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COPII Coat Assembly and Selective Export from the Endoplasmic Reticulum

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The coat protein complex II (COPII) generates transport vesicles that mediate protein transport from the endoplasmic reticulum (ER). Recent structural and biochemical studies have suggested that the COPII coat is responsible for direct capture of membrane cargo proteins and for the physical deformation of the ER membrane that drives the transport vesicle formation. The COPII-coated vesicle formation at the ER membrane is triggered by the activation of the Ras-like small GTPase Sar1 by GDP/GTP exchange, and activated Sar1 in turn promotes COPII coat assembly. Subsequent GTP hydrolysis by Sar1 leads to disassembly of the coat proteins, which are then recycled for additional rounds of vesicle formation. Thus, the Sar1 GTPase cycle is thought to regulate COPII coat assembly and disassembly. Emerging evidence suggests that the cargo proteins modulate the Sar1 GTP hydrolysis to coordinate coat assembly with cargo selection. Here, I discuss the possible roles of the GTP hydrolysis by Sar1 in COPII coat assembly and selective uptake of cargo proteins into transport vesicles.

Key words: coat protein, endoplasmic reticulum, GTPase, Sar1, vesicular transport.

In eukaryotic cells, intracellular protein transport between the organelles of the secretory pathway is mediated by vesicular carriers that are released from a donor organelle and fuse with an appropriate acceptor organelle (*1*, *2*). The formation of transport vesicles and sorting of cargo into the emerging vesicles are mediated by protein coats (*3*, *4*). Three kinds of transport vesicle have been characterized that are defined by their coat proteins: COPII-coated vesicles allow proteins to exit from the ER toward the Golgi complex (*5*); COPI-coated vesicles are responsible for retrograde Golgi-to-ER and intra-Golgi transport steps (*6*); and clathrin-coated vesicles mediate post-Golgi and endocytic transport processes (*7*). A common feature of these vesicular carriers is that most of them employ small GTPases to direct coat assembly at the donor membranes (Fig. 1). However, controversy remains about when the bound GTP is hydrolyzed and how the GTP hydrolysis is regulated. The COPII vesicle is one of the best-studied transport vesicles, since it can be generated from isolated organelle membranes (*8*), synthetic liposomes (*9*), and cargo-reconstituted proteoliposomes (*10*). Here, I review the mechanism of COPII vesicle biogenesis, in particular focusing on the roles of GTP hydrolysis by Sar1 in the coordination of coat assembly and cargo sorting.

COPII vesicle formation at the ER membrane

The COPII coat consists of the small GTPase Sar1 (*11*) and the heterodimeric protein complexes Sec23/24 and Sec13/31 that sequentially bind to the ER membrane (*8*) (Fig. 2). Assembly of the COPII coat on the ER membrane is initiated by the exchange of GDP for GTP on Sar1, which is catalyzed by the transmembrane guanine nucleotide exchange factor (GEF) Sec12 (*12*, *13*). Sec12 is strictly regulated to localize to the ER, and thereby COPII assembly is restricted to the ER (14–16). The exchange of GDP for GTP triggers the exposure of the N-terminal hydrophobic element of Sar1, which is inserted into the ER membrane (*17*, *18*). Activated Sar1-GTP then recruits the Sec23/24 complex by binding to the Sec23 portion, and the cytoplasmically exposed signal of the transmembrane cargo is captured by direct contact with Sec24 to form a so-called “prebudding complex” (*19*, *20*). Thus, the formation of the prebudding complex consisting of Sar1-Sec23/24 bound to cargo protein is the cargo-recognition step prior to coat polymerization. The prebudding complexes are then clustered by Sec13/31 to form COPII vesicles (*21*).

Additional regulatory factors, Sec16p and Sed4p, are known to contribute to the COPII assembly, although they appear to be specific for *S. cerevisiae* and closely related species. Sec16p is a hydrophobic protein that associates peripherally with the ER membrane and contains domains that can directly contact with the COPII coat (*22*). Therefore, Sec16p may act as a scaffold for assembly of the coat. Sed4p is an integral membrane protein located at the ER membrane, and deletion of the *SED4* gene retards transport from the ER to the Golgi (*23*). Although the cytoplasmic domain of Sed4p shares significant homology with that of Sec12, its GEF activity has not been demonstrated (*24*). However, this domain interacts directly with Sec16p at the ER membrane, and thus these factors seem to function together in coat assembly. Further studies are required to clarify the roles of Sec16p and Sed4p.

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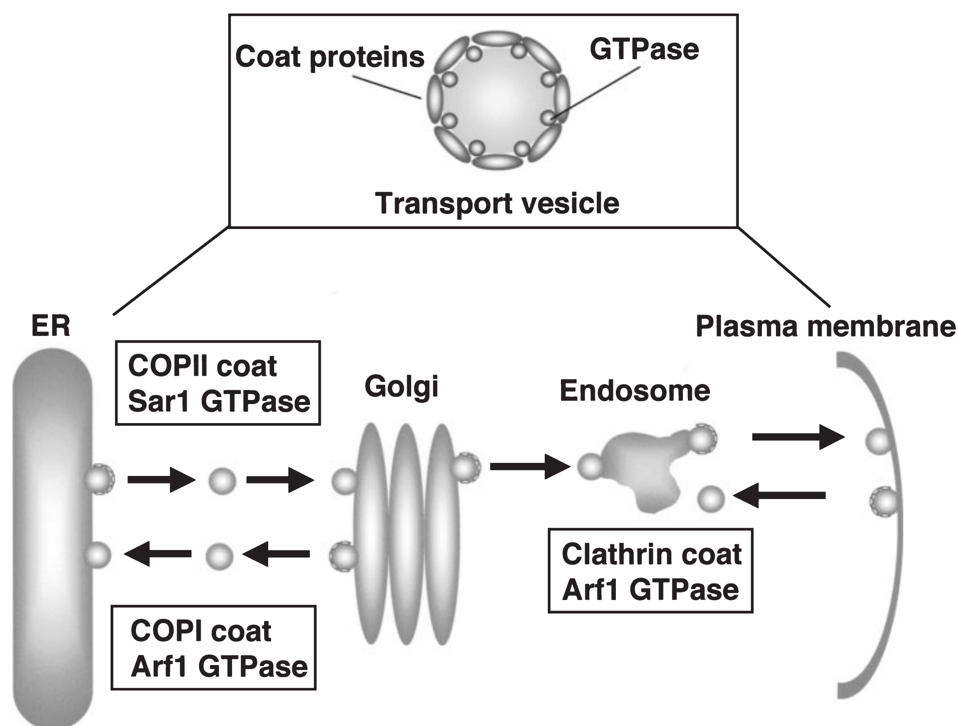

Fig. 1. **Coat proteins and GTPases in vesicular traffic.** Transport vesicles (50–100 nm in diameter) are generated by the actions of both coat proteins and small GTPases. The vesicle formation is initiated by the recruitment of cytosolic coat proteins to the surface of the donor membrane, which then induce deformation of the membrane into a coated vesicle. The COPII coat is known to mediate export from the ER to either the ER-Golgi intermediate compartment (ERGIC) or the Golgi complex, whereas the COPI coat is involved in retrograde transport from the Golgi to the ER as well as in intra-Golgi transport. The clathrin coat functions in transport between post-Golgi compartments including the trans-Golgi network (TGN), endosomes, and the plasma membrane. In all cases, activation of the small GTPase to its GTP-bound state precedes recruitment of the coat proteins to the membrane. Sar1-GTP is involved in the COPII system and Arf1-GTP participates in the COPI and clathrin systems.

ER export signals

It is now quite evident that the majority of secretory cargo proteins do not exit the ER by bulk flow, but rather are actively sorted from ER resident proteins and incorporated into the prebudding complexes. Certain integral membrane cargos are captured into the prebudding complexes through direct interactions with COPII subunits (*19*), and several lumenal secretory proteins require specific transmembrane cargo receptors to mediate the interaction with the COPII coats (*25*, *26*). The signals recognized by the COPII coat are found in the cytoplasmic domains of transmembrane cargo proteins. These signals are quite diverse, and several classes of ER export signals have so far been identified on the cytoplasmic tails of cargo proteins from various organisms (*27*). For example, vesicular stomatitis virus glycoprotein (VSV-G) (*28*), mammalian Kir2.1 potassium channel protein (*29*), and yeast Sys1p and Gap1p (*30*) possess cytoplasmically exposed diacidic (DXE) motifs that are required for transport from the ER. Dihydrophobic (FF, YY, LL, or FY) motifs are found in the cytoplasmic domains of several transmembrane cargos such as the p24 family proteins (*31*) (*32*), the Erv41/46p complex (*33*), and the ERGIC53/Emp47p family proteins (*34*, *35*). Another motif identified is the tyrosine-containing motif found in the tail sequence of the ERGIC53/Emp47p family proteins.

Like COPII, the COPI coat binds to transmembrane proteins that are retrieved from the Golgi to the ER by recognizing the C-terminal K(X)KXX motifs in their cytoplasmic tails (*36*). These motifs are able to serve as retrieval signals when transplanted to other proteins. No such a simple transplantable motif for COPII binding has so far been identified, yet many of the cargo proteins that exit from the ER require multiple signals to be packaged into COPII vesicles, such as a combination of any of the above motifs or oligomeric association (*10*, *33*, 37–40). The assembly-dependent cargo sorting at the ER exit sites might be important in terms of the ER quality control. The ER contains considerable amounts of newly synthesized unfolded or unassembled cargos, which should be segregated from secretory proteins to be exported. It is tempting to speculate that combinations of ER export signals ensure efficient exclusion of unassembled cargos from COPII vesicles. This might be the reason why the ER requires different mechanisms from the Golgi in protein sorting.

The utilization of diverse export signals also implies the existence of either multiple binding sites on the coat protein or various subtypes of COPII coat. Recent studies have suggested that both are the case. Several distinct Sec24 family members, Iss1p/Sfb2p (*41*) and Lst1p/Sfb3p (*42*) in yeast, and Sec24A-D in higher eukaryotes (*43*), are able to pair with Sec23 and bind to export signals different from those recognized by Sec24. Putative signal-binding sites on the Sec24 molecule became available when the crystal structure of the prebudding complex was determined (*18*), and binding sites for three SNARE proteins, Sed5, Bet1, and Sec22, have been mapped (*44*, *45*). These studies indicate the presence of at least three distinct signal-binding sites, termed “A-site,” “B-site,” and “Arg342,” have been identified so far. Furthermore, it is notable that one of them, which corresponds to the B-site, is conserved as a cargo-interaction domain on Lst1p. These data together suggest that the binding properties, but not specificities, are conserved between the Sec24 homologs. Moreover, recent studies have suggested that Sar1 also plays a role in cargo recognition by direct binding to the export signals (*46*).

GTP hydrolysis and cargo selection

In vitro reconstitution experiments using synthetic liposomes have defined components required for genera-

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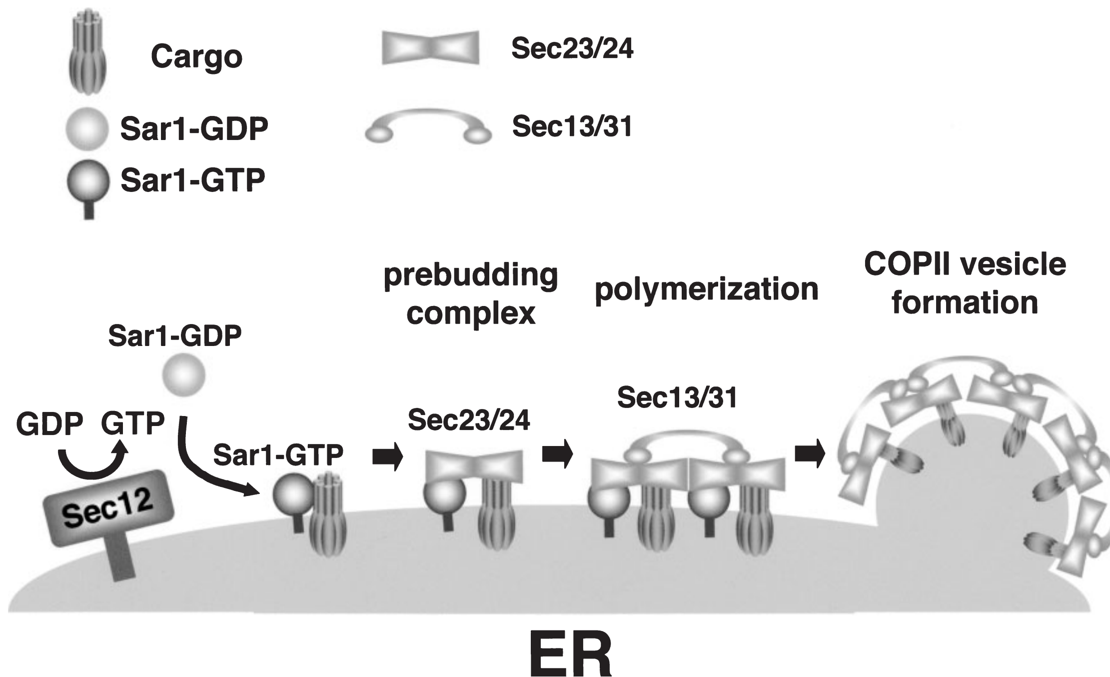

Fig. 2. **COPII vesicle formation and the selective uptake of cargo molecules.** The COPII vesicle formation is initiated by GDP-GTP exchange on Sar1 catalyzed by the transmembrane guanine nucleotide exchange factor Sec12. Activated Sar1-GTP recruits the Sec23/24 subcomplex by binding to the Sec23 portion, and the cytoplasmically exposed signal of transmembrane cargo is captured by direct contact with Sec24, forming the "prebudding complex." It is currently not clear whether the membrane-bound Sar1-GTP associates with cargo before the recruitment of Sec23/24 or lateral diffusion of Sar1-GTP-Sec23/24 complex captures cargo. These prebudding complexes are clustered by the Sec13/31 subcomplex, generating COPII-coated vesicles. The COPII vesicles then shed their coat before fusion, which is achieved by the activity of GTPase-activating protein (GAP). The Sec23 subunit is the GAP for Sar1, and this activity is further stimulated by the recruitment of Sec13/31. Thus, assembly of the complete COPII coat triggers its disassembly, but the precise timing is not known.

tion of COPII-coated vesicles from donor membranes; these are Sec23/24, Sec13/31, and GTP-locked Sar1 with a non-hydrolyzable GTP analog (*9*). Therefore, the GTP hydrolysis by Sar1 is dispensable for coat polymerization and subsequent membrane deformation. Conversely, transport vesicles shed their coats before fusion with their target membrane. This is achieved by the activity of GTPase activating protein (GAP), which accelerates GTP hydrolysis and leads to coat disassembly. In the case of COPII vesicles, GTP hydrolysis by Sar1 triggers coat disassembly and reverses the assembly process. A paradoxical implication of this mechanism is that the Sec23 subunit is the GAP for Sar1 (*47*), and the GAP activity is further stimulated by recruitment of Sec13/31 (*48*). Therefore, assembly of the complete COPII coat triggers disassembly, which compromises the capture of cargo molecules and coat polymerization. How can the prebudding complexes polymerize before the GTP hydrolysis and dissociation of the coat subunits? It is expected that a number of regulatory interactions contributed by cargo proteins or modulators of coat assembly might temporarily restrict the GTP hydrolysis by Sar1 to stabilize the prebudding complex until it is incorporated into the polymerized coat. However, it had been difficult to examine GTP hydrolysis of Sar1 during coat assembly and cargo sorting with *in vitro* budding assays using fractionated organelle membranes. Recent progress in *in vitro* reconstituted assays with cargo-reconstituted proteoliposomes has made it possible to test this issue (*49*).

Yeast Emp47p, a type-I membrane protein that cycles between the ER and the Golgi, has been proposed as a cargo receptor at the ER exit sites (*35*, *50*). This protein contains COPII-binding signals in its C-terminal tail and is packaged into COPII vesicles. When the proteoliposomes reconstituted with purified Emp47p and the ER resident protein Ufe1p were mixed with COPII proteins and guanine nucleotide, Emp47p but not Ufe1p was concentrated into synthetic COPII vesicles. The GTP hydrolysis by Sar1 as stimulated by Sec23/24 has been directly tested with the cargo-reconstituted proteoliposomes. Strikingly, when Emp47p is present on liposomes, the GTP hydrolysis by Sar1 was more efficient than without cargo, which also accelerates prebudding complex disassembly (*49*). Thus, the prebudding complex with cargo appears to have a shorter lifetime on membranes than that without cargo. In the light of this finding, it seems unlikely that cargo binding could restrict the Sar1 GTP hydrolysis to extend the lifetime of the prebudding complex. An alternative model should be developed to explain the cargo-dependent stimulation of the Sar1 GTPase activity during COPII vesicle formation.

How might the prebudding complex coordinate the Sar1 GTP hydrolysis with coat assembly? The most probable explanation is that the GTP hydrolysis causes release of Sar1 from the prebudding complex, while the

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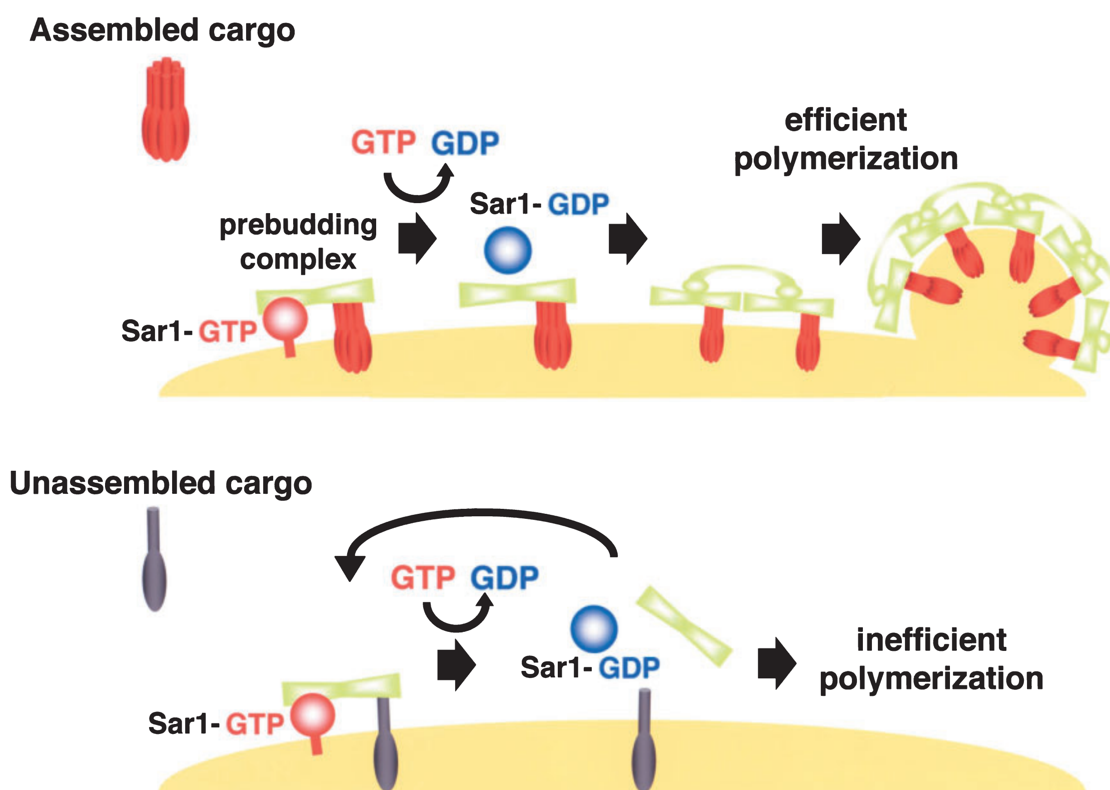

Fig. 3. **A model for GTP hydrolysis-driven proof-reading by Sar1.** The assembled cargo has a high affinity for the COPII coat because of the combined export signals. This cargo-coat association persists long enough for cargo to be incorporated into COPII coats after the Sar1 GTP hydrolysis. By contrast, the Sar1 GTP hydrolysis dissociates the weak association between the coat and unassembled cargo before polymerizing into COPII coats. Thus, the prebudding complex stabilities are biased towards the complex including assembled cargo, ensuring that the fully assembled cargo is preferentially incorporated into COPII vesicles.

other coat subunits remains assembled on the membrane; and in this case the polymerized coat may be stable enough to preserve the structure in the absence of Sar1. Alternatively, the kinetics of vesicle formation might be faster than the kinetics of Sar1 GTP hydrolysis, so that there would be time for a vesicle to form before the prebudding complex disassembly. In fact, the prebudding complexes have a relatively slow rate of GTP hydrolysis (~30 s) (*48*), which might be long enough to allow the vesicle formation. Subsequent binding of Sec13/31 stimulates the GTPase rate additional tenfold, resulting in loss of the coat.

A kinetic proof-reading model

Although it has been thought that GTP hydrolysis is not required for cargo selection or coat polymerization, a recent report has suggested that this may not be the case for certain cargos. Emp47p has been shown to form a 600-kDa homo-oligomer through the coiled-coil region within its lumenal domain in the ER, which is essential for the exit from the ER. In contrast, monomeric Emp47p lacking the coiled-coil region retains the ability to form the prebudding complex but is no longer incorporated into COPII vesicles (*10*). However, when vesicles were generated with *in vitro* budding assays in the presence of a nonhydrolyzable GTP analog, GMP-PNP, the packaging efficiency of monomeric Emp47p was significantly higher than that in the presence of GTP. The amounts of other cargos found in the vesicle fractions were comparable under the GTP- and GMP-PNP–containing conditions, indicating a preferential incorporation of monomeric Emp47p into COPII vesicles in the absence of Sar1 GTP hydrolysis (*49*). In other words, Sar1 selectively promotes exclusion of unassembled cargos from COPII vesicles by virtue of its GTP hydrolysis. The monomeric Emp47p mutant lacking the ability to assemble into an oligomer might mimic a transient state of newly synthesized unassembled Emp47p in the ER. Such an unassembled cargo should be efficiently excluded from COPII vesicles, although it has an ability to bind coat subunits via its C-terminal signal. Hence, the prolonged binding of coat proteins to unassembled cargo, achieved by the absence of Sar1 GTP hydrolysis, allows the cargo incorporation into vesicles. I propose a "kinetic proof-reading" model outlined in Fig. 3. The assembled cargo in the prebudding complex has a high affinity for the COPII coat subunit because of the combined export signals displayed on assembled subunits of the protein oligomers. This cargo-coat complex persists long enough to be polymerized into COPII vesicles even after the Sar1 GTP hydrolysis. By contrast, the weak association between the coat and a single export signal can be disrupted by the Sar1 GTP hydrolysis before clustering into COPII coats. Thus, the GTP hydrolysis of Sar1 counteracts the prebudding complex polymerization with unassembled cargo, ensuring that only the prebudding complex with fully assembled

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cargo is successfully incorporated into COPII vesicles. Further analyses will be needed to characterize the contribution of the Sar1 GTPase to the cargo selection.

Future prospects

Recent structural and biochemical studies have begun to uncover how the COPII coat captures cargo molecules and assembles on the ER membranes. The small GTPase Sar1 is likely to have a key function in the regulation of coat dynamics and cargo sorting during vesicle formation. Future studies are likely to focus on how the Sar1 GTPase regulates coat recruitment and vesicle uncoating. To draw a comprehensive picture of how the GTPase controls cargo sorting and coat polymerization, a combination of structural approaches and real-time assays that monitor coat assembly and disassembly should be informative.

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