Minireview

# Biogenesis of COPI-coated transport vesicles

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**Abstract Biosynthetic protein transport and sorting along the secretory pathway represents the last step in biosynthesis of a variety of proteins. Proteins destined for delivery to the cell surface are inserted cotranslationally into the endoplasmic reticulum (ER) and, after their correct folding, are transported out of the ER towards their final destinations. The successive compartments of the secretory pathway are connected by vesicular shuttles that mediate delivery of cargo. The formation of these carrier vesicles depends on the recruitment of cytosolic coat proteins that are thought to act as a mechanical device to shape a flattened donor membrane into a spherical vesicle. A general molecular machinery that mediates targeting and fusion of carrier vesicles has also been identified. This review is focused on COPI-coated vesicles that operate in protein transport within the early secretory pathway. Rather than representing a general overview of the role of COPI-coated vesicles, this mini-review will discuss mechanisms specifically related to the biogenesis of COPI-coated vesicles: (i) a possible role of phospholipase D in the formation of COPI-coated vesicles, (ii) a functional role of a novel family of transmembrane proteins, the p24 family, in the initiation of COPI assembly, and (iii) the direction COPI-coated vesicles may take within the early secretory pathway.**



*Key words:* Membrane traffic; Vesicular transport; Coatomer; Protein retrieval; Retrograde transport; Coated vesicle; ER–Golgi recycling

## 1. Biogenesis of COPI-coated vesicles

In all cases characterized in detail, the generation of a distinct transport vesicle from a donor membrane involves the recruitment of cytosolic coat proteins to the membrane [1,2]. The coat of COPI-coated vesicles is composed of the small GTPase ADP-ribosylation factor (ARF) [3] and of the seven subunits (α-, β-, β′-, γ-, δ-, ε- and ζ-COP [4,5]) of a stable cytosolic protein complex, coatomer [1]. As depicted in Fig. 1, the process of COPI vesicle formation can be dissected into three steps: (i) GTP-dependent binding of ARF to the donor membrane, (ii) bud formation concomitant with coat assembly due to recruitment of coatomer, and (iii) fission of a newly formed COPI-coated vesicle. A Golgi-localized guanine nucleotide exchange factor specific for ARF (ARF-NEF) initiates coat assembly by converting ARF-GDP into ARF-GTP. This step has been shown to be sensitive to the fungal metabolite Brefeldin A (BFA) [6,7]. Therefore, coat assembly is inhibited by BFA [8], resulting in inhibition of protein secretion [9]. Recently, proteins catalyzing nucleotide exchange onto ARF have been characterized at the molecular level [10–12]. Due to the nucleotide exchange, a conformational change in ARF is thought to take place that exposes a N-terminally bound myristic acid. As a consequence, ARF-GTP binds to the membrane as the first step in coat assembly. There is biochemical evidence that the stable binding of ARF to the membrane is assisted by a proteinaceous receptor [13] that remains to be identified. In the second stage of coat assembly, complete coatomer containing all seven subunits binds to the membrane [1,14]. This process depends strictly on the preceding binding of ARF-GTP [15,16] and, without any further requirement, leads to the formation of a COPI-coated bud [17,18]. In the presence of fatty acyl-CoA, periplasmic fusion drives fission of a newly generated COPI-coated vesicle [18,19]. The components that mediate this last step in vesicle biogenesis are not known.

While the general cytosolic machinery that buds COPI-coated transport vesicles from donor membranes is characterized in detail, a role for additional factors has been proposed in recent years. In the following, we discuss a possible role of phospholipase D1 (PLD1) in ARF-dependent budding of COPI-coated vesicles and the possible functions of a novel family of transmembrane proteins that has been implicated in vesicle budding.

## 2. A role for phospholipase D in the formation of COPI-coated vesicles?

PLD catalyzes the hydrolysis of phosphatidylcholine (PC) generating phosphatitic acid (PA) and choline. This process can be activated by extracellular agonists and is stimulated by guanine nucleotides [20], implicating the involvement of G-proteins. Surprisingly, a cytosolic factor that stimulates PLD activity was identified to be ARF [21,22]. PLD stimulation depends on GTP and is modulated by phosphatidylinositol-4,5-bisphosphate ($PIP_2$) [21]. Furthermore, a subpopulation of PLD was reported to be localized to Golgi membranes purified from a variety of cell lines [23]. Cell lines (e.g. PtK1 cells) have been analyzed that, in contrast to CHO cells, contain an ARF species that is tightly bound to the Golgi complex [24]. Consistently, these membranes exhibit a relatively high constitutive PLD activity. Using Golgi membranes from these cells, COPI-coated vesicles can be generated without adding exogenous ARF. [24]. Therefore, it was proposed that ARF might not be a stoichiometric coat component of COPI-coated vesicles but is required only for stimulation of PLD that, in turn, initiates coat assembly. In this concept, PA and $PIP_2$ contribute to the structural basis for coat protein recruitment whereas ARF is not required for coat binding itself. However, this view is in conflict with the following data. First, a direct interaction of ARF with the coatomer subunit β-COP

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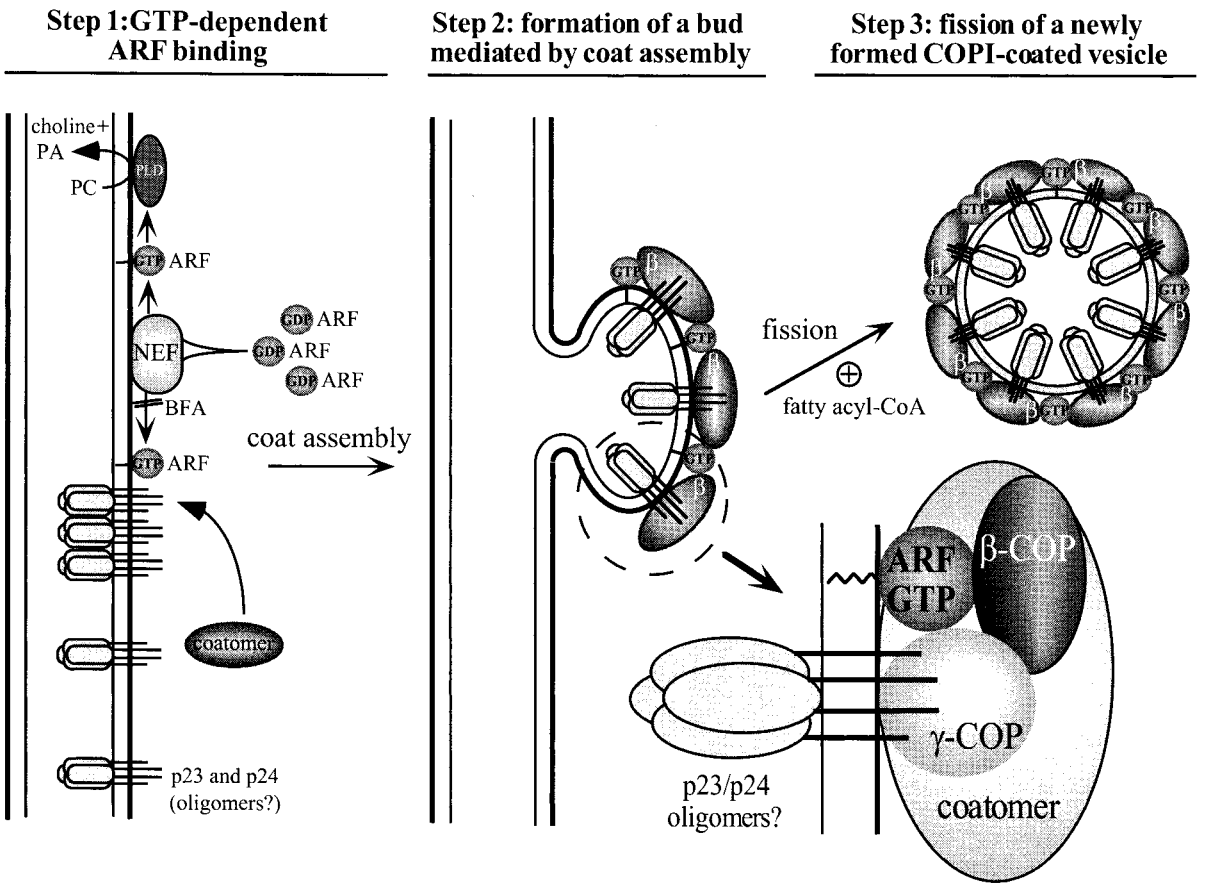


Fig. 1. Model for the biogenesis of COPI-coated vesicles. Vesicle formation is considered as a three-step procedure. (i) Following activation of ARF, coatomer becomes recruited to the membrane. Furthermore, ARF may activate PLD resulting in the generation of PA from PC. (ii) A novel family of transmembrane proteins, termed p24 family, forms a scaffold needed for efficient coat assembly. Polymerization of coatomer drives bud formation. (iii) In the last step, periplasmic fusion, a process that requires fatty-acyl-CoA, results in fission of a newly formed transport vesicle. ARF, ADP-ribosylation factor; BFA, brefeldin A; PA, phosphatidic acid; PC, phosphatidylcholine; PLD, phospholipase D. For further details see text.

has been demonstrated both in the Golgi and in purified COPI-coated vesicles [25]. Second, deletion of the *PLD1* gene in yeast results in cells growing quite normally [26], whereas deletion of every single coatomer subunit probed so far is lethal [27–30]. A second PLD activity (PLD2) with substantially distinct biochemical properties has been identified in yeast and mammals. Since this activity, in contrast to PLD1, is not able to catalyze transphosphatidylation using short-chain alcohols as acceptors it cannot be inhibited by ethanol [31]. Furthermore, this activity is almost exclusively found at the plasma membrane and cannot be stimulated by ARF [32]. Therefore, the finding that PLD1 gene disruption is not lethal cannot be explained with the presence of PLD2 activity. Third, ARF1 has been shown not only to recruit coatomer to the cisternal membranes of the Golgi but also to recruit the adaptor complex 1 (AP-1) to the trans-Golgi network [33,34]. Thus, ARF-dependent stimulation of PLD is not sufficient to explain recruitment of distinct coat proteins to distinct intracellular membranes initiated by ARF [35]. Fourth, ARF has been demonstrated to control COPI vesicle uncoating by hydrolyzing GTP [36] and, therefore, is needed as a component of the vesicles in order to allow coat disassembly prior to fusion with the appropriate target membrane. Fifth, although PA should be enriched in ARF/GTPγS-activated Golgi membranes and in purified COPI-coated vesicles, the amount of PA in Golgi membranes does not increase applying these conditions (Mark Stamnes and James E. Rothman, personal communication) and the amount of PC is not decreased in purified COPI-coated vesicles compared with their donor membranes (Britta Brügger and Felix T. Wieland, unpublished results). As both the direct contact site between ARF and β-COP [25] and the region in ARF that is responsible for PLD activation [37] have been identified, ARF mutants can now be constructed that can be analyzed with respect to their ability to stimulate PLD and to recruit coatomer. It appears possible that ARF, in addition to direct and GTP-dependent recruitment of COPI subunits to the Golgi membrane, plays a role by activation of PLD in order to rearrange the lipid environment to improve the conditions for COPI vesicle budding (see Fig. 1). An important role of lipids in the biogenesis of transport vesicles is also apparent from studies on secretory vesicle formation at the trans-Golgi network (TGN) [38,39]. Therefore, methods for sensitive and quantitative analysis of lipids like electrospray ionisation mass spectrometry [40] might be a clue for studying the role of lipids in vesicular transport.

### 3. Interactions of the COPI coat with transmembrane proteins

So far, of COPI- and COPII-coated vesicles only the coat components are well characterized at the molecular level and,

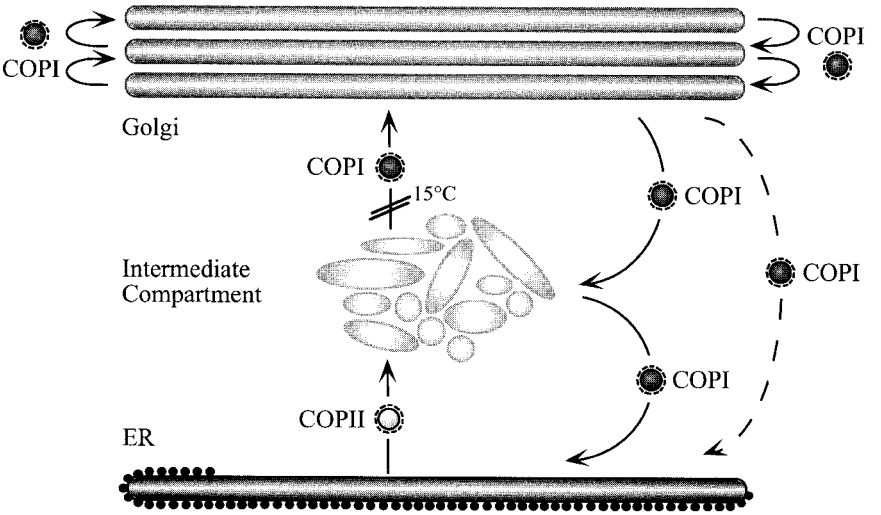


Fig. 2. Vesicular transport within the early secretory pathway of mammalian cells. While COPI-coated vesicles appear to be involved in several transport steps, COPII-coated vesicles are expected to exclusively mediate export of cargo proteins from the endoplasmic reticulum.

therefore, it has been a major question in the field whether proteinaceous coat receptors exist in the donor membrane that facilitate coat assembly. The first interaction described between COPI coat components and potential membrane components was the binding of coatomer to C-terminal KKXX motifs [41] known to function as ER retrieval signals in type I transmembrane proteins [42–44]. The identification of the subunit of coatomer that binds the KKXX motif has been a matter of controversy. On the one hand, a subcomplex containing α-, β′- and ε-COP was proposed to bind KKXX [45–47] whereas a study using photolabile peptide analogues demonstrated a direct interaction of γ-COP with the KKXX motif [48]. While the reason for this discrepancy is not yet clear, the data might reflect a rearrangement of coatomer in the course of its binding to the Golgi membrane. The KKXX motif may first contact γ-COP followed by shift of binding to an α,β′,ε subcomplex. However, it is also possible that the different experimental approaches taken contribute to these controversial findings. The interaction of coatomer with the KKXX motif has suggested a direct role of the COPI coat in retrograde transport from the Golgi to the ER, resulting in a now long-lasting discussion about the direction COPI-coated vesicles may take. This issue will be discussed later in this review.

The first membrane-spanning protein enriched in COPI-coated vesicles from mammals was identified recently and has been termed p24 according to its apparent molecular weight [49]. In yeast, the putative homologue of mammalian p24, Emp24p, was characterized [50]. p24 belongs to a family of type I transmembrane proteins with a single membrane span, a large lumenal domain and a short cytoplasmic tail. The p24 family is confined to seven structurally related homologues within yeast. Functional studies on p24 implied that it plays a role in the budding process of COPI-coated vesicles: a yeast strain carrying a temperature-sensitive allele of *N*-ethylmaleimide-sensitive fusion protein (NSF) shows accumulation of transport vesicles at the non-permissive temperature. When Emp24p is deleted in this strain, a significant reduction in vesicular structures is observed at the non-permissive temperature indicating that budding of transport vesicles is impaired [49]. Emp24p has been shown to be a component of COPII-coated vesicles indicating that p24 family members are not necessarily specific to a certain class of COP-coated vesicles. The absence of Emp24p in a yeast deletion mutant resulted in a secretion defect of only a subset of secretory proteins [50]. It has been suggested, therefore, that Emp24p plays a role in the sorting of cargo in that it serves as a cargo receptor that concentrates certain secretory proteins into COPII-coated buds. Interestingly, a subset of p24 family members contains a C-terminally located KKXX motif or a closely related sequence. A 23 kDa member of the p24 family (termed p23) carries a similar but not identical C-terminal motif, KKLIE, and has been shown to be a major membrane component of COPI-coated vesicles [51]. Immunological studies revealed that p23 is localized to the Golgi complex and concentrates into COPI-coated buds and vesicles when Golgi membranes are treated with ATP, GTPγS and cytosol, conditions that promote the formation of COPI-coated vesicles in vitro. Since p23, like other members of the p24 family, efficiently binds coatomer via its cytoplasmic tail, it is an excellent candidate for a protein involved in COPI assembly. From the above data, our current view of the interaction of coatomer with the Golgi membrane is depicted in Fig. 1. In a first step, β-COP binds to membrane-bound, GTP-activated ARF and, subsequently, coatomer interacts with the membrane via a second interface, formed by the cytoplasmic tails of p23 and p24. This binding is either mediated by the α,β′,ε subcomplex or γ-COP. The amounts of p23 present in COPI-coated vesicles compared to coatomer have been quantified and indicate a stoichiometry of 4:1 [51]. These data suggest that p23 interacts with coatomer in COPI-coated vesicles by forming an oligomer (as postulated in Fig. 1).

Although p24 does not carry a double-lysine motif, its cytoplasmic tail also binds to coatomer [47]. These results led to the identification of a second binding motif for coatomer, a double-phenylalanine motif. This (or a closely related) signal is present in all p24 family members and, in case of p24, mediates COPI binding in spite of the lack of a double-lysine motif. Interestingly, a peptide analogous to the cytoplasmic tail of p23 also depends on the double-F motif for coatomer

binding, because exchange of its double-K motif results only in reduced binding of coatomer, whereas exchange of its double-F motif completely abolishes binding, even when the double-K motif is still present. This characteristic of coatomer binding is clearly different from the binding of the ER retrieval motifs KKXX and KXKXX, where the lysines represent the essential amino acids. These data also suggest that coatomer binds to p24 proteins in two different ways either using the double-phenylalanine or double-lysine signal. In fact, coatomer subcomplexes generated from salt-dissociated coatomer interact differentially with p24 tails dependent on the presence or absence of the double-lysine motif [47]. p24 proteins bearing a double-lysine motif specifically bind an α,β′,ε subcomplex (termed B subcomplex), whereas those with solely the double-phenylalanine motif bind a β,γ,ζ subcomplex (termed F subcomplex), both in small but detectable amounts.

As mentioned above, p24 proteins were also implicated in the process of cargo sorting. This hypothesis is based on the finding that gene disruption of Emp24 in yeast results in transport defects of a subset of secretory proteins [50,52]. Furthermore, at the level of the ER, proteins destined for export are thought to become concentrated into departing transport vesicles while residents of the ER are excluded [53], indicating the existence of a machinery involved in active packaging of cargo molecules. Later in the secretory pathway, secretory proteins are not further concentrated [53,54] indicative of a bulk flow mechanism [55]. While p23 is mainly localized to the Golgi [51], some p24 proteins appear to be present in the ER as well [50,52,56] The lumenal domains of p24 proteins have a predicted propensity to form coiled-coil structures [49] known to be involved in protein–protein interactions. Therefore, p24 proteins in the lumen of the ER might be involved in the selection of soluble cargo molecules in order to mediate concentration into transport vesicles. On the other hand, protein–protein interactions mediated by coiled-coil motifs are known to be rather stable and might be the basis of hetero-oligomer formation of p24 proteins [52].

ER-resident membrane proteins that have escaped from the ER may be retrieved by a direct interaction with COPI coat proteins at the level of the Golgi. Thus, cargo might interact with coat components either directly or indirectly via cargo-coat receptors. Another family of putative cargo receptors comprises intracellular lectins like ERGIC53 [57] and VIP36 [58]. ERGIC53 has been shown to be a mannose-specific lectin [59] that constitutively cycles through the early secretory pathway implicating a role in sorting of mannose-containing cargo molecules. In contrast, VIP36 cycles between the TGN and the plasma membrane and might be involved in the sorting of GPI-anchored proteins at the level of the TGN. Like ERGIC53, p23 also cycles through the early secretory pathway [77]. However, a possible involvement of p24 proteins and members of the ERGIC53/VIP36 family of intracellular lectins in the sorting of any type of cargo molecule remains to be established on the basis of direct interactions with proteins destined for packaging into transport vesicles.

## 4. Balance of anterograde and retrograde transport between the ER and the Golgi complex

An overview of the possible roles of COP-coated vesicles in protein transport within the early secretory pathway is given in Fig. 2. COPI-coated vesicles have been identified as mediators of intracisternal protein transport through the Golgi stack reconstituted in vitro [54,60,61]. The anterograde nature of these vesicles was confirmed by the detection of appropriate markers like VSV–G-protein in COPI-coated vesicles generated from Golgi membranes that have been isolated from VSV-infected CHO cells [3,62]. More recently, transport of secretory proteins between the ER and the Golgi was also shown to involve the COPI coat [63,64].

These data were further strengthened by a detailed immunocytochemical study demonstrating that VSV–G-protein is localized in COPI-coated vesicles formed after a short release from a 15°C transport block [65], a condition that traps transported species in the so-called intermediate compartment. These data strongly suggest a direct role of COPI-coated vesicles in anterograde transport from this intermediate compartment to the Golgi apparatus. By use of an in vitro assay that reconstitutes ER to Golgi transport in semi-intact cells a sequential coupling of COPII and COPI-coats has been proposed [66]. More recently, these data were extended by the development of a two-stage in vitro assay based on the formation of transport vesicles from a microsomal fraction which are then isolated and fused with the Golgi apparatus to reconstitute ER to Golgi transport [67]. By use of mutants (either arrested in a GTP or GDP form) of both Sar1, a small GTP-binding protein similar to ARF involved in COPII coat recruitment, and ARF the authors conclude that transport of VSV–G-protein out of the ER is exclusively mediated by COPII-coated vesicles, whereas COPI is required at a later step in anterograde ER to Golgi transport. A role of COPI in anterograde biosynthetic transport is also supported by studies applying yeast genetics. Several coatomer subunits have been identified as *Sec* genes because temperature-sensitive alleles exhibit defects in secretory function at the non-permissive temperature [27,68]. More direct proof for a role of COPI in anterograde transport in yeast came from a biochemical study. In the presence of purified coat components both COPI- and COPII-coated vesicles were shown to form independently from purified ER membranes in vitro [69]. The two types of coated vesicles represent distinct populations that carry the appropriate targeting molecules needed for correct delivery to the Golgi apparatus. Moreover, formation of COPI-coated vesicles was inhibited by brefeldin A (BFA) whereas COPII vesicle biogenesis was unaffected. Both vesicle types were devoid of ER residents. Taken together, these data indicate that in yeast COPI- and COPII-coated vesicles mediate independent transport routes from the ER to the Golgi. Although typical yeast proteins destined for transport to the cell surface (i.e. yeast α-factor) were exclusively found in COPII-coated vesicles, there were several other polypeptides detected present only in COPI-coated vesicles [69]. The identity of these proteins remains to be clarified in order to decide whether a distinct set of cargo proteins is exclusively exported by COPI-coated vesicles. An alternative view considers COPII-coated vesicles as the carriers for exit of secretory cargo proteins from the ER, and COPI-coated vesicles to move bidirectionally between the ER and the Golgi, carrying ER-escaped proteins, as well as components of the machinery (e.g. targeting proteins) for both anterograde and retrograde transport [70]. However, in yeast a possible role of an intermediate compartment in ER to Golgi transport remains to be established in order to clarify the role of COPI-coated vesicles in this anterograde transport step.

Evidence for a possible involvement of COPI components in retrograde transport came from work demonstrating that coatomer can bind to the C-terminal ER retrieval motif KKXX [41] found in numerous ER-resident type I transmembrane proteins [71]. This prediction was further validated by a functional approach demonstrating that several COPI mutants are deficient in retrograde transport from the Golgi to the ER in yeast [29,45]. In addition, the mammalian KDEL-receptor was shown to be a major component of COPI-coated vesicles, indicating an involvement of these vesicles in the retrieval of lumenal ER residents [72]. It is not yet clear whether retrograde protein transport from the Golgi back to the ER involves the intermediate compartment or whether proteins can be transported directly to the ER.

Despite a long list of data that indicate a direct role of COPI-coated vesicles in biosynthetic protein transport through the early secretory pathway [3,27,54,60–69], it was proposed that COPI-coated vesicles might operate exclusively in retrograde transport from the Golgi to the ER [73]. This view is mainly based on yeast genetics and explains anterograde transport defects by an indirect mechanism assuming that mutations of COPI components in yeast or microinjection of antibodies directed against COPI components into mammalian cells may affect retrograde transport. In this view, anterograde transport would be inhibited due to a preceding breakdown of retrograde transport resulting in a depletion in the ER of machinery needed for the formation of anterograde carriers. Indeed, work based on yeast genetics has provided evidence supporting this model [74,75]. However, this model is in striking contrast with data mentioned above and has, therefore, stimulated new investigations on the role of COPI-coated vesicles. In morphological studies based on cryoimmuno-electron microscopy of whole-cell sections of pancreatic cells proinsulin was used as an anterograde marker, and the KDEL receptor was used as a retrograde marker. The results indicate that two independent populations of COPI-coated vesicles exist, either containing the anterograde or the retrograde marker molecule [76]. In mammalian cells it appears most likely that COPI-coated vesicles mediate anterograde protein transport from the intermediate compartment to the Golgi as well as through the Golgi apparatus, whereas COPII-coated vesicles mediate protein export from the ER (Fig. 2). Retrograde transport may depend exclusively on COPI-coated vesicles. In yeast, the situation is less clear, which is in part due to the lack of characterization of an intermediate compartment between the ER and the Golgi.

## 5. Conclusions

While the basic cytosolic machinery for the formation of COPI-coated vesicles has been revealed within the past 10 years, the membrane proteins involved in budding are now being defined. A major type I membrane protein of COPI-coated vesicles exhibits the characteristics expected for a Golgi-localized coatomer receptor. It will be interesting to elucidate the molecular mechanisms that underlie the interaction of the cytosolic and the membrane machinery resulting in bud formation. In addition, a role of PLD1 in this process still remains to be established by a quantitative assessment of PA in donor and COPI vesicle membranes. Specifically, it will also be interesting to establish the molecular mechanisms that allow coatomer to act as a mechanical device to deform the flattened donor membrane to produce a spherical transport vesicle. Another key issue will be to elucidate the mechanisms that underlie the packaging of cargo into anterograde and retrograde transport vesicles.

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