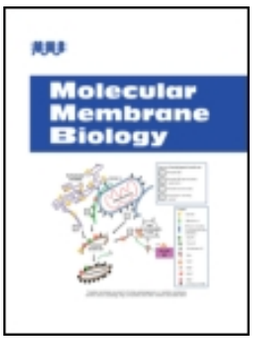

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ER-to-Golgi transport: COP I and COP II function (Review)

Rainer Duden*

Cambridge Institute for Medical Research, Department of Clinical Biochemistry, University of Cambridge, Hills Road, Cambridge CB2 2XY, UK

Summary

COP I and COP II coat proteins direct protein and membrane trafficking in between early compartments of the secretory pathway in eukaryotic cells. These coat proteins perform the dual, essential tasks of selecting appropriate cargo proteins and deforming the lipid bilayer of appropriate donor membranes into buds and vesicles. COP II proteins are required for selective export of newly synthesized proteins from the endoplasmic reticulum (ER). COP I proteins mediate a retrograde transport pathway that selectively recycles proteins from the *cis*-Golgi complex to the ER. Additionally, COP I coat proteins have complex functions in intra-Golgi trafficking and in maintaining the normal structure of the mammalian interphase Golgi complex.

Keywords: COPI, COPII, coatomer, Golgi complex, coat proteins.

Introduction

Protein secretion is an essential and evolutionarily conserved process in all eukaryotic cells, which contributes to cell surface growth. The eukaryotic exocytic pathway is organized into a series of distinct membrane-bounded compartments, namely the endoplasmic reticulum (ER), the intermediate compartment (IC) or vesicular-tubular clusters (VTCs) and the Golgi complex, through which newly synthesized proteins pass in a sequential and vectorial fashion (Palade 1975). Despite a large flux of material through the system, each compartment maintains a unique protein and lipid composition at steady-state. Traffic between these compartments is mediated by coated vesicular and tubular carriers (reviewed in Rothman and Wieland 1996, Schekman and Orci 1996, Kirchhausen 2000). Two distinct classes of evolutionarily conserved coat proteins, COP I and COP II, are crucial for directing the sequential transfer of material between the ER and the Golgi complex (see Figure 1). This mini-review focuses on progress made in the last 5 years in understanding of the diverse cellular functions of these fascinating protein machines.

COP II-coated vesicles mediate ER-to-Golgi transport

ER export of newly synthesized proteins is mediated by COP II-coated vesicles. The COP II coat was first identified in the yeast *S. cerevisiae*, using a powerful combination of yeast genetics and biochemistry (Novick *et al*. 1981, Baker *et al*. 1988, reviewed in Wuestehube and Schekman 1992, Schekman 2002). The components involved in COP II vesicle formation are essential for cell viability in yeast and conserved throughout all eukaryotes (Kaiser and Schekman 1990, Orci *et al*. 1991, Shaywitz *et al*. 1995, Stephens *et al*. 2000, and references therein). Elegant *in vitro* studies in yeast have identified the cytosolic components of the COP II coat, which comprise the small GTPase Sar1p, and two hetero-dimeric protein complexes, Sec23/24p and Sec13/31p (Barlowe *et al*. 1994, Schekman 2002, see Table 1). Vesicle budding is initiated when an ER-localized transmembrane protein, Sec12p, mediates the GTP-for-GDP exchange on Sar1p (Barlowe and Schekman 1993). GTP-loaded Sar1p then tightly binds to ER membranes, probably by embedding an N-terminal α-helix into the bilayer in a manner similar to that described for ARF1, the small GTPase acting in COP I-dependent traffic (Huang *et al*. 2001). Membrane binding of Sar1p-GTP allows recruitment of the Sec23/24 complex and subsequently of the Sec13/31p complex, which induces coat polymerization and membrane deformation into buds and vesicles (Barlowe *et al*. 1994). After budding, Sec23p acts as a Sar1p-specific GTPase activating protein (GAP), helping Sar1p to hydrolyse its GTP, which in turn triggers vesicle uncoating. The Sec13/31p complex, once recruited to the membrane and having induced membrane curvature, leads to increased Sar1p-GTPase activity mediated through the action of Sec23p. This built-in GTPase-activating protein makes the COP II coat intrinsically unstable (Antonny *et al*. 2001). Non-hydrolyzable analogues of GTP have, therefore, been used to isolate and characterize COP II-coated vesicles (Barlowe *et al*. 1994, see Figure 2(*A*) and (*B*)).

Sar1p-GTP, Sec23/24p and Sec13/31p constitute the minimal machinery for vesicle formation, since they suffice to form 60–70 nm COP II coated vesicles even from liposomes (Matsuoka *et al*. 1998). Biochemical studies indicate that Sec13/31p is a heterotetramer consisting of two copies each of Sec13p and Sec31p, thus displaying molecular properties consistent with its ability to trigger coat polymerization. X-ray crystallography, deep-etch rotary shadowing and electron microscopy have recently been used to visualize the structure of COP II subunits and the surface structure of COP II-coated vesicles. The Sec23/24p complex resembles a bow tie and the Sec13/31p complex has a flexible structure of 24–30 nm in length comprising a terminal bilobed globular structure bordering a central rod (Lederkremer *et al*. 2001, Bi *et al*. 2002). The surface view of vesicles revealed a coat built with polygonal units (Matsuoka *et al*. 2001).

Another protein essential for COP II vesicle formation is encoded by the *SEC16* gene. Sec16p is a 240 kDa hydrophilic protein which is essential for yeast viability and

*To whom correspondence should be addressed.
e-mail: rd217@cam.ac.uk

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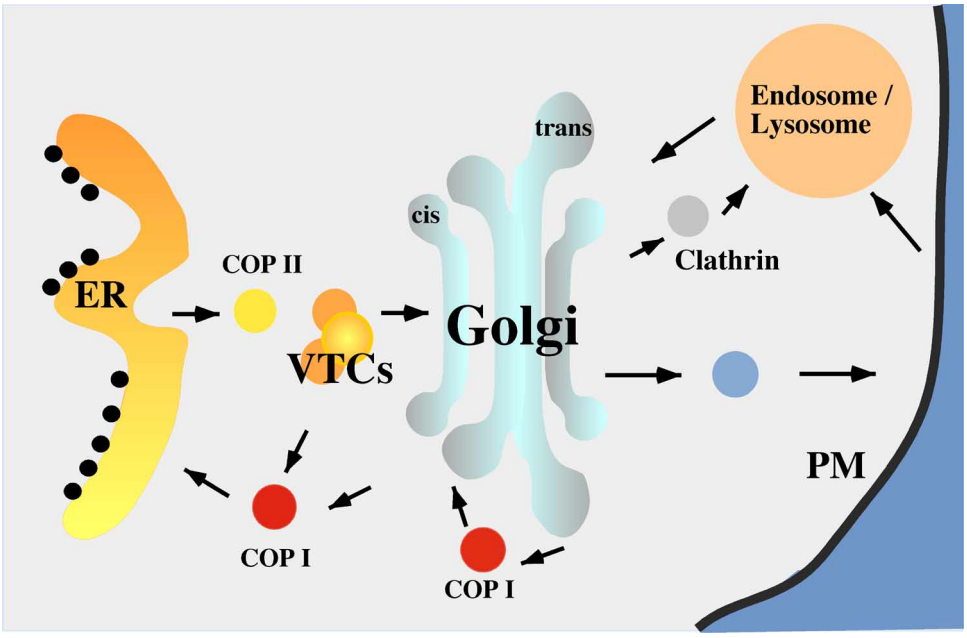

Figure 1. Schematic depiction of secretory membrane traffic pathways. Vesicular traffic pathways from the endoplasmic reticulum (ER) to vesicular-tubular clusters (VTCs), to and within the Golgi complex, to the endosome/lysosome system and to the plasma membrane are indicated (simplified scheme). Transport between the ER and the *cis*-Golgi is regulated by the COP II and COP I vesicle coats. VTCs = vesicular-tubular clusters. PM = plasma membrane. For details see text.

its function has been shown to be strictly required for COP II-dependent vesicle budding from the ER *in vivo* (Kaiser and Schekman 1990). However, Sec16p is tightly and peripherally bound to ER membranes and, thus, is not one of the cytosolic proteins required to drive COP II budding from ER membranes *in vitro* (Supek *et al*. 2002). Sec16p directly interacts with several components of the COP II coat, namely Sar1p, Sec23p, Sec24p and Sec31p (Espenshade *et al*. 1995, Supek *et al*. 2002, and references therein). Sec16p may organize an initial step in the assembly of the COP II

Table 1. Components of the COP II coat, COP I coat and its regulators.

Subunits	Description
COP II	
Sar1p	24 kDa small GTPase of the Ras family, with sequence similarity to ARF-type GTPases; exchange of bound GDP for GTP is mediated by the integral ER membrane protein Sec12p; Sar1p-GTP associates with ER membranes.
Sec23 complex	400 kDa complex comprising Sec23p and Sec24p. Sec23p (85 kDa) is a Sar1p-specific GTPase-activating protein. Sec24p (105 kDa) is required for the binding of the Sec13 complex to ER membranes. There are two proteins homologous to Sec24p in the yeast genome: Lst1p (alias Sfb3p) and Iss1p (see text and Peng *et al*. 2000, Miller *et al*. 2002).
Sec13 complex	700 kDa complex comprising Sec13p (34 kDa) and Sec31p (150 kDa). Both proteins contain several WD repeats implicated in protein–protein interactions.
Sec16p	240 kDa hydrophilic protein which is tightly peripherally associated with ER membranes. Sec16p is thought to be an 'organizer' of the COP II coat since it interacts with several COP II subunits.
Sec12p	Guanine nucleotide exchange factor for Sar1p. Transmembrane protein resident in the ER.
COP I	
ARF1	20 kDa small GTPase; N-terminally myristoylated; GTP-bound form associates with membranes; GDP-bound form is predominantly cytosolic.
Coatomer	Evolutionarily conserved $\sim$700 kDa complex consisting of seven distinct subunits: α-, β-, β'-, γ-, δ-, ε- and ζ-COP (with molecular masses of: 160, 110, 102, 97, 57, 36 and 20 kDa). The genes encoding subunits of yeast coatomer are *RET1* (α), *SEC26* (β), *SEC27* (β'), *SEC21* (γ), *RET2* (δ), *SEC28* (ε) and *RET3* (ζ) (for references see Gaynor *et al*. 1998). A sub-complex containing α-, β'- and ε-binds peptides containing the KKXX ER retrieval motif *in vitro* (Cosson and Letourneur 1994). β-, γ-, δ- and ζ-COP show weak but siginificant homology to subunits of clathrin adaptors.
ARFGAP	In yeast, two ARFGAPs, Glo3p and Gcs1p, are involved in COP I-dependent Golgi-to-ER traffic (see text). In mammalian cells, the Gcs1p orthologue is named ARFGAP1 (Cukierman *et al*. 1995). Glo3p orthologues are present in mammalian genomes, but their functions have not been characterized yet.
ARFGEF	ARFGEFs are not part of the COP I coat, but required to enable membrane binding of Arf1p through loading it with GTP. In yeast, Gea1p and Gea2p, and possibly Sec7p are involved in COP I dependent trafficking. For details see text.

coat, thereby potentiating the action of COP II proteins to bud transport vesicles.

In mammalian cells and yeast, the dynamics of COP II-coated ER exit sites have been studied by time lapse fluorescence microscopy of living cells, using chimeras of a COP II subunit with the naturally fluorescent protein, GFP (Green Fluorescent Protein) (Hammond and Glick 2000, Stephens *et al*. 2000, Bevis *et al*. 2002). The majority of COP II labelling appeared as kinetically stable 'spots' tightly associated with ER membranes (see Figure 2(*c*)). Secretory cargo (e.g. VSV-G-GFP) was found to segregate from these sites and be transported to the Golgi complex without any apparent association with COP II. The current model is that COP II vesicles rapidly uncoat after budding, allowing them to fuse with each other and generating the VTC membrane structures (which are also called 'intermediate compartment' (IC)). These pleiomorphic structures containing secretory cargo then move towards the Golgi complex in a saltatory fashion along tracks defined by microtubules. Although COP II coat proteins rapidly cycle on and off the membrane during the COP II budding cycle, they get recruited back to the same domains on the ER membrane, thus defining kinetically stable ER exit sites.

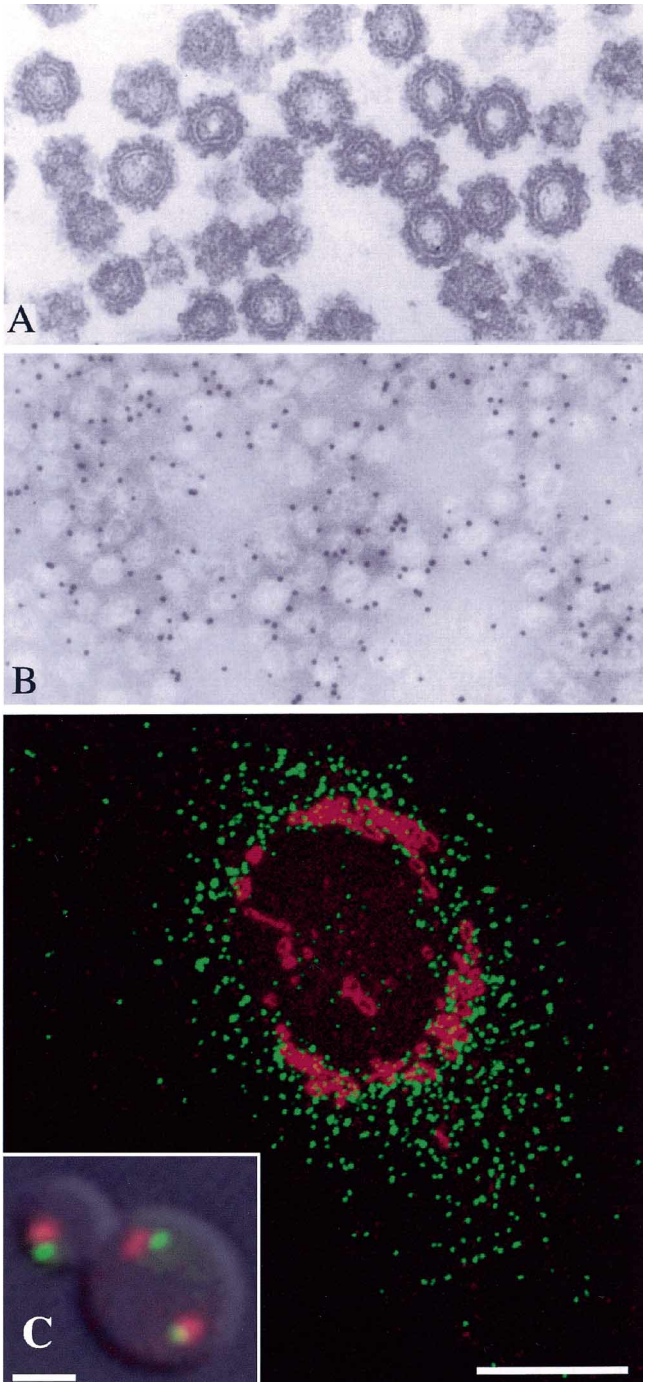

Figure 2

Cargo selection into COP II vesicles

A 'quality control' mechanism ensures that only correctly folded proteins may exit the ER and most ER-resident proteins are efficiently retained. However, the processes by which secretory proteins are segregated away from ER resident proteins during vesicle formation are still a matter of debate (see Warren and Mellman 1999). There is good evidence that at least certain cargo destined for ER exit is selectively captured by direct interactions with COP II proteins. COP II vesicles produced *in vitro* from yeast membranes in the presence of the minimal machinery described above are capable of packaging a large set of soluble and transmembrane cargo proteins, including the pre-cursor of the pheromone α-factor, and the v-SNAREs Bet1p, Bos1p and Sec22p (Barlowe *et al*. 1994, Matsuoka *et al*. 1998), which are necessary for the targeting and fusion of these vesicles with Golgi membranes (see Rothman 2002).

The Sec24p subunit of the coat is involved in recruitment of cargo into the vesicles. Cargo-containing 'pre-budding complexes' can be isolated from ER membranes after the addition of Sar1p and Sec23/24p, suggesting that this partial coat can recruit proteins destined for incorporation into vesicles (Aridor *et al*. 1998, Kuehn *et al*. 1998). Furthermore, the Sec23/24p heterodimer, and in some cases Sec24p alone, can bind to cytoplasmic domains of a variety of cargo molecules. For instance, the v-SNAREs Bet1p and Bos1p can interact with Sec23/24p in a Sar1p-dependent fashion (Springer and Schekman 1998) and the Golgi t-SNARE

Figure 2. (*A*) Purified COP II vesicles produced *in vitro* in an incubation with the non-hydrolyzable GTP-analogue GMP-PNP. A compact electron-dense coat is visible on virtually all equatorially sectioned vesicles in this tannic acid-stained, Epon embedded section (103 770 ×). (*B*) Immuno-staining on a cryosection (68 400 ×) of a COP II vesicle pellet with an antibody against Sar1p. (*A*) and (*B*) are reproduced with permission from Barlowe *et al*. (1994), *Cell*, **77**, 895–907. (*C*) Physical arrangement of ER exit sites and the Golgi complex in mammalian cells and a yeast. A mammalian NRK cell stained for ER exit sites (the COP II component Sec13, green) and the *cis*-Golgi complex (giantin, red). The inset shows a *Pichia pastoris* yeast cell expressing GFP-tagged markers for transitional ER (Sec13p, green) and the *cis*-Golgi complex (visualized with the Golgi marker Sec7p, red). Note the huge physical distance of ER-to-Golgi transport in mammalian cells compared to yeast cells. Scale bars, 5 μm. Images courtesy of Ben Glick. Reproduced with permission from Reynaud and Simpson (2002), *EMBO Reports*, **3**, 828–833.

Sed5p directly binds Sec24p, even in the absence of Sec23p and Sar1p (Peng *et al*. 1999). Further evidence for a role of Sec24p in cargo selection stems from the observation that Lst1p, a Sec24p homologue in the yeast genome, is required for efficient ER export of the plasma membrane ATPase, Pma1p (Roberg *et al*. 1999, Shimoni *et al*. 2000). Yeast mutants lacking Lst1p accumulate Pma1p in the ER, which results in a pH-sensitive growth phenotype (Roberg *et al*. 1999). Lst1p is present in a complex with Sec23p, similar to the Sec23/24p complex. Vesicles generated with the Sec23/Lst1p complex replacing Sec23/24p are deficient in a distinct sub-set of cargo molecules, including the v-SNAREs Bet1p, Bos1p and Sec22p. Consistent with the absence of any SNAREs in them, these vesicles are unable to fuse with Golgi membranes (Miller *et al*. 2002). Unlike Sec24p, Lst1p is unable to bind to Bet1p *in vitro*, which suggests a direct correlation between cargo binding and recruitment into vesicles. Consistent with the latter observation, over-expression of Lst1p cannot compensate for loss of the essential Sec24p function (Peng *et al*. 2000). Yeast cells possess three Sec24p homologues and higher eukaryotes express at least four isoforms (see Table 1). It is thought that this variation in the Sec24p subunit provides COP II vesicles with a broader specificity for cargo selection, as well as greater flexibility in vesicle size, which is probably important to accommodate bulky and/or oligomeric cargo (Shimoni *et al*. 2000).

The specificity of the cargo–coat protein interaction suggested the existence of sorting signals in the cytoplasmic domains of membrane cargo proteins. Indeed, two such ER export signals have been identified in mammalian cells. First, a di-phenylalanine motif present in ERGIC-53, a lectin-like receptor for glycoproteins, is crucial for its efficient ER-to-Golgi transport (Appenzeller *et al*. 1999, Nufer *et al*. 2002). This motif is also present in the conserved p24 family of proteins, which have been implicated as cargo receptors (Dominguez *et al*. 1998). Secondly, a di-acidic sorting signal Asp-X-Glu (D-X-E, where X is any amino acid) for efficient ER-to-Golgi transport has been identified in the C-terminus of the vesicular stomatitis virus glycoprotein (VSV-G) and the potassium channel proteins, Kir1.1 and Kir2.1 (Nishimura and Balch 1997, Ma *et al*. 2001). In the case of VSV-G, the motif has been shown to function in concentrating this cargo molecule into COP II coated structures. The motif is functional both in mammalian cells and in yeast (e.g. in the transmembrane protein Sys1p) and directly interacts with the Sec23/24p complex. Mutations in this signal abolish binding of COP II proteins and result in significant ER retention of the mutant proteins (Votsmeier and Gallwitz 2001).

Soluble cargo may get incorporated into COP II vesicles either by default (also named 'bulk flow') or through selective interactions of the coat with membrane receptors that bind to this cargo. It has been demonstrated that certain soluble secretory proteins, e.g. the yeast pheromone α-factor, are concentrated $\sim$20-fold in COP II vesicles relative to bulk flow markers or phospholipids (Malkus *et al*. 2002). Convincing candidates for export receptors for soluble proteins have been identified. Erv29p, a conserved transmembrane protein, was found to be directly required for packaging glycosylated α-factor pre-cursor (gpαF) into COP II vesicles (Belden and Barlowe 2001). *In vivo*, export of gpαF is saturable and dependent upon the expression level of Erv29p. *In vitro*, an Erv29p–gpαF complex can be isolated from ER-derived vesicles. Erv29p, thus, has all the properties expected of a shuttle receptor that aids export of gpαF and probably other soluble cargo (Belden and Barlowe 2001). Furthermore, Erv29p carries a di-lysine motif which is required for its subsequent Golgi-to-ER retrieval via COP I coated vesicles (which are discussed below), to be available for another transport round. Undoubtedly, further cargo receptors and cargo adaptors involved in the selective COP II-dependent ER export pathway will be discovered in the next few years.

An interesting remaining question is whether there may be special COP II vesicles for special cargo proteins. Muniz *et al*. (2001) have shown by immunoisolation and density gradient analysis from yeast that GPI-anchored proteins, such as Gas1p, are transported in different vesicles from those containing the amino acid permease Gap1p or gpαF. It remains to be determined whether or not these vesicles utilize different sub-types of COP II vesicle coat proteins, or combinations thereof, that may be adapted to deal with different cargo.

COP I-coat proteins mediate a Golgi-to-ER retrieval pathway

The fusion of membrane carriers with the *cis*-Golgi complex, resulting in the delivery of secretory cargo, presents the cell with the problem that escaped ER proteins and, most importantly, the machinery proteins, e.g. SNAREs utilized in the vesicle fusion step, are now in the wrong location and must be returned to the ER. This retrograde transport step is mediated by COP I-coated vesicles and tubules. Additionally, the COP I coat is thought to be involved in intra-Golgi traffic and in maintaining the normal structure of the mammalian Golgi complex.

The COP I coat was first identified in a mammalian cell-free assay that reconstitutes intra-Golgi transport (reviewed in Rothman and Wieland 1996, Schekman and Orci 1996, Rothman 2002). Golgi-derived COP I coated vesicles accumulate and can be purified from such *in vitro* reactions in which transport is blocked with the non-hydrolyzable GTP analogue, GTP-γ-S (Orci *et al*. 1986, Serafini *et al*. 1991). The abundant coat present on these vesicles is composed of the small ras-like GTPase ARF1 and coatomer, a cytosolic 700 kDa protein complex comprising seven stoichiometric subunits: α-, β-, β'-, γ-, δ-, ε- and ζ-COP. As for COP II, all components of the COP I coat are conserved from mammals to yeast (Serafini *et al*. 1991, Waters *et al*. 1991, Hosobuchi *et al*. 1992, for review see Gaynor *et al*. 1998). Each of the genes encoding coatomer subunits in yeast is essential for cell viability, except SEC28 encoding ε-COP. ε-COP functions as a structural component of coatomer, stabilizing α-COP and, thus, coatomer at elevated temperatures (Duden *et al*. 1998).

Coatomer, and ARF in its GTP-bound form, are the only cytosolic proteins necessary to produce COP I-coated vesicles from Golgi-enriched membranes or from chemically

defined liposomes and, thus, constitute the minimal machinery to deform the lipid bilayer into buds and vesicles (Orci *et al*. 1993, Spang *et al*. 1998). Uncoating of COP I vesicles is initiated when ARF hydrolyzes its bound GTP, which releases ARF and coatomer back into the cytosolic pool and allows fusion of the vesicle with its target membrane to occur (Rothman 2002, and references therein).

In mammalian cells, the COP I coat is predominantly associated with the *cis*-side of the Golgi complex and VTC or Intermediate Compartment (IC) structures scattered throughout the cytoplasm (Duden *et al*. 1991, Oprins *et al*. 1993, Scales *et al*. 1997, see Figure 3). Using fluorescence microscopy of living cells, it has been demonstrated that VTC structures in the vicinity of the ER, while moving towards the Golgi complex, rapidly acquire COP I proteins (Stephens *et al*. 2000, Presley *et al*. 2002). These pleiomorphic structures containing secretory cargo no longer harbour COP II coat proteins. The function of COP I on the anterograde-directed VTCs is thought to be the sorting of cargo for transport back to the ER, since retrograde cargo proteins like the KDEL-receptor ERD2, but not anterograde cargo like VSV-G, cosegregate with COP I (Shima *et al*. 1999).

The strongest evidence for a role of COP I coat proteins in retrieval of proteins to the ER stems from experiments in yeast. Cargo transported in COP I vesicles in mammalian cells and yeast includes type I membrane proteins harbouring a di-lysine motif, KKXX or KXKXX, i.e. lysines in either -3

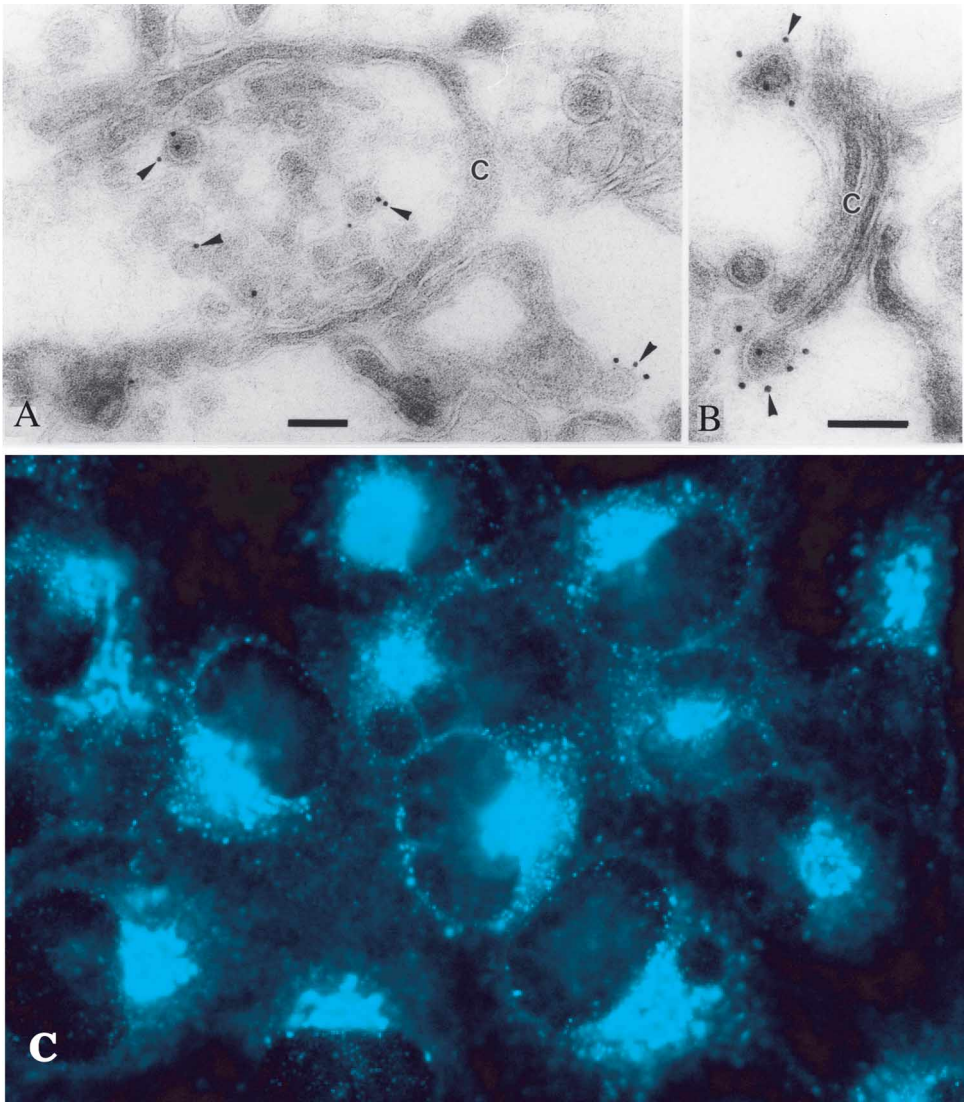

Figure 3. (*A*) and (*B*) Localization of the COP I coat by immunoelectron microscopy on GTP-γ-S-treated rat liver Golgi fractions. Ultra-thin frozen sections stained with a β-COP anti-serum ('EAGE') is shown in an overview (*A*) and in an enlarged area of the Golgi complex (*B*). β-COP/COP I is preferentially found on coated vesicles close to Golgi cisternae (arrowheads in (*A*)) and on budding profiles (arrowheads in (*B*)). (*C*) = Golgi cisternae. Scale bar = 100 nm (*C*) Live mammalian ldlF cells expressing ε-COP-CyanFluorescentProtein (CFP). ε-COP-CFP gets incorporated into coatomer in these cells and, thus, can serve as an *in vivo* marker for the visualization of COP I localization (see e.g. Shima *et al*. 1999). Note that bright perinuclear structures (i.e. Golgi complex) and VTCs scattered in the cytoplasm are labelled. (*A*) and (*B*) are reproduced with permission from Duden *et al*. (1991), *Cell*, **64**, 649–665.

and -4, or -3 and -5 positions from the carboxyterminus (with X = any amino acid). Proteins harbouring this motif are mostly ER-localized at steady-state but many achieve this localization in a dynamic fashion, through continuous cycling between the ER and the Golgi complex. The di-lysine motif is necessary and sufficient to target reporter proteins into the retrograde Golgi-to-ER pathway *in vivo*, and coatomer can directly interact with such motifs *in vitro* (Jackson *et al*. 1993, Cosson and Letourneur 1994, Gaynor *et al*. 1994). A genetic selection for yeast mutants with specific defects in KKXX-dependent trafficking yielded mutations in α-, γ-, δ- and ζ-COP, which provided strong evidence for an essential function of COP I in retrieval (Letourneur *et al*. 1994, Lewis and Pelham 1996). In particular, *ret1-1*, an α-COP mutant, displayed a strong *in vivo* KKXX-retrieval defect even at permissive temperature and coatomer from *ret1-1* cells has lost the ability to interact with the KKXX-motif *in vitro*. Anterograde traffic is virtually unaffected in this mutant, even upon shift to restrictive temperature (Letourneur *et al*. 1994).

Consistent with this data, in mammalian cells microinjection of anti-COP I antibodies inhibits the Golgi-to-ER retrieval of the lectin-like molecule ERGIC-53, a VTC marker protein and of the KDEL-receptor ERD2 (Majoul *et al*. 1998, Girod *et al*. 1999). However, microinjection of antibodies against β-COP also blocks ER-to-Golgi transport of a model anterograde vesicle cargo, the glycoprotein of Vesicular Stomatitis Virus (VSV-G) (Pepperkok *et al*. 1993). Based on the evidence from yeast, the current consensus model is that this inhibition of anterograde transport observed upon inhibition of COP I is indirect, owing to the fact that anterograde and retrograde pathways are tightly coupled. Continued anterograde traffic in the secretory pathway requires a functional COP I-mediated Golgi-to-ER retrieval pathway (reviewed in Gaynor *et al*. 1998).

Cargo selection into COP I vesicles: role of cytoplasmic domains and sorting motifs

Similar to the COP II coat, it is thought that the COP I coat components selectively recruit intended cargo into the budding vesicle. Retrograde cargo proteins carrying a di-lysine motif bind directly to coatomer. Luminal ER proteins bearing a KDEL-motif, like the chaperones BiP and PDI, are recognized in the *cis*-Golgi by a multi-spanning transmembrane protein, the KDEL-receptor (encoded by the ERD2 gene in yeast) and are targeted into COP I vesicles as a receptor–ligand complex (Sönnichsen *et al*. 1996, Aoe *et al*. 1997, Majoul *et al*. 2001).

Intact coatomer, which is a stable heptameric complex, functions as the building block of the COP I coat in intact cells. Nevertheless, specific functions of individual subunits within coatomer have been discerned experimentally. A stable α-, β'-, ε-COP sub-complex of coatomer can be obtained in high salt buffers and may be purified from both yeast and mammalian cytosol. *In vitro*, this sub-complex can bind to the di-lysine trafficking motif KKXX (Cosson and Letourneur 1994, Lowe and Kreis 1995). Significantly, the point mutations in four different alleles of *ret1* identified in the selection, including *ret1-1*, cluster within 85 residues in or close to an amino-terminal domain containing WD40 repeats, providing strong genetic evidence for a role of this domain of α-COP in KKXX-motif binding (Letourneur *et al*. 1994, Schröder-Kohne *et al*. 1998). In support of this, a strong KKXX-signal, KKYL, isolated in a systematic screen for ER trafficking signals in mammalian cells can directly bind to α-COP in the yeast two-hybrid assay (Zerangue *et al*. 2001). These data highlight α-COP as an excellent candidate for the 'KKXX-receptor'. Additionally, photo-crosslinking data indicate that a second KKXX binding site within coatomer may be present on γ-COP (Harter and Wieland 1998). Another Golgi-to-ER retrieval motif that requires critical aromatic residues has recently been identified, e.g. in the cytoplasmic domain of the ER-resident protein, Sec71p. This novel motif specifically interacts with δ-COP (Cosson *et al*. 1998).

Interestingly, the remaining four subunits of coatomer, β-, γ-, δ- and ζ-COP, display weak but significant sequence homology with subunits of the hetero-tetrameric adaptor complexes of clathrin-coated vesicles (reviewed in Kirchhausen 2000). This strongly suggests that there may be similarities in structure or function between the two types of coat. Indeed, the domain interactions within the β-, γ-, δ- and ζ-COP sub-complex of coatomer, as studied using the yeast two-hybrid system, are highly reminiscent of clathrin adaptor complexes (Faulstich *et al*. 1996, Eugster *et al*. 2000). A β-/δ-COP sub-complex can bind to rat liver Golgi membranes in an ARF and GTP-γ-S dependent fashion and β-COP directly interacts with ARF (Zhao *et al*. 1997, Pavel *et al*. 1998, Eugster *et al*. 2000).

Again reminiscent of COP II, it is thought that the molecular basis of efficient initiation of COP I vesicle budding involves a (at least) bivalent interaction of coatomer with membrane-bound ARF-GTP and cytoplasmic tails of cargo or putative cargo receptors (see Figure 4). This phenomenon would directly couple uptake of cargo to transport vesicle formation (Bremser *et al*. 1999). Alternatively, the sorting of some COP I cargo proteins, e.g. SNAREs and the KDEL-receptor, likely involves direct interactions of their cytoplasmic domains with ARFGAPs, which then triggers recruitment of coatomer (Rein *et al*. 2002, Yang *et al*. 2002).

Role of regulatory proteins acting on ARF in COP I-dependent trafficking

ARF by itself has very poor GTP-hydrolysis and GTP-for-GDP exchange activities *in vitro* and, thus, needs helper proteins to accomplish its functional GTP/GDP cycle (Donaldson and Jackson 2000). Golgi-localized, tightly membrane-associated guanine nucleotide exchange proteins (ARFGEFs) catalyse the exchange of GTP for GDP on ARF1, i.e. these proteins may determine the correct membrane site where the GTP-bound form of ARF1 and coatomer get recruited (Donaldson and Jackson 2000, Zhao *et al*. 2002). In yeast, Gea1p and Gea2p provide overlapping function in COP I-mediated retrograde Golgi-to-ER trafficking (Peyroche *et al*. 2001, Spang *et al*. 2001). In mammalian cells so far the best candidate for an ARFGEF relevant for

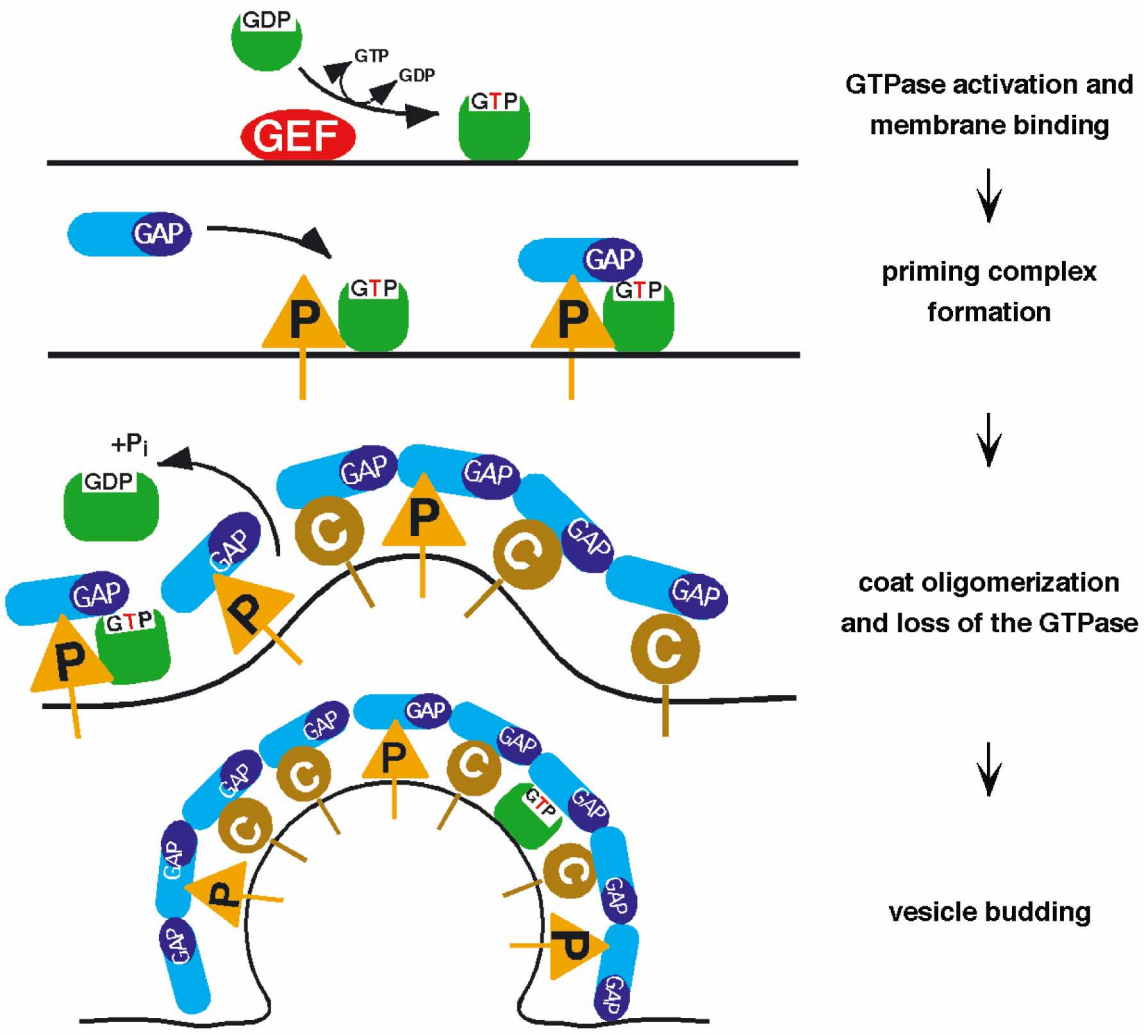

Figure 4. Schematic model of vesicle budding. Basic principles conserved between COP I and COP II budding have emerged (see Springer *et al*. 1999 for further details). For both coats, a small GTPase is recruited to the appropriate donor membrane by its specific GEF. Next, the GTP-bound form of the GTPase, i.e. Arf1-GTP or Sar1-GTP, is recognized together with a membrane protein (e.g. a v-SNARE or a cargo receptor) by either a coat component or a GAP protein (e.g. ARFGAP1 binds the KDEL-receptor in the case of COP I). This complex on the membrane may act as a 'primer' (P) for further coat recruitment. Priming complexes associate laterally, enabling further cargo recruitment and deformation of the lipid bilayer into a bud and vesicle. GTP-hydrolysis is then a pre-requisite for vesicle uncoating. Reproduced with permission from Springer *et al*. (1999), *Cell*, **97**, 145–148.

the COP I-mediated pathway is a protein named GBF1 (Kawamoto *et al*. 2002, Zhao *et al*. 2002).

GTPase-activating proteins acting on ARF1 (ARFGAPs) complete the cycle by catalysing the hydrolysis of GTP to GDP, which deactivates ARF1 and triggers uncoating of the vesicle. Two yeast ARF-GAPs, Gcs1p and Glo3p, provide an overlapping essential function in retrograde Golgi-to-ER transport (Dogic *et al*. 1999, Poon *et al*. 1999).

In mammalian cells, ARFGAP1, the Gcs1p orthologue, is localized to the Golgi complex and its function has been extensively studied in *in vitro* experiments (Cukierman *et al*. 1995, Yang *et al*. 2002, and references therein). It has been observed that vesicles formed *in vitro* in the presence of non-hydrolyzable GTP analogues or using a constitutively active ARF1 mutant (Q71L-ARF1) are depleted of cargo, compared to vesicles formed in the presence of GTP (Lanoix *et al*. 1999, Malsam *et al*. 1999, Pepperkok *et al*. 2000, Yang *et al*. 2002). This was a strong indication that GTP-hydrolysis plays a role in cargo inclusion into COP I vesicles, implying that ARFGAPs may somehow function in cargo sorting as well as in vesicle uncoating.

Indeed, convincing evidence supporting a direct role of ARF-GAPs in the sorting of certain transmembrane proteins, namely the KDEL-receptor and v-SNAREs, has been reported (Aoe *et al*. 1997, Lanoix *et al*. 1999, 2001, Rein *et al*. 2002). Recently, using purified components, it has been shown that on COP I vesicles formed *in vitro* with GTP the 'classic' ARFGAP, named ARFGAP1, is a stoichiometric component of the coat. GTP-γ-S prevents recruitment of ARFGAP1 to nascent vesicles and has an inhibitory effect on COP I vesicle formation *in vitro* (Yang *et al*. 2002). ARFGAP1 can bind to the cytoplasmic tail of the KDEL-receptor ERD2 (Yang *et al*. 2002). There was a striking correlation between the amount of ARFGAP1 present on the

vesicles and the amount of ERD2 present in the vesicles, suggesting that ARFGAP1 is crucial for sorting of ERD2–KDEL ligand complexes into COP I coated structures (Aoe *et al*. 1997, Yang *et al*. 2002, and references therein). Further, *in vivo*, using live cell imaging and Fluorescence Resonance Energy Transfer (FRET) techniques, it has been shown that increased ligand occupancy of the KDEL-receptor ERD2 correlates with increased sorting of ERD2 into COP I coated vesicles (Majoul *et al*. 2001). ARFGAPs may also have a critical role for the incorporation of v-SNAREs, as it has been shown *in vitro* using purified components from yeast that coatomer binding to v-SNAREs requires the action of ARFGAPs (Rein *et al*. 2002). Thus, unexpectedly, recent data indicate that the principal mechanisms by which the GAPs for the COP I and COP II coats regulate vesicular traffic are much more similar than originally thought (see Figure 4).

It seems clear that the bulk of Golgi-to-ER trafficking is COP I-mediated. Intriguingly, however, at least for certain cargo, there appear to be also COP I-independent routes of Golgi-to-ER transport. For example, the Golgi-to-ER transport of Shiga toxin or Shiga-like toxin, and of certain Golgi-resident glycosylation enzymes cannot be effectively blocked with experimental treatments that do block the recycling of ERD2 or the VTC marker ERGIC-53 (Girod *et al*. 1999). This suggests the presence of an alternate Golgi-to-ER pathway that may not require COP I, but the molecular details remain to be defined (reviewed in Storrie *et al*. 2000, Matanis *et al*. 2002).

Functions of COP I coat proteins within the Golgi complex

While a crucial function of the COP I coat in Golgi-to-ER retrieval is firmly established, the exact roles of COP I in intra-Golgi transport are still controversial. COP I may function in intra-Golgi retrograde transport of Golgi enzymes (Harris and Waters 1996, Love *et al*. 1998, Lanoix *et al*. 1999, 2001) and perhaps also in transport of anterograde cargo within the Golgi complex (Orci *et al*. 1997). This topic is outside the scope of this text, but has been covered in a recent review (Pelham and Rothman 2000). Intra-Golgi traffic is also discussed in detail in the accompanying review by Elsner *et al*. (2003).

Further, in ways that are incompletely understood, COP I function is essential for maintaining the normal structure of the interphase Golgi complex in mammalian cells. Strong evidence for this stems from a temperature-conditional ε-COP mutant cell line (ldlF cells) which shows severely fragmented Golgi membranes rapidly upon shift to the non-permissive temperature (Guo *et al*. 1994). Further, the drug brefeldin A, which inactivates ARF by inhibiting the ARF guanine nucleotide exchange factors (ARFGEFs) also causes dramatic and very rapid (within minutes) morphological changes in Golgi structure in mammalian cells. These observations probably reflect a breakdown of the Golgi complex under COP I-deficient conditions. Given that removal of COP I from Golgi membranes leads to fusion of the Golgi membranes with the ER and, thus, the collapse of the Golgi complex, it is possible that COP I may play an important role in covering membrane components, e.g. SNAREs, which when exposed can mediate membrane fusion. In support of this, microinjection of antibodies against β-COP prevents the brefeldin A-induced fusion of the Golgi complex with the ER. The role of COP I function in the Golgi complex is currently subject to intensive investigation in several laboratories.

Conclusions and perspective

Considerable new insights into the COP II coat have recently been obtained using sophisticated combinations of biochemistry, biophysics, live cell imaging, X-ray crystallography, single particle analysis and high resolution electron microscopy techniques. A similarly broad set of techniques is currently being applied to the COP I coat in several laboratories. So far, no X-ray crystallographic information on any of the COP I coat subunits is available. In the next few years, one can confidently expect to learn more about the regulation and dynamics of the COP I and COP II coats in space and time, using *in vitro* and *in vivo* approaches that resolve these phenomena in real time. The basic machinery for vesicle formation has been well defined and common principles that govern COP I and COP II vesicle formation have been described, but much remains to be learned about molecular and structural details of these processes. For example, the role of lipids in coat recruitment is not yet understood, and many regulatory proteins, including docking factors, cargo adaptors and receptors, and possibly factors acting in vesicle uncoating, are still elusive.

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