coat is simple. Upon GDP to GTP exchange, Sar1p binds to the ER and promotes COPII coat assembly. Subsequent GTP hydrolysis by Sar1p causes it to dissociate and the coat to disassemble [2,3]. However, two factors complicate the issue: these are the polymeric nature of the coat and the fact that the GTPase activating protein (GAP) of Sar1p, Sec23p, is a subunit of the coat. Nucleotide exchange and GTP hydrolysis must be spatially and temporally organized to coordinate coat assembly with cargo selection and coat disassembly with vesicle fission.

The transmembrane protein Sec12p promotes nucleotide exchange on Sar1p [40]. The restriction of Sec12p to the ER membrane localizes COPII budding. A role for a kinase in the recruitment of Sar1p to the ER membrane has been suggested [41,42], however the target of this phosphorylation, perhaps Sec12p, has not been established. In mammalian cells, the fast (< 1 min) turnover of COPII-labeled areas and the morphological effect of Sar1p and GTP on permeabilized cells suggest the presence of discrete ER export sites with high exchange activity [5•,23•]. Because of the constitutive function of COPII vesicles, it is possible that Sec12p is more or less continuously active.

Sec23p promotes GTP hydrolysis on Sar1pGTP [43]. The formation of a complex with Sar1pGDP, Sec23/24p and the phosphate analog BeF_x suggests some mechanistic analogy with other GAP proteins [44•]. However, Sec23/24p alone is not an efficient GAP. On liposomes the lifetime of the Sar1p-GTP–Sec23/24p complex is relatively long ($t_{1/2}$ ~ 30 seconds) [44•]. Thus, the formation and diffusion of prebudding complexes, including Sar1pGTP, Sec23/24p and membrane proteins, may not be compromised by the GTPase reaction. In contrast, the GAP activity of Sec23/24p is increased by one order of magnitude in the presence of Sec13/31p, such that the complete COPII coat disassembles in a few seconds [44•]. This suggests that GTP hydrolysis by Sar1p occurs after the incorporation of prebudding complexes into the coat through the bridging action of Sec13/31p (Figure 3). Thus, the GTPase reaction may depend not only on the interplay between COPII proteins and cargos within an elementary coat unit, but also on the lateral relationship between the units. As in polymers containing nucleotide-binding proteins (i.e. tubulin and dynamin [45,46]), GTP hydrolysis may be spatially organized. An intriguing possibility would be the formation of a stabilizing ring of Sar1pGTP at the coat periphery (Figure 3). Although GTP hydrolysis appears not to be required for cargo selection and COPII bud formation and fission from native ER membranes [1], the regulation of the GTPase process may invoke proteins that have not yet been identified.

Conclusions

We now know that the COPII coat is responsible for direct capture of membrane cargo proteins and for the physical deformation of the ER membrane that accompanies the formation of a sharply curved transport vesicle. However, our picture of the COPII coat remains incomplete because none of the COPII components have been crystallized. Although this goal will almost certainly be achieved in the near future, we are for the time being restricted to models from which testable hypotheses about polymerization and cargo selection may be made. One may ask how the coat subunits are orientated relative to one another and to the membrane surface. The discovery of Sec24 homologues should help to define specific domains for cargo capture, although at this point the influence of physical factors such as protein cargo size and lipid environment should not be underestimated. Finally, the short intrinsic lifetime of the COPII coat makes the question of its dynamics and regulation in relation to cargo capture and vesicle fission a challenging problem ripe for investigation.

Update

Recent work has further addressed the molecular basis of ER export of some important classes of membrane proteins. For inwardly rectifying potassium channels, the ER export motif identified by Ma *et al*. [24•] has been also identified by another group [47•]. For G-protein-coupled receptors, a recent study highlights the importance of a cytosolic motif adjacent to the last transmembrane segment [48•]. This motif forms an amphipathic helix, which lies at the membrane surface. Mutagenesis of hydrophobic residues within this motif impairs ER exit of the dopamine D1 receptor [48•]. Drip78, an ER resident protein, interacts with this motif to control the export of the D1 receptor [48•]. Finally, for major histocompatibility class (MHC) I molecules, an active concentration mechanism occurs in the ER after the antigenic peptide loading step [49]. Because MHC class I molecules do not seem to contain an export motif, their exit from the ER should result from interaction with transport receptors [49].

Acknowledgements

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- •• of outstanding interest

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