Vesicular transport: the core machinery of COPI recruitment and budding

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Summary

Vesicular transport is the predominant mechanism for exchange of proteins and lipids between membrane-bound organelles in eukaryotic cells. Golgi-derived COPI-coated vesicles are involved in several vesicular transport steps, including bidirectional transport within the Golgi and recycling to the ER. Recent work has shed light on the mechanism of COPI vesicle biogenesis, in particular the machinery required for vesicle formation. The new findings have allowed us to generate a model that covers the cycle of coat recruitment, coat polymerization, vesicle budding and uncoating.

Key words: Coatomer, ARF, p24 proteins, Vesicular transport, Coat assembly, Protein secretion, Golgi

Introduction

Three kinds of transport vesicle have been functionally characterized at a molecular level and can be defined by both their membrane origin and their coat proteins (Kirchhausen, 2000). Clathrin-coated vesicles are formed from both the plasma membrane and the trans-Golgi network and mediate vesicular trafficking within the endosomal membrane system (Schmid, 1997). COPI-coated vesicles and COPII-coated vesicles are transport intermediates of the secretory pathway (Rothman and Wieland, 1996; Schekman and Orci, 1996; Barlowe, 1998). COPII vesicles emerge from the endoplasmic reticulum (ER) in order to export newly synthesized secretory proteins towards the Golgi (Schekman and Orci, 1996; Barlowe, 1998). COPI vesicles instead appear to be involved in both biosynthetic (anterograde) and retrograde transport within the Golgi complex (Orci et al., 1997), as well as mediating the recycling of proteins from the Golgi to the ER (Cosson and Letourneur, 1994; Letourneur et al., 1994; Sönnichsen et al., 1996).

COPI vesicles have been used widely as a model system to study the molecular mechanism of transport vesicle biogenesis since they can be generated from purified Golgi membranes in vitro and isolated in appreciable amounts (Malhotra et al., 1989; Rothman, 1994). Here, we discuss the basic molecular components of COPI vesicles and their coordinated interplay in coat assembly and disassembly in the context of results obtained from various in vitro systems that reconstitute COPI vesicle biogenesis.

Recruitment of COPI to Golgi membranes

As depicted in Fig. 1, the coat structure of COPI vesicles consists of the heptameric coatomer protein complex, and the small GTPase ADP-ribosylation factor 1 (ARF1) (Rothman and Wieland, 1996). Coatomer and GDP-bound ARF1 (ARF1-GDP) are soluble cytosolic factors that are recruited to Golgi membranes in a GTP-dependent manner (Donaldson et al., 1992a; Palmer et al., 1993), and this initiates COPI-dependent vesicle budding (Rothman, 1994). GTP loading of ARF1 is catalyzed by a guanine-nucleotide-exchange factor (GEF) that is inhibited by the fungal metabolite brefeldin A (BFA) (Donaldson et al., 1992b; Helms and Rothman, 1992). BFA causes a redistribution of COPI components to cytoplasm, a breakdown of COPI vesicle formation and a redistribution of Golgi enzymes into the ER (Lippincott-Schwartz et al., 1989; Donaldson et al., 1990; Orci et al., 1991). Several ARF-specific GEFs have been described at the molecular level and can be classified as BFA resistant or BFA sensitive (Jackson and Casanova, 2000). Currently, which of the BFA-sensitive ARF-GEFs promotes COPI vesicle formation is not clear.

Nucleotide exchange on ARF1 has widely been regarded to be the initial step of COPI coat assembly (Rothman, 1994). However, recent data demonstrate that ARF1-GDP interacts with the Golgi in a specific manner (Gommel et al., 2001) and that this interaction precedes nucleotide exchange. On the basis of crosslinking studies as well as ARF1-binding experiments employing native Golgi membranes, p23, a type I transmembrane protein known to play a key role in COPI coat assembly (Sohn et al., 1996; Bremser et al., 1999) was identified as an ARF1-GDP receptor (Gommel et al., 2001). p23 belongs to the p24 protein family, members of which share common structural features such as double lysine or double arginine residues in their cytoplasmic tails (for a review, see Emery et al., 1999). While the first member was identified in 1991 (Wada et al., 1991), the existence of a family of related proteins was reported in 1995 by Rothman and colleagues (Stamnes et al., 1995). Currently, six family members have been identified in higher eukaryotes, whereas eight are known in yeast (Emery et al., 1999). The identification of p23 as an ARF-GDP receptor (Gommel et al., 2001) is consistent with recent in vivo studies demonstrating energy transfer between p23-CFP and ARF1-YFP in living cells, an interaction detected only under conditions that allow ARF-mediated GTP hydrolysis (Majoul et al., 2001). Peptide-mapping studies

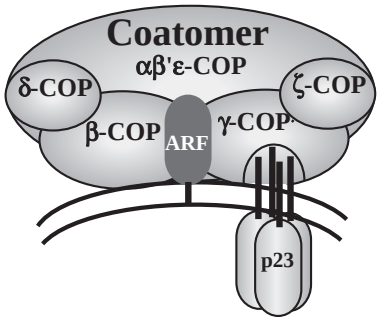

Fig. 1. Coat protein composition of COPI vesicles. The COPI coat consists of a heptameric protein complex termed coatomer and the small GTPase ARF1. Established subcomplexes as well as known interactions between the coat components are indicated.

revealed that the p23-interacting domain is located within the C-terminal 22 residues of ARF1 (Gommel et al., 2001).

Since GEF-catalyzed nucleotide exchange on ARF1 can take place in the presence of liposomes lacking any proteinaceous factors (Beraud-Dufour et al., 1999), the ARF1-GDP-p23 interaction cannot be a prerequisite for nucleotide exchange. However, in the context of a native membrane, p23 might direct ARF1-GDP to subdomains of the Golgi that are active in COPI vesicle formation and thus to its GEF. In addition, ARF-mediated GTP hydrolysis is required during early stages of the budding process to allow efficient uptake by COPI vesicles of various cargo molecules (Nickel et al., 1998b; Malsam et al., 1999; Pepperkok et al., 2000). Interestingly, ARF-mediated GTP hydrolysis is differentially affected by members of the p24 protein family and this is implicated in their sorting into distinct classes of COPI vesicle (Goldberg, 2000). Thus, ARF1-GDP is likely to be produced continuously during the budding process. Since it is only ARF-GTP that stably interacts with the membrane [through an exposed myristic acid residue covalently attached to the amphipathic N-terminus of ARF1 (Goldberg, 1998)], some mechanism must efficiently retain ARF1-GDP in the budding zone. Since p23 belongs to the core machinery of COPI budding (see below), the observed interaction between ARF1-GDP and p23 may well have such a function.

Upon nucleotide exchange, ARF1-GTP dissociates from p23 (Gommel et al., 2001). As a result, two binding sites for coatomer are generated, on ARF1-GTP (Zhao et al., 1997; Zhao et al., 1999) and p23 (Sohn et al., 1996; Dominguez et al., 1998). p23, and p24, another member of the p24 protein family (Stamnes et al., 1995), exist in various oligomeric forms (Füllekrug et al., 1999; Gommel et al., 1999; Marzioch et al., 1999), which might be important for COPI vesicle formation (see below). Despite the abundance of p23 and p24 in the Golgi, coatomer binding strictly depends on the preceding activation and membrane recruitment of ARF1 (Donaldson et al., 1992a; Palmer et al., 1993). In principle, at least two scenarios would be consistent with this observation. First, activated and thus membrane-associated ARF1 could simply change the equilibrium between cytosolic and Golgi-associated coatomer pools on the basis of its GTP-dependent direct interaction with coatomer (Zhao et al., 1997; Zhao et al., 1999). In this case p24 proteins in the Golgi would be constitutively active in binding coatomer; however, ARF-GTP recruitment would efficiently redistribute coatomer from the cytosol to the membrane. A second possibility would be that binding of ARF-GTP to the Golgi initiates a process that converts p23/p24 oligomers from an inactive state into a state active with regard to coatomer binding activity. Such a mechanism is supported by the fact that ARF-GDP binds to p23 and, upon nucleotide exchange, dissociates from p23 (Gommel et al., 2001), suggesting that multiple cycles of ARF-GDP-p23 complex formation, nucleotide-exchange-mediated dissociation and GTP-hydrolysis-mediated regeneration of ARF-GDP might act on the oligomeric status of p23/p24 complexes. A possible structural basis would be an ARF-GTP-dependent rearrangement of p23/p24 oligomers – for example, the conversion of homooligomers into heterooligomers. Such a process could be driven by cycles of GDP-for-GTP exchange on ARF and GTP hydrolysis (Fig. 2, grey box), a process known to be required for later stages of COPI vesicle biogenesis (Nickel et al., 1998b; Malsam et al., 1999; Pepperkok et al., 2000). It is of note that both working models are based on a bivalent interaction of coatomer with the membrane as well as explain why binding to Golgi membranes of coatomer strictly depends on the preceding activation and membrane binding of ARF1 (Donaldson et al., 1992a; Palmer et al., 1993).

COPI polymerization

Following recruitment of the coat components, a mechanical force is needed to convert the flattened donor membrane into a spherical transport vesicle. Ultrastructural studies (Orci et al., 1986; Malhotra et al., 1989) indicate that coatomer and ARF1-GTP polymerize to form a defined macromolecular structure that has a particular geometry likely to govern the size of COPI vesicles. Through interactions between the coat and the underlying membrane, coat protein polymerization must have a profound impact on the shape of the membrane, resulting in the formation of a vesicular structure.

How exactly is coat polymerization triggered and does this process involve a conformational change in the coat protein itself? These questions have been addressed by in vitro experiments employing purified coatomer and synthetic peptides that correspond to the cytoplasmic domains of the coatomer-binding proteins p23 and p24 (Sohn et al., 1996; Dominguez et al., 1998). As a control peptide, the cytoplasmic domain of the yeast protein Wbp1 (te Heesen et al., 1992) was used in these studies because it is structurally and functionally related to some p24 proteins in its C-terminal sequence and binds coatomer. In the case of Wbp1, an ER-resident protein, coatomer binding allows its retrieval from the Golgi to the ER (Cosson and Letourneur, 1994; Letourneur et al., 1994) and thus makes the protein cargo rather than machinery. The various peptides were designated p23-CT, p24-CT and Wbp1-CT (with CT for cytoplasmic tail) in order to reflect their origin. Low concentrations of preformed homodimers of p23-CT and p24-CT peptides promote aggregation of soluble coatomer (Reinhard et al., 1999). By contrast, dimeric Wbp1-CT has no such effect, which is consistent with a non-machinery nature of Wbp1. Dimeric p23-CT and p24-CT spontaneously form stable tetramers with a defined secondary structure as determined by mass spectrometry and NMR (Fligge et al., 2000; Weidler et al., 2000). This observation is

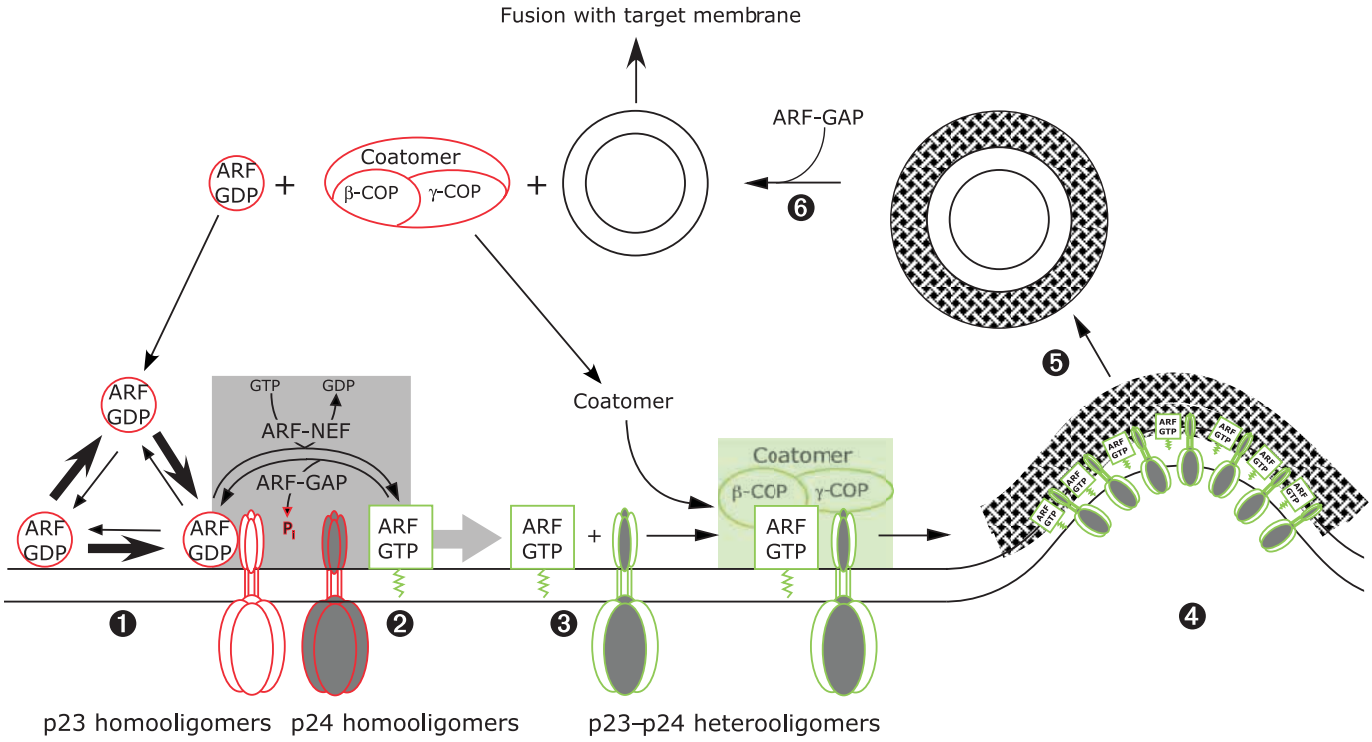

Fig. 2. The core machinery of COPI recruitment to membranes, coat polymerization, vesicular budding and uncoating. Recruitment of coat proteins is initiated by ARF-GDP binding to p23 (1). Upon nucleotide exchange, ARF-GTP dissociates from p23 resulting in its stable association with the membrane (2). Multiple cycles of GTP hydrolysis and GDP to GTP exchange are likely to occur (grey box, see text for details), possibly causing rearrangements of p23/p24 oligomers (3). The products of these processes are ARF-GTP and presumably a p23/p24 heterooligomer, which triggers coatomer binding and coat polymerization (4). Following budding (5), the catalytic domain of ARF-GAP is sufficient to trigger uncoating (6). Active components are shown in green; inactive components are shown in red.

consistent with the 4:1 stoichiometry of p23-CT to coatomer determined in aggregates (Reinhard et al., 1999); the same ratio is also found in native COPI-coated vesicles (Sohn et al., 1996). Interestingly, limited proteolysis revealed that coatomer aggregation is accompanied by a conformational change in γ-COP, the coatomer subunit that directly contacts p23 (Harter and Wieland, 1998; Reinhard et al., 1999). This study has established two conformations of coatomer: that of the soluble coatomer; and that of coatomer in COPI-coated vesicles or aggregates. Coatomer aggregation in the presence of tetrameric p23-CT thus appears to be related to COPI coat polymerization on native membranes, which suggests that the interaction of p24 proteins with coatomer is a critical trigger of COPI coat assembly.

Interestingly, preformed p23-CT–p24-CT heterodimers appear to stimulate coatomer aggregation even more efficiently than do p23- or p24 homodimers (C. Reinhard and F.T.W., unpublished). This observation might indicate that a specific configuration of p23/p24 oligomers is required for the initiation of COPI coat assembly (Fig. 2). As discussed above, binding of coatomer to Golgi membranes requires prior activation and membrane binding of ARF1. Therefore, it is conceivable that rearrangements of p23/p24 oligomers occur during the overall process of COPI coat assembly, which might be driven by multiple cycles of GDP-for-GTP exchange on ARF1 and ARF1-mediated GTP hydrolysis, a process known to be essential for loading of cargo molecules into COPI vesicles (Nickel et al., 1998b; Malsam et al., 1999; Pepperkok et al., 2000). However, it has to be pointed out that other models would explain ARF-dependent coatomer binding to Golgi membranes equally well (see previous section) and, therefore, at the present time this model remains entirely speculative.

A minimal machinery sufficient for COPI coat assembly, budding and uncoating

The in vitro budding assay that reconstitutes COPI vesicle biogenesis makes use of native Golgi membranes with a complex mixture of proteins and lipids. But are the factors described above sufficient to drive COPI vesicle formation? To address this question, a COPI budding assay employing purified coat proteins and liposomes with a defined molecular composition as donor membranes has been developed (Bremser et al., 1999). To simulate the presence of p24 protein cytoplasmic tails on the surface of these liposomes, synthetic peptides corresponding to their cytoplasmic domains were attached to a phospholipid to serve as a membrane anchor (Martin and Papahadjopoulos, 1982). When such lipopeptide-containing liposomes are incubated with purified ARF1, coatomer and GTP, numerous densely coated vesicles with a diameter of about 50 nm are observed by biochemical and ultrastructural analyses. A statistical analysis established a product precursor relationship demonstrating that the coated vesicles are indeed generated from large donor liposomes (Bremser et al., 1999).

Liposome-derived COPI-coated vesicles are closely related

to authentic Golgi-derived COPI-coated vesicles with regard to density, size and morphology. Strikingly, their formation does not depend on the lipid composition of the donor bilayer. Liposomes composed of only egg yolk phosphatidylcholine (PC) are sufficient for COPI vesicle formation, provided that the p23 lipopeptide is present. These data suggest that specific lipids are not essential for COPI recruitment to membranes. Interestingly, Spang et al. have reported experimental conditions that promote formation of COPI vesicles from protein-free liposomes, establishing a role for ARF-GTP and coatomer in shaping the donor membrane (Spang et al., 1998). The lipid composition used in this work, however, is highly unlikely to exist in a biological membrane. By contrast, p24 proteins not only bind to coatomer and alter its conformation but also are abundant residents of the intermediate compartment (IC) and the Golgi (Sohn et al., 1996; Rojo et al., 1997; Dominguez et al., 1998), the intracellular sites of COPI vesicle biogenesis (Griffiths et al., 1995; Orci et al., 1997). Therefore, we propose that the minimal machinery for COPI vesicle formation consists of p23/p24, ARF-GTP and coatomer (Fig. 2). While it is certainly possible that specific lipids (such as acidic membrane lipids) might influence the rate of vesicle budding under physiological conditions (De Camilli et al., 1996; Roth and Sternweis, 1997), they do not appear to be essential components of the core machinery required for the formation of COPI vesicles.

ARF-dependent GTP hydrolysis has been demonstrated to initiate COPI vesicle uncoating (Tanigawa et al., 1993). At the time, ARF1-specific GTPase-activating proteins were not characterized in molecular terms. The first ARF1-GAP open reading frame was identified by Cassel and colleagues followed by the characterization of its domain structure (Cukierman et al., 1995). Interestingly, the catalytic domain of ARF1-GAP alone is sufficient to uncoat synthetic COPI vesicles formed from p23-lipopeptide-containing liposomes in the presence of ARF1-GTP and coatomer (C. Reinhard and F. T. Wieland, unpublished). Although previous results demonstrated a requirement for GTP hydrolysis in the uncoating reaction (Tanigawa et al., 1993), the ability of the catalytic ARF-GAP domain to uncoat liposome-derived COPI vesicles indicates that this activity is sufficient to convert coated vesicles into naked vesicles. These data are consistent with Goldberg's finding that a tripartite complex of ARF1, ARF1-GAP and coatomer controls ARF-mediated GTP hydrolysis (Goldberg, 1999). As illustrated in Fig. 2, this observation adds to our picture of the core machinery needed to mediate a full round of COPI membrane recruitment, vesicular budding and coat removal, the latter process being a prerequisite for fusion with target membranes of transport intermediates.

In conclusion, available data support the view that p23 and p24 mediate COPI recruitment and coat assembly under physiological conditions. As p24 proteins are localized to the early secretory pathway (Sohn et al., 1996; Rojo et al., 1997; Dominguez et al., 1998), this would also be a plausible explanation for the observation that COPI-coated vesicle budding appears to be restricted to the IC and the Golgi (Griffiths et al., 1995; Orci et al., 1997). However, this view has been challenged by yeast genetic experiments in which all known p24 proteins were knocked out in a single strain. Severe transport phenotypes could not be observed and the cells exhibited a morphologically normal endomembrane system (Springer et al., 2000). These findings are even more surprising because a p23-knockout in mice is embryonically lethal at the earliest possible stage (Denzel et al., 2000), indicating that in mice p23 is essential. It is not yet clear why yeast cells have access to some kind of alternative mechanism, or whether additional factors cause lethality in a developing multicellular organism in the absence of p23. One could address this problem by studying protein transport in mammalian cells by employing RNA interference to temporarily inhibit p23 and p24 protein expression.

Future perspectives

The in vitro assays described here have revealed a core machinery mediating a complete cycle of COPI membrane recruitment, coated vesicle formation and uncoating. We have not taken into account the manifold and complex aspects of COPI vesicle function in the context of a living cell here. These, for example, include the uptake by COPI vesicles of specific sets of SNARE proteins required for targeting and fusion (Söllner et al., 1993; Weber et al., 1998; Nickel et al., 1999; McNew et al., 2000), with a recent paper by Spang and co-workers providing a possible mechanism for coupling of SNARE sorting and COPI coat assembly (Rein et al., 2002). Other aspects include the bidirectional nature of COPI vesicles (Orci et al., 1997) as well as a functional role for cytoskeletal and tethering elements in COPI transport (Orci et al., 1998; Sönnichsen et al., 1998; Fucini et al., 2000; Seemann et al., 2000; Valderrama et al., 2000; Valderrama et al., 2001; Shorter et al., 2002). Moreover, a large number of additional factors have been implicated in the overall process of COPI-dependent transport, including heterotrimeric G-proteins, Rab2, PKC, CDC42, Arp2/3, mAbp1 and chimaerins (Stow and Heimann, 1998; Fucini et al., 2000; Tisdale, 2000; Wu et al., 2000; Luna et al., 2002; Wang and Kazanietz, 2002). A major challenge for future work will be for us to integrate the partial reactions involving these factors to provide a complete picture of the function and regulation of this fascinating process.

We would like to stress that this review is focused on the molecular mechanisms of COPI vesicle biogenesis and, therefore, would like to apologize to those authors who have publications relevant to the overall process of COPI-dependent transport that were not cited in this article. Moreover, in many cases, review articles were cited instead of the original literature owing to space limitations. Work in the laboratories of the authors is supported by grants from the German Research Foundation (W.N., B.B. and F.T.W.), the Human Frontiers Science Program Organization (F.T.W.) and the Peter und Traudl Engelhorn Foundation (B.B.).

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