# Vesicle coating and uncoating: controlling the formation of large COPII-coated carriers

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### Abstract

The basic mechanisms underlying the formation of coated vesicles are now defined in considerable detail. This article highlights recent developments in our understanding of the problem of exporting large macromolecular cargo such as procollagen from the endoplasmic reticulum and discusses the implications that this has for cell and tissue organisation and human disease.

## Introduction and context

Transport of molecules between intracellular membrane-bound compartments is vital for organelle homeostasis and secretion. Genetic and biochemical studies provided the basis for the vesicular transport hypothesis, which begins with protein sorting, vesicle budding, vesicle targeting, and finally vesicle fusion [1]. The first membrane-trafficking step in the secretory pathway, the export of secretory cargo from the endoplasmic reticulum (ER), requires the coat protein II (COPII) coat complex [2]. COPII is a multi-protein complex that is built up sequentially through Sar1-dependent recruitment of other cytosolic components to the membrane. In metazoans (and the yeast *Pichia pastoris* but notably not *Saccharomyces cerevisiae* [3]), COPII assembly occurs at specialised zones of ER membrane that are defined by electron microscopy (EM) and called transitional ER [2,4]. Together with the nascent budding structures, the transitional ER and other COPII-labelled profiles go to make up an ER exit site (ERES), a term originating largely from light microscopy observations. The small guanine triphosphate (GTP)-binding protein Sar1 is recruited to the ER membrane following guanine diphosphate (GDP)/GTP exchange by its guanine nucleotide exchange factor (GEF), Sec12. Sar1-GTP is stabilised on the membrane via insertion of an N-terminal alpha helix into the lipid bilayer; this can drive deformation of the membrane and even fission [5] as well as result in recruitment of Sec23-Sec24. Typically defined by *in vitro* reconstitution assays, Sar1 in association with cargo and Sec23-24 has been termed the pre-budding complex. Within this pre-budding complex, Sec23 provides Sar1 GTPase-activating protein (GAP) activity and Sec24 provides a cargo-binding function [6]. The GAP activity of Sec23 is balanced at this pre-budding stage by the GEF activity of Sec12 [7]. The Sec13-Sec31 complex is recruited to form the outer layer of the coat and can further increase the GAP activity of Sec23 on Sar1 [8]. This results in rapid coat disassembly to form the mature vesicle; inherent to this mechanism is that coat disassembly is programmed into coat assembly.

The formation of vesicles at transitional ER presents a specific problem relating to the export of large secretory cargo from the ER; macromolecules measuring hundreds of nanometres must be accommodated within coated vesicles and these are typically described as carriers of 60-80 nm in diameter; such issues are not so apparent for COPI-mediated pathways, and much unusually large endocytic cargo is internalised via clathrin-independent mechanisms or following only partial assembly of a clathrin lattice [9,10]. It is important to note that the classical image of 60-80 nm coated vesicles arises largely from *in vitro* reconstitution assays performed in the presence of non-hydrolysable analogues of GTP (for example, [11]). The very presence of coated vesicles in

mammalian cells remains controversial; while they are detectable [12], it is likely that only very few are present at any one time due to their rapid uncoating.

Premature disassembly of the coat is prevented by ongoing guanine nucleotide exchange on Sar1 catalysed by the ER-localised transmembrane protein, Sec12 [7,13]. Sec12 is excluded from the vesicle itself, which means that, once independent of the ER, the coat is stabilised only by coat-cargo interactions [13]; GTP hydrolysis therefore ultimately leads to coat disassembly. The presence of Sec12 in the ER membrane would maintain an active pool of Sar1-GTP at the bud neck [7], and from such *in vitro* reconstitution experiments (elegantly described in [14]), one can infer that nascent budding structures will contain more Sar1-GTP at the neck of a budding vesicle than within the core of the coat, which is likely to have key implications for vesicle fission [5,15]. Further factors are likely to be essential; for example, recruitment of Sar1 to mammalian membranes is ATP-dependent [16]. In all cases, the COPII complex plays an integral role in the selection of cargo, and the features described above have key implications for the generation of both small and large transport carriers. Notably, intimate control of coating and uncoating is required to facilitate the incorporation of atypically large cargo.

## Major recent advances
### Cranio-lenticulo-sutural dysplasia and chylomicron retention disease
Recent work has shown that highly efficient coupling of Sec23-Sec24 to Sec13-Sec31 is required for export of collagen [17-19]. A mutation (F382L) in one of the two Sec23 genes in humans, *sec23a*, causes cranio-lenticulo-sutural dysplasia (CLSD) [20], a disorder characterised by craniofacial development defects. Mutation of *sec23a* in zebrafish also causes craniofacial development defects [18]. In these *crusher* mutant fish, chondrocytes contain extended ER compartments containing accumulated extracellular matrix proteins, notably collagen II, the major component of cartilage extracellular matrix, which is normally packaged as 300 nm procollagen bundles that exit the ER [18]. Accumulation of intracellular collagen is also seen in cells from CLSD patients. Recent data from the labs of Schekman, Orci, and Goldberg provide a detailed mechanistic explanation for the defects in COPII function seen in CLSD and *crusher* mutant zebrafish [17,21]. Elegant biochemical and structural data from the Goldberg lab show that the F382 residue sits on the surface of Sec23A where Sec31 binds as an extended polypeptide [21]; this region of Sec31 also contacts Sar1. Consistent with this failure to efficiently couple the inner and outer layers of the COPII coat in *sec23a* mutants, the gross phenotypes of collagen

accumulation and craniofacial development defects are recapitulated by morpholino suppression in zebrafish of Sec13 [19]. Sec13 suppression in human fibroblasts drastically inhibits secretion of procollagen, but not the transport of small cargo. This suggests that the efficient and concerted assembly of the full coat is essential for transport of large cargoes [19].

Intriguingly, the two Sar1 proteins are expressed by many cell types. Sar1A and Sar1B have differing affinities for the Sec13-Sec31 complex [17], and Sar1A but not Sar1B was shown to partially rescue the F382L-Sec23A phenotype present in CLSD patients [17]. A prediction from the crystal structure that contains elements of Sar1, Sec23-Sec24, and Sec31 [21] is that Sar1 and Sec31 interact at residues that differ between the two Sar1 isoforms. As highlighted previously [14], the structure of cages formed with Sec13-31 alone shows a structure composed of squares and triangles [22] rather than the hexagons and pentagons that are seen in the clathrin coat. This, coupled with relatively simple inter-subunit contacts, would make Sec13-Sec31 inherently able to accommodate a wide range of cargo sizes [14,22].

Another human disorder, chylomicron retention disease (CMRD), is caused by mutations in *sara2*, which encodes Sar1B; loss of Sar1B causes severe fat malabsorption and retention of chylomicron-like particles in membrane-bound compartments [23]. Chylomicrons are large lipoprotein particles (hundreds of nanometres in diameter) that, like collagen, require the formation of large carriers for export from the ER. This supports the theory that Sar1B could be required selectively for transport of chylomicrons due to its weaker affinity for Sec13-Sec31 and thereby facilitate the formation of more flexible COPII cages [24]. However, as stated above, Sar1A can rescue the budding defect caused by the F382L mutation of Sec23A [17], at least *in vitro*, so Sar1B cannot be required specifically for large cargo export. While the tissue-specific defects seen in CLSD and CMRD could result from differences in the expression profile of Sar1 isoforms, it remains possible that additional cargo-specific factors are involved.

### A role for specific cargo receptors
Many cargo receptors have now been identified for a variety of processes, including ER-Golgi intermediate compartment (ERGIC)-53, Erv29p/Surf-4, and Emp24p [25-28]. While these transmembrane cargo adaptors can also engage the COPII complex, no concrete data exist to suggest that these play a major role in modulating the kinetics of COPII vesicle formation to facilitate large-carrier formation. This might reflect the fact that these proteins mediate the export of small freely diffusible

cargoes. Recently, TANGO1 was characterised as a cargo receptor that could fulfil such a role for at least one macromolecular cargo [29]. TANGO1 was identified in a screen for regulators of transport and organisation of the ER/Golgi interface [30] and has been shown to be a collagen VII-binding protein that also links to the Sec23-Sec24 layer of the COPII coat [29]. Suppression of TANGO1 blocks the secretion of collagen VII from cells but not that of collagen I, showing that it is not a general collagen receptor within the ER. It also differs from other known cargo receptors in that it localises to ERESs at a steady state [29]. TANGO1 is expressed ubiquitously and yet collagen VII is more restricted in its expression pattern [31]. This suggests that TANGO1 could act as a transport adaptor for other cargoes or play other roles relating to COPII function in other tissues.

TANGO1 could also play a role in the global organisation of ERES [29], but it remains unclear whether this relates to a direct effect on COPII dynamics or results from an accumulation of collagen within the ER following TANGO1 suppression. This latter possibility [29] is consistent with data showing that Sec13 suppression also leads to an accumulation of collagen within the ER and perturbation of ERES organisation [19]. However, this effect on COPII organisation was also seen in HeLa cells, which secrete very little collagen [19]. The role of TANGO1 is further complicated by the presence of an alternative truncated form of the protein (called TANGO or melanoma inhibitory activity family, member 3 [MIA3]) that is secreted [32] and modulates cell adhesion [33]. Expression of TANGO/MIA3 is reported to be down-regulated in colon and hepatocellular carcinomas [34], which could also relate to a defect in epithelial organisation resulting from a loss of collagen VII secretion [35].

### Future directions

Control of coat formation is clearly a key aspect to ensure incorporation of large cargo; the other key aspect, of course, is vesicle uncoating. The role of cargo receptors in the export of these macromolecular cargoes remains unclear. One can hypothesise that the accumulation of GTP-loaded Sar1 at bud sites could direct both membrane deformation and, through concerted local GTP hydrolysis, fission [5,15] (Figure 1). This could require selective stabilisation of Sar1-GTP at the neck of nascent buds by Sec16 [36] or TANGO1 [29] (Figure 1). This would be consistent with these proteins being excluded from budding vesicles as has been shown for TANGO1 [29]. The fission step, in particular, remains ill defined here. A key future direction is to study how these factors, as well as the isoforms of core COPII subunits (such as Sar1A versus Sar1B), affect the kinetics and thermodynamics of COPII assembly.

**Figure 1. Possible mechanisms to couple coat protein II (COPII) assembly to the export of collagen**
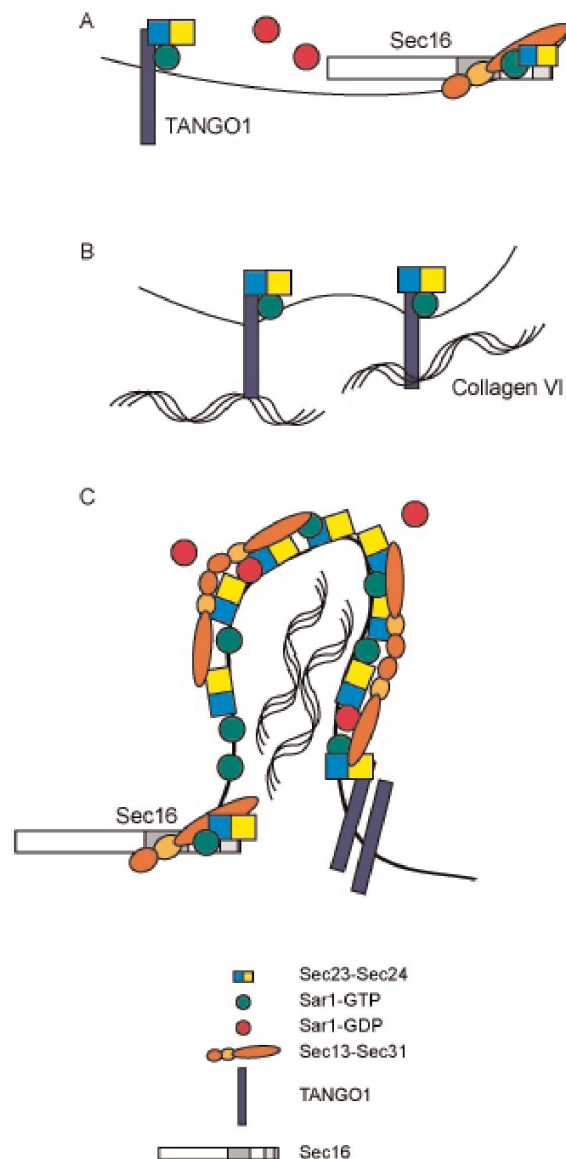


**(A)** TANGO1 and Sec16 both have the capacity to interact with COPII components to increase the local pool of 'active' COPII. Such clustering of Sar1-GTP could induce membrane curvature to initiate bud formation. **(B)** TANGO can also interact with collagen VII. However, it is not clear whether TANGO1 can engage both collagen VII and Sec23-Sec24 simultaneously. **(C)** Together, these properties provide a potential mechanism to couple the incorporation of cargo with the organisation of COPII assembly. Whether either Sec16 or TANGO1 can modulate the kinetics of GTP hydrolysis by Sar1, or other aspects of COPII assembly, remains to be determined. Furthermore, it is not known whether the engagement of cargo by TANGO1 has any impact on COPII dynamics. Assembly of Sec13-Sec31 is also required for efficient export of collagen I, indicating that coordinated assembly of COPII is central to the export of collagen from the endoplasmic reticulum. GDP, guanine diphosphate; GTP, guanine triphosphate.

For larger carriers, the question remains open as to whether completion of coat formation is absolutely required [37] or whether partial coats analogous to those seen during endocytic entry of vesicular stomatitis virus can form [9]. One could envisage that the loss of assembled coat at regions of buds more distal to the neck could occur before the completion of scission. Data support the idea that the amount of Sar1-GTP is different within the 'older' part of the coat compared with that at the bud neck, the 'newer' part [7,14,38]. This would, despite transient stabilisation by interaction with cargo [13], ultimately result in the loss of Sec23-Sec24 and Sec13-Sec31. This could explain the presence in EM sections of apparent export carriers that are not coated with COPII in the vicinity of bona fide COPII-coated export sites [37]. Further immunolabelling and EM tomography of relevant cell types in an unperturbed state, or even tissue sections, would likely resolve this. Complete cage assembly could be necessary to encapsulate macromolecular cargo and this would be consistent with a selective defect in collagen secretion following knockdown of Sec13-Sec31 [19]. The use of metazoan model systems such as zebrafish has the potential to provide key physiologically relevant insights here.

In summary, recent data indicate that tight control over coated vesicle formation is required to direct the incorporation of large cargo into nascent buds; such control necessarily extends to the process of uncoating. Directed coat assembly, coupled with spatial restriction of GTPase activity and the activity of cargo receptors such as TANGO1, provides a concerted mechanism to ensure efficient incorporation of these larger cargoes into cells. From these data, it is becoming clear that the fine-tuning of the kinetics and thermodynamics of COPII assembly is essential for the export of many atypical secretory cargo molecules from the ER, and this has clear implications for human development, health, and disease.

## Abbreviations

CLSD, cranio-lenticulo-sutural dysplasia; CMRD, chylomicron retention disease; COPII, coat protein II; EM, electron microscopy; ER, endoplasmic reticulum; ERES, endoplasmic reticulum exit site; ERGIC, ER-Golgi intermediate compartment; GAP, GTPase-activating protein; GEF, guanine nucleotide exchange factor; GDP, guanine diphosphate; GTP, guanine triphosphate; MIA3, melanoma inhibitory activity family, member 3.

## Competing interests

The authors declare that they have no competing interests.

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