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SPOTLIGHT

Want to leave the ER? We offer vesicles, tubules, and tunnels

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Export from the ER is COPII-dependent. However, there is disagreement on the nature of the cargo-containing carriers that exit the ER. Two new studies from Shomron et al. (2021. *J. Cell Biol.* https://doi.org/10.1083/jcb.201907224) and Weigel et al. (2021. *Cell*. https://doi.org/10.1016/j.cell.2021.03.035) present a new model, where COPII helps to select secretory cargo but does not coat the carriers leaving the ER.

An analogy for ER-to-Golgi transport is day-to-day logistics, whereby we have developed different types of carriers to accommodate cargo of varying shapes, quantities, and sizes. Following the same logic, we could assume that our cells are equipped with carriers of different shapes and sizes to shuttle small or bulky cargo or different cargo quantities out of the ER. Although this appears straightforward, the molecular details of ER-to-Golgi transport has been the subject of intensive debate.

Early work on secretory trafficking from the 1960s noted that secretory proteins leave the ER at ribosome free regions, which were termed transitional elements (or transitional ER; 1). These transitional elements were postulated to give rise to "transport vesicles with fuzzy coats," which mediate transport from the ER (1). Sec23 was found to be enriched at these transitional elements (2) and was later shown to be part of the COPII complex that mediates export from the ER in small vesicles (3). Since then, the standard model for export from the ER was that small and round COPII vesicles (60–80 nm in diameter) leave the ER from transitional elements, now referred to as ER exit sites (ERES; Fig. 1 A). This model was later expanded to include pre-Golgi intermediates such as the ER-Golgi-intermediate compartment (ERGIC) generated from the homotypic fusion of COPII vesicles (4).

Despite its wide acceptance, experimental evidence for the presence of COPII vesicles at ERES in intact cells was scarce. The main evidence for COPII vesicles came from in vitro assays from which such vesicles were isolated. Furthermore, the relatively small size of COPII vesicles (60–80 nm) could not explain transport of bulky cargo such as type I collagen (with a length of 300 nm). A major challenge to the vesicular transport model came from a paper by Mironov et al. (5), who presented evidence that ER-derived carriers are large uncoated saccules that mature toward the Golgi. Notably, round and small COPII vesicles as carriers mediating the ER export were absent during synchronized transport of type I collagen and VSVG-ts045 (a temperature-sensitive mutant of the vesicular stomatitis virus glycoprotein). Both types of cargo rely on retention in the ER at 40°C followed by export upon lowering the temperature (and addition of ascorbic acid in the case of type I collagen). Supporters of the conventional vesicular transport model argued that the size of type I collagen as well as the large quantities of molecules queuing to leave the ER simultaneously might have led the ER export machinery to adapt to this situation, thereby leading to formation of the observed carriers. Further support for the vesicle transport model came from work using 3D electron tomography showing that ERES are domains that are continuous with the ER, which are surrounded by COPII vesicles (6). The electron tomography was performed in both chemically fixed as well as high-pressure-frozen cells in the absence of secretory cargo overexpression or synchronized trafficking waves. Because ERES exhibited budding profiles coated with COPII, it was concluded that the COPII vesicles bud as coated carriers from ERES. Despite this support for the vesicular transport model, it was becoming increasingly clear that ER-to-Golgi transport cannot be explained solely by small COPII vesicles. Thus, the idea that different types of carriers operate in the ER-Golgi route began to ripen in the community.

In yeast, cis-Golgi compartments were shown to touch ERES to "pick up" secretory cargo (7). Whether this "hug and kiss" model involves fusion of the cis-Golgi with budded COPII vesicles or whether it forms a "tunnel" between the ERES and the cis-Golgi remains unclear. In support of the existence of tunnels is work from the Malhotra group showing that collagen transport might occur via recruitment of ERGIC membranes to ERES enriched in procollagen (8). The observation that a tunnel is formed between the ERES and ERGIC necessitates the preexistence of an ERGIC

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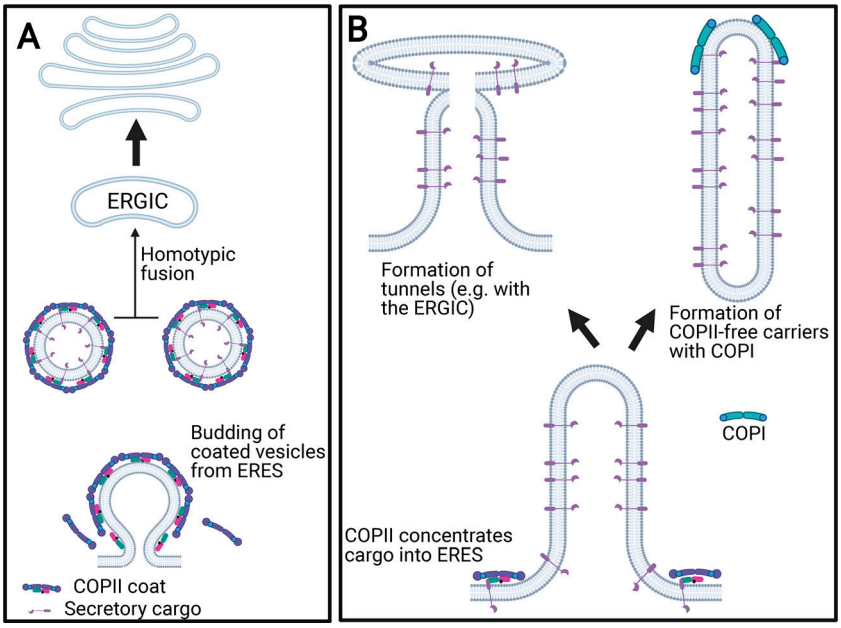

Figure 1. **Depiction of different possible modes of ER export. (A)** The classical (vesicular) transport model that includes budding of a coated vesicle followed by homotypic fusion of COPII vesicles. **(B)** ERES can give rise to tubular structures, which either depart as tubules followed by COPI recruitment or may form tunnels with distal compartments.

membrane container. As discussed, the ERGIC might form by homotypic fusion of COPII vesicles, and thus the tunnel model proposed by Raote and Malhotra (9) can easily be reconciled with the vesicle transport model.

In this issue, Shomron et al. (10) present a major challenge to the vesicle transport model. They suggest that COPII complexes only decorate the neck of an ERES, where they solely serve to concentrate cargo into transport containers. This confirms earlier papers showing that COPII mediates concentrative ER export (11, 12). Strikingly, Shomron et al. observe with live imaging that secretory cargo enters a tubule that segregates from COPII at the level of ERES, indicating that the departing transport carrier is not coated. Furthermore, COPII was confined to the neck of the tubular carrier. This finding agrees with previously observed (noncoated) saccules that leave the ER (5). A concurrent study from Weigel et al. (13) reached a similar conclusion. To overcome prior difficulties associated with fixation, low sampling, and thick sections, they aimed at imaging ERES in living cells by combining focused ion beam scanning electron microscopy with cryo-structured illumination microscopy. Furthermore, they used the retention using selective hook technology (14) to perform synchronized cargo release experiments, thus avoiding problems associated with temperature shifts. In agreement with Shomron et al., they show that ERES give rise to a network of tubules that contain secretory cargo devoid of COPII components. Again, COPII components were only found to localize to the neck of these tubules, implicating that the main role of COPII is to concentrate cargo into carriers. They also showed that ERES are structures continuous with the ER (confirming the earlier data from 3D electron-tomography; 6) that adapt in size to accommodate the load of secretory cargo (again confirming earlier work by others; 15, 16).

Another interesting finding by both groups was that the tubule acquired COPI as it moved toward the Golgi (10, 13). Therefore, they independently conclude that this presents evidence for a role of COPI in anterograde ER-to-Golgi transport, which challenges the classical model whereby COPI is thought exclusively to mediate retrograde transport from the Golgi back to the ER. It remains unclear what role COPI would precisely play in anterograde transport. Simply because the tubular membrane container is positive for COPI does not necessarily mean that COPI regulates anterograde transport. Carriers need tethering factors such as p115/Uso1, which are recruited by Rab1 to deliver their content to the next compartment. No role for COPI is known in this process. An alternative explanation for the recruitment of COPI to the ER-derived carriers is that this marks the beginning of retrograde transport back to the ER. This is supported by the observation that a mutant of ERGIC-53 (LMAN1) that does not bind COPI is capable of leaving the ER and without exhibiting any defect in anterograde transport (17). Strikingly, this mutant ERGIC-53 mislocalizes to the plasma membrane because it cannot use COPI for retrograde transport (16). Thus, recruitment of COPI might contribute to the maturation of the forward moving membrane carrier by retrieving back ER proteins.

Altogether, it appears that several types of carriers (tubules, saccules, tunnels, and coated vesicles) may coexist and operate along the ER-to-Golgi route. The papers by Shomron et al. and Weigel el al. do not cancel or revoke the other models of trafficking. Rather, they add a new model and show us how diverse and flexible this trafficking route is. It is possible that our cells are equipped with all types of carriers, which cells use depending on the size, quantity, or type of cargo, as well as on the cellular and the environmental context. This diversity might confer robustness of the ER-to-Golgi transport pathway. This might explain why different groups reached sometimes opposing conclusions. For instance, papers that relied on waves of synchronized trafficking or on bulky cargo might have shifted the balance toward a certain type of carrier. Most cells contain several hundred ERES, with some of them at several microns' distance to the Golgi. It is therefore possible that different types of carriers might operate in a manner depending on the type of ERES. Future work will clarify and reconcile all these open questions.

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