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Coat Proteins and Vesicle Budding

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The trafficking of proteins within eukaryotic cells is achieved by the capture of cargo and targeting molecules into vesicles that bud from a donor membrane and deliver their contents to a receiving compartment. This process is bidirectional and may involve multiple organelles within a cell. Distinct coat proteins mediate each budding event, serving both to shape the transport vesicle and to select by direct or indirect interaction the desired set of cargo molecules. Secretion, which has been viewed as a default pathway, may require sorting and packaging signals on transported molecules to ensure their rapid delivery to the cell surface.

Eukaryotic cells have an elaborate network of organelles, many of which are in constant and bidirectional communication through a flow of small transport vesicles. For each organelle a specific mechanism exists to capture and package certain proteins and lipids that are destined for transport to a receiving compartment. In return, the receiving compartment accepts proteins that are meant to remain, or to be passed to another station, and then retrieves for recycling other proteins that belong in the donor organelle. Among the recycled proteins are structural components of the traffic pathway that must be used repeatedly to sustain transport. The most remarkable feature of this process is that selectivity is achieved in spite of the fluid nature of the membrane. In the absence of specific mechanisms to recognize and sequester proteins destined for transport and retrieval, communicating organelles would quickly lose their identity, succumbing to the lateral diffusional mobility of membrane proteins embedded within the bilayer. The evidence that we summarize in this review suggests that membrane identity is maintained by the selective capture into coated vesicles of proteins destined for transport.

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Three Paradigms of Vesicle Bud Formation

Three models have contributed to our understanding of the mechanism of vesicle budding. The first is fashioned on the example of enveloped viruses that bud from

the cell surface or into an intracellular compartment (*1*). In this example, a cytoplasmic nucleoprotein particle adheres to a virally encoded membrane or peripheral protein and deforms the membrane, clustering viral and possibly specific cellular proteins into the forming capsid. A cellular and topologically inverse analog to this could be cargo proteins that form a particle in the lumen of an organelle and recruit membrane proteins to bud a specific vesicle into the cytoplasm. The formation of a regulated secretory granule is thought to depend on the production of its contents, though it is not known if the cargo dictates the sorting process (*2*). This model probably does not apply to most intracellular traffic. Endocytosis and constitutive transport early in the secretory pathway do not require the presence of cargo molecules (*3*).

The second model of budding involves a consideration of the lateral and transverse organization of lipids within a membrane. Sheetz and Singer in their membrane bilayer couple hypothesis suggested that a local change in the surface area of the two monolayers could lead to membrane curvature, inducing the formation of a bud (*4*). Such changes in the amount of surface area could occur by transbilayer movement of phospholipids or by lipid covalent modification on one surface leading to a change in the lipid packing density. Alternatively, the lateral organization of lipid domains may drive membrane vesiculation. Model phospholipid membranes bud spontaneously under conditions in which transbilayer movement or covalent modifications cannot occur (*5*). Laterally segregated domains within these model membranes may experience a lipid boundary tension that is relieved by constriction of the boundary interface, resulting in the budding of a patch of bilayer (*6*).

Ample evidence exists to support a role for lipids in the protein sorting and budding events (*7*). Lipids attached directly to protein cargo may determine the lateral segregation of this class of molecules into transport vesicles. In polarized epithelial cells a subset of vesicles that bud from the trans Golgi complex are enriched in glycolipid-anchored (glycosylphosphatidylinositol, GPI) proteins that are destined to reside on the apical plasma membrane (*8*). The GPI anchors are believed to partition into a sphingoglycolipid "raft" that may dictate protein sorting into apically directed vesicles (*9*). Such anchors and glycolipid rafts may influence protein sorting and vesicle budding directly, or they may do so indirectly through an association with cytoplasmic structural proteins.

The last and by far the best supported model for bud formation posits a role for cytoplasmic coat proteins. Although lipids, and in some instances cargo, may serve to define the site of bud emergence, it is almost certainly through the action of coat proteins that membrane constituents are segregated and the bilayer is mechanically deformed to produce a transport vesicle. The experimental systems and structural and functional studies that define these coat proteins form the basis of the remainder of this review.

Coat Proteins: The Experimental Systems

A growing list of distinct coat protein complexes have been observed or isolated and associated with endocytic or secretory processes. The first and structurally best characterized coat protein is clathrin, which is involved in receptor-mediated endocytosis and in the transport of lysosomal or vacuolar proteins from the trans Golgi network (*10*). Several other nonclathrin coats have been described in the past 10 years. These include COPI and COPII, involved in vesicle traffic early in the secretory pathway (*11, 12*); a striated coat on caveolae, which are involved in the sequestering and internalization of folate and other receptor molecules, and possibly also in apical vesicle traffic in polarized cells (*13*); a lace-like coat surrounding buds and vesicles at the trans Golgi network (*14*); a neuronal-specific coat molecule with sequence homology to a subunit of COPI (*15*); and an immunological variant of COPI associated with vesicle traffic between endosomal membranes (*16*).

Clathrin

In receptor-mediated endocytosis, clathrin-coated vesicles capture receptor proteins, either with or without ligand bound indirectly through an interaction with the plasma membrane adaptor protein complex [α-adaptin, β-adaptin, AP50, and AP17 (*17*)]. The COOH-terminal, cytoplasmically exposed tail of receptors, such as the low density lipoprotein (LDL) receptor, have sorting determinants that recruit the adaptor protein to a coated pit (*18*). Clathrin, a triskelion structure of three heavy and three light chains, assembles in the pit, initially in the form of a planar surface comprising primarily hexagonal facets of a polygonal lattice. The network serves to enrich receptors destined for internalization and to exclude surface proteins designed to remain at the cell surface. Some receptors, such as the LDL receptor, are constitutively included within the pit, whereas others, such as the insulin and epidermal growth factor receptors, are mobilized only in response to ligand (*19*). By a progressive rearrangement of the triskelion contacts, possibly involving adenosine triphosphate (ATP) hydrolysis and a protein chaperone such as heat shock cognate (Hsc70) protein (*20*), the polygonal lattice acquires pentagonal facets that impart curvature to form a coated bud.

Dynamin, a peripheral guanosine triphosphatase (GTPase) protein initially distributed about the surface of a nascent clathrin-coated bud, localizes to the bud–plasma membrane junction possibly to effect vesicle closure (*21, 22*). Dynamin function was first defined by the characterization of a temperature-sensitive paralytic mutant of *Drosophila* called *shibire* (*23*). Neurosecretory cells in the *shibire* mutant accumulate long-necked coated pits that fail to generate coated vesicles (*24*). Purified dynamin has the capacity to polymerize into a coiled collar that may operate to constrict the neck of the bud before vesicle closure and release (*22*). Dynamin activity may be regulated by a kinase-phosphatase cycle (*25*). In the inactive state dynamin could form a collar at the bud–plasma membrane junction and allow further recruitment of membrane receptors. Once a full complement of receptors is acquired, or some physiologic signal is activated, covalent modification of dynamin would initiate the assembly or disassembly event that leads to membrane fission.

In the trans Golgi network, clathrin-coated vesicles capture receptors involved in traffic of lysosomal or vacuolar proteins. Recruitment in this location is mediated in part by a distinct adaptor complex (β′-adaptin, γ-adaptin, AP47, and AP19) that serves the same role as the plasma membrane adaptor. Membrane proteins such as the mannose-6-phosphate receptor are responsible for both the traffic of endogenous lysosomal precursor proteins and the capture of appropriate ligands for receptor-mediated endocytosis. Clearly, distinct or shared signals on molecules that engage the intracellular and cell surface pathways must be displayed on the cytosolic face of the membrane for access to the adaptor complexes (*26*). Recently, a trans Golgi network protein (TGN38) known to cycle to and from the plasma membrane has been shown to have a tyrosine-based localization signal that interacts with the medium-size protein subunits of both clathrin adaptor complexes (AP47 and AP50) (*27*). Examples such as this give the clear impression that protein sorting and cargo packaging during budding will be governed by a series of cognate protein interactions.

Although numerous physiological and morphological results have led to our current view of clathrin-mediated processes, biochemical analysis with cell-free budding reactions has proved more refractory. Three approaches have met with some success, but none has yet yielded a purified, functional protein. In one assay, plasma membrane

fragments are fixed to a glass support and then monitored morphologically or biochemically for binding of adaptor proteins and clathrin and for the formation of coated buds (*28*). Although the expected sequence of binding events is reproduced by this assay, the approach is cumbersome as a routine assay to detect and purify new molecules. A more easily quantifiable alternative assay measures stages in the envelopment of transferrin bound to its receptor in preparations of plasma membrane fragments (*29*). Transferrin bound to receptor initially is accessible to exogenous proteins and small molecules but progressively becomes sequestered and inaccessible. The complete reaction requires cytosol, hydrolyzable ATP and guanosine triphosphate (GTP), dynamin, adaptor proteins, and clathrin (*30*). A complete resolution of the protein requirements for this reaction will be most revealing.

Short of the full budding event, several laboratories have explored the biochemical requirements for recruitment of adaptor proteins to isolated membrane vesicles. Such binding requires cytosol and GTP but does not lead to a productive recruitment of clathrin into coated vesicles (*31, 32*). Binding of Golgi and plasma membrane adaptor complexes is enhanced by the use of a nonhydrolyzable analog of GTP, guanosine 5′-O-(3-thiotriphosphate) (GTP-γ-S). However, the specificity of binding may not be reproduced with GTP-γ-S because the plasma membrane adaptor becomes misappropriated to a trans Golgi location (*33*). Interestingly, the binding of the TGN adaptor is blocked by brefeldin A, a drug known to inhibit the activation by nucleotide exchange of the small GTP-binding protein adenosine diphosphate (ADP)–ribosylation factor (ARF) (*31, 34*). Indeed, purified ARF1 protein facilitates adaptor complex binding to membranes much as it does to recruit other coat proteins to produce distinct transport vesicles (see below). As we shall see, the interaction between a small GTP-binding protein, a coat protein subunit, and a membrane receptor or target may inform the entire process of cargo capture and budding.

Coatomer

Until about 10 years ago, clathrin was viewed as the single essential carrier for all vesicular traffic. Two things changed this picture. First, a viable yeast mutant missing the clathrin heavy chain was shown to grow slowly but to secrete proteins at a normal rate (*35*). Second, the morphologic observation that not all Golgi-associated vesicles were covered by clathrin, and that a cell-free reaction that reproduces vesicular traffic within the Golgi complex was shown not to depend on clathrin (*11*). Instead, when the transport reaction is inhibited by incubation of isolated Golgi membranes with cytosol and GTP-γ-S, numerous nonclathrin-coated vesicles are found to populate all of the Golgi cisternae. Immunoelectron microscopy showed that these vesicles carry vesicular stomatitis virus membrane G protein (VSV G), which is the reporter glycoprotein used to monitor protein traffic in the cell-free reaction. The coat on GTP-γ-S–inhibited vesicles appears fuzzy but dense, completely unlike the regular polyhedral lattice characteristic of clathrin.

Large-scale isolation of coated vesicles formed in the presence of GTP-γ-S revealed a set of stoichiometric coat subunits (α, 160 kD; β, 110 kD; β′, 102 kD; γ, 98kD; δ, 61kD, ε, 31 kD; and ζ, 20 kD) and ARF (*36*). The β subunit (β-COP) had already been identified as a major peripheral membrane protein of the Golgi apparatus whose membrane attachment is influenced by exposure of cells to brefeldin A (*37*). Thus, the coat is seen as a complex recruited from the cytoplasm to Golgi membranes directed by the brefeldin-sensitive activation of ARF protein. A soluble complex comprising the coat assembly protomer, called coatomer, was isolated and together with ARF and GTP-γ-S (or GTP) the entire process of Golgi vesicle budding has been reproduced with pure components (*38*).

Coatomer and ARF clearly are required to form vesicles in the cell-free system; however, their role in glycoprotein transport has been difficult to establish. For example, brefeldin A blocks coated vesicle formation but does not inhibit the progressive glycosylation of VSV G protein that marks its passage among Golgi cisternae (*39*). Brefeldin-insensitive transport is sensitive to inhibitors of membrane fusion but is resistant to inhibition by GTP-γ-S. Reactions with ARF-depleted or coatomer-depleted cytosol show a similar inhibition of vesicle formation, but not of VSV G transport (*40*). Thus, protein transport may be uncoupled from vesicle formation in the in vitro reaction. To explain this conundrum, Elazar *et al.* proposed a vesicle budding-fusion couple hypothesis (*41*). According to this view, during the biosynthesis of the vesicle fusion machinery, fusogenic proteins must be concealed by coat proteins to prevent premature fusion among Golgi cisternae. Removal of coat proteins or ARF gives the appearance of protein transport but would actually represent the unphysiological fusion of Golgi membranes. The sequential assembly and disassembly of a coated vesicle ensures that only transported proteins and not entire Golgi compartments are consumed by membrane fusion.

Although ARF and GTP (or GTP-γ-S) are required to recruit coatomer to Golgi membranes, and ARF is highly concentrated on coatomer coated vesicles (*37*), ARF-GTP may not make up the sole coatomer binding site. ARF has been shown to activate a Golgi-localized phospholipase D (PLD) in a reaction that is stimulated by phosphatidylinositol-4,5 bisphosphate (PIP_2), a lipid cofactor known to stimulate GTP hydrolysis by ARF (*42*). Furthermore, PIP_2 and the product of PLD action on phosphatidylcholine, phosphatidic acid, have recently been found to bind pure coatomer to synthetic phospholipid vesicles (*43*). Thus, ARF may augment coatomer recruitment through PLD to provide an environment suitable to the formation of a coated vesicle.

An essential role for coatomer and ARF in protein traffic in vivo is supported by numerous genetic and inhibitor studies. In yeast, the genes for most of the coatomer subunits and three ARF genes have been obtained (*44–46*). Mutations that delete any one of the coatomer subunits or two of the ARF isozymes are lethal and block protein traffic. Temperature-sensitive mutations in the β′ and γ subunits display conditional secretory defects. In mammalian cells, a mutation *ldlF* affects the ε-COP subunit and results in a secretory and Golgi stability defect (*47*). Finally, microinjection of a specific β-COP antibody into virally infected tissue culture cells blocks VSV G protein transport into the Golgi complex (*48*).

Implicit in the data presented thus far is the assumption that coatomer generates an anterograde or forward-directed transport vesicle. However, Letourneur *et al.* (*46*) have found evidence to suggest that the principal role of coatomer may be to drive the formation of retrograde transport vesicles responsible for retrieval of endoplasmic reticulum (ER) resident proteins from the Golgi complex. Such proteins often terminate in a KKXX (K is lysine) motif that is known to ensure recycling back to the ER. Simple binding experiments show that the α and β′ subunits of coatomer cause the intact complex to bind to an immobilized KKXX-terminated protein chimera (*49*). A genetic selection for yeast mutants (retrieval, *ret*) that allows a KKXX-terminated membrane protein chimera to be exported to the cell surface yielded mutations in the α, β′, and γ coatomer subunits (*46*). Curiously, many of the mutations obtained in this selection confer temperature-sensitive growth with no corresponding defect in secretion. One interpretation of these results is that *ret* mutations specifically cripple the interaction of COP subunits with retrieval sequences with no effect on the capacity of coatomer to create anterograde vesicles. Indeed, certain alleles of coatomer subunits (*sec21-1*, γ-COP) block both retrieval and secretion. However, at least part of the secretion defect of such a mutation may

result from the failure to retrieve ER membrane proteins that are essential to target anterograde vesicles to the Golgi complex (*50*). In this regard, the overproduction of one of the proteins required for targeting (Sec22p) partially suppresses the growth defect of *ret1-1* and *sec21-1* mutant cells (*45*). Several such targeting proteins have been identified in yeast (Sec22p, Bos1p, and Bet1p), and none has the usual KKXX retrieval signal (*51*).

COPII

Independently of the results suggesting a role for coatomer in membrane traffic between the ER and Golgi, evidence emerged concerning a set of yeast Sec proteins required for vesicle budding from the ER. Genetic experiments identified a set of interacting gene products involved in the production of 60-nm vesicles observed to mediate protein transport from the ER in yeast [*SEC12*, *SEC13*, *SEC16*, *SEC23* (*52*)]. A cell-free reaction that reproduces the transport of a radioactive secretory protein, yeast α-factor precursor, from the ER to the Golgi complex depends on functional forms of several of these gene products (*53*, *54*). The vesicle budding step in this transport reaction is measured by observing the packaging of glycosylated α-factor precursor (gpαF) into small vesicles that separate from the much larger donor ER membrane (*54*).

Each of the cytosolic proteins required for budding was purified to yield a reconstituted reaction that reproduces all of the salient features of the physiological event (*55*, *56*). Specifically, the reconstituted reaction produces functional transport vesicles that are capable of transferring gpαF to the yeast Golgi complex by a process of targeting and fusion that uses a distinct set of Sec proteins (*56*). In addition, a highly selective protein sorting event accompanies the formation of the transport vesicles. Proteins destined for transport to the Golgi complex, including other cargo molecules such as the major GPI-linked protein in yeast (Gas1p) and two amino acid permease precursors (Hip1p and Gap1p), and vesicle-targeting proteins such as Sec22p, Bos1p, and Bet1p, are efficiently packaged and concentrated in transport vesicles (*12*, *50*, *57*). In contrast, proteins designed to function and remain in the ER, such as membrane components of the polypeptide translocase (Sec61p) and the soluble lumenal chaperone (Kar2p, the yeast BiP) are not packaged (*56*, *58*). Thus, although retrieval from the Golgi complex of lumenal and ER membrane resident proteins is known to be essential in yeast, the primary means of sorting is achieved right at the moment of vesicle budding, long before the action of the retrieval system (*59*).

The purified Sec proteins required for budding and sorting comprise a set of five subunits including a monomeric, small GTP-binding protein, Sar1p, and two heterodimeric complexes, Sec23p-Sec24p and Sec13p-Sec31p (*55*, *56*). In addition, an integral membrane glycoprotein, Sec12p, remains in the ER to serve as a landmark, guiding the budding machinery to the proper compartment (*60*). The bud site choice and key aspects of the budding mechanism are regulated by GTP binding and hydrolysis. Sar1p is recruited to the ER membrane by transient interaction with the NH_2-terminal, cytoplasmically exposed domain of Sec12p (*61*). This domain is a Sar1p-specific guanylate nucleotide dissociation factor that promotes exchange of guanosine diphosphate (GDP) for GTP, allowing the active form of Sar1p to bind to the ER membrane (*62*). Sar1p then recruits the Sec23p and Sec13p complexes to produce functional transport vesicles. GTP, which is the only nucleotide required for budding, is consumed by hydrolysis either during or after the completion of budding. This event is stimulated by the Sec23p subunit, which serves as a Sar1p-specific GTPase-activating protein (GAP) (*63*). The GAP function of Sec23p appears not to be its only role, because transport vesicle formation does not require GTP hydrolysis (*12*). However,

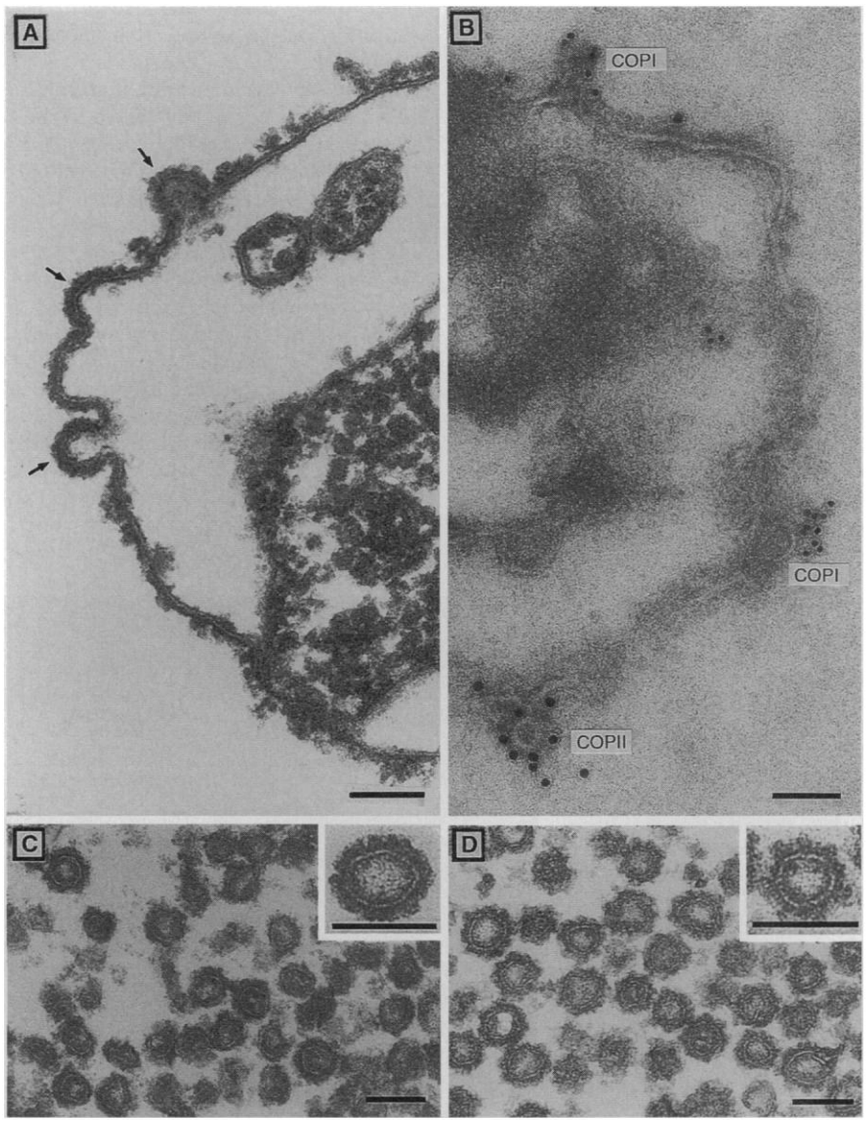

Fig. 1. Composite image of yeast nuclei showing (**A**) the nuclear envelope with coated buds (arrows) and (**B**) the immunolabeling of Sec21p-myc (COPI) and Sec13p (COPII) coat components. The morphology of purified fractions of (**C**) COPI and (**D**) COPII vesicles. The inserts show the respective vesicle type at high magnification. For the conventional electron microscopy images in (A), (C), and (D), samples of the nuclear vesicle pellet were processed as previously described (*11*). Immunolabeling was done on cryosections prepared according to (*83*), with goat antibody to rabbit (Sec13p and dihydrofolate reductase = COPII) or goat antibody to mouse (Sec21p-myc = COPI) immunoglobulin G coupled to gold particles of different sizes (COPI = 10 nm; COPII = 15 nm) on the same section. Scale bars indicate 100 nm.

vesicles formed in the presence of the nonhydrolyzable GTP analog 5′-guanylyl-imidodiphosphate (GMPPNP) are inert in regard to targeting and fusion to the Golgi complex.

In spite of the genetic and physiological evidence supporting a role for coatomer in anterograde transport from the ER, the reconstituted ER budding reaction neither contains nor is stimulated by the addition of coatomer and ARF (*64*). Nevertheless, two features of the reconstituted reaction are consistent with a coatomerlike process. Both reactions involve small GTP-binding proteins, and within this extended family, Sar1p and ARF are the closest relatives (*65*). Both reactions proceed in the presence of nonhydrolyzable GTP to produce targeting and fusion-inhibited vesicles (*12, 66*).

Close inspection of ER-derived transport vesicles reveals a coat complex not previously found elsewhere that comprises the set of Sec proteins necessary to drive vesicle formation (Fig. 1) (*12*). Vesicles formed in the presence of GMPPNP retain Sar1p, and the coat thus impedes access of the vesicle membrane to targeting sites on the Golgi complex. GTP vesicles fail to retain Sar1p, but the other Sec protein subunits remain at least transiently associated, being shed before vesicle docking on the Golgi. The superficial similarity of these coated vesicles belies a completely distinct polypeptide composition from the coatomer coat (Fig. 1). To emphasize the similarities, yet distinguish the vesicle types, we refer to coatomer-coated vesicles as COPI, and to ER-derived transport vesicles as COPII vesicles. Although COPI and COPII coats appear much less regularly arrayed than the clathrin polyhedron, rotary shadowed images of frozen-etched samples reveal a subunit profile consisting of a cluster of 2- to 4-nm protein particles on the surface of a COPII vesicle (Fig. 2).

When a budding reaction is conducted in the presence of crude cytosol in place of the purified Sec proteins, three additional proteins, Sec7p, Sec16p, and Ypt1p, become associated with transport vesicles (*67*). Sec7p and Ypt1p may become associated during the budding event, however, they are required only later in the targeting of vesicles to the Golgi complex (*68*). Sec16p associates with COPII vesicles by virtue of interactions with Sec23p in the coat and Sed4p, a Sec12p homolog located in the ER membrane. Although the *SEC16* gene product clearly is required for vesicle budding from the ER in vivo, it seems not to be required in the cell-free reaction (*69*). Sec16p may play an essential regulatory role that is bypassed in the current formulation of the in vitro system.

Mammalian equivalents of the COPII subunits have been detected by molecular cloning and immunological cross-reaction (*70*). Immunolocalization studies with Sec23p, Sec13p, and Sar1p reveal a concentration of these proteins surrounding the buds and vesicles emerging from the ribosome-free transitional face of the ER in pancreatic acinar and β cells. This distribution closely approximates the location expected for proteins that act in the formation of anterograde transport vesicles. Functional studies, with mutant forms of mammalian Sar1p introduced into permeabilized CHO mammalian cell preparations, are consistent with a direct role for COPII in transport from the ER but not in transport within the Golgi complex (*71*). In contrast, COPI subunits are found near the cis face of the Golgi complex and in a region of the ER, called CRER (coatomer-rich ER), on the opposite face of the cisterna that gives rise to transport vesicles (*72*).

How then do COPI and COPII organize the traffic of vesicles passing to and from the Golgi complex? The simple view that COPII handles anterograde budding and COPI mediates retrograde budding was tested by inspecting a pure ER membrane for its capacity to form vesicles with isolated or mixed fractions of coat proteins, Sar1p, and ARF. Yeast nuclei, which represent ~30% of the total ER membrane, are observed to form both COPI and COPII vesicles that emerge by budding from the outer membrane of the nuclear envelope (Fig. 1) (*64*). The two coats form buds completely independently of each other to produce a mixture of COPI and COPII vesicles (Fig. 1). Both vesicles are free of ER resident proteins but contain the same set of targeting molecules (Sec22p, Bos1p, and Bet1p) and several other major, but unidentified, polypeptides, suggesting that each is designed to sort and transport proteins to the Golgi complex. In contrast, budding of COPI but not COPII vesicles is inhibited by brefeldin A, demonstrating that the ARF-specific nucleotide exchange activity is represented in a bona fide ER membrane. Furthermore, at least several of the visible polypeptide constituents of the COPI and COPII membranes are distinct, suggesting that each membrane may be responsible for the transit of some common and some distinct cargo proteins. The rules that govern cargo capture by the COPI and COPII coats represent attractive areas for genetic and biochemical exploration.

COPI and COPII vesicles generated from the yeast nuclear envelope each contain a large number of protein species, and yet the anecdotal evidence suggests that only COPII vesicles carry the major cargo proteins. The α-factor precursor Gas1p, the amino acid permeases Hip1p and Gap1p in yeast, and VSV G protein in mammalian cells are packaged exclusively by the COPII coat (*12, 57, 71*). It remains possible that further inspection will reveal bona fide cargo molecules that use COPI for exit from the ER; however, another possibility, depicted in Fig. 3, accounts for the available evidence. A restricted set of membrane proteins may travel to and from the cis Golgi cisterna in a COPI vesicle. The major role of this limb of the secretory pathway would be to recover escaped proteins that belong in the ER.

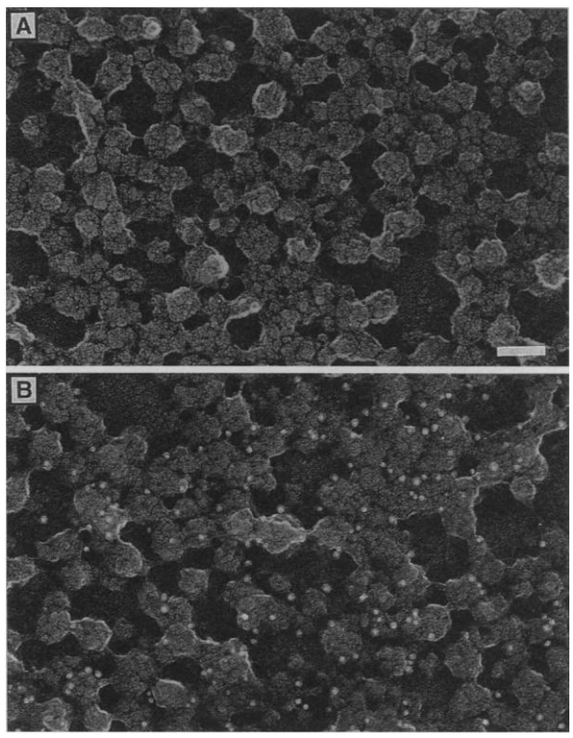

Fig. 2. (**A** and **B**) Purified COPII vesicles after quick freeze–deep etch processing and rotatory shadowing. The subunit constitution of the coat is visible on several vesicles. Immunolabeling of the vesicle fraction with antibody to Sar1p was performed before freeze-etch processing in (B). The protein A–gold particles appear as white dots on the dark background of the reversely printed negative. Scale bar for (A) and (B) is 100 nm.

However, among the molecules necessary for retrograde transport are integral membrane targeting proteins that would ensure the correct docking of vesicles at the ER. Such molecules must themselves be retrieved to the cis Golgi cisterna for the retrograde pathway to be sustained. Segregation of such membrane proteins into COPI vesicles away from the regular cargo in a COPII vesicle may be necessary to organize the pathway into distinct anterograde and retrograde limbs. One prediction of this model is that a COPI-specific membrane protein would continue to cycle between the ER and Golgi complex in a COPII mutant cell.

Secretion: Selective or Default?

The selective capture of secretory cargo by COPII or COPI vesicles highlights an issue that has been the subject of debate for at least 10 years. During this period, numerous studies pointed to the possibility that secretory proteins may not have any special signals to ensure their rapid transit through the secretory pathway. The emerging view was that only those proteins retained within an intracellular compartment would display a signal necessary for proper localization. Space limitations preclude a full review of the merits and deficiencies of the experiments that led to the default hypothesis. In any case, a combination of established facts and new evidence point increasingly to a role for positive sorting information on secreted proteins.

Several old observations are consistent with a role for distinctive sorting signals on exported proteins. Various secretory proteins demonstrate characteristic half-times of transit from the ER to the Golgi complex (*73*). One interpretation of this result is that secretory proteins may comprise families with respect to sorting receptors, each of which has a different pace of transit between the ER and Golgi. Deletion of the COOH-terminal cytosolic tail of VSV G protein causes the trimers, which assemble at control rates, to remain in the ER much longer than normal (*74*). Likewise, point mutations within secreted proteins such as yeast invertase and the immunoglobulin light chain produce proteins that fold properly and exhibit normal functional attributes but which display significantly retarded rates of transport from the ER (*75*). Finally, although resident ER lumenal proteins, such as BiP, are retrieved by a COOH-terminal localization signal (Lys-Asp-Glu-Leu in mammals; His-Asp-Glu-Leu in *Saccharomyces cerevisiae*), deletion of this signal produces a fully functional molecule that nevertheless is only very slowly transported out of the ER (*76*).

A number of new results encourage the search for sorting signals. Qualitative and quantitative immunoelectron microscopic studies reveal a concentration of serum albumin and VSV G protein at putative exit points of the ER and within transport vesicles bound for the Golgi complex (*77*). Such concentration of these molecules and exclusion of resident proteins may result from positive or negative recognition of cargo molecules. However, in the in vitro vesicle budding analysis, yeast gpαF within the lumen of the nuclear envelope is packaged essentially exclusively by COPII and not by COPI vesicles (*64*). Thus, a soluble cargo molecule, with access to two distinct carriers, favors one over the other. Although other explanations are possible, the case for a positive signal attracting gpαF to the COPII membrane clearly is most compelling.

If signals exist, what do they comprise and how are they deciphered? Two recent examples highlight possible signals and receptors. Emp24p, a major integral membrane protein of COPII vesicles, facilitates the transport of a subset of secretory proteins in yeast (*78*). Deletion of the *emp24* gene is not lethal, but at least two cargo molecules experience a severalfold delay in transit out of the ER in null mutant cells. A family of *emp24* genes has been detected, and each member may serve to recognize and capture an overlapping set of cargo molecules. Deletion of multiple genes of this family may further retard and enlarge the range of molecules that experience a delay in transport.

One example of a potential signal is the *N*-glycan chain of glycoproteins. In polarized epithelial cells, some surface proteins are exported apically whereas others are transported to the basolateral surface (*26*). Cytosolic signals are known to influence basolateral determination and GPI anchors to dictate apical delivery (*79*). Some secreted proteins may exit from both surfaces (*80*). Chimeric constructs that include a site for attachment of *N*-glycan chains confer the apical sorting decision on a protein that would otherwise distribute equally between the two paths (*81*). Perhaps correspondingly, a major protein transported between the ER and Golgi, ERGIC53, has been shown to possess lectin activity, thus this molecule could enhance the traffic of glycoproteins from the ER (*82*).

Coat-Mediated Sorting

For each of the coat complexes we have described, evidence suggests that cargo capture is dictated by an interaction between a coat subunit and a peptide determinant exposed on the cytosolic face of the donor organelle. Specific binding may be facilitated by the action of the small GTP-binding proteins, Sar1p, or one of the ARF isozymes. A similar mechanism is invoked to explain the role of Ras in the coupling of receptor and effector molecules.

Our working model of the mechanism by which COPII recruits cargo and shapes a bud is shown in Fig. 4. Sar1p is recruited directly to the ER membrane by virtue of its functional interaction with Sec12p, an ER resident protein. The activated species, Sar1p-GTP, then recruits the Sec23p complex to form a binary complex free to diffuse within the plane of the ER membrane, sampling potential partners by collisional encounters. Favorable interaction may transfer the Sec23p complex to a protein now marked for transport. Unfavorable interaction, such as with an ER resident protein, could trigger premature GTP hydrolysis or have no consequence. Sar1p-GTP hydrolysis, stimulated by the GAP activity of Sec23p, recycles Sar1p-GDP to the cytosol

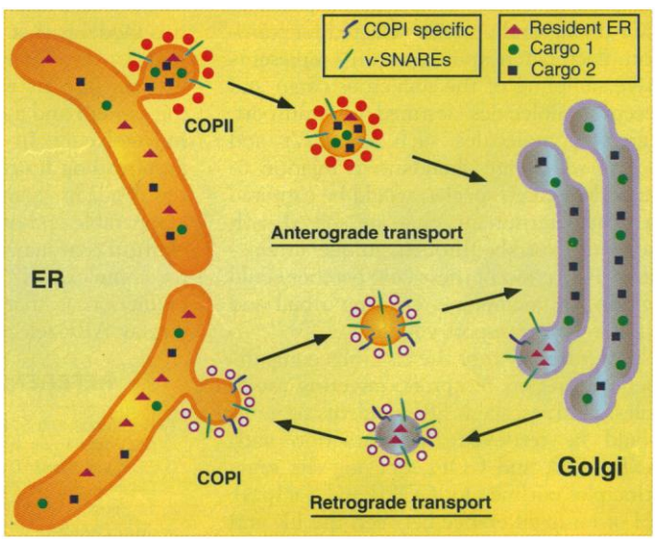

Fig. 3. Proposed roles of COPI and COPII in vesicle traffic between the ER and cis Golgi. COPII vesicles are shown transporting normal cargo molecules, a low number of escaped resident ER proteins, and anterograde targeting membrane proteins [v-SNARE, vesicle-bound soluble NSF (*N*-ethylmaleimide–sensitive factor) attachment protein receptor]. COPI vesicles are shown mediating a cycle of transport including a retrograde limb that returns escaped ER proteins and v-SNAREs to the ER, and an anterograde limb that carries the same v-SNAREs and COPI-specific transport factors back to the cis Golgi.

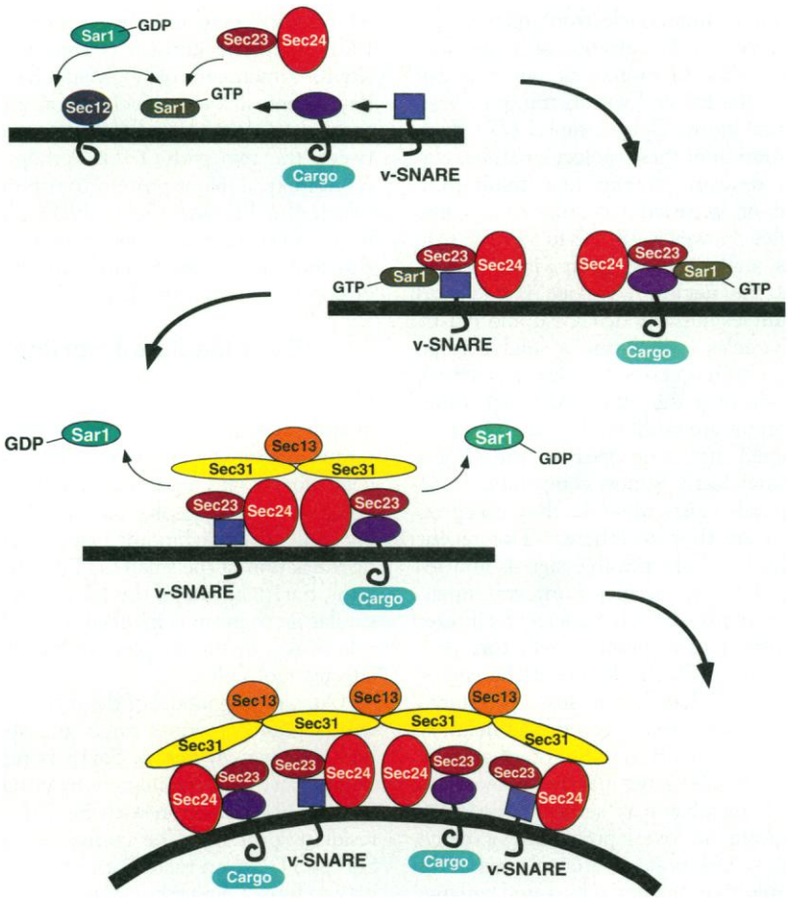

Fig. 4. A model for COPII-mediated cargo sorting and vesicle budding. Sar1p acquires GTP by nucleotide exchange facilitated by Sec12p on the ER membrane. Sar1p-GTP then recruits Sec23p-Sec24p to the ER to form a surveillance complex that recognizes cargo or SNARE proteins that are designated for packaging. Sar1p hydrolyzes GTP under the influence of the Sec23p subunit, producing Sar1p-GDP that dissociates from the membrane, allowing additional cycles of Sec23p-Sec24p recruitment and SNARE or cargo protein acquisition. Proteins designated for transport are clustered by a multivalent interaction of Sec23p-Sec24p and Sec13p-Sec31p to form a patch, a bud, and ultimately a COPII coated vesicle.

for another round of activation and cargo recruitment. Sec23p-activated cargo or adaptor molecules may then be clustered by multivalent interaction with the Sec13p complex to form a concentrated patch of COPII coat and proteins selected for transport. Each patch would contain a representative sampling of the numerous cargo and receptor molecules destined for transport. Targeting molecules, such as Sec22p and Bos1p, which are abundant in relation to individual cargo species, would be captured by coat determinants that are shared with cargo or possibly through unique interactions. Accretion of these coat patches could deform the membrane, creating a bud and ultimately a transport vesicle.

After transport to the cis Golgi compartment, secretion receptors, targeting molecules, and escaped ER resident proteins would be retrieved by interaction with ARF, GTP, and COPI, obeying the same principles outlined for COPII and Sar1p. A pH or ionic difference between the ER and cis Golgi would dictate the selection of anterograde and retrograde cargo.

This model applies equally to the formation of clathrin vesicles, and possibly to members of the less well characterized family of coat proteins. The novel feature of this model is that it invokes a proofreading function for the subunits of the coat that interact directly with the small GTP-binding protein and a cargo, receptor, or targeting molecule. In the case of COPII, this proofreading function may be provided by the Sec23p complex. Accordingly, the comparable proofreading function for a clathrin coat may be contained in the adaptor complexes. If so, a simple and testable prediction is that adaptor complexes will display ARF-selective GAP activity.

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Phosphoinositides as Regulators in Membrane Traffic

Pietro De Camilli,* Scott D. Emr, Peter S. McPherson, Peter Novick

Phosphorylated products of phosphatidylinositol play critical roles in the regulation of membrane traffic, in addition to their classical roles as second messengers in signal transduction at the cell surface. Growing evidence suggests that phosphorylation-dephosphorylation of the polar heads of phosphoinositides (polyphosphorylated inositol lipids) in specific intracellular locations signals either the recruitment or the activation of proteins essential for vesicular transport. Cross talk between phosphatidylinositol metabolites and guanosine triphosphatases is an important feature of these regulatory mechanisms.

In eukaryotic cells, the distinct composition of the different intracellular compartments is maintained despite continuous intercompartmental transport of membrane and lipid components. This homeostasis depends on vesicular carriers that mediate traffic by means of vectorial transfer of selected membrane and lumenal cargoes. A general framework has been proposed to explain the selective incorporation of proteins from donor membranes into carrier vesicles as well as the specific targeting and fusion of each class of vesicle with the appropriate target component (*1*). Membrane and lumenal proteins are incorporated into vesicles through direct or indirect interactions with coat proteins that are assembled on the cytoplasmic surface of the donor membrane (*1*, *2*). Self-assembly of the coat forces an increase in membrane curvature in a localized region until a coated vesicle bud, anchored by a narrow stalk, has formed. In at least some cases, additional factors are required to sever the vesicle neck and generate a free vesicle (*3*). After transport, the vesicle sheds its coat proteins and the uncoated vesicle docks with the target membrane through a cascade of molecular interactions, including the binding of membrane proteins of the vesicle (termed v-SNARES) with membrane proteins of the target membrane (termed t-SNARES) (*1*). Formation of the v- and t-SNARE complex is then followed by the fusion event that completes the transport reaction.

This framework raises a number of questions, many of which can be viewed as problems of regulation. For example, what regulates vesicle formation? What makes this process vectorial? What regulates coat assembly and disassembly? Because v-SNAREs and other membrane proteins must be recycled back to the donor compartment, what distinguishes the forward vesicles from the recycling vesicles that carry the same proteins but are directed toward a different target compartment?

Any model of vesicular traffic must include mechanisms to guarantee temporal and spatial specificity, because without such regulators, vesicular traffic would result in the rapid homogenization of all cellular compartments. Both proteins and lipids participate in this regulation. Among proteins, a major role is played by guanosine triphosphatases (GTPases). Among lipids, growing evidence suggests a key function for phosphatidylinositol (PtdIns) and its phosphorylated derivatives, collectively referred to as phosphoinositides (PIs). Unlike the head group of other phospholipids, the inositol ring is a highly versatile substrate that can be modified at several positions. Phosphorylation of the inositol ring of PtdIns at one or a combination of positions (3′, 4′, or 5′) generates a set of five unique stereoisomers that appear to function as regulators of vesicular transport reactions, the cytoskeleton, and cell growth. Here, we review results that implicate PIs and their metabolites in vesic-

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