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VIEWPOINT

Tailored assemblies of COPII proteins in secretion

Vivek Malhotra[1,2,3]

Export of secretory cargoes from the endoplasmic reticulum (ER) requires COPII proteins, which were first identified for their ability to coat small vesicles that bud from the ER. Recent data indicate that COPII proteins can also organize into a collar at the necks of tubules, as well as phase-separate into liquid-like condensates. Thus, COPII assemblies seem to be tailored to accommodate variations in the size and quantities of cargo secreted.

The endoplasmic reticulum (ER) exit sites (ERES) and COPII proteins

It is estimated that a third of the human genome encodes proteins that are synthesized in the ER. Most of these proteins exit the ER for the Golgi apparatus, and from there, they are targeted to various destinations within the cell, including the plasma membrane and the extracellular space. In metazoans, the cargoes exiting the ER are collected at specialized sites or domains called ERES. The metazoan-specific ERES resident proteins TANGO1, cTAGE5, and TANGO1-Short (TANGO1 family) are required for the formation of ERES and for collecting "bulky" cargoes (such as collagens and chylomicrons) for their export (Raote and Malhotra, 2021). How cells compartmentalize the ER into TANGO1 family–containing sites in the plane of the membrane is not fully understood. The unicellular eukaryote *Saccharomyces cerevisiae* lacks obvious ERES proteins of the TANGO1 family, but whether there is a rudimentary form or a transient exit domain that functions analogously to its metazoan counterpart cannot be ruled out.

By contrast, the COPII proteins that also function in cargo export—Sar1 (small GTPase), Sec23 and Sec24 (inner coat), and Sec13 and Sec31 (outer coat)—are conserved from yeast to humans. COPII proteins are recruited to ERES. Regardless of the cell type, COPII proteins are well-suited for the export of secretory proteins that can be accommodated into "small" vesicles (~60 nm average diameter). Extensive reviews cover intricate details of COPII-mediated production of small coated vesicles. In brief, Sar1 is recruited to the ER in its GTP-bound form. This event initiates a cascade whereby Sar1-GTP recruits the inner layer proteins Sec23/Sec24, followed by the recruitment of the outer layer proteins Sec13/Sec31. The interaction between the outer and inner layers stimulates the GTPase-activating activity of Sec23, resulting in the hydrolysis of Sar1-GTP. These steps parallel the process of ER deformation and the formation and release of small vesicles. Sec16, a peripheral protein on the cytoplasmic face of the ERES, which binds TANGO1, plays a key role in scaffolding COPII proteins (Miller and Schekman, 2013).

It is evident that small COPII vesicles are inadequate in size for exporting large cargo molecules, such as collagens, extracellular matrix components, and ApoB-containing lipid particles. Furthermore, specialized cells encounter the challenge of secreting substantial amounts of other proteins, such as mucins, antibodies, and albumin. How cells manage such hefty secretory loads underscores a fundamental challenge in eukaryotic cell physiology.

Proteins exiting the ERES are destined for the cis-Golgi cisterna. A recent significant discovery is a protein called TFG (Trk-fused gene), which is essential for organizing the ERES-Golgi interface (Peotter et al., 2019; Qui et al., 2024; Wegeng et al., 2024, *Preprint*). It is suggested that the porous condensates of TFG allow the trafficking of COPII vesicles while excluding COPI vesicles from entering the porosities. This raises the question of how cells utilize the TFG system for trafficking bulky, collagen-loaded transport intermediates, which are not carried in the standard COPII vesicles.

COPII proteins collar the neck of cargo-filled tubules at ERES

COPII proteins have long been shown to coat the surface of the newly formed cargo-filled bud, which ultimately separates from the ER to produce a small COPII-coated vesicle. Recent data show that COPII proteins are located in a collar at the neck of a tubule emerging from the ER (Hutchings et al., 2018; Shomron et al., 2021; Weigel et al., 2021; Saxena et al., 2023, *Preprint*). TANGO1 mediates the tethering and fusion of the ERES with ERGIC to create a tunnel between these two compartments. The deposition of collagen into this long tubule from the ER lumen is followed by fission at the neck to release a collagen loaded saccule, which matures into a cis-Golgi and so on. There is no transport vesicle per se in this step of the secretory pathway (Raote and Malhotra, 2021). Assembly of COPII components into a collar at the neck of these

[1]Centre for Genomic Regulation, The Barcelona Institute of Science and Technology, Barcelona, Spain; [2]Universitat Pompeu Fabra, Barcelona, Spain; [3]ICREA, Barcelona, Spain.

Correspondence to Vivek Malhotra: vivek.malhotra@crg.es.

tubules requires TANGO1, a proposition supported by super-resolution imaging (Weigel et al., 2021). This arrangement of COPII proteins is especially relevant for the export of bulky cargoes that cannot be accommodated inside small vesicles, as has been discussed extensively (Raote and Malhotra, 2021).

COPII proteins assemble into liquid condensates

New data reveals that COPII proteins undergo phase separation, and this assembly is also important for cargo export (van Leeuwen et al., 2022; Gallo et al., 2023; Wang et al., 2023). As summarized below, these new developments have relevance in furthering our understanding of the trafficking of diverse secretory cargoes.

Catherine Rabouille and colleagues reported that amino acid starvation of *Drosophila* S2 cells and mammalian cells (Zacharogianni et al., 2014; Zhang et al., 2021; van Leeuwen et al., 2022) resulted in the recruitment of soluble ERES components into a membrane-less compartment they termed Sec bodies. Reportedly, Sec bodies contained Sec16, Sec23, Sec24, and Sec31, but with Sar1 notably absent. Upon replenishing cells with amino acids, soluble ERES components relocated to their original site on the ER. Rabouille and colleagues also noted that Sec16 and specific isoforms of Sec24 (Sec24AC, Sec24CD) contained a higher content of low complexity sequences (LCS), also called intrinsically disordered regions (IDRs) compared to other proteins in the early secretory pathway and the *Drosophila* proteome. Interestingly, the LCS were predominantly located in the N-terminus of the Sec24 family of proteins. While Sec24AB and Sec16 were required for Sec body formation, Sec24CD, despite its high LCS content, was not involved. Their findings suggested that Sec bodies form prior to ER traffic cessation, as a means to regulate trafficking dynamics (van Leeuwen et al., 2022). These studies established that COPII components are capable of assembling into liquid condensates.

Assembly of COPII condensates is a regulated process

Lucas Pelkmans and colleagues recently demonstrated that under normal growth conditions, COPII assembly into liquid phase condensates is a regulated process (Gallo et al., 2023). They observed that the serine/threonine protein kinase DYRK3 interacts with several SEC proteins and is enriched at the ERES. Given the role of DYRK3 in nuclear speckle condensates, alterations in ERES size upon DYRK3 manipulation suggested a similar effect on COPII phase separation. DYRK3 inhibition or depletion perturbed ERGIC-Golgi organization and impaired traffic, measured using the VSV-G protein. Sec16A was identified as a target of DYRK3 with its phosphorylation by DYRK3 influencing its phase-separation properties, resulting in Sec16A condensates containing Sec23A, Sec16, TFG, GM130, p115, Sec12, and TANGO1. Loss of DYRK3 resulted in a gel-like state, blocking traffic, while its presence maintained a liquid-like state conducive to VSV-G trafficking (Gallo et al., 2023). These data indicate that DYRK3 is an active component of the machinery required for export from the ERES and does so by keeping the COPII components in a liquid like state.

Xiao-Wei Chen and colleagues uncovered another intriguing aspect to the understanding of COPII condensates. They observed that the size of the puncta of condensed SEC24A-EGFP (endogenously tagged using CRISPR knock-in methodology) on the ER surface in cells expressing the N-terminal fragment of APOB was larger than in cells expressing a standard transmembrane protein (Wang et al., 2023). Ordinarily, APOB is assembled as a lipid particle in the ER lumen, and the APOB-containing lipid particle is then exported from the ER, with SURF4 acting as the cargo receptor. In APOB N-terminal–expressing cells, SEC24A-enriched puncta exhibited properties of a liquid condensate. But how does the luminally located APOB N-terminal peptide affect the propensity of the cytoplasmic Sec24 A to undergo apparent phase separation? The N-terminus of SEC24 is enriched in IDRs, which are well separated from its ordered domains that interact with SEC23. These studies on the condensate formation of SEC23A/24A were replicated in vitro with purified proteins. The condensates demonstrated selectivity, excluding soluble GFP, but absorbing Sar1b. Importantly, when supplied, SEC13/31 were absorbed into preformed condensates of SEC23/SEC24, limiting their further growth to ensure the bioactivity of enriched COPII components (Wang et al., 2023). Previously, the authors showed in a mouse model that impairing Sar1b function affected COPII function and reduced lipid levels in the circulation. Notably, viral exposure of Sar1b LKO (liver-specific knock-out) mice significantly increased triglyceride and low-density lipoprotein cholesterol levels. Interestingly, adenovirus infection did not restore diminished plasma lipids in SURF4 LKO mice, confirming COPII function as the target hijacked by adenovirus. The authors noted a significant enlargement of COPII puncta in adenovirus-infected primary hepatocytes, with a fourfold increase in integrated signals of SEC24A on condensed puncta, along with similar increases in SEC31A and SURF4 levels. Cytosol from adenovirus-infected hepatocytes significantly promoted the formation of SEC23/SEC24 liquid condensates in vitro, suggesting the existence of a condensation-promoting factor. Investigation led them to identify Mn^{2+} as this factor. In vitro, the effect of 10 µM Mn^{2+} was evident and became more pronounced at 250 µM Mn^{2+}. It is important to note that, normally, the cellular levels of Mn^{2+} are in the range of 10–50 µM. The significance of the high Mn^{2+} concentrations in promoting SEC23/SEC24 condensate assembly needs to be addressed. Regardless, they successfully captured SEC23/SEC24 on Mn^{2+}-coupled beads, with Mn^{2+} binding predominantly in the structured regions of SEC23/SEC24, not the IDRs or SEC13/31.

Taken all together, these data reveal that Sec16, Sec23, and Sec24 proteins can assemble into a liquid condensate. Sec16 assembly into a condensate is influenced by nutrient deprivation and by DYRK3 kinase.

Sec23 and Sec24 are influenced by Mn^{2+}-dependent changes in their organization into a condensate. The intracellular store of Mn^{2+} for this condensation reaction is shown to be mitochondria, but how mitochondria are influenced to participate in the condensates of COPII at ERES remains mysterious. Another unexplained issue is why this process of COPII condensates is not required for the secretion of the most abundant secretory cargo, albumin (Wang et al., 2023). Is this condensation a cell-type-specific event, or do ERES differ in some way that permits COPII proteins to exist in various forms for simultaneous export of diverse cargoes (Saxena et al., 2023, *Preprint*)?

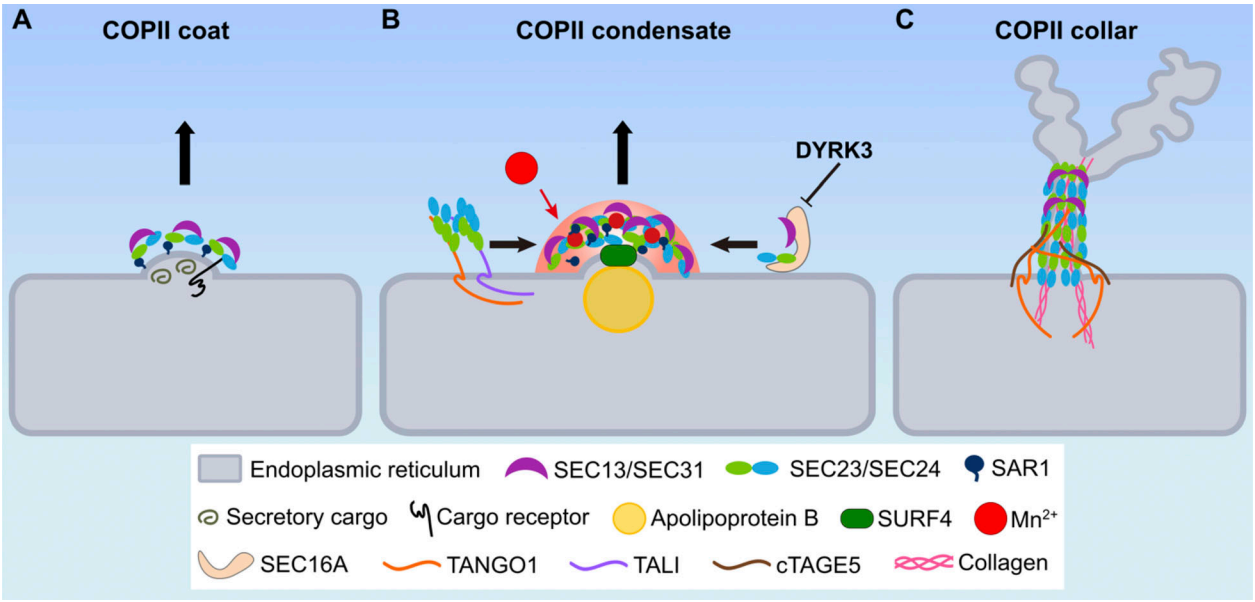

Figure 1. **COPII-mediated cargo export in the early secretory pathway. (A)** COPII proteins fully coat nascent buds emerging from the ER, ultimately facilitating the generation of cargo-loaded vesicles for initiating the intracellular transport. **(B)** COPII proteins employ the condensation mechanism, in a manner regulated by Mn^{2+}, to catalyze the efficient export for bulky yet abundant cargos from the ER. SEC16A and TANGO/TALI that reside in the ERES harbor multiple COPII-interacting domains, thereby locally condensing cytosolic COPII proteins and potentially triggering phase separation. **(C)** COPII proteins collars at the neck of tubular structures, which are organized by TANGO1, for transporting bulky cargos like collagens.

How do condensates contribute to the formation of transport intermediates?

The well-organized assembly of COPII proteins at both structural and functional levels has led to a general understanding of how the inner coat molds the membrane into a bud and facilitates the collection of ligand-bound cargo receptors. GDP-to-GTP conversion in Sar1 initiates the assembly of COPII proteins at ERES, and GTP hydrolysis occurs concomitantly with production of a COPII-coated vesicle. The production of a vesicle encompasses many subprocesses, including cargo capture, changes in membrane curvature, and finally, the fission of a cargo-filled container from the ER membrane (Miller and Schekman, 2013). But how exactly do condensates function in this process? Which step is affected by this form of COPII assembly?

Certain proteins known to control membrane shape possess both structured and IDRs, suggesting that they may cooperate in shaping cellular membranes. Adhesion among IDRs tends to create convex curvature, while repulsion leads to concave curvature (Yuan et al., 2021). It is crucial to assess the properties of the IDRs in COPII proteins to determine if they possess an inherent capacity to alter membrane curvature. However, whether this capability is both necessary and sufficient for generating transport intermediates for the export of bulky cargo, such as APOB-containing particles, remains uncertain.

Surprisingly, cells are viable and can secrete without Sar1

Anjon Audhya and colleagues present data challenging the dogma that Sar1 is essential for COPII recruitment to the ER and vesicle biogenesis (Kasberg et al., 2023). Earlier reports have shown that loss of both SAR1A and SAR1B in Caco-2/15 cells is not lethal, but causes a defect in trafficking of chylomicrons (Sane et al., 2019). Regarding the effects on ERES exit sites, Audhya and colleagues demonstrate that the Sec23-24 complex is recruited to the ER in cells that lack Sar1A and are markedly depleted of Sar1B, forming a liquid condensate sufficient to traffic secretory cargoes to the Golgi compartment, albeit at a slower rate than in normal cells. This raises questions about how cells export cargoes without Sar1. The levels of Sec16 and TANGO1 increase in Sar1-deficient cells. In these cells, TANGO1, which is known to bind Sec23, likely recruits the inner coat Sec23/Sec24 complex to the exit sites (Kasberg et al., 2023). The slow GTPase-activating activity of Sec23 in the absence of Sar1 might generate an export route, albeit slowly, out of the ER. Another recent report describes the production of COPII-coated vesicles from yeast microsomal membranes with purified COPII proteins in the absence of GTP hydrolysis. The authors suggest the presence of a fission factor in the microsomal membranes. These observations then raise the additional issue of the mechanism of membrane fission without Sar1 and without GTP hydrolysis. Further experimental validation is needed to elucidate this potential mechanism of cargo export in Sar1- and GTP hydrolysis-independent COPII vesicle production (Pyle and Zanetti, 2024, *Preprint*).

The next steps?

The wealth of data accumulated over the last three decades has reinforced the concept of sequential and layered assembly of COPII proteins, initiated by the recruitment of Sar1-GTP to the ER membrane. However, while COPII-coated vesicles play a role in intracellular transport, other COPII-dependent structures appear to be utilized for the secretion of large and abundant proteins such as collagens, lipoprotein particles, and mucins. The discovery of proteins from the TANGO1 family and advancements in super-resolution microscopy

have brought forth new avenues to address these challenges. We are now confronted with three potential scenarios: COPII proteins fully coating nascent buds emerging from the ER until separation via fission; COPII proteins remaining at the neck of tubules/tunnels; and COPII proteins undergoing phase separation (Fig. 1). Not to be overlooked is the intriguing possibility that cells may assemble COPII proteins even in the absence of Sar1. These developments present both challenges and exciting opportunities to elucidate the significance and mechanisms of these newly proposed COPII assemblies in ER cargo export.

In conclusion, while COPII components are undoubtedly crucial for secretion, it is evident that there is no one-size-fits-all procedure for cargo export from the ER. Cells seem able to employ various assemblies of COPII proteins to tailor transport routes, ensuring the secretion of the right quality and quantity of essential proteins according to physiological demands. This perspective makes it easier to understand that cells utilize these proteins as collectors and gatekeepers at the exit site to balance what should leave and what should stay.

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