COPII and COPI Traffic at the ER-Golgi Interface

Protein traffic is necessary to maintain homeostasis in all eukaryotic organisms. All newly synthesized secretory proteins destined to the secretory and endolysosomal systems are transported from the endoplasmic reticulum to the Golgi before delivery to their final destinations. Here, we describe the COPII and COPI coating machineries that generate carrier vesicles and the tethers and SNAREs that mediate COPII and COPI vesicle fusion at the ER-Golgi interface.

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Secretory Compartments of the Endoplasmic Reticulum-Golgi Interface

In eukaryotic cells, secretion involves movement of cargoes through a linear assembly of membrane-bound compartments. The secretory pathway is comprised of structurally distinct organelles and membrane-bound transport intermediates that facilitate transport between them. The advent of live imaging and the identification of specific compartmental markers have changed the face of the secretory pathway. Currently, the compartments of the secretory pathway, beginning with the site of cargo synthesis, include the endoplasmic reticulum (ER), ER exit sites (ERES), the ER-to-Golgi intermediate compartment (ERGIC), the Golgi complex, and the trans-Golgi network (TGN) (FIGURE 1). In some cell types, endosomes are also traversed by cargo proteins and thus also are considered secretory compartments.

ER and ERES

All newly synthesized secretory and plasma membrane (PM) proteins and proteins that reside in the ERGIC, Golgi, endosomes, and lysosomes are translated on the cytoplasmic surface of the rough ER (RER) and are either inserted into RER membrane or translocated into the RER lumen. The advent of new organellar markers and live imaging of cargo movement established that proteins are exported from the RER only from specialized ERES (FIGURE 2A). ERES are morphologically defined as smooth projections of the ER that are coated with COPII coat components (see below). ERES give rise to COPII-coated vesicles. Mammalian cells generally have 50–70 ERES of ~0.5–μm diameter randomly scattered along the ER with some concentration in an area adjacent to the Golgi (67, 165). ERES are relatively stable, persisting for >10 min. Thus these sites are likely to generate numerous COPII vesicles during their existence. ERES-generated COPII vesicles transport cargo to the next compartment in the secretory pathway, the ERGIC.

ERGIC

The ERGIC (also known as vesicular-tubular clusters or pre-Golgi intermediates) is a collective name given to pleiomorphic compartments composed of vesiculo-tubular profiles that are found adjacent to ERES (FIGURE 2B). Although the exact nature of the ERGIC is still a matter of debate, the ERGIC is distinct in biochemical composition from the ER and the Golgi (150) and is not continuous with either the ER or the Golgi (26). Individual ERGIC units seem to communicate laterally through rapidly forming and dissipating tubules and vesicles (17).

Live imaging of the ERGIC marker ERGIC53 shows that individual ERGIC are stable compartments that remain close to ERES, without net movement toward the Golgi (17). In contrast, live imaging of secretory cargo shows separation of large (typically 1 μm in diameter) anterograde-destined carriers containing cargo from ERGIC53-labeled stable ERGIC. Cargo-containing carriers track on microtubules toward the microtubule organizing center (MTOC) and the Golgi complex (70, 128). Thus it appears that ERGIC is a stationary sorting compartment that gives rise to relatively large anterograde-destined transport intermediates.

In addition to generating anterograde carriers for cargo transport to the Golgi, the ERGIC sorts proteins into at least two additional pathways: recycling proteins are sent back to the ER and resident ERGIC proteins are retained within the ERGIC. Sorting into the recycling pathway is most clearly understood and is mediated by the COPI coat, which facilitates formation of COPI vesicles (described in detail below). It is less clear whether COPI also participates in the formation of the large anterograde carriers and/or protein retention
within the ERGIC. However, it is clear that COPI is recruited to the anterograde carriers as they translocate from the peripheral ERGIC toward the MTOC (166), suggesting that COPI-coated vesicles continuously bud from the carriers.

**Golgi**

The Golgi complex exhibits distinct morphologies in different organisms. In mammals, the Golgi is organized into a single ribbon of stacked cisterna (50, 84). In most model organisms, including the worm *C. elegans*, the fly *D. melanogaster*, and the plant *A. thaliana*, the Golgi exist as discrete stacked elements (Golgi mini-stacks) scattered throughout the cell (51). In the yeast *S. cerevisiae*, the Golgi exist in discrete tubules scattered throughout the cell (2).

Irrespective of morphology, the Golgi in all higher eukaryotes is composed of closely packed membrane cisterna with a defined polarity, *cis* facing the ER, *trans* facing the opposite side, and *medial* in between (FIGURE 2C). The number of cisterna varies in different organisms and in distinct tissues, with most cells having four to seven cisterna. The polarity of the Golgi is most clearly reflected in the localization of various glycosyl-transferases. Enzymes such as the early acting mannosidase II are detected on the *cis*-side, whereas the late-acting galactosyl-transferase is detected in the *trans*-cisterna (95, 136, 176, 178, 183).

The Golgi complex is flanked on the *cis*-side by a tubular *cis*-Golgi network (CGN) and on the *trans*-side by the TGN. The CGN may consist of fused and concentrated transport intermediates approaching the Golgi from the ER. The relationship between the ERGIC, anterograde carriers, and the CGN is unclear. The current model is consistent with carriers originating from the ERGIC, tracking on microtubules toward the MTOC and fusing there with each other to generate a new

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**FIGURE 1. Intracellular transport pathways**

The scheme depicts the compartments of the secretory and endosomal pathways. Transport steps are indicated by arrows. Colors indicate the known coats: COPII (blue), COPI (green), and clathrin (red). Secretory cargos are synthesized in the ER, exit the ER at ERES in COPII-coated vesicles, and are transported to ERGIC. Cargos are sorted from ERGIC into anterograde carriers that move them to Golgi. After passage through the Golgi, cargos are sorted at the TGN for delivery to the PM, early and late endosomes, and in some cells to secretory granules. A COPI-mediated recycling pathway retrieves proteins from the Golgi and ERGIC and returns them to the ER.
cis-cisterna. The TGN consists of tubular elements that are distinct from the trans-Golgi cisterna. The TGN appears to be a complex compartment consisting of distinct subdomains that contain different coiled-coil proteins called golgins (43, 44, 62). From the TGN, transport intermediates can either traffic directly to the PM or can deliver cargo to the endosomal system or to secretory granules (FIGURE 1).

Transport through the Golgi has been extensively studied and even more extensively debated. Multiple models have been considered and the one gaining the most recognition is the cisternal maturation model (63, 127, 168, 188). In this model, anterograde carriers formed from ERGIC translocate toward the MTOC and accumulate at the cis-Golgi region where they fuse into a network that subsequently becomes the cis-Golgi cisterna. Newly formed cis-cisterna progressively move toward the trans-side as they remodel by losing early acting Golgi enzymes and acquiring late-acting ones (24). This model implies that the secretory cargo remains inside the original membrane compartment throughout transit, while the compartment itself undergoes complete remodeling through the loss of resident early Golgi proteins and the acquisition of late ones (167). Remodeling occurs through packaging of Golgi proteins into COPI vesicles that move in the retrograde direction from trans- to cis-Golgi cisterna. In this model, the full complement of Golgi resident proteins is continuously recycled to the more proximal compartment.

**Increasing Complexity of the Trafficking Machinery**

The last few decades have witnessed an explosive growth in defining distinct stages of traffic and identifying molecules that regulate each stage of transport. The functions of coat complexes, Sar/ARF GTPases and their activating guanine nucleotide exchange factors (GEFs), and deactivating GTPase activating proteins (GAPs), tethering factors, and soluble N-ethylmaleimide sensitive factor receptors (SNAREs) in membrane trafficking are clearly established (FIGURE 3). Below, we summarize the general understanding of this basic machinery responsible for cargo traffic at the ER-Golgi interface.

**Vesicle Formation at the ER-Golgi Interface**

COPII- and COPI-coated vesicles are operational at the ER-Golgi interface. The formation of both types of vesicles is mechanistically conserved and in both cases involves coat complexes, Sar1/ARF GTPases, their GEFs, and their GAPs.

**Anterograde COPII vesicles.** Cargo sorting for exit from the ER occurs exclusively at ERES and is...
mediated by the COPII coat, which binds and concentrates secretory cargoes into vesicles (9, 19, 93, 142, 143, 177, 204). COPII recruitment is mediated by the active GTP-bound form of the small GTPase Sar1. Like all GTPases, Sar1 exhibits low intrinsic GDP-to-GTP exchange, and in cells this process is catalyzed by the Sec12 GEF (11, 143, 189). Sec12 is a membrane protein localized to the ER, which restricts Sar1 activation to the ER. Active Sar1 directly binds the Sec23/Sec24 heterodimeric subcomplex of

![Diagram of vesicular transport](http://physiologyonline.physiology.org/)

**FIGURE 3. Steps of vesicular transport**

Protein traffic within the cell involves the packaging of cargo proteins into vesicles that bud from donor membranes, move to the acceptor membranes, and then tether and fuse with acceptor membranes to release their cargo. This process involves activation of a GTPase on a donor membranes by a GEF (step 1), recruitment of a coat and an ARF GAP (step 2), and cargo sorting/concentration (step 3) into a nascent bud. Coat polymerization is believed to deform the membrane, and following scission (step 4) the vesicle is pinched off from the donor membrane. After moving into the proximity of the acceptor membranes, the coat dissociates (step 5), and the vesicle attaches to acceptor membrane via tethers and subsequently fuses through a SNARE-mediated mechanism (step 6). Transmembrane and soluble cargo retain their topology during transport.
the COPII coat, which in turn recruits the Sec13/Sec31 heterotetrameric subcomplex to the Sec23/Sec24 core. Sar1, Sec23/24, and Sec13/31 are the minimal components required to generate COPII vesicles in vitro (106). Sec12 is required in addition to reconstitute GTP-dependent COPII vesicle budding (59). There are two Sar1 paralogs (Sar1a and Sar1b), two Sec23 paralogs (Sec23a and Sec23b), four Sec24 paralogs (Sec24a–d), a single Sec13 paralog, and two Sec31 paralogs (Sec31a and Sec31b). The exact functional relationships between these components to make distinct COPII vesicles remain to be defined.

Sorting of transmembrane cargo proteins into COPII vesicles is mediated by the Sec24 subunit of COPII, and different isoforms of Sec24 have been shown to bind different sorting motifs and thus expand the range of cargos that can be exported out of the ER. Sec24 binds to the cytoplasmic tails of transmembrane cargo proteins and sequesters them into the nascent vesicles. Sec24 isoforms a and b (but not isoforms c and d) bind the DXE motif and also the LXXL/ME motif on cargo proteins. Sec24 isoforms c and d bind the IXM motif (101).

Soluble cargo proteins within the ER lumen are exported from the ER by binding to cargo receptors of the ERGIC-53 family, the p24 family, or the Erv family that facilitate their sorting into COPII vesicles. These transmembrane receptors contain lumen-exposed domains that bind cargo proteins and cytoplasmic domains that interact with COPII components for inclusion into COPII vesicles.

ERGIC-53 is a single-pass transmembrane protein with a large NH₂-terminal luminal domain that binds glycosylated cargo proteins (6, 82). Additional members of the ERGIC-53 family include ERGL, VIP36, and VIPL that display distinct binding preferences for high mannose containing glycoproteins (80). The function of these receptors as cargo transporters implies that they bind cargo within the ER and release it within the ERGIC/Golgi before recycling back to the ER. The regulation of cargo binding and release appears to be mediated by a decrease in intraluminal pH between the ER (~7.2) and the Golgi (~6.4), decreased Ca²⁺ levels, and possibly phosphorylation (68, 169).

The p24 family members are similar to ERGIC-53 and contain a luminal NH₂-terminus, a single transmembrane segment, and a small cytoplasmic tail that binds to COPII components (169). The p24 proteins are ubiquitous and, in mammals, consist of 8–10 isoforms with distinct expression patterns and possibly different cargo binding preferences (170).

The Erv family members are multi-spanning transmembrane proteins with varying topologies (reviewed in Ref. 41). The types of cargoes they bind vary, and Erv29 appears to bind preferentially soluble proteins, whereas both Erv14 and Erv26 appear to traffic preferentially transmembrane proteins. A recent review provides more detail on the function of the ERGIC-53, p14, and Erv classes of cargo receptors (41).

The critical roles of cargo receptors are underscored by diseases associated with mutations in various receptors. Mutations in ERGIC-53 cause autosomal recessive bleeding disorders due to defects in trafficking of coagulation factors V and VI (205, 206). Deletion of only the p23 family member of the p24 family in mice causes embryonic lethality (42). Interestingly, deletion of all eight members of the p24 family in yeast maintains cell viability despite trafficking defects of select cargoes (16, 145). Clearly, the trafficking of essential cargos by distinct p24 proteins is essential for metazoan development, even if the removal of all p24 proteins can be tolerated in a single eukaryote cell grown under laboratory conditions.

Importantly, all the cargo receptors in the ERGIC-53, p24, and Erv families continuously cycle between the ER and the ERGIC/Golgi. All contain cytoplasmically exposed motifs for interactions with COPI components for inclusion into COPII vesicles, as well as sorting motifs recognized by COPI components for inclusion into recycling COPI vesicles.

In contrast to the cargo receptors outlined above that are packaged into COPII vesicles, additional factors that do not enter COPII vesicles participate in traffic of specific cargos, such as the large, >300 nm in length fibrillar collagens. TANGO1 (transport and Golgi organization) factor (also called melanoma inhibitory activity member 3 or MIA3) is a transmembrane protein localized to ERES that was initially shown to be required for efficient exit of collagen VII from the ER (139). TANGO1 binds collagen VII within the ER lumen and links it to the Sec23/24 subunits of COPII within the cytosol and thus loads collagen VII into nascent COPII vesicles. The essential function of TANGO1 is underscored by the severe chondrodysplasia and death at birth of homozygous TANGO1-null mice (193). TANGO-null mice show defects in trafficking of all collagens examined (I, II, III, IV, VII and IX), but traffic of other components of extracellular matrix, such as fibronectin or aggrecan, is not impaired, indicating that TANGO1 is a cargo-selective “loader.” Recently, TANGO has been shown to interact with cTAGE5 (cutaneous T-cell lymphoma associated antigen 5), a transmembrane protein localized to ERES (140). Like TANGO1, cTAGE5 binds Sec23/24 subunits of COPII and is required for efficient secretion of collagen VII. Thus cTAGE5 might be a “co-loader” of collagen into COPII vesicles. Neither TANGO nor cTAGE5 homologs are detected in
yeast, suggesting that they do not represent the core COPII vesicle budding machinery.

Sorting of cargo into COPII vesicles is also regulated by the Sec23 subunit, which acts as a GAP for Sar1 and may proofread cargo sequestration into nascent vesicles (19). Sar1 GTPase activity is further stimulated by binding of Sec13/Sec31, which may ensure that complete COPII coats only assemble after sorting of cargoes into the nascent vesicle. GAP activity may also participate in the eventual disassembly of the COPII coat, although the details of uncoating remain under investigation.

Membrane deformation leading to the formation of COPII vesicles is likely facilitated by Sar1 and the Sec23/24 complex and then globally stabilized by the Sec13/31 complex. Sar1 has been shown to deform membranes in vitro (87) and has been suggested to facilitate vesicle scission in a manner analogous to that of dynamin, which mediates scission of clathrin-coated vesicles (7, 129). Vesicle separation requires the amphiphilic NH₂-terminal helix of Sar1 that inserts into the membrane and initiates membrane constriction (21, 87). The size of COPII vesicles varies and in vivo appears to range from 50 to 90 nm. The size is probably regulated by the Sec13/Sec31 subcomplex because Sec13/Sec31 heterotetramers alone can self assemble into various size cages (58). The potential to form different sizes of COPII vesicles may be important for accommodating different size cargo that needs to exit the ER.

The formation of ERES and packaging of cargo into COPII vesicles is regulated by additional factors. Depletion or inactivation of the peripheral membrane protein Sec16 causes a decrease in the recruitment of COPII components to ERES and causes cargo accumulation within the ER (74, 75, 171). Sec16 has been shown to directly bind all four subunits of the COPII coat and is believed to stabilize COPII on membrane during COPII vesicle formation (48, 61, 152, 171). Sec16 targets to ERES independently of COPII (74). Sec16 appears to function together with its direct interactor TFG1 (TRK-fused gene) (194). TFG1 also localizes to ERES, and its targeting depends on Sec16 since depletion of Sec16 inhibits TFG1 recruitment to ERES. Depletion of TFG1 causes cargo accumulation within the ER (194). Both Sec16 and TFG1 are general traffic facilitators and are likely to influence the trafficking of multiple if not all cargo out of the ER. Their function is fundamentally different from that of TANGO and cTAGE5, which are cargo-specific regulators of trafficking.

Another factor that may function to stabilize the COPII coat is p125A, a soluble protein that is stably associated with the Sec13/Sec31 heterotrimer in the cytosol (121). P125A also binds Sec23, but this interaction appears to occur exclusively on the membrane. p125A is enriched at ERES, and depletion of p125A does not block traffic but causes delays in ER export of multiple cargos. Thus p125A has been proposed to stabilize the binding of the Sec13/Sec31 outer coat to the sec23/sec24 inner coat and may have a general role in regulating COPII function.

Additional factors that control ERES structure and COPII vesicle formation include PCTAIRE kinase (126), p38 MAP kinase (185), phosphatidylinositol (155), phosphatidylinositol-4 kinases (23), and the calcium binding ALG-2 (18). However, the mechanisms through which these components regulate ERES and COPII vesicle formation remain under investigation.

The integrity of the COPII coating machinery is essential for proper development and physiology. Loss of the Sar1b isoform expression or function causes accumulation of large (200–400 nm) chylomicrons within the ER (157). Mutations in the SEC23A gene cause cranio-lenticulo-sutural dysplasia due to defects in packaging of collagens into COPII vesicles (57, 179). In contrast, mutations in the SEC23B gene cause congenital dyserythropoietic anemia type II, an autosomal recessive disease probably due to incorrect glycosylation or trafficking of components required for erythropoiesis (20, 149). The distinct phenotypes caused by mutations in the highly related Sec23a and Sec23b underscore the high level of specialization of the COPII export machinery for specific cargoes in specific cell types. Similar to the loss of Sec23a in humans, defects in Sec24d cause skeletal defects in zebra fish due to inhibition in the trafficking of type II collagen and matrilin (119, 141).

**Retrograde COPII vesicles.** COPII appears to be required at multiple stages of ER-to-Golgi transport. Initially, COPII was proposed to form vesicles for anterograde (forward) cargo transport (8, 122). However, subsequent studies implicated COPI in retrograde (from Golgi back to ER, and intra-Golgi) retrieval of proteins (90, 154, 164). In the currently accepted model, COPII vesicles may form from the ERGIC, from anterograde carriers as they move toward the Golgi, and from the Golgi cisterna. Such COPII vesicles may traffic all the way back to the ER or may transport cargo to the more proximal compartments (from trans- to medial-Golgi, from medial- to cis-Golgi, from cis-Golgi to ERGIC, and from ERGIC to ER).

The COPI coat is composed of heptameric (α, β, β’, γ, δ, ε, ζ) complexes called coatmers composed of two subcomplexes: a trimeric complex composed of α, β’, ε, and a tetrameric complex composed of γ, δ, ζ, and β (49, 187). Structural analysis of the α, β’, ε trimeric complex shows that it contains motifs similar to those in the Sec13/Sec31 complex that forms the outer layer of COPII-coated vesicles.
vesicles (86). Thus, the α, β, ε trimeric complex is proposed to form the outer layer of the COPI coat. This is also supported by the findings that the γ COP subunit has structural similarity to α and β adaptin proteins (71) that form the inner core of the clathrin coat. Thus the present understanding is that the γ, δ, ζ, β tetrameric complex forms the inner core and that the α, β, ε trimeric complex forms the outer layer of the COPI coat.

Two distinct isoforms of the γ (γ1 and γ2) and ζ (ζ1 and ζ2) subunits have been identified (22). The isoforms assemble into heptamers with the other five subunits, and analyses of coatomers in 3T3 cells indicate that ~50% of coatomers contain the γ1ζ1 isoforms, ~30% contain γ2ζ1, ~20% contain γ1ζ2, and <5% contain γ2ζ2 (14).

Significantly, the different γ and ζ isoforms (and by extension the COPI coats containing these proteins) show distinct subcellular localization (109). The majority (~70%) of COPI coats containing γ1 are localized within the cis-Golgi, whereas the majority (>60%) of COPI coats containing γ2 are found in the trans-Golgi. The majority (~80%) of COPI coats containing ζ2 are found in the cis-Golgi. The localization of ζ1-containing COPI coats has not been determined as yet due to the lack of isoform-specific antibodies. This differential distribution of γ and ζ subunits occurs within the context of almost equal distribution of the β subunit, which shows 55% localization to the cis-Golgi and 45% to the trans-Golgi (109). This raises the key question of whether the different COPI coats serve different transport routes and traffic different cargo between different compartments.

Structurally and functionally distinct COPI vesicles have been described by numerous immunoelectron microscopic studies. Analysis of COPI vesicles in the scale-producing alga Scherffelia dubia shows two types of COPI vesicles: those with a light interior and a thin ~11-nm coat, and those with darker content and a thicker ~18-nm coat composed of an inner layer and an outer diffuse halo (47). Significantly, the “thin” COPI vesicles are found exclusively in the proximity of the cis-Golgi and in the space between the cis-Golgi and the ERES, suggesting that they recycle materials from the cis-Golgi to the ER. In contrast, the “thick” COPI vesicles localize exclusively adjacent to medial- and trans-Golgi, suggesting that they mediate retrograde trafficking within the Golgi. Only the “thick” COPI vesicles contain the Golgi enzymes sialyl-transferase and mannosidase I. The implication of this finding is that COPI recycling to the ER occurs exclusively from the cis-Golgi, never from medial- or trans-Golgi.

The existence of a population of COPI vesicles that shuttles from the Golgi to the ER that is distinct from intra-Golgi COPI vesicles is also apparent in mammalian cells because immunoelectron microscopy shows that COPI vesicles containing the KDEL receptor are distinct from COPI vesicles carrying the Golgi SNARE GOS28 (122). The separation of ER-destined COPI vesicles from intra-Golgi COPI vesicles is also supported by in vitro assays reconstituting COPI vesicle budding that show the formation of a population of COPI vesicles containing p24 family members p24α2, p24β1, p24β1, and p24γ3 (ER-destined vesicles) and a population of COPI vesicles containing mannosidase II and the GOS28 SNARE (intra-Golgi vesicles) (85). The packaging of p24 proteins into COPI vesicles that are separate from COPI vesicles that carry mannosidase II has been confirmed in another study (100). These two populations of COPI vesicles could be further distinguished by their different tethering specificity (discussed below).

The involvement of COPI vesicles in anterograde trafficking, although currently out of vogue, is supported by experimental evidence. Immunoelectron microscopy showed COPI vesicles containing the anterograde cargos proinsulin and the viral glycoprotein VSV-G (122, 123). However, analogous analysis by a different group did not detect VSV-G in COPI vesicles (105). An in vitro assay reconstituting COPI vesicle budding from rat liver Golgi generated COPI vesicles that also contained the anterograde cargo polymeric IgA receptor (100). The complexity of the COPI vesicle populations remains to be explored, and the development of means to selectively isolate specific populations of COPI vesicles coupled with proteomic analyses is likely to define the cargos and the directions of distinct COPI classes.

COPI is recruited to membranes by activated ARFs. Based on amino acid sequence similarity, the six mammalian ARFs have been subdivided into three classes (79). Class I [ARF1, ARF2 (this ARF has been lost in humans), and ARF3] and class II [ARF4 and ARF5] localize to compartments of the secretory pathway and function therein. The sole class III ARF (ARF6) appears to be involved in endocytosis and actin dynamics (40).

COPI appears to be recruited to membranes by class I and II ARFs, as COPI dissociates from all cellular membranes when a GEF that activates these ARFs is inhibited by treatment with the inhibitor Brefeldin A (see below). However, it is likely that different ARFs regulate COPI recruitment to distinct compartments. This hypothesis is suggested by the slightly different localizations of the ARFs. For example, ARF1 seems more generally distributed, whereas ARF4 appears to be concentrated at the ERGIC (37). Additionally, siRNA-mediated depletion of different ARFs results in distinct effects on trafficking (184). ARFs recruit COPI coatomers by interacting directly with the β,
Like all small GTPases, ARFs cycle between a GDP-bound inactive state and a GTP-bound active state. ARF activation is mediated by a family of GEFs characterized by a conserved Sec7 domain (76). The ~200-amino acid Sec7 domain was initially identified in the yeast protein Sec7p, and it alone is sufficient for exchange of GDP for GTP on ARF in vitro. Eukaryotic ARF GEFs are divided into five families based on overall structure and domain organization: GBF/BIG, cytohesins, EFA6, BRAGs, and F-box.

GBF1 is the sole ARF activator required for COPI recruitment to membranes and COPI vesicle formation. Inactivation of GBF1 with the specific inhibitor Golgicide (GCA) (138) or RNAi-mediated depletion of GBF1 (103, 175) causes COPI dissociation from membranes. GBF1 has been shown to activate ARF1, ARF4, and ARF5, but not ARF3, despite strong similarity to ARF1 (102). GBF1 has been localized to ERGIC and Golgi where it co-localizes with COPI. GBF1 interacts with Rab1b, and this interaction appears to promote GBF1 recruitment to membranes (110). It is possible that GBF1 activates distinct ARFs at distinct compartments to facilitate formation of vesicles coated with different coatomers and containing different cargoes.

Sorting of type I transmembrane proteins that contain NH$_2$ termini within the ER lumen and COOH termini within the cytoplasm into COPI vesicles for recycling to the ER is mediated by two highly conserved motifs within the COOH terminus of their cytoplasmic domains: WXXW/Y/F and KXXX. These motifs are recognized by the $\alpha$, $\beta'$, $\gamma$, and $\delta$ subunits of COPI subcomplexes that mediate their incorporation into nascent COPI vesicles (38).

COPI-binding motifs are absent in all known Golgi glycosyl-transferases, all of which are type II proteins with cytosolic NH$_2$ termini. Thus glycosyltransferases must utilize a distinct way to be incorporated into recycling COPI vesicles. Recent studies showed that the cytosolic tail of the Kre2 $\alpha$-1,2-mannosyl transferase of the medial-Golgi binds Vps74p and that this interaction is required for the Golgi localization of this enzyme (148, 181). Importantly, Vps74p is not required for the Golgi localization of other Golgi proteins such as the multi-spanning Ret1p and Tvp15p. Thus Vps74 appears to be a COPI vesicle “loader” specific for only glycosyl transferases. Vps74 appears to bind the semi-conserved motif of F/L-L/V/I-X-X-RK that is found in the cytoplasmic NH$_2$ termini of the known 16 Golgi glycosyltransferases (181). Mutation of the FLS motif in Kre2p to AAA abolished Vps74 binding and abolished Kre2p localization to the Golgi, but it remains to be determined whether Vps74 sorts all glycosyl transferases into COPI vesicles. Vps74 links the glycosyl transferases to the COPI coat by binding to the Sec26p ($\beta$-COP) and the Ret2p ($\delta$-COP) of the coatamer (181). A mammalian homolog of Vps74p is GMx33a and GMx33b (also called GOLPH3, GPP34, and MIDAS), shown previously to bind Golgi glycosyl transferases (160, 195).

In addition to transmembrane proteins that recycle back to the ER by either directly interacting with the COPI machinery or using the Vps74 linker, soluble proteins that contain the KDEL COOH-terminal motif also are retrieved via COPI vesicles. The KDEL sequence is recognized by the KDEL receptor, a seven-span transmembrane protein that continuously cycles between the ER and the Golgi (180). KDEL receptor directly interacts with COPI components (99). Three human homologs of KDEL receptor exist (ERD21, ERD22, and ERD23) and appear to have overlapping but distinct substrate preferences (132). Cargo binding by KDEL receptors is regulated by differences in the luminal pH within the Golgi (binding is promoted by the relatively low pH of ~6.4) and the ER (dissociation is promoted by the higher pH of ~7.2) (144).

The formation of COPI vesicles also appears to involve the activity of ARF GAPs (78, 89, 96, 97, 117, 118, 131, 188). ARF GAPs are characterized by a conserved GAP domain of ~70 amino acids that includes a zinc finger motif and an invariant arginine residue. There are more than 30 genes with a predicted GAP domain in the mammalian genome. Most GAPs are large, multi-domain proteins that, in addition to inactivating ARFs, may function as scaffolds to regulate the assembly of large protein complexes. ARFGAPs are recruited to budding COPI vesicles by direct binding to active ARF, cytoplasmic tails of cargo proteins, and the coatamer (ARFGAP binds directly to the $\gamma$ and $\beta'$ subunits of COPI). These interactions generate a complex consisting of cargo, coat, ARF, and ARFGAP. ARFGAP activity appears to be stimulated by cargo and coat proteins and leads to increased GTP hydrolysis by ARF and release of ARF from the complex. This coordination between cargo, coats, and ARFGAP ensures that only vesicles containing cargo proteins are produced (64, 96). It remains unclear whether ARFGAP1 remains as a component of mature COPI vesicles, but it is accepted that GTP hydrolysis by ARF is insufficient to cause coat dissociation. The mechanisms that regulate COPI vesicle uncoating remain to be characterized. The existence of multiple GAPs to regulate ARF activity and the currently accepted model that GAPs do not persist on budded COPI vesicles is quite different from the Sec23 subunit of COPII coat that functions
as GAP for Sar1 and is incorporated into budded COPII vesicles.

A number of mammalian ARFGAPs have been localized to the Golgi including ARFGAP1 and the closely related ARFGAP2/3, ASAP2, ARAP1, and SMAP2. It remains to be determined whether distinct ARFGAPs regulate the formation of distinct COPII vesicles containing different sets of proteins.

Defects in COPI function cause disease or developmental abnormalities, highlighting the essential role of this coating machinery. A single amino acid substitution of Ile 422 to Thr in the \( \delta \) COP subunit close to the cargo-binding site causes coat-color dilution and ataxia due to degeneration of Purkinje cells in the cerebellum in mice (197). The mutation disrupts intracellular trafficking in melanocytes, but the exact cargoes that are mistargeted in the melanocytes and the brain remain to be identified. More importantly, the phenotypic severity varies in different tissues, suggesting that the trafficking of some cargoes is more affected or that some cargoes are more important to the animal’s well being.

Similar differences in phenotypic severity are observed in *Drosophila* with loss-of-function mutations in \( \gamma \) COP (65). Although \( \gamma \) COP activity is essential for embryonic viability, the most pronounced defects are observed in the morphogenesis of the epidermis and tracheal tubes. The tracheal defect correlates with defects in the secretion of the protein Piopio. This again stresses that phenotypic effects of removing components of general traffic machinery are surprisingly specific.

The function of \( \zeta \) COP isoforms has been examined indirectly in a study showing transcriptional silencing of the \( \zeta 2 \) isoform in a number of cancer cell lines of diverse origin (158). Interestingly, this makes the tumor cells exquisitely dependent on \( \zeta 1 \) function. Although normal cells show normal growth after depletion of either \( \zeta 1 \) or \( \zeta 2 \) (but not when both isoforms are depleted), tumor cells are killed when only \( \zeta 1 \) is depleted because they do not have \( \zeta 2 \). This suggests that \( \zeta 1 \) and \( \zeta 2 \) can compensate for each other’s function in cells in culture, although the effects of inactivating \( \zeta 1 \) or \( \zeta 2 \) in animals remain to be determined. Importantly, the strict dependence on \( \zeta 1 \) in cancer cells raises the exciting opportunity for developing anti-cancer therapeutics based on the solved structure of \( \zeta \) COP (202).

Additional regulators of COPI that cause disease also have been identified. Scy1-like (Scy1l) is a member of the Scy-like family of catalytically inactive protein kinases and has been shown to bind \( \beta \) COP (28). The binding of the cytosolic Scy1l to \( \beta \) COP is through a COOH-terminal RKLD sequence, similar to the KXXX motif present in transmembrane proteins recognized by the COPII machinery. Scy1l co-localizes with COPI in cells, and depletion of Scy1l causes defects in trafficking of the KDEL receptor. Mice with null mutations in the Scy1l gene exhibit neuromuscular atrophy and spinocerebellar ataxia (146).

The *Drosophila* homolog of GBF1 called Garz is essential for embryonic viability (FlyBase). Tissue-specific silencing of Garz within the salivary gland causes a severe disruption in gland development and alteration in polarity due to arrest in surface deployment of cell-cell and cell-matrix proteins required for morphogenetic events (174).

**Tethering and Fusion of COPII and COPI Vesicles**

The final stages of cargo delivery to the target membrane involve at least two events: recognition between the membranes of the cargo carrier and the target compartment, and overcoming the thermodynamic barrier to membrane fusion. The initial carrier-membrane interaction appears to be mediated by tethers, whereas the subsequent fusion of the bilayers is facilitated by SNAREs.

**Tethers.** A large family of tethering factors has been characterized (173, 192). Tethers are very diverse but can be divided into a group of coiled-coil proteins (known as golgins) (130) and a group of multi-subunit complexes (120). The coiled-coil tethers p115 (yeast Uso1p), GM130 (yeast Bug1), giantin, golgin-84, and CASP, and the multi-subunit complexes Dsl1, TRAPPI, TRAPPII, and COG are all involved in ER-Golgi trafficking.

**Tethering COPII vesicles.** Initial insight into the tethering mechanisms for COPII vesicles came from yeast. The yeast Uso1p was shown to tether COPII vesicles to yeast Golgi in an in vitro reconstructed system, and Uso1p is required for COPII vesicle tethering in vivo (10, 33, 34). Uso1p and its mammalian homolog p115 (described below) are large peripherally membrane-associated proteins that exist in vivo as parallel homodimers with two globular heads and a long coiled-coil tail (115, 151, 199). Interestingly, the tails consist of four coiled-coil motifs separated by proline-rich “hinges” that have been proposed to allow the accordion-style collapse of the tethers, perhaps to bring the vesicle into close proximity to the target membrane.

Recruitment of Uso1p to COPII vesicles appears to be regulated by the active form of the small GTPase Ypt1p (33). Uso1p presumably links the COPII vesicle to Golgi membrane by binding to a Golgi protein, but the exact binding partner on the Golgi is uncertain. Defects in Uso1 can be suppressed by overexpression of Ypt1 and the known ER-to-Golgi SNAREs (Bet1, Bos1, Sec22, and Ykt6), and the mammalian p115 binds SNAREs, raising the possibility that Uso1p might link COPII vesicles to Golgi membranes by binding to a Golgi SNARE.
In addition to Uso1p, other proteins participate in COPII vesicle tethering. Recent studies indicate that yeast Grh1p, a relative of GRASP65 (described below) also associates with COPII vesicles by binding the Sec23p/Sec24p subunits of the COPII coat (15). Grh1p appears to facilitate COPII vesicle tethering because Grh1p depletion inhibits tethering in an in vitro reconstituted assay (15). The exact mechanism by which Grh1p functions is unknown, but Grh1p binds the coiled-coil protein Bug1p (which is structurally similar to GM130, a known GRASP65 interactant), and it is possible that this interaction is involved in tethering. Bug1p is also required for COPII vesicle tethering in vitro (15). As is the case for Uso1p, the identity of the binding partner on the Golgi for Grh1p and Bug1p is unknown. It also remains to be determined whether Uso1p, Grh1p, and Bug1p function together in a common pathway or in separate pathways to facilitate COPII vesicle tethering. Based on findings from the mammalian system (see below), it is likely that they act together.

The mechanism for tethering COPII vesicles in mammalian cells is likely to involve p115, GM130, and GRASP65. However, the target of COPII vesicles in mammalian cells is less clear, and it is possible that COPII vesicles tether to pre-Golgi compartments, possibly ERGIC and maybe also the Golgi (3, 112, 196).

p115 localizes to COPII vesicles, and p115 recruitment to COPII vesicles is promoted by the active form of the mammalian Ypt1 homolog Rab1 (3). p115 also localizes to ERGIC and the Golgi, and this association is extremely dynamic (25). p115 binds the Golgi-localized coiled-coil tether GM130 (92), and it is possible that this interaction facilitates the tethering of COPI vesicles to Golgi membranes. GM130 is also present on ERGIC (104) and could mediate COPII vesicle tethering to that compartment. GM130 is a peripherally associated protein that is bound to Golgi membranes by the Golgi reassembly stacking protein of 65 kDa (GRASP65) (12). GRASP65 is N-myristoylated, and this mediates the tight association of GRASP65 with membranes (12). Thus p115 on incoming COPII vesicles may bind to GM130/GRASP65 tightly associated with ERGIC and Golgi membranes to tether COPII vesicles.

In addition to coiled-coil tethers, COPII vesicle tethering in yeast also involves the heptameric TRAPPI complex (30). TRAPPI is unique because it acts as a Rab GEF (32). Yeast TRAPPI activates Ypt1, whereas mammalian TRAPPI activates Rab1 (186). Yeast TRAPPI appears to tether ER-derived COPII vesicles through a direct interaction between the Bet-3 subunit of TRAPPI and the Sec23 subunit of COPII (30). The participation of the coiled-coil tethers and the heptameric TRAPPI appears to be sequential: TRAPPI binds to Sec23p on budded COPII vesicles and activates Ypt1/Rab1 on the vesicles, and this recruits Uso1p/p115 to the vesicles (30). Uso1/p115 then tethers COPII vesicles to Golgi, presumably by interacting with Bug1p/Grh1p/GM130/GRASP65.

**Tethering COPI vesicles.** Tethering COPII vesicles is significantly more complex because, unlike COPII vesicles that originate from a single compartment (the ER) and deliver cargo to a single compartment (the Golgi), COPII vesicles bud from many compartments and fuse with many compartments. Tethering of COPII vesicles to the ER appears to be mediated by the trimeric Dsl1 complex composed of the soluble proteins Dsl1, Dsl3, and Tip20 (107). Dsl1 localizes to the ER by stably associating with the ER SNAREs Ufe1, Use1, and Sec20 (107). Dsl1 has been shown to interact with the α and ε subunits of the trimeric subcomplex of the coatamer that forms the outer layer of the COPI coat (4, 5, 73, 133, 182). Thus interaction between Dsl1 and the outer layer of the COPI coat is optimal for “catching” recycling COPII vesicles and facilitating their fusion with the ER.

Intra-Golgi COPII vesicles appear to be tethered by multiple tethers, depending on their destination. The p115 tether has been proposed to mediate COPII vesicle tethering to cis- and perhaps medial-Golgi because p115 is restricted in its localization to those Golgi compartments (116). In support of the role at that level of the Golgi, p115 binds the cis-Golgi protein GM130 (stably associated with Golgi membranes through GRASP65) and the medial-Golgi transmembrane coiled-coil protein giantin (91, 163). p115 binds the β-COP subunit of COPI (66) and thus could directly tether COPII vesicles derived from medial-Golgi and to cis-localized GM130 and COPII vesicles derived from trans-Golgi to medial-localized giantin.

Golgin-84 is a transmembrane coiled-coil protein localized to medial-Golgi (13, 46). Overexpression or depletion of golgin-84 causes fragmentation of the Golgi (46) and causes a partial mislocalization of Golgi proteins into adjacent cisterna and accumulation of untethered COPII vesicles (161). However, injection of soluble golgin-84 into cells does not affect the retrograde movement of ERGIC53 from the cis-Golgi to the ER (100). Together, the data suggest that golgin-84 mediates tethering exclusively within the Golgi. Golgin-84 binds CASP, a coiled-coil protein localized to medial-Golgi (60). COPII vesicles generated in an in vitro budding assay contain golgin-84 and can be recovered on CASP-coated glass beads (100), strongly suggesting a tethering function.

Both p115 and golgin-84 appear to intersect with the octameric COG tethering complex (161, 162). COG localizes to the Golgi where it mediates the tethering of COPI vesicles that traffic in the
pears similar to the TRAPPII complex from yeast in mammalian TRAPPII has been described and appears within the endosomal system (31). A single TRAPPII complex appears to be largely at the TGN are TRAPPII-specific (36, 200). In yeast, the role of six of which are shared with TRAPPI and three that TRAPPII appears to be composed of nine subunits, COPI vesicle tethering within the Golgi. Yeast plex, TRAPPII, has been suggested to facilitate deleterious. fully assembled octameric COG complex are even a small decrease in cellular levels of the ical phenotype (54, 83). Overall, it is obvious that of COG subcomplexes caused a milder neurological disease with a fatal outcome in early infancy (94). Similarly severe phenotypes were observed in patients with a mutation in Cog7 that significantly decreased overall COG levels (111, 203). A 76-amino acid truncation of the COOH-terminus of Cog8 that prevents the assembly of heptameric COG complex but allows the assembly of COG subcomplexes caused a milder neurological phenotype (54, 83). Overall, it is obvious that even a small decrease in cellular levels of the fully assembled octameric COG complex are deleterious.

In addition to COG, another multi-subunit complex, TRAPPII, has been suggested to facilitate COPI vesicle tethering within the Golgi. Yeast TRAPPII appears to be composed of nine subunits, six of which are shared with TRAPPI and three that are TRAPPII-specific (36, 200). In yeast, the role of TRAPPII complex appears to be largely at the TGN and within the endosomal system (31). A single mammalian TRAPPII has been described and appears similar to the TRAPPII complex from yeast in that it also contains the TRAPPII-specific Trs130, Trs120, and Trs65 subunits (137). Mammalian TRAPPII localizes to the ERGIC and the Golgi, and within the Golgi appears enriched on COPI-coated buds and vesicles but not on cisternal elements. Depletion of Trs130 disrupts Golgi morphology and leads to the accumulation of vesicles adjacent to the Golgi. It also inhibits trafficking of VSV-G at the disrupted Golgi, suggesting that TRAPPII function is required within the Golgi after ER to Golgi step of transport. Importantly, the Trs130 and the Trs120 subunits of TRAPPII bind γ1 COP. This suggests that TRAPPII could directly link COPI vesicles to target membranes. However, TRAPPII has GEF activity for Rab1, and it is possible that TRAPPII functions within the Golgi to activate Rab1 (to allow recruitment of Rab1-dependent tethers such as p115), and is not a bona fide tether. All tethers are either long proteins or large assemblies that appear perfect for “catching” vesicles from a long distance. Coiled-coil tethering factors have been proposed to act by assembling tethering bridges that originate from vesicles and from acceptor membranes, whereas multi-subunit tethering factors localize to and act predominantly on the acceptor membranes (98). The model of tethers as molecular bridges has been refined, and it is possible that tethers interact with other components of the fusion machinery to directly facilitate fusion. Specifically, tethers have been shown to directly bind SNAREs and to facilitate the formation of SNARE pins as a prelude to fusion (45, 153, 156). Thus tethers are believed to accelerate a rate-limiting step in SNARE complex assembly. Tethers also bind Rabs, and this interaction may increase tethering specificity or position the Rab in close proximity to the SNAREs and promote their pairing. The exact role of Rabs in fusion is still debated, but the requirement for Rab function is absolute (29, 88). Tethers might represent molecular platforms that coordinate the function of Rabs and SNAREs (and perhaps other factors) in fusion.

SNAREs. Membrane-membrane fusion during vesicular traffic is facilitated by members of a large family of SNAREs (27, 52, 72). Members of the SNARE family are found on vesicles (v-SNAREs) and on target membranes (t-SNAREs), and fusion of the two opposing membranes occurs through pairing of cognate SNAREs. Assembly of SNARE complexes involves the formation of a 4-α-helix bundle, where one helix is contributed by the v-SNARE and three by t-SNAREs (53, 190). The interaction of v- and t-SNAREs on the vesicle and target membrane is described as the trans-configuration or trans-SNARE. This configuration brings the two membranes into sufficient proximity for membrane fusion. After membrane fusion, the v- and t-SNAREs are still together but now reside within the same membrane, a conformation referred to as cis or
cis-SNARE. The cis-SNARE complexes are disassembled in an ATP-dependent process through the action of the N-ethylmaleimide-sensitive factor (NSF) (113, 114, 124, 191, 201). This disassembly step regenerates the cellular pool of free SNAREs and allows repeated rounds of transport.

It is extremely difficult to define the membranes in which individual SNAREs function in ER-Golgi traffic because these proteins continuously cycle between compartments (39, 69, 77, 198, 207). A complex of syntaxin-5, Sec22b, membrin, and Bet1 appears to facilitate membrane fusion at early steps, probably catalyzing fusion of COPII vesicles with the ERGIC. A distinct complex composed of syntaxin-5, GOS28, Bet1, and Ykt6 acts later, perhaps to facilitate fusion of anterograde carriers at the cis-Golgi. A third complex composed of syntaxin-5, GOS28, Ykt6, and GS15 acts late within the Golgi, perhaps facilitating intra-cisternal traffic.

Future Directions

Despite intense interest and experimentally derived insights, many questions remain about protein trafficking and activity of proteins involved in this process. Some of the more intriguing points are listed below.

1) It remains unknown how many different types of COPII vesicles bud from the ER and whether different cargoes are packaged into different COPII vesicles. Bioinformatics of mammalian proteomes suggest that of the ~25,000 predicted open reading frames, ~11% (2,750) encode soluble secretory proteins and ~21% (5,250) encode transmembrane proteins destined for compartments of the secretory and endosomal systems (35, 81). Although not all genes are expressed in all cells, it is reasonable to predict that a typical cell must efficiently export thousands of proteins out of its ER by packaging them into COPII vesicles. How different cargos are handled for export in different cell types remains to be elucidated.

2) It is also unknown how many distinct types of COPII vesicles are generated from the Golgi and the ERGIC, and how all these vesicles are correctly targeted to the compartment that is proximal to their compartment of origin.

3) The increased complexity of the mammalian secretory pathway is paralleled by the evolution of isoforms for most of the key players, and there are multiple isoforms of the GEFs, GAPs, Sar/ARFs, and COPII and COPI subunits. However, how these different isoforms function in different tissues, different stages of traffic, and trafficking of select cargos remain to be determined.

4) The timing of coat disassembly and the in vivo triggers for uncoating remain to be characterized. Interestingly, the interactions between COPII and COPI coats and tethers (p115, Dsl1, COG, and TRAPP) suggest that tethering occurs before vesicles uncoat. Furthermore, Dsl1 has been shown to bind to sites involved in interactions between COPI subunits that stabilize the COPI coat (134, 147, 208). Thus Dsl1 might initiate the uncoating of COPI vesicles tethered to the ER. This raises the question of whether other tethers also may aid in uncoating, how coats are shed while bound to tethers, and how the uncoated vesicles are prevented from escaping.

5) Finally, one of the key questions in membrane trafficking involves upstream regulatory mechanisms. The secretory pathway responds to organismal demands for secretion during events such as development, immune challenge, lactation, etc. The signaling and transcriptional mechanisms that facilitate amplification of the secretory pathway are under active investigation, and analysis of early acting transcription factors required for the development of the salivary glands (an organ with extreme secretory capacity) in Drosophila uncovered a key role for a complex of homeotic transcription factors Scr/Exd/Hth (Sex Comb Reduced/Extra-denticle/Homothorax) (1). This complex directly regulates the CrebA transcription factor that alone is sufficient and necessary for upregulation of at least 34 genes involved in the biogenesis of the secretory pathway and secretion (56). CrebA not only induces expression of protein machinery involved in secretion at the ER-Golgi interface but also more than 20 known or predicted cargo proteins. Microarray analysis of HeLa cells expressing Creb3L1 (Creb3L1 and Creb3L2 are the mammalian orthologs of Drosophila CrebA) shows upregulated expression of genes involved in Golgi vesicle transport and secretion (56). Thus Creb3L1 can turn a HeLa cell that is not specialized for secretion into a cell that amplifies the secretory pathway. The important role of the CrebA-/Creb3-like transcription factors in regulating the secretory pathway is also underscored by the recent finding that a missense mutation in Creb3L2 (a zebra fish ortholog of CrebA/Creb3) decreases the expression of Sec23a, Sec23b, and Sec24d subunits of the COPII coat (108).

Recent advances have revealed significant insight into the complexity of membrane trafficking. Continuing molecular, structural, biophysical, and reconstitution studies in vitro and in vivo will further our understanding of how the trafficking machinery functions to facilitate efficient and high-fidelity transport of cargo through multiple secretory compartments.

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