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Coat flexibility in the secretory pathway: a role in transport of bulky cargoes

Joshua Hutchings and Giulia Zanetti

Membrane trafficking in eukaryotic cells is a highly dynamic process, which needs to adapt to a variety of cargo proteins. The COPII coat mediates ER export of thousands of proteins with a wide range of sizes by generating coated membrane vesicles that encapsulate cargo. The process of assembly and disassembly of COPII, regulated by GTP hydrolysis, is a major determinant of the size and shape of transport carriers. Here, we analyse our knowledge of the COPII coat architecture and its assembly/disassembly dynamics, and link coat flexibility to the role of COPII in transport of large cargoes. We propose a common mechanism of action of regulatory factors that modulate COPII GTP hydrolysis cycle to promote budding.

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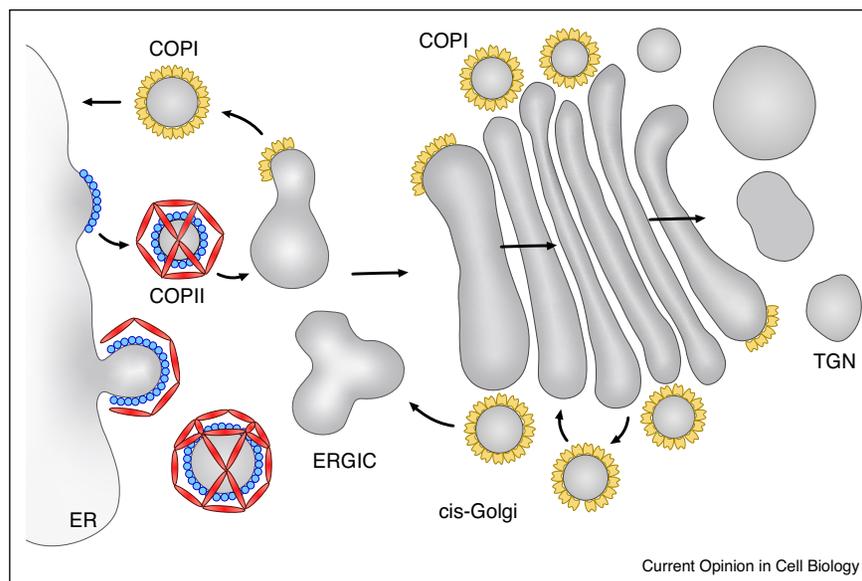
Introduction

Compartmentalisation in membrane-bound organelles is a fundamental hallmark of eukaryotic cells. Regulated trafficking of lipids and proteins between topologically equivalent compartments is essential to maintain organelle identity and sustain cellular functions. To mediate trafficking, cytosolic coat proteins assemble on membranes and promote budding and transport of cargo between compartments. It is becoming increasingly clear that one of the fundamental characteristics of coats is their ability to achieve and control flexibility and plasticity of membrane budding, as observed for the COPII [1–4], COPI [5,6], and clathrin [7] archetypical coats. This review will focus on the COPII coat, discussing its flexible architecture and the functional importance this has in general protein export and in secretion of bulky cargoes.

The COPII coat is responsible for transport of thousands of newly synthesised proteins between the endoplasmic reticulum (ER) and Golgi apparatus. It is constituted by a set of essential cytosolic factors that dynamically assemble into two concentric layers, forming an inner and outer coat around budding membranes. COPII is highly conserved from yeast to human, suggesting that analogous molecular mechanisms for membrane remodelling and cargo capture act across species. Nevertheless, the spatial organisation of COPII traffic routes is to some extent species-specific. COPII-coated carriers form in defined cup-shaped areas of the smooth ER named ER exit sites (ERES, reviewed in Ref. [8]). While in most organisms cargo travels directly from the ER to *cis*-Golgi cisternae, in metazoa COPII carriers are first delivered to an ER-Golgi intermediate compartment (ERGIC), from where cargo moves on to the *cis*-Golgi in COPI-coated vesicles (Figure 1) [9]. COPII carriers travel short-range in a cytoskeleton-independent manner, as indicated by a plethora of ultrastructural work showing ERES in proximity to *cis*-Golgi cisternae or ERGIC (e.g. Refs. [5,10*,11]). Live-cell fluorescence in yeast has shown that *cis*-Golgi compartments are highly mobile and can reach out to the ERES to ‘pick up’ budding vesicles, in what has been proposed as the ‘hug and kiss’ model for short-range transport [12].

Coat assembly starts when the small GTPase Sar1 is activated by its ER-resident GEF, Sec12 [13]. Nucleotide exchange leads to Sar1-GTP exposing an N-terminal amphipathic helix which inserts into the ER outer membrane leaflet, initiating coat recruitment and membrane bending [14]. Sec23–Sec24 heterodimers bind to ER-associated Sar1 to form the inner coat, which performs multiple functions. First, it acts as an adaptor, with Sec23 directly interacting with Sar1 and with outer coat components and regulators [15,16], and Sec24 binding cargo or cargo receptors that harbour specific ER-exit motifs [17]. Second, Sec23 acts as a GTPase-activating protein (GAP) for Sar1, triggering destabilisation of the assembling coat and vesicle scission [18]. Third, the inner coat serves as a structural scaffold: Sec23 and Sar1 mediate lateral assembly of inner coat subunits into lattices, reinforcing membrane curvature through interaction with phospholipids [1,15,19*]. Together with Sar1, Sec23 recruits heterotetramers of Sec13–Sec31, 30 nm long rod-shaped complexes that assemble to form outer coat ‘cages’ [1,20,21]. These serve as a scaffold, reinforcing and stabilising membrane curvature, and also actively interact with the inner coat to accelerate its catalytic activity and promote its lattice assembly [16]. We have recently shown that the presence of the outer coat is in fact required to induce inner coat assembly [19*].

Figure 1



An overview of the secretory pathway in metazoan cells.

Budding from the ER to the ERGIC is mediated by the double-layered COPII coat (inner layer in blue and outer layer in red), while ER retrieval is mediated by the COPI coat (yellow). Transport throughout the Golgi is mediated by the COPI coat.

The COPII coat's flexible architecture

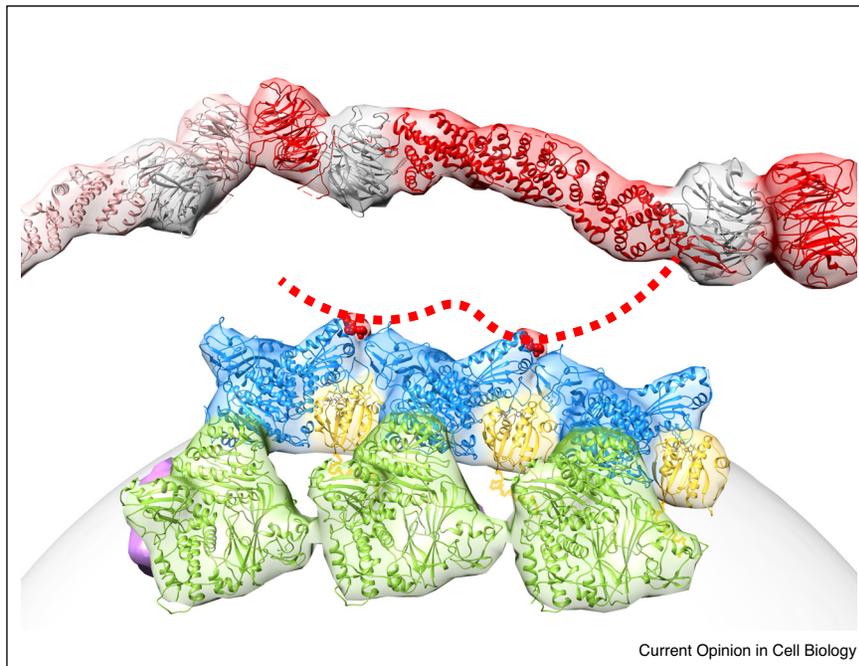
Studies on the architecture of the assembled COPII coat, together with X-ray structures of individual components, have provided molecular models for the whole coat, shedding light on how flexibility is achieved. Single-particle cryo-EM of *in vitro*-assembled outer coat proteins showed how these arrange in X-shaped vertices to form polyhedral cages, which can adopt different diameters (from 60 to 100 nm) through variations of angles at vertices and rearrangements around hinge regions [20,21]. *In vitro* reconstitution approaches have later provided pictures of the full COPII coat assembled on membranes, showing a much wider range of coat shapes and sizes [2]. Cryo-tomography and subtomogram averaging of coated membranes have confirmed that the same outer coat interactions previously seen in cage vertices can lead to a tubular coat arrangement [1]. These studies also resolved the inner coat lattice around tubules, revealing that inner coat subunits assemble through tight apposition of Sec23 and Sar1 in neighbouring heterotrimeric [1]. We were recently able to determine the structure of the assembled inner coat at 4.9 Å resolution using the latest developments in cryo-tomography and subtomogram averaging [19,22]. This revealed the molecular details of inner coat subunit interactions and provided direct evidence of Sar1 amphipathic helix insertion in the membrane outer leaflet, showing how it contributes to membrane deformation. Furthermore, our recent work [19] together with earlier X-ray crystallographic studies from the Goldberg lab [16,23], revealed important insights

into the relationship between the inner and outer coat layers. Extensive contacts with the inner coat are mediated by the Sec31 proline-rich C-terminal region: this contains a 'catalytic peptide' that binds to Sec23 and Sar1 near the GTP pocket, accelerating Sec23 GAP activity by 10-fold [16]. It also encompasses multiple triple-proline motifs that concomitantly bind Sec23 at the interface between inner coat subunits, 'gluing' them together [19,23] (Figure 2).

Dynamic assembly and disassembly of COPII

Both the inner coat and the outer coat therefore concomitantly establish a range of interactions that contribute to coat assembly, while inducing its disassembly by accelerating GTP hydrolysis. This renders the COPII coat intrinsically short-lived and flexible. This is important for delivery of cargo and recycling of coat components, and potentially also for regulation of coat formation by other factors, as discussed below. It must be noted that *in vitro* reconstitution experiments were performed in the presence of non-hydrolysable GTP analogues to prevent uncoating, therefore providing a snapshot of the COPII coat in the absence of disassembly. It is expected that GTP hydrolysis would lead to coat destabilisation through detachment of Sar1 from the membrane, and that in normal conditions inner coat lattices would therefore not be as persistent and extended as seen *in vitro*, consistent with ultrastructural data showing COPII surrounding tubules as well as vesicles [2,24,25].

Figure 2



Structure of the COPII coat.

Inner coat assembly is mediated by the Sec23 (blue) and Sar1 (yellow) subunits, when Sar1 is in the GTP-bound state. Sec24 (green) mediates incorporation of most cargo proteins (pink). Sec31 (red) templates inner coat assembly by binding at the interface between inner coat subunits through PPP motifs in its disordered C-terminal region. Sec13 (grey)-Sec31 rods themselves assemble to form cages.

Overall, biochemical and structural studies show that a high number of scattered weak and dynamic interactions contribute to coat assembly, and suggest how interplay between coat assembly and disassembly can remodel membranes in a flexible manner. In our recent *in vitro* study, we show that using COPII mutants partially defective in outer coat polymerisation supports budding with non-hydrolysable GTP analogues, but not with GTP, that is, productive membrane deformation with weak-assembly mutants occurs only when coat disassembly and turnover are not allowed [19^{*}]. Moreover, it has previously been shown in budding yeast that defects in COPII (namely the lethal depletion of the outer coat protein Sec13) can be tolerated in the absence of GPI-anchored proteins in the inner ER leaflet, supporting the idea that membranes with different properties are more or less easily remodelled by COPII [26]. Therefore, the equilibrium between coat assembly and disassembly might be a determinant of budding for membranes with different resistance to bending, and might be an important regulatory platform for secretion (Figure 3).

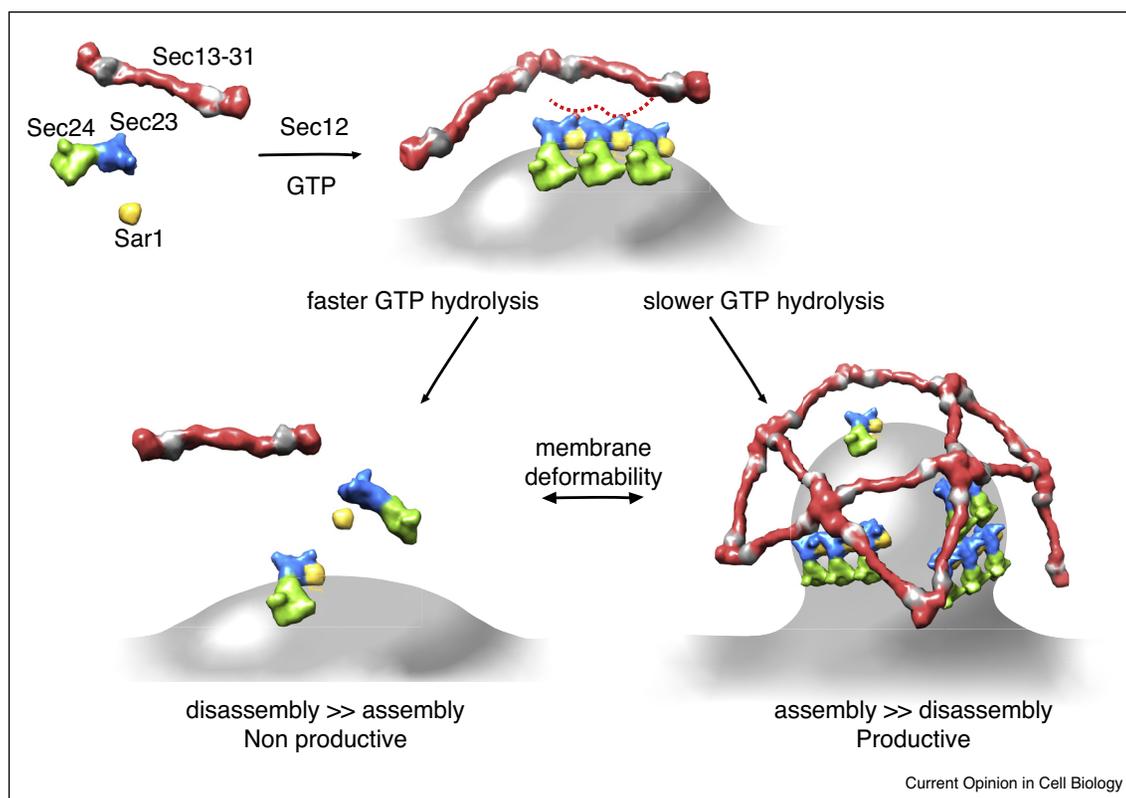
Despite significant advances in our mechanistic understanding of COPII-mediated membrane remodelling, major questions remain outstanding: given the great potential for variation of coat architecture seen *in vitro*, how are vesicle size and shape determined and regulated *in vivo*? What is the physiological importance of the coat's

dynamic flexibility? As discussed below, several factors have been identified that interfere with coat assembly and/or disassembly, possibly playing a role in regulating membrane remodelling as required by the cell. These include scaffolding factors required to initiate and organise COPII budding at ERES (Sec16, TFG, Sec23IP), and receptors for large cargoes (TANGO1, cTAGE5, and TALI). These factors all contain proline-rich regions that bind the inner coat in competition with Sec31. While basic COPII molecular mechanisms are highly conserved, some of these additional regulatory factors seem to have emerged and differentiated in higher eukaryotes and might determine species-specific differences in the spatio-temporal organisation of COPII budding [27].

COPII organising factors

Sec16 is an essential secretion factor conserved in all eukaryotes. It localises at ERES and interacts with many COPII components (reviewed in Ref. [28]). As shown by X-ray crystallography, it forms complexes with Sec13 similar to the Sec13/31 outer rods [29]. Like Sec31, it also contains a proline rich region at the C-terminus, which competitively interacts with Sec23 [30]. Sec16 co-localises with Sar1 at ER membranes, and is associated with Sar1 localisation at the rims of budding vesicles [31]. While long thought to be a scaffolding factor that templates COPII assembly, more recent experiments suggest a regulatory role for Sec16. In

Figure 3



COPII assembly/disassembly equilibrium as a major determinant of budding.

Assembly on membranes is initiated upon GTP exchange on Sar1. Coat assembly leads to GTP hydrolysis. In our model, the speed of GTP hydrolysis determines whether the resulting ratio between assembly and disassembly of inner and outer coat is sufficient to productively remodel membranes into smaller carriers. We propose this might be dependent on how easily deformable the membrane is (e.g. on initial curvature, or type of cargo).

budding yeast, Sec16 is shown to be recruited by COPII through an important interaction with Sec24 [30,32], whilst in humans Sec16 is recruited by TANGO1 [33]. Recruitment of Sec16 to the forming bud has been shown to retard GTP hydrolysis on Sar1 [30], and its regulatory function is also supported by the observation that redistribution of Sec16 to the cytosol accelerates COPII turnover in *Pichia pastoris* [32]. Recent evidence also suggests that Sec16 alternative splicing might play a role in its regulatory mechanisms [34]. Sec16 probably acts by retarding vesicle scission, while at the same time inducing and stabilising inner coat polymerisation at the budding site. The inhibition of GTP hydrolysis might be necessary to bend flat membranes at the initial stages of budding, and only after membrane deformation is established Sec31 might take the place of Sec16 to promote the coat dynamics necessary to complete the budding process (Figure 3).

TFG (Trk-fused gene) is a metazoan COPII-regulation factor that also harbours a proline-rich C-terminal region which competes with Sec31 for binding to the inner coat [35]. Binding of TFG to Sec23 is thought to promote

release of Sec31 and uncoating, TFG can therefore be thought as a regulator that comes in later in the COPII cycle [36]. Its function appears to be mediated by clustering COPII vesicles between the ER and ERGIC, minimising their dispersion into the cytosol and retaining them after outer coat disassembly, a prerequisite for fusion with ERGIC membranes [36–38]. Recently, it has been shown that TFG functions in a calcium-dependent manner through the association with the Ca⁺⁺ binding protein ALG-2, providing evidence for Ca⁺⁺ levels as an important regulator of COPII budding [39].

Sec23IP, also known as p125, is a member of the phospholipase A1 family and it also contains a Sec23 PPP interaction motif [40,41]. Sec23IP also binds Sec13–31 and is involved in PI4P-mediated regulation of COPII budding at ERES [42,43], although the mechanisms and physiological roles of Sec23IP remain to be established [27].

Bulky cargo receptors

In higher eukaryotes, some cargo are significantly large, and are thought to pose a challenge on the COPII

machinery for transport. Procollagens (PCs) assemble into >300 nm fibrils in the ER and constitute the most abundant secretory cargo [44]. Despite canonical COPII carriers are in the order of 100 nm diameter, plenty of biochemical and genetic evidence unambiguously shows that PC ER export is COPII dependent [45–51]. TANGO1 has been identified in *Drosophila* as a factor that promotes collagen secretion by interacting with COPII [52]. Vertebrates also express TANGO1 splice variants, including a long isoform (TANGO1L) essential for procollagen secretion, as well as a short (TANGO1S) isoform [53,54]. Other vertebrate TANGO1 homologues, namely cTAGE5 and TALI, are also involved in secretion of PC or other large cargoes [55–57]. Several studies have contributed to define mechanisms of TANGO1 action. TANGO1 comprises an intraluminal portion, whose SH3 domains concentrate fully assembled PC trimers by direct interaction with the dedicated chaperone HSP47 [52,58]. On the cytoplasmic side, TANGO1 comprises a C-terminal PRD domain that interacts with Sec23 through triple proline motifs at the same site as the outer coat Sec31 [19*,23*,52]. Cytoplasmic coiled-coil domains mediate TANGO1 self-assembly and, in metazoa, lateral interaction with other members of the TANGO1 family such as cTAGE5, facilitating assembly into rings that are seen to encircle COPII [10*,55,59*,60]. In addition, one of the coiled-coil domains harbours a segment that is necessary both for ring formation and for recruitment of ERGIC membranes through interaction with a tethering complex, a mechanism thought to provide extra membrane to the growing PC-containing bud [60,61]. cTAGE5 also binds Sec12 to concentrate activated Sar1 at ERES, leading to more persistent assembly of COPII at the site of large cargo budding [56,62]. Importantly, however, the GTP-locked mutant of Sar1 blocks procollagen trafficking, reiterating the importance of GTPase regulation in COPII assembly dynamics [62].

In *Drosophila*, TANGO1 is proposed to be a general secretion regulator, although it is clear that bulky cargo have a lower tolerance to TANGO1 depletion than ‘standard’ cargo [10*]. Given that *Drosophila* lack many of the vertebrate receptors like cTAGE5, TALI and TANGO1S, TANGO1 alone may fulfil these multiple roles in procollagen secretion. In vertebrates, TANGO1S can compensate for TANGO1L depletion in general protein secretion, likely attributed to important interactions with Sec16 [54]. More recently it has been suggested that the effect of TANGO1 depletion on the secretory pathway is attributable to retention of bulky cargoes that blocks exit routes [62].

In addition to TANGO1 and its metazoan homologues, Sedlin (a component of the TRAPP complex) has been shown to be recruited by TANGO1 and affect PC secretion by modulating Sar1 GTPase cycle [63]. Importantly, point mutations in COPII subunits and specific paralogues affect

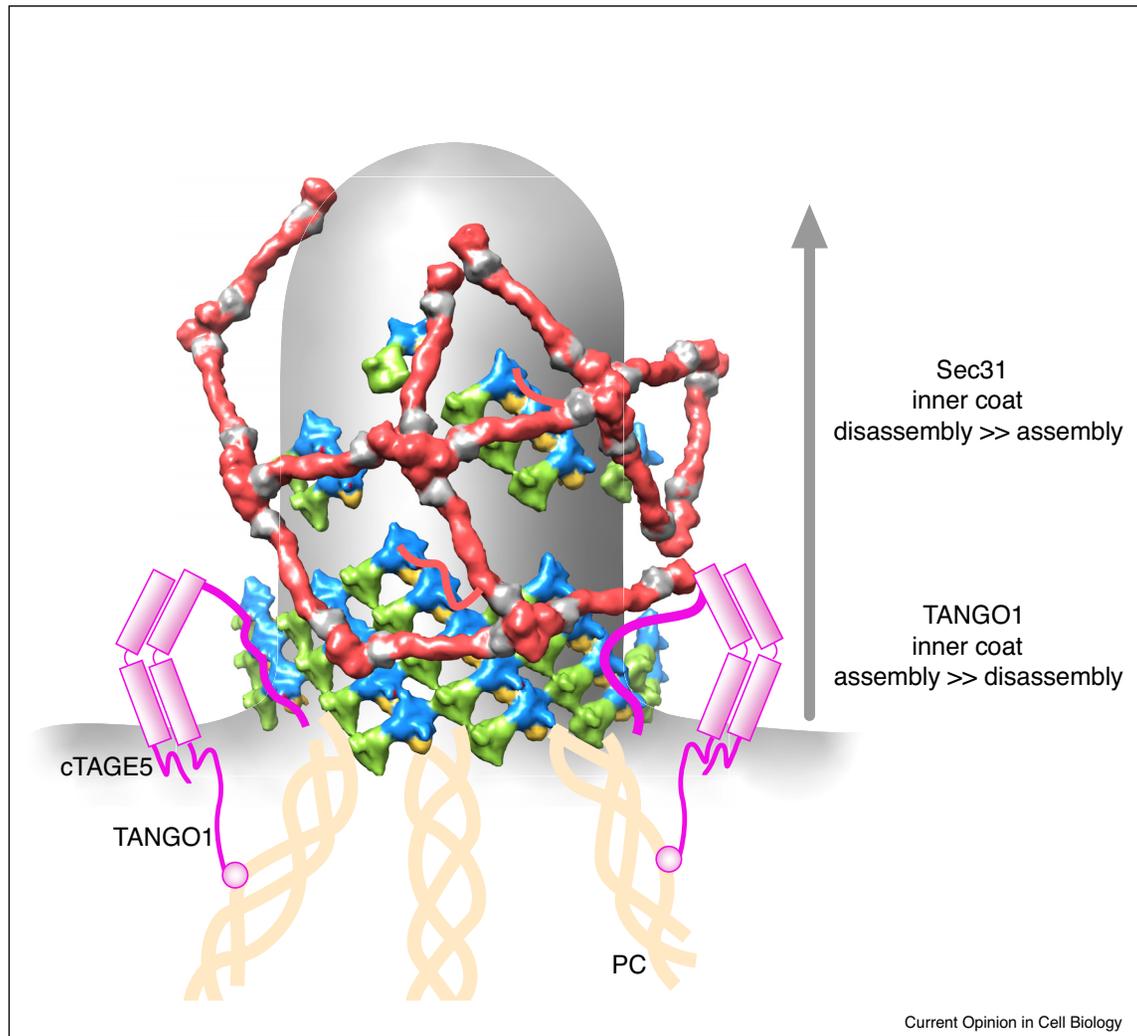
large cargo secretion in human disease and in animal models, again by imposing modulations on the Sar1 GTP hydrolysis cycle [24,47,64,65]. This reinforces the hypothesis that bulky cargo secretion requires ad-hoc regulation of the COPII assembly and disassembly cycle.

Mechanisms for secretion of bulky cargoes

A number of hypotheses have been put forward to explain how PCs and other large cargoes are transported by the COPII machinery. The prevalent view is that COPII forms large or tubular carriers with the help of its dedicated cargo receptors. Large carrier formation is supported by a series of studies that have shown that ubiquitylation of Sec31 leads to formation of COPII punctae with diameters exceeding 1 μm [48,49]. This is mediated by the ubiquitin-ligase Cul3 and the KLHL12 adaptor, together with Ca^{2+} -associated co-adaptors PEF1 and ALG-2: interfering with this system leads to absence of large carriers and a block in PC secretion [48,49]. KLHL12-induced large punctae have been shown by STORM microscopy to be hollow structures that contain PC [50*]. While these studies have been conducted in experimental systems with KLHL12 and PC overexpression, it must be noted that similar PC containers have been detected in Soas2 cells in native conditions [50*]. *In vitro* reconstitution of budding from PC-containing microsomes suggests that TANGO1 might be transported into such large carriers rather than being retained in the ER, together with PC, its chaperone HSP47 and, interestingly, Sec12 [51].

Formation of large COPII-coated carriers is not the only model that has been put forward to explain COPII-mediated export of large cargo. A recent paper has reported the lack of extra-large (>1 μm) COPII coated PC carriers, although mobile punctae of 300–400 nm diameter were seen with STED fluorescence, consistent with potential PC transport carriers [66]. It has been proposed that COPII is only needed to concentrate cargo at ERES and possibly trigger membrane tubulation, but without further coating budding membranes [67*]. In an alternative hypothesis PC fibrils, which are in fact more flexible than traditionally thought [44], could potentially curl into vesicles within the canonical COPII pathway [66]. None of these views is incompatible with – or distinguishable from – a general scenario where coat disassembly at the ‘far’ end of large buds occurs before coat assembly and detachment from the ER are complete, and where naked PC-containing carriers diffuse to the Golgi or ERGIC to deliver their content (as depicted in the model proposed in Ref. [66]). Given the size of putative transport carriers, only recent super-resolution fluorescent studies have started targeting the appropriate resolution ranges. Electron microscopy will in the end be required to define carriers ultrastructure, and so far there has been very little direct evidence that shows the shape and size of PC carriers [50*]. Whether it is to coat larger or tubular carriers, or to encapsulate a particularly difficult and abundant molecule, it is clear that PC and other large cargoes impose special requirements on the COPII machinery. We propose that PC secretion factors such as

Figure 4



Model for COPII-mediated Procollagen ER export.

When large cargo needs to be transported, COPII assembly is stabilised at the base of the budding carrier by rings of TANGO1 that promote inner coat assembly through multiple PPP motifs binding at the interface between Sec23 subunits. As the carrier grows, Sec31 displaces ER and procollagen-bound TANGO1 and eventually promotes disassembly of the coat. This is an elaboration of the model proposed in Ref. [23], where we account for our recent finding that PPP bridges between inner coat subunits.

TANGO1 function at least partly by regulating the GTP hydrolysis cycle and allowing the inner coat lattice scaffold to expand and locally promote tubulation of ER membranes (Figure 4).

Conflict of interest statement

Nothing declared.

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