New insights into protein secretion: TANGO1 runs rings around the COPII coat

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In this issue, Liu et al. (2017. *J. Cell Biol.* https://doi.org/10.1083/jcb.201611088) and Raote et al. (2017. *J. Cell Biol.* https://doi.org/10.1083/jcb.201608080) use super-resolution microscopy to visualize large COPII-coated endoplasmic reticulum (ER) export carriers. Rings of TANGO1 surround COPII, implicating TANGO1 in organizing ER exit sites and in regulating COPII coat dynamics and geometry.

TANGO1 is a transmembrane ER protein that localizes to ER exit sites (ERES), where the COPII coat generates carriers for exporting secretory cargoes to the Golgi (Barlowe and Miller, 2013; Malhotra and Erlmann, 2015). Mammalian cells contain multiple TANGO1-related species, including a short form called TANGO1S as well as cTAGE5, which forms a complex with TANGO1 (Maeda et al., 2016). Mammalian TANGO1 and its relatives are specifically involved in the secretion of large cargoes such as collagens (Saito et al., 2009; Malhotra and Erlmann, 2015). Collagen fibrils can be 300- to 400-nm long and do not fit inside the originally described 60- to 90-nm COPII vesicles, suggesting that the TANGO1 family evolved to adapt COPII to the needs of metazoans.

TANGO1 binds to collagens through its luminal domain, but unlike traditional ER export receptors, TANGO1 stays behind at ERES rather than entering COPII carriers (Saito et al., 2009). Nevertheless, both TANGO1 and cTAGE5 interact with COPII in a way that sheds light on their functions. COPII coat assembly begins with Sec12, a guanine nucleotide exchange factor that brings the small GTPase Sar1 to the ER membrane. Sar1-GTP recruits the Sec23/Sec24 complex to form the inner layer of the coat. Then Sec23 interacts with Sec31 and recruits the Sec13/Sec31 complex to form the outer layer of the coat. Intriguingly, Sec23 stimulates the GTPase activity of Sar1, and this stimulation is enhanced by Sec31, implying that the COPII coat is unstable because of loss of Sar1-GTP (Barlowe and Miller, 2013). TANGO1 and cTAGE5 may inhibit this GTP hydrolysis reaction because they bind Sec23 in a manner that precludes Sec31 binding (Saito et al., 2009; Ma and Goldberg, 2016).

The mutually exclusive binding of Sec23 to either Sec31 or TANGO1/cTAGE5 implies that TANGO1/cTAGE5 are unlikely to be present within the fully assembled COPII coat. Instead, structural data suggest that TANGO1/cTAGE5 bind at the edge of the COPII lattice, where they cluster the inner coat subunits in a way that favors the formation of a cylindrical COPII tube (Ma and Goldberg, 2016). COPII is flexible enough to generate a variety of structures that include small spherical coats, large spherical coats, and tubular coats (Barlowe and Miller, 2013; Zanetti et al., 2013). It seems that TANGO1/cTAGE5 influence not only the dynamics but also the geometry of COPII assembly.

An untested but plausible idea is that the lattice edge is the exclusive location of COPII assembly and disassembly events. Analogies can be made to a microtubule, in which hydrolysis of GTP by assembled tubulin subunits creates a polymer that is intrinsically unstable but can shrink only at the ends. Similarly, hydrolysis of GTP by Sar1 may create a COPII lattice that is intrinsically unstable but can shrink only at the edge. Just as microtubules are stabilized by end-binding proteins, the COPII lattice may be stabilized by edge-binding proteins such as TANGO1/cTAGE.

This view is supported by the elegant new studies in this issue of mammalian TANGO1 from Raote et al. and of *Drosophila melanogaster* TANGO1 from Liu et al. Conventional fluorescence microscopy cannot resolve the distribution of proteins in and around the nascent COPII coat, so Raote et al. (2017) turned to stimulated emission depletion microscopy to image collagen export. They used cultured fibroblasts in which collagen accumulated in the ER while other membrane traffic events proceeded normally. Meanwhile, Liu et al. (2017) used structured illumination microscopy to visualize TANGO1 at ERES in *Drosophila* and to characterize the effects of knocking down TANGO1 expression. Both papers report that TANGO1 is found at the edges of ERES, in rings that surround COPII. The TANGO1 rings have diameters on the order of 200 to 1,000 nm and are thought to represent individual ER export carriers. Therefore, it seems likely that in animal cells ER export often involves carriers that are much larger than the traditionally studied COPII vesicles.

TANGO1/cTAGE may not be the only COPII edge-binding proteins. The TANGO1 family is found only in vertebrates and some invertebrates, suggesting that other components play a more conserved role in stabilizing the edge of the COPII lattice. One candidate is Sec16, a peripheral membrane protein that localizes to ERES and suppresses GTP hydrolysis by Sar1 (Barlowe and Miller, 2013). It was hypothesized that Sec16 promotes COPII vesicle formation by preserving a ring of Sar1-GTP at the edge of the COPII lattice (Bharucha et al., 2013; Fig. 1). Consistent with this idea, yeast Sar1 was recently reported to localize in a Sec16-dependent manner to the edges of COPII structures (Kurokawa et al., 2016), and Liu et al. (2017)

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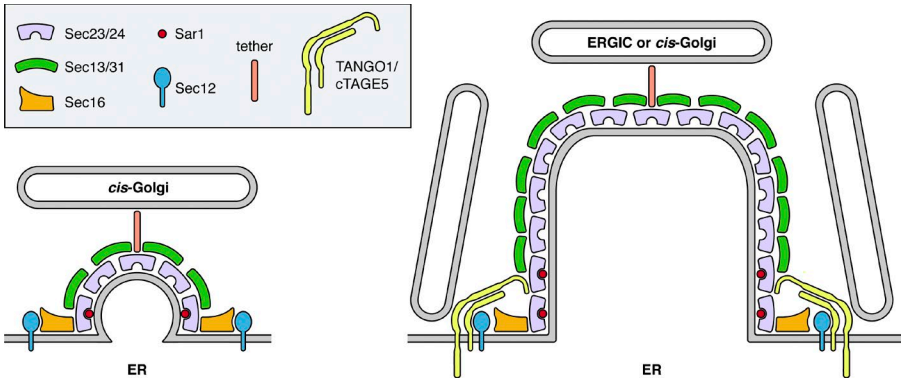

Figure 1. **Postulated arrangement of components that influence COPII assembly and tether ERES to post-ER membranes.** (left) Formation of a COPII vesicle by conserved proteins. The ER and ERGIC/Golgi membranes are shown in gray. The COPII coat consists of the purple and green components on the membrane surface. At the base of the budding vesicle, Sec16 suppresses GTP hydrolysis by Sar1 to create a stabilizing ring of Sar1-GTP. In some organisms, Sec16 also binds Sec12 to increase the local concentration of Sar1-GTP. The nascent vesicle is linked to Golgi membranes by conserved tethers. (right) Formation of a large COPII-coated carrier with the aid of TANGO1/cTAGE5. The TANGO1/cTAGE5 complex cooperates with Sec16 to stabilize the COPII lattice and to alter its geometry. cTAGE5 also binds Sec12, whereas TANGO1 binds ERGIC or Golgi membranes. The luminal domain of TANGO1 has additional interactions that are not depicted. *Drosophila* contains TANGO1 but not cTAGE5. See the text for details.

observed that *Drosophila* Sec16 colocalizes with rings of TANGO1. In addition to stabilizing existing Sar1-GTP in the lattice, COPII edge-binding proteins ensure a local supply of fresh Sar1-GTP by binding to Sec12 (Fig. 1). In the yeast *Pichia pastoris*, Sec16 recruits Sec12 to ERES (Bharucha et al., 2013), and in mammalian cells, cTAGE5 plays the same role (Saito et al., 2014; Fig. 1). A ring of Sar1-GTP at the edge of the COP II lattice will stabilize the lattice and allow it to grow and will be available at the final stage of COPII carrier formation, when Sar1 promotes membrane fission (Barlowe and Miller, 2013). This basic sequence of events is expected to occur during ER export in all eukaryotes, with TANGO1/cTAGE modulating COPII assembly to make carriers suitable for the unique secretion requirements of animal cells.

Is the TANGO1 family only needed to export large secretory cargoes? Studies of mammalian cells have yielded evidence for such specificity (Malhotra and Erlmann, 2015). However, the presence of multiple TANGO1 family members complicates the analysis. A further complication is that mammalian cells also contain an oligomeric Sec16-binding protein called TFG, which localizes to ERES and assists in the ER export of collagens (Johnson et al., 2015; McCaughey et al., 2016). *Drosophila* is an attractive system for studying TAN GO1 function because it lacks TFG and contains only a single TANGO1 species. Liu et al. (2017) observed that although *Drosophila* TANGO1 is especially important for collagen secretion, it is needed for efficient secretion of all secretory cargoes examined. Although these data from *Drosophila* are seemingly at odds with those from mammalian cells, it may be that *Drosophila* has solved the problem of secreting large cargoes by using TANGO1 to make "generic" ER export carriers that transport a wide range of cargoes. In contrast, mammalian cells use TANGO1 and its relatives selectively based on the secretory needs of different cell types.

TANGO1 interacts not only with COPII but also with cargoes. The luminal domain of TANGO1 binds collagens with the assistance of Hsp47, suggesting that TANGO1 helps package collagens into nascent export carriers (Malhotra and Erlmann, 2015; Ishikawa et al., 2016). Yet, TANGO1S can partially substitute for full-length TANGO1 despite lacking the luminal domain (Maeda et al., 2016). This point is emphasized by the new analyses of *Drosophila*: Liu et al. (2017) report that a mutant form of TANGO1 lacking the luminal domain rescued the defects observed after knocking down the full-length protein. Thus, interaction with secretory cargoes may be a secondary function of TANGO1.

Potentially more important is an interaction of TANGO1 with post-ER membranes. In most eukaryotes, the cis face of the Golgi is next to ERES, but in mammalian cells, an ER–Golgi intermediate compartment (ERGIC) is next to ERES and is the first destination of secretory cargoes after they exit the ER (Barlowe and Miller, 2013). The cytoplasmic portion of mammalian TANGO1 contains a tethering domain that binds to ERGIC membranes (Santos et al., 2015; Fig. 1). This interaction has been proposed to recruit ERGIC elements that fuse with the ER to supply membrane for large export carriers. Alternatively, Liu et al. (2017) show that *Drosophila* TANGO1 is needed to maintain the association of ERES with the Golgi, suggesting that TANGO1 plays an organizational role by linking ERES to post-ER membranes. Moreover, the expression level of *Drosophila* TANGO1 regulates the size and number of ERES (Liu et al., 2017). Additional components are likely to be at work because TFG has been implicated in the higher-order organization of mammalian ERES (Johnson et al., 2015; McCaughey et al., 2016) and because an ERES–Golgi association is seen in organisms that lack both TANGO1 and TFG (Fig. 1). It is too early to conclude whether the interaction of TANGO1 with post-ER compartments serves to recruit membranes for export carrier growth or to create an ERES architecture suitable for exporting large cargoes, or both. Regardless of the mechanism at work, TANGO1 is a multifunctional protein that helps coordinate the production of specialized COPII-dependent export carriers.

The most mysterious aspect of TANGO1 function is the specific nature of the export carriers that carry collagens and other large cargoes out of the ER. Geometric considerations imply that collagen enters such carriers end on (Raote et al., 2017), but the visualized carriers are wider than the COPII-coated tubes that have been described in vitro (Zanetti et al., 2013). Are the physiological ER export carriers uniformly coated with COPII? Do they actually pinch off to form vesicles, or do they form transient continuities that enable large cargoes to move directly from the ER into an ERGIC or Golgi compartment (Malhotra and Erlmann, 2015; Liu et al., 2017)? Raote et al. (2017) found that TANGO1 can be visualized in

two configurations that probably reflect distinct stages in the ER export process. These studies are pushing fluorescence microscopy to its limits. For the next level of analysis, correlative fluorescence and electron microscopy will be needed to obtain a high-resolution picture of the carriers generated in vivo by COPII and TANGO1.

More generally, the emerging data about TANGO1 highlight how little we know about certain aspects of ER export. A working model is that, in all eukaryotes, regulatory components operate at the edge of the COPII lattice to control the rates of coat assembly and disassembly and to help define coat structure (Fig. 1). These conserved processes need to be better described before we can understand how animal-specific ERES proteins such as TANGO1 modify the properties of the COPII lattice. Similarly, tethering of ERES to post-ER membranes may be universal and involve conserved proteins (Fig. 1). The tethering mechanism needs to be investigated to understand how animal-specific ERES proteins such as TANGO1 customize ER export complexes. Studies of TANGO1 and its relatives are prompting us to revisit fundamental questions about the earliest events in the secretory pathway.

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