



Yujie Li (Autor)

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Max-Planck-Institut Für Biophysikalische Chemie

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Telefon: +49 (0)551 54724-0, E-Mail: info@cuvillier.de, Website: <https://cuvillier.de>

1 INTRODUCTION

1.1 Vesicular transport in eukaryotic cells

Protein transport in exo- and endocytosis in eukaryotic cells is mediated by vesicular carriers. The exocytic pathway plays an essential role in many biological processes of living cells. The fundamental role is to maintain the dynamic structure and the function of the plasma membrane and other intracellular membranes. Cells use this pathway to secrete the majority of extracellular proteins. Neuronal cells also utilize this pathway for regulated release of neuropeptides and neurotransmitters. Endocytosis is a process whereby cells absorb materials (fluid, organic and inorganic molecules) from outside cell membranes.

Since the pioneering work of George Palade (Palade, 1975), more and more details have been known about the vesicular transport. The general scheme of these pathways is outlined in Fig.1.1. The passage of proteins through exocytic and endocytic pathways involves an orderly progression through a series of membrane-bound compartments. Proteins destined for the exocytic pathway are initially targeted to the endoplasmic reticulum (ER) where several posttranslational modifications occur. The first vesicular transport step along the exocytic pathway is from the ER to the *cis*-Golgi compartment. The proteins are further modified during the passage through medial Golgi compartments. Upon reaching the *trans*-Golgi network (TGN), the proteins are sorted and packaged into distinct vesicles destined for post-Golgi structures, either the plasma membrane or the endosomal/lysosomal compartments (vacuole in yeast). In the endocytic pathway, proteins are internalized into early endosomes, and transported via late endosomes to the lysosome/vacuole. Protein traffic occurs in both directions along the exocytic and endocytic pathways. Retrograde transport retrieves escaped “resident” proteins and components of the transport machinery and bring them back to their original compartments. All of these steps are highly regulated and balanced so that a large amount of cargo can flow through without affecting the integrity and steady-state composition of the cells’ organelles.

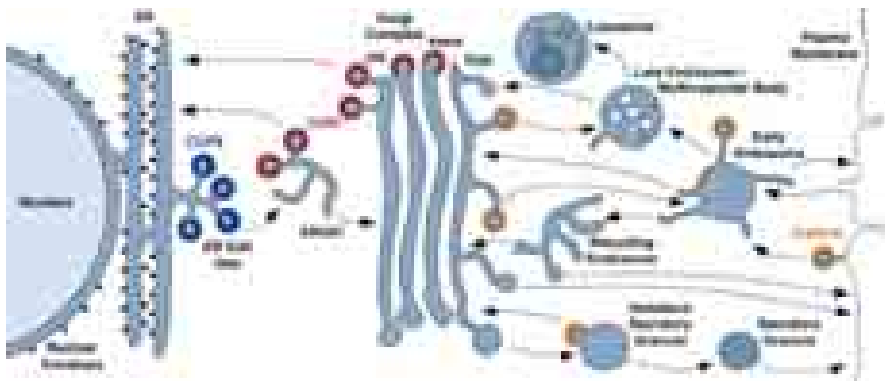


Fig. 1.1 Intracellular transport pathways

The scheme depicts the compartments of the secretory, lysosomal/vacuolar, and endocytic pathways. Transport steps are indicated by arrows. Colors indicate the known or presumed locations of COPII (blue), COPI (red), and clathrin (orange). (from Bonifacino and Glick, 2004)

The transport of cargo molecules between the organelles of the secretory pathway is mediated by transport vesicles. There are three major types of coated vesicles which differ in their coat proteins (shown in Fig.1.1 by different colors). Each type is used for different transport steps: clathrin-coated vesicles mediate various post-Golgi and endocytic vesicular trafficking steps (Kirchhausen, 2000); COPII vesicles are responsible for the anterograde ER-to-Golgi transport; COPI vesicles mediate the retrograde transport from the Golgi to the ER and the transport between the cisternae of the Golgi (Rabouille and Klumperman, 2005). The process of vesicular transport is divided into three main steps: vesicle budding, tethering/docking, and vesicle fusion with the target membrane. In general, as shown in Fig.1.2, vesicles bud from a “donor” compartment by a process that selectively incorporates the cargo into the forming vesicles while excluding resident proteins. The budding process involves the coat proteins, the monomeric GTPases of the dynamin and ARF family members, as well as adaptor proteins. The vesicle loses its coat due to many events including inactivation of the small GTPase, phosphoinositide hydrolysis and the action of uncoating enzymes. The naked vesicles subsequently move to a specific “acceptor” compartment, possibly traveling along the cytoskeleton (Kamal and Goldstein, 2000). They become tethered to the acceptor compartment with the help of Ypt/Rab-GTPases and tethering factors, and finally fuse with the appropriate target membrane to unload the cargo molecules.

Members of the SNARE, Ypt/Rab and Sec1/Munc18 (SM) protein families appear to direct and regulate the vesicle docking and fusion steps.

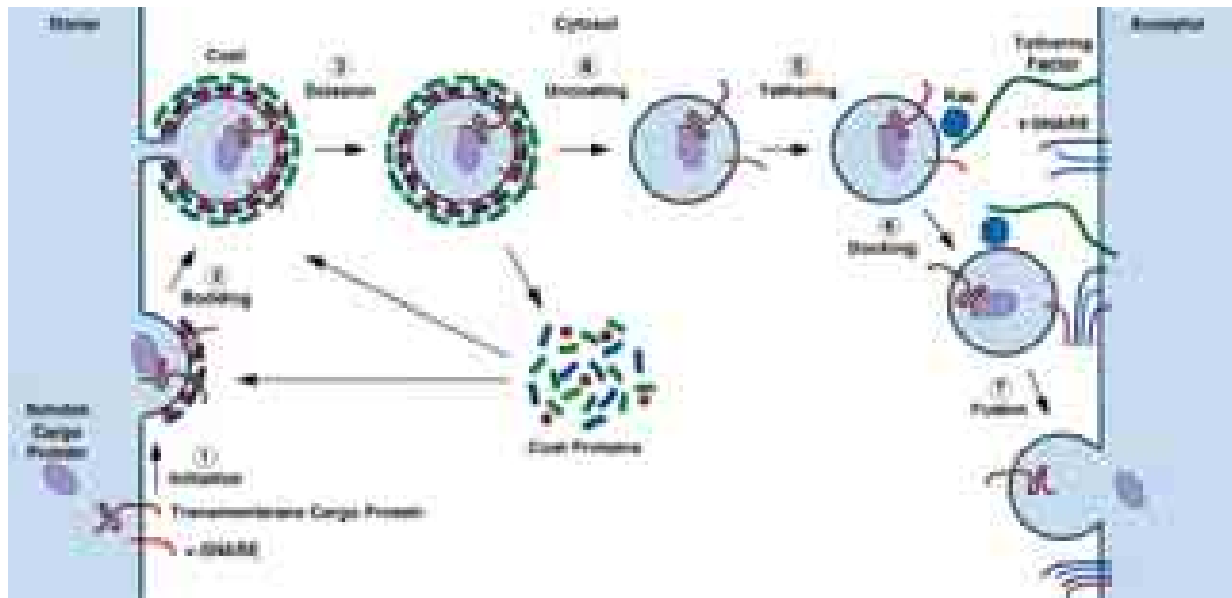


Fig.1.2 Transport vesicle formation and fusion

(1) Initiation of coat assembly. (2) Budding. Coat proteins associate with the donor membrane. Cargo becomes concentrated and membrane curvature increases. (3) Scission. (4) Uncoating. The vesicle loses its coat because of the inactivation of the small GTPase or other events. (5) Tethering. The naked vesicle moves to the acceptor compartment and becomes tethered to the acceptor membrane with the aid of tethering factors and Rab/Ypt-GTPases. (6-7) Docking and fusion. The SNAREs form a four-helix bundle (trans-SNARE complex) and then drive membrane fusion. (from Bonifacino and Glick, 2004)

1.2 Bi-directional protein transport between the ER and the Golgi

The endoplasmic reticulum consists of an elaborate and dynamic tubular and cisternal network that is continuous with the outer nuclear membrane. It has a central role in lipid and protein biosynthesis. The rough ER (regions of ER coated by membrane-bound ribosomes) is the place for protein synthesis and regarded as the entry point of the newly synthesized proteins into the secretory pathway. The smooth

ER (regions of ER that lack bound ribosomes) is proposed to function especially in lipid metabolism. It is sometimes called transitional ER because it contains the ER exit sites from which vesicles carrying the newly synthesized proteins and lipids bud off for transport to the Golgi apparatus. The Golgi apparatus functions as a processing and sorting station in the early secretory pathway. Under the electron microscope, the Golgi apparatus is typically seen as a stack of *cis*, *medial* and *trans* cisternae surrounded by numerous tubules and vesicles.

It is generally accepted that vesicular transport between the first two compartments of the secretory pathway, the ER and the Golgi, occurs in two directions: anterograde (ER-to-Golgi) transport delivers newly synthesized cargo proteins to the Golgi via COPII vesicles, whereas retrograde (Golgi-to-ER) transport retrieves ER residents and other machinery components back to the ER via COPI vesicles.

1.2.1 COPII vesicles

In *S. cerevisiae*, the core COPII components are the small Ras-like GTPase Sar1p and the two heterodimeric protein subcomplexes Sec23p/Sec24p and Sec13p/Sec31p (Barlowe et al., 1994). In addition, several regulatory proteins participate in COPII assembly, (Table 1.1), including Sec12p, a guanine nucleotide exchange factor (GEF) for Sar1p (Barlowe and Schekman, 1993), Sed4p, a Sec12p homolog that maybe a putative inhibitor of the Sar1 GTPase (Saito-Nakano and Nakano, 2000), and Sec16p, a putative scaffold protein which was proposed to organize and stabilize COPII coat assembly (Espenshade et al., 1995; Shaywitz et al., 1997).

1.2.2 COPI vesicles

In contrast to COPII coat proteins, there are seven COPI subunits (α , β , β' , δ , γ , ϵ , ζ) divided into two subcomplexes: the F subcomplex is composed of β , δ , γ and ζ -COPs, of which the β and γ subunits display homology to the AP complex appendage domains; and the B subcomplex, within which the α and β' subunits contain WD40 repeats similar to clathrin terminal domains (Table 1.2). When the two subcomplexes come together, cargo can be recruited into the budding vesicles (Takatsu et al., 2001). Just as Sar1p for COPII, Arf1p is the small GTPase for COPI. However, compared with

Sar1p, several exchange factors and GTPase-activating proteins exist for Arf1p (Table 1.2).

Table 1.1 Components of COPII ER export machinery

Yeast proteins	Mammal orthologs	Main properties	Functions
Sar1p	Sar1a Sar1b	Small GTPase of the Ras family	Recruits coat components to membranes in a GTP-dependent Manner; N-terminal helix initiates membrane curvature and completes the fission of a COPII vesicle.
Sec23p	Sec23A Sec23B	Sar1p GTPase binding subunit; GTPase-activating protein (GAP) for Sar1p.	Has GAP activity for Sar1p; The subcomplex is involved in cargo recognition and membrane curvature selection.
Sec24p Sfb2p Sfb3p	Sec24A Sec24B Sec24C Sec24D	Cargo binding subunit	
Sec13p Sec31p	Sec13 Sec31A Sec31B	Sec13p and Sec31p both have WD40 repeat motif and are components of the membrane-distal layer of COPII coat.	Induces coat polymerization
Sec12p	Sec12 (FERB)	Guanine nucleotide exchange factor (GEF) for Sar1p	ER-localized GEF leads to the recruitment of Sar1p and coat components to the ER.
Sed4p	unknown	Sec12 homolog devoid of Sar1p-GEF activity	Putative Sar1-GAP inhibitor
Sec16p	unknown	Large peripheral protein associated with the cytosolic face of the ER; Interacts with Sec23p/24p and Sec31p.	Stabilizes Sec23/24p complex and stimulates vesicle budding

Table 1.2 COPI components in yeast and mammalian cells

Coat	Main properties	Functions
F-subcomplex: β , δ , γ , ζ B-subcomplex: α , β' , ϵ		
Arf1p	Small GTPase of Ras family	Recruits COPI coatmer to membrane in a GTP-dependent manner
α COP / Ret1p	Contains WD40 repeats; binds KxKxx, KKxx motifs.	Recruits cargo and accessory factors
β COP	Binds Arf1p, has weak sequence identity to β -adaptin; has appendage domain like large AP subcomplex.	Binds to diacidic cargo motifs; binds to KDEL receptor; recruits cargo and accessory factors via appendage domain but not yet identified.
β' COP / Sec26p	Contains WD40 repeats; binds KxKxx motifs.	Recruits cargo
γ COP/ Sec21p	Binds members of the p24 family; has appendage domain like large AP subcomplex; interacts with Arf1.	Recruits cargo; recruits cargo and accessory factors (ArfGAP) via appendage domain.
δ COP / Ret2p	Has weak sequence identity to μ -adaptin	Binds accessory proteins (Dsl1p) via acidic tryptophan motif
ϵ COP / Sec28p	Interacts with Arf1	May stabilize the complex
ζ COP / Ret3p	Has weak sequence identity to σ -adaptin	Stabilizes the interaction between β - and γ -COP
Glo3p	GTPase-activating protein for Arf1p; binds β' WD40 motif	Activates Arf GTP hydrolysis to promote coat disassembly
Gea1p / Gea2p	Guanine nucleotide exchange factor for Arf1p	

1.3 Budding

As described above, proteins destined for residence at the plasma membrane, in endosomes or lysosomes move through the early compartments (the ER, and the Golgi apparatus) of the secretory pathway, and transfer of proteins between the ER and the Golgi apparatus is mediated by COPI and COPII transport vesicles. Vesicle budding involves a set of events. Generally, a small GTPase acts as molecular switch to turn on coat assembly. When activated, the small GTPase drives the initial recruitment of the coat which is then followed by the incorporation of coat components. Cargo molecules are recognized, and coat polymerization eventually drives the release of the vesicles from the donor membrane.

1.3.1 Coat assembly

The vesicle budding process starts with the activation of the small GTPases: Sar1p for COPII and Arf1p for COPI (Fig.1.3). Both proteins cycle between a soluble GDP-bound inactive form and an active GTP-bound, membrane-associated form. Sec12p, the guanine nucleotide exchange factor for Sar1p, is an integral membrane protein located at the ER (Barlowe and Schekman, 1993). There are two guanine nucleotide exchange factors for Arf1p in yeast, Gea1/2p, which are cytosolic proteins and may localize to the membrane with lipid- or protein-binding domains or interact with specific transmembrane proteins (Garcia-Mata et al., 2003). Under the influence of the specific GEF, which exchanges GDP for GTP, small GTPases are activated. The exchange of GDP for GTP is accompanied by translocation of Sar1p/Arf1p from the cytosol to the membrane and exposure of an amphipathic α -helix, which acts as a membrane anchor during coat assembly (Antonny et al., 1997; Goldberg, 1998; Huang et al., 2001).

During COPII vesicle formation, membrane-bound Sar1p-GTP leads to the recruitment of Sec23p/Sec24p to the membrane to form a prebudding complex which might assemble and capture cargo and their receptors. Sec24p was demonstrated to be responsible for the majority of integral membrane cargo recognition and multiple cargo binding sites have been discovered within this protein (Peng *et al.*, 1999; Votsmeier and Gallwitz, 2001; Miller *et al.*, 2003; Mossessova *et al.*, 2003). Sec23p directly binds to Sar1p and stimulates its slow intrinsic GTPase activity by supplying an "arginine finger" in the catalytic site (Bi et al., 2002). Interestingly, recent work

uncovered a novel role for Sar1p in COPII vesicle generation: the N-terminal helix of Sar1p seems to directly initiate membrane curvature and complete the vesicle fission. In support of this, mutations in the Sar1p N-terminal α -helix diminish membrane curvature and are defective in vesicle formation but do not affect the coat recruitment and cargo capture (Lee et al., 2005).

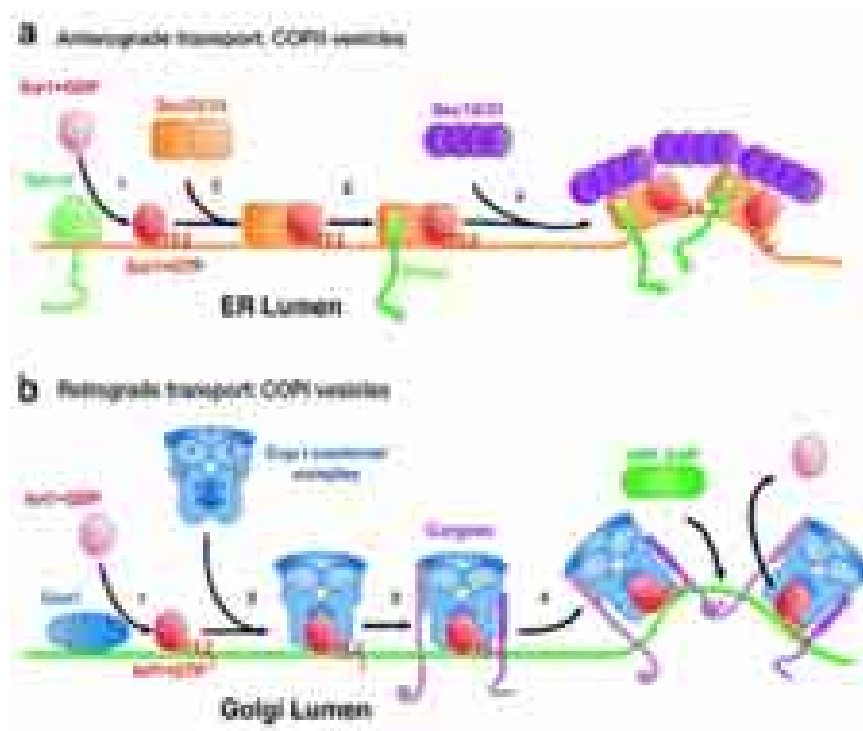


Fig.1.3 Coat assembly and vesicle budding. Schematic representation of COPII (a) and COPI (b) vesicle formation and cargo capture during anterograde and retrograde transport between ER and Golgi. (from Lee *et al.*, 2004)

Sec13p/Sec31p complex binds and thus forms the outer layer of the coat. Electron microscopy analysis of Sec13/31p suggests a flexible, elongated assembly

(Lederkremer *et al.*, 2001; Matsuoka *et al.*, 2001). Sec13p/31p may act as a structural scaffold to gather adjacent Sec23/24p complexes into a coat lattice, thus concentrating cargo proteins and propagating membrane curvature. The binding of Sec13p/Sec31p complex also further enhances the Sec23p-mediated GAP activity to approximately ten-fold (Antonny *et al.*, 2001). Although GTP hydrolysis is not required in protein sorting mediated by the COPII coat, it is an essential aspect of coat disassembly. Upon GTP hydrolysis, a conformational change in Sar1p causes release of Sar1p-GDP and the Sec23p/Sec24p, Sec13p/Sec31p complexes from the membrane. Thus, GTP hydrolysis accelerated by the action of the Sec23 GAP on Sar1p; or further enhanced by the polymerisation of the coat induced by Sec13/31p, is required to produce an uncoated vesicle exposing SNARE proteins to a target membrane for the further tethering and fusion events.

SEC16, an essential gene in *S.cerevisiae*, encodes a large hydrophilic protein that associates tightly with the ER membrane. It is a candidate scaffold protein and COPII-interaction partner to ligate both COP heterodimers (Sec23p/24p and Sec13p/31p) (Espenshade *et al.*, 1995). Sec16p may nucleate the Sar1-GTP-dependent initiation of COPII assembly and serve to stabilize a coat assembly intermediate without regulating Sar1p-GTP hydrolysis (Supek *et al.*, 2002). Sec16p may therefore act as a tether to prevent premature discharge of coat subunits before the completion of vesicle fission from the ER membrane.

The budding of COPI vesicles is quite similar to that of COPII vesicles in that the coat recruitment is initiated by GDP-GTP exchange on Arf1p, mediated by the Arf1p exchange factors Gea1/2. Membrane-bound Arf1p then recruits the preassembled heptamer complex, which contains both the F-subcomplex and the B-subcomplex. However, this assembly is a one-step assembly in contrast to the sequential assembly of the Sec23p/24p and Sec13p/31p subcomplexes during the generation of COPII vesicles.

1.3.2 Cargo selection

Cargo proteins are selected and actively accumulated into COPI and COPII vesicles. Cargo selection and coat assembly are dependent on each other. The sorting