Transport from the endoplasmic reticulum to the Golgi

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Two crucial aspects of transport vesicle function have recently been reconstituted using purified proteins and chemically defined lipid bilayers. The reconstituted steps are the assembly of a polymeric protein coat on the cytosolic surface of the membrane, and bilayer fusion based on the pairing of proteins in the vesicle and target membrane. These advances now set the stage to address major unresolved questions of how vesicle budding and vesicle fusion are regulated, how specific cargo molecules are incorporated into vesicles, and how vesicles find their target membrane.

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Abbreviations

COP	coatomer protein
ER	endoplasmic reticulum
GFP	green fluorescent protein
GST	glutathione *S*-transferase
ts	temperature-sensitive
SNARE	soluble *N*-ethylmaleimide-sensitive fusion protein attachment-protein receptor
t-SNARE	target SNARE
v-SNARE	vesicular SNARE
VSVG	vesicular stomatitis virus glycoprotein
VTC	vesicular-tubular cluster

Introduction

Proteins are transported from the endoplasmic reticulum (ER) to the Golgi complex by carrier vesicles that are formed from the membrane of the ER and that selectively fuse with the *cis*-Golgi membrane. The function of these vesicles involves two key steps: firstly, the assembly of a polymeric protein coat on the cytosolic surface of the membrane which sets the curvature of the membrane and determines which membrane proteins are accepted by the vesicle, and secondly, the pairing of vesicle proteins with proteins in the *cis*-Golgi which allows the vesicle and target membranes to fuse. It is generally accepted that most, if not all, of the forward vesicular traffic from the ER to the Golgi involves vesicles that are coated with a set of proteins known as coatomer protein (COP) II [1]. A different vesicle coat protein complex known as coatomer protein I (COPI) mediates retrograde transport from the Golgi to ER [2] and probably also forward transport through the Golgi complex [3]. The COPII proteins were first identified by genetic studies in yeast and most are defined as the product of SEC genes. It is now clear that their functions are conserved since mammalian counterparts to the yeast genes have been identified and have been shown to be involved in ER-to-Golgi transport [4–6]. In addition, many of the proteins responsible for COPII vesicle fusion with the membrane of the *cis*-Golgi have also been found to be conserved from yeast to mammals [7,8].

In this review we shall discuss recent advances in understanding how COPII vesicles are assembled, how cargo molecules are packaged into vesicles, and how vesicles that are derived from the ER selectively fuse with the Golgi.

Assembly of COPII vesicles

The COPII gene products required for vesicle formation have been defined morphologically [9] and by cell-free assays for vesicle budding [1,10–12]. Partially purified ER membranes stripped of peripheral proteins by extraction with urea will bud transport vesicles when supplied with GTP and concentrated cytosol [13]. The requirement of this assay for added cytosolic proteins can be satisfied by a 700 kDa complex of Sec31p/Sec13p, a 400 kDa complex of Sec23p/Sec24p, and the small GTPase Sar1p. The vesicles thus produced are encapsulated in an electron-dense protein coat containing Sec23p/Sec24p, Sec31p/Sec13p, and Sar1p [1].

The GTP hydrolysis cycle catalyzed by Sar1p is intimately connected to vesicle budding. Sec12p, an integral membrane protein of the ER, accelerates exchange of GTP for GDP on Sar1p, whereas Sec23p accelerates GTP hydrolysis by Sar1p [14,15]. The following outline for COPII coat biogenesis emerges from these observations: Sec12p activates Sar1p on the ER membrane by catalyzing nucleotide exchange, and Sar1p·GTP would in turn recruit Sec23p/Sec24p and Sec31p/Sec13p protein complexes to assemble on the membrane. After coat formation is complete, GTP hydrolysis and accompanying coat disassembly would then be activated by Sec23p [15,16].

Sec16p is a 240 kDa protein required for the formation of ER-to-Golgi transport vesicles *in vivo* [9,17] and *in vitro* [18]. Sec16p is found to be tightly associated with both the ER membrane and the vesicles that have budded from the ER [17]. Urea extraction does not remove Sec16p from the ER membranes explaining why exogenously added Sec16p is not required to drive vesicle budding from urea extracted microsomes. Each of the COPII proteins Sec23p, Sec24p, Sec31p, and the Sec12p homolog Sed4p, binds to a different site along the length of the Sec16p molecule [17,19,20••]. Given that Sec16p resides on the ER membrane before formation of a coat and given the extensive contacts between Sec16p and other coat components, Sec16p may serve as a scaffold that organizes the recruitment or assembly of COPII coat components from the cytosol.

A synthetic approach to define the composition of the membrane required to support COPII vesicle formation has culminated in the reconstitution of vesicle budding using only synthetic liposomes and purified proteins [21••]. Sar1p is added to liposomes combined with the nonhydrolyzable GTP analog GMP-PNP (5′guanylyl imidodiphosphate), thus bypassing any requirement for the nucleotide exchange activity of Sec12p (which is not incorporated in the liposomes). After Sar1p·GMP-PNP has bound to membranes the Sec23p/Sec24p complex and then the Sec31p/Sec13p complex bind in sequential order. In the presence of these defined COPII components, coated buds and coated vesicles form, eventually converting as much as 30% of the input liposomes into protein-coated membrane bilayers. The coated vesicles formed from synthetic lipids differ from vesicles formed from native ER membranes in a number of respects. The synthetic vesicles are less uniform in size, have a lower buoyant density, and they require five fold higher concentrations of COPII proteins for their formation than the corresponding native vesicles. These differences suggest that the ER membrane contains proteins that organize and facilitate the assembly process. The Sec16 protein with its capacity to bind to both the Sec23p/Sec24p and Sec31p/Sec13p complexes may influence coat assembly by either reducing the minimum concentration of soluble coat subunits needed to nucleate a coat polymer or by setting the relative stoichiometry of the coat subunits. As described below, a number of different integral membrane proteins that are packaged into vesicles are known to contact coat subunits and the interactions with these cargo proteins may also influence when and where coat polymerization will be initiated.

The use of synthetic membranes for vesicle budding has allowed the lipid requirements for vesicle budding to be explored. The binding of Sar1p·GMP-PNP to phospholipids is relatively insensitive to membrane lipid composition, but the subsequent binding of the Sec23p/Sec24p and Sec31p/Sec13p complexes requires a minimum of about 10% acidic phospholipids for effective binding [21••]. This finding anticipates that there will be physiologically important consequences of lipid composition for COPII coat function. Experiments showing a requirement for sphingolipids in the sorting of proteins with a glycophosphatidylinositol (GPI) anchor into COPII vesicles shows that lipid composition of the membrane can dramatically affect the partitioning of cargo molecules into COPII vesicles [22,23].

How are cargo proteins segregated into vesicles?

There are thought to be two different mechanisms within the ER for the proper segregation of cargo molecules into vesicles. The first mechanism is the so-called quality control system, revealed by the observation that incorrectly folded proteins or components of multisubunit complexes expressed in stoichiometric excess are usually not transported out of the ER [24]. Quality control is thought to be fundamentally a retention process whereby incompletely folded proteins are kept from entering budding vesicles because they remain bound to a stationary resident of the ER such as BiP, a luminal member of the Hsp70 family.

It is now clear from quantitative immunocytochemistry and an evaluation of cargo partitioning during *in vitro* COPII budding reactions that many cargo proteins are concentrated approximately 10-fold into COPII vesicles [25,26]. This implies a second mechanism for cargo segregation based on positive sorting signals in the cargo molecules. By analogy to the clustering of transmembrane receptor proteins into clathrin-coated pits, partitioning of membrane proteins into vesicles could be a consequence of an affinity of the cargo molecules for the membrane-proximal surfaces of the COPII coat proteins. Two predictions follow from the idea that the COPII coat is an agent for cargo selection. First, there should be *cis*-acting targeting sequences in the cytosolic portions of membrane proteins that specify their entry into COPII vesicles. Second, it should be possible to detect specific protein–protein interactions between membrane cargo proteins and components of the COPII coat. The past year has seen substantial progress in experimental confirmation of both of these expectations.

A number of transmembrane proteins that are transported out of the ER contain the motif Asp-X-Glu (where X is any amino acid) in their cytosolic carboxy-terminal domains. Mutational studies of this di-acidic motif in the context of the cytoplasmic tail of the vesicular stomatitis virus glycoprotein (VSVG) showed that mutation of either of the acidic residues to alanine reduced by fivefold the rate of transport of VSVG from the ER [27•]. A different motif that also specifies exit from the ER has been identified in proteins that recycle within the early part of the secretory pathway. The p24 family of putative cargo receptors for vesicular trafficking proteins carry paired phenylalanine residues near the carboxyl terminus that are important for efficient forward transport from the ER to the Golgi [28,29•]. *In vitro* binding studies using peptide analogs of the carboxy-terminal tail of p24 show an interaction with Sec23p from a cytosolic extract (presumably in complex with Sec24p) that depends on the paired phenylalanine residues [29•]. A similar analysis of the ERGIC-53 protein, a lectin-like molecule that constitutively cycles between the ER and the Golgi, identified paired phenylalanine residues at the extreme carboxyl terminus of the cytoplasmic domain that are required for forward transport [30•]. Peptides based on the ERGIC-53 tail were shown to bind to Sec23p in cytosolic extracts and this binding only occurred when the paired phenylalanine residues were intact.

Complexes containing both cargo proteins and COPII subunits have been detected in microsomal extracts derived from both yeast and mammalian cells. Sar1p (expressed as a glutathione *S*-transferase [GST] fusion protein) and the Sec23p/Sec24p complex can assemble onto washed yeast

microsomal membranes. After detergent solubilization of these membranes, several different types of cargo proteins were found to copurify with Sar1p–GST, including polytopic membrane proteins (amino acid permeases), transmembrane proteins (Sec22p and Emp24p), and the soluble luminal protein pro-—factor, whereas ER-resident proteins Kar2p and Sec61p were excluded from the complex [31••]. The association of cargo proteins with Sar1p–GST required the addition of Sec23p/Sec24p. When the Sec31p/Sec13p proteins were also added the cargo-containing complex was no longer detected, presumably because pre-budding complexes had been converted into completed vesicles. In parallel experiments using mammalian microsomes, Sar1p–GST and Sec23p/Sec24p bound to microsomal membranes and formed a complex that contained the cargo protein VSVG but not the ER-resident protein ribophorin [32••]. The pre-budding complexes can be thought of as the equivalent of the coated-pit stage in the formation of endocytic vesicles, since they contain a partially assembled coat and are enriched in cargo proteins. In this regard, it will be of interest to determine to what extent the pre-budding complexes that have been isolated serve as direct precursors to completed vesicles. It will also be of interest to determine the size, the complexity, and the morphology of the pre-budding complex. Finally, now that *cis*-acting sequences for export from the ER have been identified, it should be possible to determine whether these sequences specify incorporation of cargo into the pre-budding complexes or whether they are required for another aspect of cargo loading.

Soluble luminal cargo proteins could be segregated into vesicles by indirect contact with the COPII coat via integral membrane protein adaptors. Candidate adaptors for pro-α-factor have been identified in cross-linking studies of the pre-budding complex, but these factors remain poorly defined [31••]. The p24 family of proteins have also been suggested to act as cargo adaptors since these proteins cycle between the ER and Golgi [28] and yeast mutants of Epm24p, a member of the p24 family, impair transport of invertase and the cell surface protein Gas1p from the ER [33]. It has not yet been possible, however, to detect a specific association between a p24 protein and a cargo molecule. Given the abundance of the p24 proteins, their oligomeric assembly into high molecular weight complexes [29•,34], and their effect upon a variety of different aspects of COPII vesicle function [35], it seems likely that the p24 proteins have a more general role in cargo segregation than acting as receptors for specific cargo proteins. In thinking about what types of membrane proteins could serve as cargo receptors, perhaps too much emphasis has been placed on a requirement that the putative adaptor proteins be confined to recycle between the ER and Golgi; recycling would only be necessary if the luminal cargo protein were in great stoichiometric excess over the adaptor. For luminal proteins of modest abundance any of the many membrane proteins that are unidirectionally transported to the cell surface should be able to fulfill the function of a cargo adaptor.

What is the intermediate that mediates membrane traffic between the endoplasmic reticulum and the Golgi complex?

The mechanism by which completely formed COPII vesicles arrive at the Golgi apparatus remains to be determined. Initially, it was thought that vesicles might diffuse randomly from their site of budding and that targeting would be achieved by capture of the vesicle on chance collision with the *cis*-Golgi. The recent visualization of a cargo molecule in living cells, however, has now shown us that this widely accepted view may be incorrect [36••,37••].

The fate of the temperature-sensitive (ts) viral glycoprotein ts045 VSVG was monitored en route from the ER to the Golgi complex by tagging this marker protein with the green fluorescent protein (GFP). At 40°C, this ts045 VSVG–GFP glycoprotein misfolds and accumulates in the ER. Upon a shift down to 32°C it rapidly refolds, exits the ER and is transported to the cell surface. When ts045 VSVG–GFP was monitored during a shift from 40°C to 32°C, it concentrated in a pre-Golgi intermediate (also called vesicular-tubular clusters or VTCs) composed of vesicles and small tubules [38,39]. The relationship between the VTCs and the *cis*-Golgi has never been clearly established; however, the ability to visualize ts045 VSVG in transit to the plasma membrane now demonstrates that the VTCs move proteins toward the Golgi along microtubules. These findings imply that cargo is transported between the ER and Golgi apparatus via a large transport intermediate rather than by small vesicles.

Immunofluorescence microscopy data indicated that, as ts045 VSVG exits the ER, it appears to be concentrated at specific export sites in association with COPII proteins [37••]. The COPII vesicles then cluster and fuse to form the heterogeneous VTCs and the COPII coat is exchanged for a COPI coat. Although the role of COPI in these events is unclear, the exchange of COPII for COPI may provide a mechanism for controlling the interaction of the VTCs with microtubules. Alternatively, COPI may function to retrieve molecules from the VTCs to the ER, or in anterograde transport from the VTCs to the Golgi. Further experiments will be needed to test these possibilities.

How do vesicular-tubular clusters form and find their target?

Soluble *N*-ethylmaleimide-sensitive fusion protein attachment-protein receptors (SNAREs) are cytoplasmically oriented membrane proteins that reside on vesicular carrier (v-SNARE) and target organelles (t-SNARE). The SNARE hypothesis, originally put forth by Rothman and colleagues [7], postulates that the specificity of membrane traffic is mediated by the pairing of a v-SNARE with its cognate t-SNARE. The SNARE hypothesis is an attractively simple idea but the actual process by which COPII vesicles are targeted appears to be more complicated. An implication of the studies described above is that ER-derived vesicles first cluster and then fuse with each other

to form the VTCs. The VTCs then target and fuse with the Golgi. These recent findings are consistent with the observation that ER-derived yeast vesicles formed *in vitro* are immunopurified as a cluster of small vesicles [40], and that the active form of syntaxin 5 (a t-SNARE) is on vesicles rather than the Golgi [41•]. That Bet1p, which appears to be a t-SNARE (based on homology to SNAP-25), resides on ER-derived vesicles rather than the target membrane supports the proposal that ER-to-Golgi vesicles undergo homotypic fusion prior to heterotypic fusion with the Golgi [42].

How a transport intermediate or vesicle is targeted to the correct membrane is a key question that remains unanswered. While the SNAREs are sufficient for membrane fusion [43•], recent findings imply they do not determine the specificity of membrane traffic. For example, the t-SNARE is not localized to specific sites on the target membrane [44,45], and the v-SNARE can pair with more than one t-SNARE [46,47]. If the SNAREs do not provide an ER-derived vesicle (or the VTCs) with direction, what component of the secretory apparatus plays this role? One candidate is the recently identified transport protein particle (TRAPP). TRAPP is a large novel complex (~800 kDa) of nine subunits that resides on the *cis*-Golgi where it functions in the targeting and fusion of vesicles [48••]. Genetic and biochemical data support the hypothesis that TRAPP acts upstream of the SNAREs in vesicle targeting. While the role of TRAPP in vesicle targeting is unknown, an attractive possibility is that it serves to capture transport vesicles at specific sites on the Golgi, bringing the SNAREs in proximity with each other to form a prefusion complex.

Genetic data suggest that TRAPP acts in conjunction with other components of the secretory apparatus, such as Usolp and the small GTP-binding protein Ypt1p [49]. Uso1p (the yeast homologue of mammalian p115), a cytoplasmic factor that tethers vesicles to the Golgi *in vitro* [50,51], may interact with specific proteins on the vesicle as well as with TRAPP on the Golgi. Ypt1p, which has been proposed to act upstream of the SNAREs [52,53], may directly interact with one or more of the subunits of TRAPP. It was recently proposed that the t-SNARE Sed5p is the effector of Ypt1p [54]. An important prediction of this hypothesis is that Sed5p, as an effector of Ypt1p, should selectively bind to the GTP-bound form of Ypt1p. The nucleotide state of Ypt1p during interactions with Sed5p has not yet been examined.

The SNAREs, which have been shown to function at different stages of anterograde and retrograde membrane traffic, resemble each other [8,55,56]. In contrast, the highly conserved subunits of TRAPP are not homologous to other known components of the secretory apparatus, including the exocyst, a multiprotein complex exclusively required for post-Golgi secretion [57]. The SNAREs, which appear to act primarily in membrane fusion [43•], may be related to one another, as the mechanism of endoplasmic membrane fusion is highly conserved at each transport step; however, complexes whose role it is to target transport vesicles (or transport intermediates) may not resemble other vesicle receptors that bind to a different class of vesicles.

Conclusion

In summary, we have described how a vesicle buds from the ER and how it targets and fuses with the Golgi apparatus. Both events require protein–protein interactions that are regulated by small GTPases. Precisely how these events take place will be the subject of future studies for years to come.

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