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Review

The COPI system: Molecular mechanisms and function

R. Beck [a,1], M. Ravet [b,1], F.T. Wieland [c,*], D. Cassel [b,*]

[a] Yale University School of Medicine, Department of Cell Biology, 333 Cedar Street, SHM C-232, New Haven, CT 06520, USA
[b] Department of Biology, Technion-Israel Institute of Technology, Haifa 32000, Israel
[c] Biochemistry Center, Heidelberg University, Im Neuenheimer Feld 328, D-69120 Heidelberg, Germany

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ABSTRACT

Transport of membranes and proteins in eukaryotic cells is mediated by vesicular carriers. Here we review the biogenesis and functions of COPI vesicles, carriers that operate in the early secretory pathway. We focus on mechanisms mediating coat recruitment, uptake of cargo, vesicle budding and fission, and finally dissociation of the coat. In this context, recent findings on the interplay between machinery and auxiliary proteins in COPI vesicle formation and function will be discussed. Specifically, we will weigh the pros and cons of recent data on roles of the small GTP binding protein Arf1, of Arf1GAPs, and lipids during COPI carrier formation.

1. Introduction

Vesicular carriers were first proposed in mammalian cells by Palade and colleagues [1]. In subsequent years, three types of coated carriers have been characterized in detail with regard to their structural components and functions: clathrin-coated vesicles that mediate transport in the late secretory pathway and the endocytic pathway (reviewed in [2]), COPI-coated vesicles that function in the early secretory pathway (reviewed in [3,4]), and COPII vesicles that export proteins from the endoplasmic reticulum (ER) (reviewed in [5]). Some structural and mechanistic similarities that exist between the three systems have been reviewed [6,7]. Here we focus on recent advances in the understanding of the components and mechanisms underlying the formation of COPI vesicles. These carriers were first isolated in a cell-free system, following incubation of Golgi-enriched membranes with cytosol in the presence of a hydrolysis-resistant derivative of GTP, GTPγS [8,9]. Analysis of the coated vesicles initially led to the identification of four large protein subunits (α-, β-, γ- and δ-COP) [10,11]. A complex containing these and additional subunits was subsequently purified from the cytosol and was termed coatomer (from coat protomer) [12]. The molecular identification of additional coatomer subunits, β′-COP [13,14], ε-COP [15] and ζ-COP [16], complemented the set of constituents of a heptameric coat complex. Analysis of the interactions between subunits has led to a model of the organization of the coatomer complex [17,18]. This organization is comparable to that of the heterotetrameric adaptins in complex with clathrin, where the β, γ, δ and ζ subunits referred to as the F subcomplex correspond to adaptin subunits. The remaining coatomer subunits α, β′ and ε comprise the B subcomplex [19]. Subunits of the F subcomplex display weak but significant sequence similarities to corresponding adaptin subunits. Functionally, however, the coatomer architecture seems to be more complex, as the binding of different types of membrane proteins involves subunits from both the F- and the B-subcomplexes (see "cargo sorting" below) [7,20–22].

The process of COPI vesicle biogenesis is controlled by Arf1, a small GTPase of the Ras superfamily, originally identified as a cofactor that is required for the action of cholera toxin in mammalian cells [23]. A guanine nucleotide exchange factor (GEF) containing a Sec7 domain [24] induces the exchange of bound GDP to GTP in Arf1. GBF1, a large Sec7 domain-containing GEF is the major exchange factor involved in COPI vesicle biogenesis [25,26]. Following nucleotide exchange, Arf1 undergoes a conformational change, leading to the exposure of a myristoylated N-terminal amphipathic helix that provides stable membrane anchorage [27–29]. Arf exchange only takes place on membranes as activation in the cytosol would lead to the energetically unfavorable exposure of a hydrophobic patch (the myristoylated amphiphatic helix) to an aqueous surrounding. Following nucleotide exchange,

* Corresponding authors.
E-mail address: felix.wieland@bzh.uni-heidelberg.de (F.T. Wieland).
[1] These authors contributed equally.

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Arf1-GTP recruits coatomer to the membrane [30–32]; real-time in vitro assays have revealed that Arf1 activation is rate-limiting, and is followed by an almost instantaneous binding of coatomer [33].

Coatomer recruitment to the Golgi also involves members of a family of 23–24 kD type-I transmembrane proteins (the p24 family) that are sorted into COPI vesicles where they become a major constituent [34,35]. Their short cytoplasmic tails bind directly to coatomer [35,36], thereby increasing the efficiency of vesicle formation [37].

After the formation of a COPI-coated vesicle, the coat must be shed to allow for vesicle fusion with the target membrane. The uncoating reaction depends on GTP hydrolysis by Arf1 [38], catalyzed by Arf1 GTPase-activating proteins (ArfGAPs). In addition to recruiting and releasing coatomer, as described above, an Arf-GEF/GAP mediated cycle of GTP exchange and hydrolysis was proposed to play a role in regulating the uptake of cargo into COPI vesicles [39–43].

2. Function of COPI in the early secretory system

The best characterized function of the COPI system is the retrograde transport of luminal and membrane proteins in the ER-Golgi segment of the secretory pathway [3,44,45]. Soluble cargos such as escaped resident ER glycoproteins are captured by adaptor transmembrane proteins that communicate with the COPI coat at their cytosolic aspect. A major, if not exclusive, adaptor function is provided by KDEL receptors that recognize a four-letter code presented at the carboxy terminus of many ER-resident proteins [46,47]. Membrane protein cargo, e.g., a subunit of the ER-localized oligosaccharide transferase complex, can be retrieved by direct interaction with coatomer [45]. COPI-dependent transport may also play a role in the correct steady-state distribution of proteins within the Golgi stack [48,49], reviewed in [50]. Retrograde transport of Golgi enzymes is a key element in the cisternal maturation mechanism, whereby cisternae mature progressively by receiving processing enzymes from a later cisterna [47,51–53]. According to this mechanism, cargo progression through cisternae would not require anterograde intra-Golgi transport, but rather represent "bulk flow", a mechanism that had been proposed earlier [54]. While there is also evidence for anterograde transport between Golgi cisternae by COPI vesicles [41,55,56], this mechanism cannot account for the transport of large cargo such as procollagen. A third mechanism for cargo progression through the Golgi involving partitioning and exchange between cisternae has recently been proposed [57].

Retrograde traffic through the COPI system also contributes to an ER quality control mechanism that ensures the correct assembly of certain multisubunit membrane proteins [58]. Such protein assemblies include subunits with a diarginine signal whose interaction with coatomer causes ER retrieval/retention. Upon subunit assembly, the arginine signals become masked, thus allowing for ER exit [59,60].

Evidence for COPI-mediated retrieval of membrane proteins is often based on analysis of their processing by Golgi glycosyltransferases (e.g., [61–63]). Whenever Golgi-type glycosylation cannot be detected, it is difficult to distinguish ER retrieval from ER retention. In one report, a carboxy dilysine signal (KKAA) considered as a canonical signal for ER retrieval through the COPI system was found to cause ER retention rather than retrieval of reporter protein; whether the COPI coat was involved in this process remains unclear [64].

The COPI system also plays a role in the transport of biosynthetic cargo to the Golgi apparatus. Whereas the initial phase of ER exit is mediated by COPII-coated carriers, subsequent transport is carried out by tubulovesicular intermediates (the ERGIC) that are associated with the COPI coat [65–69]. Transition from COPII- to COPI-coated carriers is mediated by the activation of Arf1 by GBF1. Pharmacological agents and genetic manipulations that inhibit Arf1 activation were shown to inhibit ER to Golgi transport [70–75]. It should be noted, however, that inhibition of Arf1 activation may also affect other Arf effectors thought to function in the early secretory system, such as phospholipid-modulating enzymes and tethers [76]. More direct evidence for a role of coatomer in ER to Golgi transport was provided by the demonstration that VSVG transport to the Golgi is inhibited by antibodies directed against the β-COP subunit of coatomer [70,77]. In *Saccharomyces cerevisiae*, a block of anterograde transport is observed in mutants of COPI subunits [78–80].

Two recent studies examined the consequences of reducing β-COP expression in mammalian cells. β-COP depletion in HeLa cells led to the merging of ERGIC, Golgi, TGN and recycling endosomes but not ER, and prevented transport beyond the merged compartment [81]. In another study, β-COP depletion was found to decrease surface expression of the cystic fibrosis transmembrane conductance regulator (CFTR) [82]; interestingly, CFTR was found to co-immunoprecipitate with coatomer, indicating that COPI trafficking may play an active role in CFTR transport. However, the interpretation of coatomer disruption studies is complicated by the resulting perturbations of the structure of organelles of the early secretory system, and by possible indirect effects that may result from the inhibition of COPI-dependent recycling of trafficking components.

Transport between the ER and the Golgi apparatus involves cytoskeleton-based membrane translocation [83–85]. A possible connection between the coatomer coat and the cytoskeleton is provided by the finding that coatomer interacts with CDC42, which in turn regulates the recruitment of the microtubule motor dynein [86,87]. Further studies should address the precise role of interactions between coat components and cytoskeletal elements in ER to Golgi transport.

3. Cargo sorting

3.1. Sorting of soluble proteins

The lumen of the ER contains a variety of soluble proteins that perform essential functions related to protein folding and assembly. Munro and Pelham were the first to have noticed that these ER residents are distinguished from secreted proteins by the presence of a C-terminal KDEL sequence (HDEL in yeast) that prevents their secretion [88]. The finding that KDEL-proteins can be modified by post-ER enzymes indicated that these proteins are retrieved from Golgi cisternae [89,90]. Genetic screens in yeast identified ERD2 as the receptor mediating the retrieval of KDEL-proteins (KDELR) [46], and orthologues have since been characterized in vertebrates, including three human isoforms [91–93]. Vectorial transport of KDEL-proteins was suggested to exploit pH differences between the Golgi and the ER [94]. Proteins with a KDEL motif interact with a KDELR in the *cis*-Golgi, and the complex is then transported to the ER. There, the higher pH results in its dissociation, allowing the empty receptor to be recycled and participate in another round of transport.

Following the discovery of the KDEL signal, many variants of this sequence have been described and were shown to cause ER localization. In humans, a distinction between the various signals is achieved by differential specificities of the three KDELRs. Although some overlap exists, it appears that each KDEL receptor mediates the retrieval of a subgroup of soluble ER proteins [93].