Minireview

COPII: a membrane coat that forms endoplasmic reticulum-derived vesicles

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Abstract Vesicle budding from the endoplasmic reticulum (ER) has been reconstituted with washed membranes and three soluble proteins: Sec13 complex, Sec23 complex and the small GTPase Sar1p. The proteins that drive this cell-free vesicle budding reaction form an ~10 nm thick electron dense coat on ER-derived vesicles. Although the overall mechanism of membrane budding driven by various cytoplasmic coats appears similar, the constituents of this new membrane coat are molecularly distinct from the non-clathrin coat (COP) involved in intra-Golgi transport and the clathrin-containing coats. The new vesicle coat has been termed COPII.

Key words: Intracellular transport; Vesicle budding; Coat protein; Endoplasmic reticulum

1. Introduction

Intracellular transport reactions between membrane-bound compartments of the secretory pathway are mediated by vesicles that bud from a donor compartment and fuse with a specific acceptor compartment [1]. A general mechanism for the production of vesicle intermediates is thought to involve protein complexes that site-specifically coat membranes and pinch-off coated vesicles of a uniform dimension. Once vesicles are released, the coat is disassembled exposing a set of membrane-bound proteins that promote vesicle fusion [2]. Two well characterized examples of coated vesicle intermediates are the clathrin-coated vesicles that mediate transfers between the plasma membrane, endocytic and *trans*-Golgi compartments [3] in addition to COP-coated Golgi-derived vesicles that mediate intra-Golgi [4] and Golgi to endoplasmic reticulum (ER) transport [5]. Recently, a new type of coated vesicle intermediate has been identified that transports proteins from the ER to the Golgi complex and is composed of proteins distinct from the clathrin or COP-membrane coats [6]. This membrane-bound coat has been termed COPII, and is the subject of this review.

2. Genetic and biochemical analysis of vesicle budding from the ER

A combined genetic and biochemical approach in the yeast *Saccharomyces cerevisiae* continues to provide a wealth of information concerning intracellular trafficking in eukaryotic cells. An initial screen for temperature-sensitive *sec* mutants identified 23 complementation groups that were defective at various steps of the secretory pathway [7]. Further studies have discovered over 50 genes in yeast that are involved in intracellular transport [1,8]. A subset of these genes is required for protein transport from the ER to the Golgi complex and many insights concerning transport mechanisms have come from sequence analysis of these cloned genes. This collection of thermosensitive strains has also been instrumental in establishing a cell-free assay that faithfully reproduces ER to Golgi transport. [9,10]. The dissection of this cell free transport assay, together with genetic and morphologic analyses of *sec* mutant strains, has divided ER to Golgi transport into three distinct steps: vesicle budding, targeting and fusion [10,11]. The first step in this transport reaction, vesicle budding, has been reconstituted in vitro with washed membranes and three purified soluble proteins: Sar1p, Sec13 complex and Sec23 complex [12]. In the presence of GTP, these proteins satisfy the requirement for cytosol in the production of ER-derived vesicles. The vesicles formed in this reaction are authentic by two stringent criteria: first, they are competent for fusion with the Golgi apparatus; second, their protein composition is distinct for the donor membrane fraction in that they are highly enriched for cargo protein over resident ER protein [6,12,13].

3. Sec proteins that drive vesicle budding coat ER-derived vesicles

The salient features of proteins required for reconstitution of vesicle budding from the ER are summarized in Table 1. Sar1p is a 21 kDa GTPase that shares significant amino acid sequence identity with the ARF family of GTPases [14]. The exchange of nucleotide bound to Sar1p is catalyzed by Sec12p, an integral membrane glycoprotein on the cytosolic face of the ER [15]. The 400 kDa Sec23 complex contains two polypeptides: Sec23p, an 85 kDa protein that is a Sar1p-specific GTPase-activating protein (GAP), and an associated 105 kDa protein termed Sec24p [16,17]. The GAP activity associated with the Sec23p subunit is equivalent when in a purified monomeric form or complexed with the Sec24p protein. Functional Sec13 complex migrates as 700 kDa particle and is composed of two proteins; Sec13p, a 34 kDa polypeptide and a 150 kDa polypeptide termed Sec31p [13,18]. Both Sec13p and Sec31p contain repeats of a sequence known as the WD-40 motif (a conserved ~40 amino acid stretch terminating in the residues Trp Asp). Although several proteins have now been identified that contain the WD-40 consensus sequence, the function of this motif

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remains obscure [19]. Thus, with the exception of the GTPase Sar1p, the proteins required to reconstitute vesicle budding from the ER do not share overall sequence homology with previously described coat proteins involved in membrane budding from the Golgi complex [20] or the plasma membrane [21].

ER-derived vesicles produced in vitro with purified Sar1p, Sec13p complex and Sec23p complex are released as freely diffusible intermediates that can be resolved from larger ER membranes by differential centrifugation. An inspection of this crude reaction product by thin-section electron microscopy reveals an enriched population of 60 nm vesicles covered by an ~10 nm thick electron-dense coat [6]. Further purification of these vesicles from the soluble factors used in their synthesis can be achieved by gel-filtration chromatography or centrifugation on density gradients. The peripherally bound coat structure is preserved if vesicles are isolated by gel filtration on a Sephacryl 1000 column and in this single step, a homogeneous collection of coated vesicles may be obtained. The protein composition of these Sephacryl-purified vesicles assessed by polyacrylamide gel electrophoresis reveals that Sec13p, Sec31p, Sec23p and Sec24p are the abundant polypeptides contained in this preparation. Additionally, immunoelectron microscopy of fixed cryosections documents the uniform distribution of Sec23p and Sec13p in these purified coated vesicles. Thus, proteins required for vesicle budding are also retained on isolated vesicles and form a coat structure on ER-derived vesicles [6].

In addition to the protein coat, the Sephacryl-purified vesicles contain a minor set of membrane-bound proteins that are characteristic of uncoated ER-derived vesicles [13]. An uncoated form of ER-derived vesicles may be obtained by flotation of the crude reaction product on Nycodenz density gradients. The gradient conditions apparently dissociate peripherally bound membrane proteins and reveal a set of polypeptides (approx. 12) that are membrane embedded [13]. Unlike Sephacryl-purified vesicles, Sec13p, Sec31p, Sec23p and Sec24p are not detected on gradient-purified vesicles. By electron microscopy, these vesicles appear ~50 nm in diameter with irregular membrane surfaces and no apparent electron-dense coat [13]. Proteins required for vesicle fusion, such as Sec22p and Bos1p (v-SNARES) [22,23], are enriched on gradient-purified vesicles [13], however, the polypeptides observed by protein staining have not been identified and it remains to be determined if they correspond to known v-SNARES. The membrane-bound proteins contained on these uncoated ER-derived vesicles could represent four classes of molecules. First, the proteins may be required for the formation and packaging of the vesicle intermediate. Second, the constituents could function in site-specific targeting of the vesicle to the Golgi complex (such as v-SNARES). Third, the proteins may catalyze fusion of vesicle and Golgi membranes. Fourth, the constituents could be abundant secretory or membrane proteins in transit to other cellular locations.

Table 1

Native protein (size)	Subunits (size)	Notable features
Sar1p (21 kDa)	Sar1p (21 kDa)	small GTPase
Sec13 complex (700 kDa)	Sec13p (34 kDa) Sec31p (150 kDa)	repeated WD-40 motif repeated WD-40 motif
Sec23 complex (400 kDa)	Sec23p (85 kDa) Sec24p (105 kDa)	Sar1p specific GAP

4. A role for GTP hydrolysis

The small GTPase Sar1p and GTP participate in the reconstituted vesicle formation reaction. However, the hydrolysis of bound GTP by Sar1p is not a prerequisite for vesicle formation in vitro since non-hydrolyzable analogs, such as GTPγS or GMP-PNP, substitute for GTP in this reaction. Although GMP-PNP may serve as a source of guanine nucleotide for vesicle budding, vesicles formed with this analog differ from GTP synthesized vesicles in at least three respects First, maximum vesicle release from ER membranes in the presence of GMP-PNP requires elevated concentrations of Sec23 complex and Sec13 complex, perhaps an indication that these proteins are consumed in the budding reaction. Second, isolated GMP-PNP vesicles retain tightly bound protein coat and Sar1p whereas isolated GTP vesicles do not contain Sar1p and the coat is apparently destabilized. Lastly, isolated GTP produced vesicles are functional intermediates in ER to Golgi transport and fuse with the Golgi complex, in contrast to GMP-PNP vesicles that are not active in this fusion reaction [6]. It should be noted, however, that the failure of vesicles produced in the presence of non-hydrolyzable analog to fuse with the Golgi can be reversed by a procedure that removes bound Sar1p (and presumably coat structure) from vesicles [24]. Thus, GMP-PNP vesicles fail to fuse because the coat is not shed and not due to the formation of a defective vesicle. The role of GTP hydrolysis by Sar1p is comparable to a growing number of GTPases involved in diverse cellular processes, where the GTP-bound form initiates an event and GTP hydrolysis serves as a resetting mechanism for additional cycles [25].

5. Model

A model for the formation of COPII-coated vesicles is presented in Fig. 1. Sec12p catalyses guanine nucleotide exchange, thereby activating Sar1p at a vesicle formation site on the cytosolic surface of the ER (step 1). Membrane-bound and activated Sar1p then recruits the Sec23 and Sec13 complexes forming a coat structure that drives the budding event (steps 2 and 3). Hydrolysis of GTP bound to Sar1p is stimulated by the Sec23p subunit of the Sec23 complex in a reaction that must be regulated or timed such that the coat is not prematurely shed. Although GTP hydrolysis is not a prerequisite for vesicle release, the precise timing of this event is unclear. Once GTP is hydrolyzed, Sar1p is released from the vesicle, initiating the disassembly of the COPII complex (step 4). Finally, the disassembly of the coat structure is required to expose targeting proteins on the transport vesicle that mediate vesicle fusion with the Golgi complex. This model is entirely consistent with in vivo results where loss of function mutation in either *SAR1*, *SEC13*, *SEC31*, *SEC23*, or *SEC24* produces characteristic defects in export from the ER. Furthermore, the expression of a mutant form of Sar1p that mimics an activated GTP-bound state results in a dominant lethal phenotype as might be expected if COPII proteins could be assembled on ER-derived vesicles but not recycled [26].

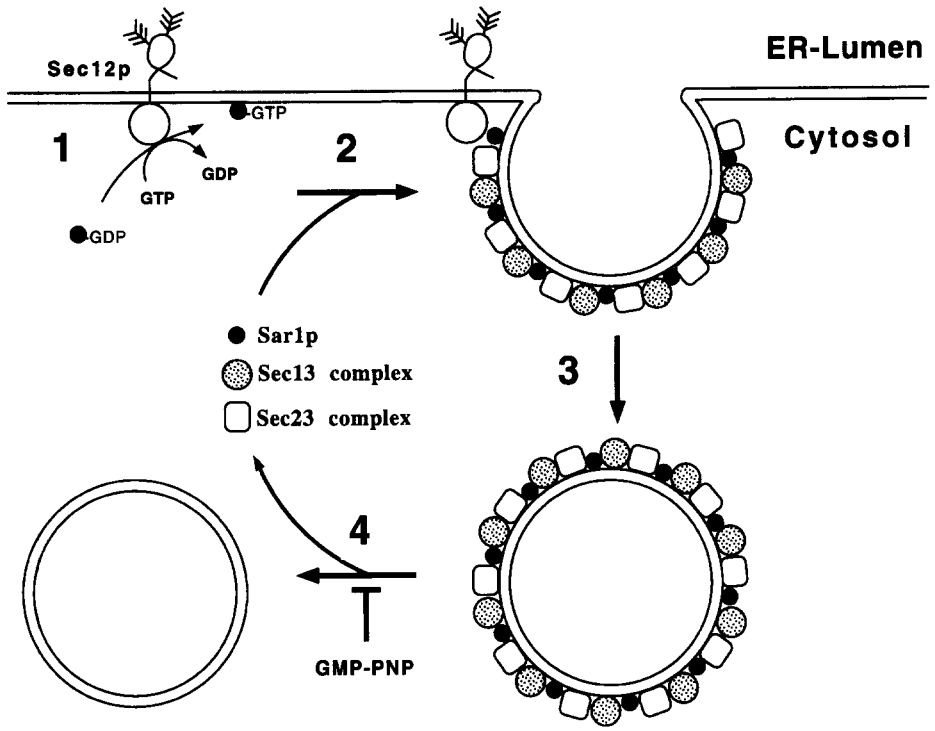

Fig. 1. Model for the synthesis of COPII-coated, ER-derived transport vesicles. Non-hydrolyzable analogs of GTP, such as GMP-PNP, inhibit vesicle uncoating. Refer to the text for more details.

6. Summary

Although the model for COPII-mediated vesicle formation (Fig. 1) presents a reasonable outline, some details have not been rigorously tested and many fundamental questions remain. For example, how are cargo molecules selected and/or resident proteins excluded during the budding event? What regulates the Sec12p nucleotide exchange activity? In the process of inducing membrane curvature, are coat proteins binding to membrane proteins and/or lipids? Additionally, little is known about the membrane fission event, in which lipid bilayers are joined to resolve the budded intermediate from the membrane surface (step 3, Fig. 1). The charged phospholipid surfaces of the inner membrane leaflet make spontaneous fusion of bilayers unfavorable, thus there are likely to be additional membrane proteins that are required to catalyze this reaction.

The mechanisms of intracellular transport appear fundamentally conserved from yeast to mammals [27]. Homologues to several of the COPII proteins have now been identified and studied in different species and appear to function similarly in vesicular transport between the ER and Golgi complex [28–31]. Not only do all eukaryotes possess similar transport pathways, but the basic mechanism of vesicle biogenesis by different cytoplasmic coats is also thought to be conserved [2]. Indeed, the production of COPII-coated ER-derived vesicles is reminiscent of the non-clathrin-coated (COPI) vesicles formed from Golgi membranes. COPI-coated vesicles are formed in vitro by incubation of Golgi membranes with coatomer (a seven-subunit ~800 kDa protein complex), the small GTPase ARF, GTP and palmitoyl CoA [4]. In both COPI and COPII formation, small GTPases initiate coat assembly and subsequent to vesicle formation, GTP hydrolysis is required for disassembly of these coats to yield fusion-competent vesicles. The small GTPases that initiate COPI and COPII formation, ARF and Sar1p, respectively, share significant amino acid sequence identity and are more closely related to each other than to other members of the small GTPase family. However, the other constituents of the COPI and COPII coats do not share significant sequence identity. One potentially interesting similarity is the presence of a repeated WD-40 sequence motif in the β' subunit [32] and the α subunit [20] of COPI that is also observed in the Sec13p and Sec31p subunits of COPII. It remains to be determined what function this motif may serve in coated vesicle formation.

On a cellular level, the contribution of each coat structure (COPI, COPII and clathrin) in specific intracellular transport steps continues to be a matter for debate. The primary site of COPII function appears to be in ER to Golgi transport although a requirement for this coat structure in other transport steps cannot be excluded. Genetic and biochemical evidence suggests that COPI mediates protein transport from the ER to the Golgi as well as between Golgi cisternae [2,32,33]. However, in vitro formation of ER-derived vesicles does not require any of the COPI proteins [6], suggesting the requirement for COPI may occur after vesicle biogenesis or may arise through some sort of indirect requirement. Interestingly, there is now compelling evidence for COPI function in retrieval of integral membrane proteins from the Golgi to the ER [5,34]. But again, these result do not exclude a direct role for COPI in ER to Golgi or intra-Golgi transport. Finally, the number of distinct membrane coats involved in intracellular trafficking remains in question but appears to be on the rise [35]. Indeed, much of the

excitement in the next few years is likely to come from resolution of current debate over the number and role of each membrane coat.

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