JCB Journal of Cell Biology

VIEWPOINT

Protein transport by vesicles and tunnels

Ishier Raote[1] and Vivek Malhotra[1,2,3]

Palade's corpus placed small vesicles as the sole means to transport proteins across stable distinct compartments of the secretory pathway. We suggest that cargo, spatial organization of secretory compartments, and the timing of fission of cargo-filled containers dictate the design of transport intermediates that can be vesicles and transient direct tunnels.

Palade's secretory pathway

From the early 1960s there were disagreements on the identity of transport carriers along the secretory pathway. Mollenhaur and Claude (1, 2) independently claimed short continuities or tunnels, whereas Palade, based on his findings, favored a vesicular mode of transport. Palade's model became universally accepted and laid the foundation of our understanding of the secretory pathway. Newly synthesized secretory proteins that enter the ER are transferred to the Golgi apparatus and then released to the extracellular space (3). Following Palade's schema, vesicles are described as uniform structures created by sculpting and extracting a small portion of the donor membrane enriched in cargo. We now know vesicles formed by the COPII coat proteins export anterograde cargo at the ER while COPI coat produce retrograde vesicles at Golgi.

Studies over the past four decades have revealed that assembly of the COPII coat occurs at specialized subdomains called ER exit sites (ERES). It is initiated through activation of the small GTPase Sar1 by its ER resident guanine-nucleotide exchange factor Sec12. The GDP-to-GTP transition of Sar1 exposes an N-terminal amphipathic α-helix, which inserts it into the ER membrane. Membrane-bound Sar1-GTP recruits Sec23-Sec24 heterodimers, which form an inner coat at a membrane bud. Subsequently, this prebudding complex recruits Sec13-Sec31 heterotetramers, providing the outer layer of a coat for a bud. The coat proteins act as a GTPase activating protein to inactivate Sar1, which then promotes fission of a complete vesicle (4). COPII coats, according to this scheme, sculpt ER membrane into a vesicle of reasonably uniform size and shape.

A sequence of events and activities, analogous to those involved in COPII biogenesis, produce a COPI vesicle of remarkably similar dimensions at the Golgi.

These mechanisms for vesicle biogenesis unveiled mechanistic confirmation of Palade's model by providing evidence for how a secretory cargo protein can be packaged into discrete vesicles formed from a donor compartment and then trafficked to the target compartment. COP-coated carriers formed by these mechanisms are indubitably important mediators of protein trafficking through the secretory pathway. However, as secretory cargoes increase in size and complexity through evolution, mechanisms for their export from the ER have adapted concomitantly. The discovery of the TANGO1 family of metazoan proteins that include cTAGE5, TALI, and TANGO1-Short, which are required for secretion of complex cargoes such as procollagens and apolipoproteins, has revealed an alternate mechanism for creating a transport intermediate, prompting us to propose a significant revision of Palade's original model of vesicular transport and return to the idea of tunnels as important conveyors of cargo in the secretory pathway.

Our current definition of intermediates in the secretory pathway is based on their morphology as described in the classical electron microscopic images generated by Palade and colleagues. We propose that in order to define a secretory intermediate we also need to take into account its mechanism of formation. Yes, a tunnel should be open at both ends (the morphological definition) but, critically, a tunnel is a structure formed by connecting two discrete, preexisting compartments that continue to retain their individual identities. A tunnel conjures up an image of a long tube open at both ends, but based on this definition, tunnels in the secretory pathway are continuities between any adjacent compartments, including the ER and the ER–Golgi intermediate compartment (ERGIC) and ERGIC and Golgi, and sequential cisternae within a Golgi stack or even between endosomal structures after the TGN. Based on the distance between the two compartments, a tunnel therefore might be a long tube or just an aperture between them. A tunnel could resemble a contact site where two compartments are held together at a single site, but unlike a contact site, a tunnel is a site where luminal material is transferred between the compartments.

TANGO1 creates a tunnel

TANGO1 family proteins can initiate ERES formation by recruiting Sec12 and hence Sar1. Additionally, TANGO1 and cTAGE5 bind to Sec16 and serve as scaffolds or stabilizers, binding and organizing COPII coat machinery (Sec23A), to link a functional export site to assembled,

[1]Centre for Genomic Regulation, The Barcelona Institute of Science and Technology, Barcelona, Spain; [2]Universitat Pompeu Fabra, Barcelona, Spain; [3]Institució Catalana de Recerca i Estudis Avançats, Barcelona, Spain.

Correspondence to Ishier Raote: ishier.raote@crg.eu; Vivek Malhotra: vivek.malhotra@crg.eu.

export-competent cargoes (5). In binding to Sec23A, TANGO1 could competitively inhibit the binding of the outer coat Sec13/31, providing a kinetic delay to carrier fission to regulate the dimension of a carrier (5, 6). Unlike other cargo receptors, TANGO1 does not appear to leave the ER, binds Sec23A instead of Sec24, and controls the Sec23A GTPase activating protein activity for Sar1-GTP and therefore the timing of the fission event (6).

Rather than molding a carrier from ER membrane as in the conventional model of COPII vesicle formation, TANGO1 tethers post-ER membranes (ERGIC/Golgi) that fuse at ERES to create an export route for procollagens (7). During this process of cargo transfer, the ERGIC and ER are maintained as two distinct compartments. In other words, TANGO1 creates a tunnel between the ER and the ERGIC. ERGIC is a composite of vesicles and tubules; if the ERGIC membrane anchored by TANGO1 is a sufficiently large tubule, then no further membrane is needed and the final container is the ERGIC and not a vesicle budding from the ER. Accordingly, procollagen transfers from ERES directly to ERGIC without any detectable stand-alone containers (8). A large (procollagen-containing) ERGIC tubule/carrier, stretching several hundred nanometers, could reach the site of its fusion with the Golgi long before cargo is fully packaged and the carrier is severed at the ER. At this stage, fusion with the Golgi would lead to a tubule of ERGIC linking the ER to the Golgi (7, 9). The arrival site at the Golgi apparatus is marked by COPII binding tethers. As a coat assembles at an ERES bud, if this is sufficiently close to the Golgi entry sites, there may well be direct interaction between the ERES and the Golgi tethers to bring the two compartments closer together.

Although mechanistically unexplored, but related to this suggestion that close apposition of compartments leads to the formation of tunnels, data show that cargo flow in the Golgi is mediated by continuities/tunnels between closely apposed cisternae (10). Similarly, we suggest that a tunnel forms at ERES closer to the Golgi, bounded by COPII and COPI collars acting, with cargo receptors, as gatekeepers to control bidirectional transport of cargoes and residents. This arrangement is likely used to sort proteins at this site (a) retaining residents, (b) targeting terminally misfolded proteins for degradation, and (c) selecting appropriate cargoes for export.

With upwards of 120,000 molecules of TANGO1, and a few hundred ERES per mammalian cell (11, 12), there could be hundreds of molecules of TANGO1 at each exit site, serving to concentrate ever larger amounts of ERES machinery and to link it to cargo in the ER. Accordingly, targeted reduction in levels of TANGO1 in various organisms and systems leads to procollagen accumulations in the ER, with a marked reduction of ERES machinery at these accumulations. This TANGO1-dependent membrane organization and close apposition of neighboring compartments in traffic from the ER generates a hub of tethering and fusion events between ERES, ERGIC, and Golgi membranes. If ERES and ERGIC membrane need to be recruited and organized to export procollagen, one would expect procollagen export to gradually and spontaneously concentrate at sites with the highest concentration of these proteins, which is close to the Golgi. The converse will also be true, procollagen via TANGO1 recruits ERGIC and Golgi. This positive feedback would therefore reinforce the apposition of Golgi and procollagen export sites (for example, as suggested by images in McCaughey et al. [8]), thereby ensuring traffic occurs predominantly via tunnels.

TANGO1-mediated creation of a transport intermediate defies the accepted principle, laid down in accordance with Palade's schema, by which cells create small COPI and COPII vesicles.

Can mega containers mediate transport?

As a general principle, when cargo packaging is complete, the container is severed from the maternal compartment and when the container reaches the target membrane it fuses. With small cargoes and ERES that are distant from the Golgi (for example, greater than the diameter of a standard COPII vesicle), fission likely precedes fusion yielding a coated container transiently separated from both the donor and the acceptor membrane (Fig. 1 A). With larger cargoes such as procollagens, a newly forming container can reach the target membrane and likely fuse before its separation from the donor compartment, thereby creating a tunnel. But if the distance were even greater, fission would precede and result in the production of a mega container (Fig. 1 B). Mironov et al. reported the en bloc protrusion of ER membrane to form a mega carrier for procollagen (13). Gorur et al. have also described procollagen-loaded COPII-coated mega carriers; whether these are containers for secretion is unclear (14, 15), but these vesicles could also be generated by recruiting and fusing ERGIC to TANGO1 sites, followed by packing of procollagens, and the subsequent fission to generate a mega transport carrier. In other words, the timing of the fission and fusion control the morphological definition of a transfer intermediate.

In organisms without a clear TANGO1 orthologue, a tunneling mechanism would be transient and driven by the tethers that anchor ER and Golgi (Fig. 1). Indeed, these mechanisms of cargo export have been debated in the plant secretory pathway (16).

Conclusions and perspectives

Tunnels for transferring secretory cargo from the ER to the Golgi were presented in the early 60s, but the proposal met with little interest and was largely ignored. The discovery that TANGO1 creates a tunnel for cargo transfer by holding together two compartments has rekindled interest in tunnels as a mode of cargo transport. A tunnel could form either by lengthening a forming bud until it encounters its target compartment or by direct attachment of two apposed compartments (a near-zero length tunnel). This represents a panoptic view of some of the recent alternatives to vesicle-mediated transfer that involve romantic innuendo, including the kiss-and-run (17), hug-and-kiss (18), or kiss-and-stay modes of material transfer. Recent advances in live, superresolution imaging of organellar interactions will be instructive in describing the morphological features of these various cargo carriers (19, 20).

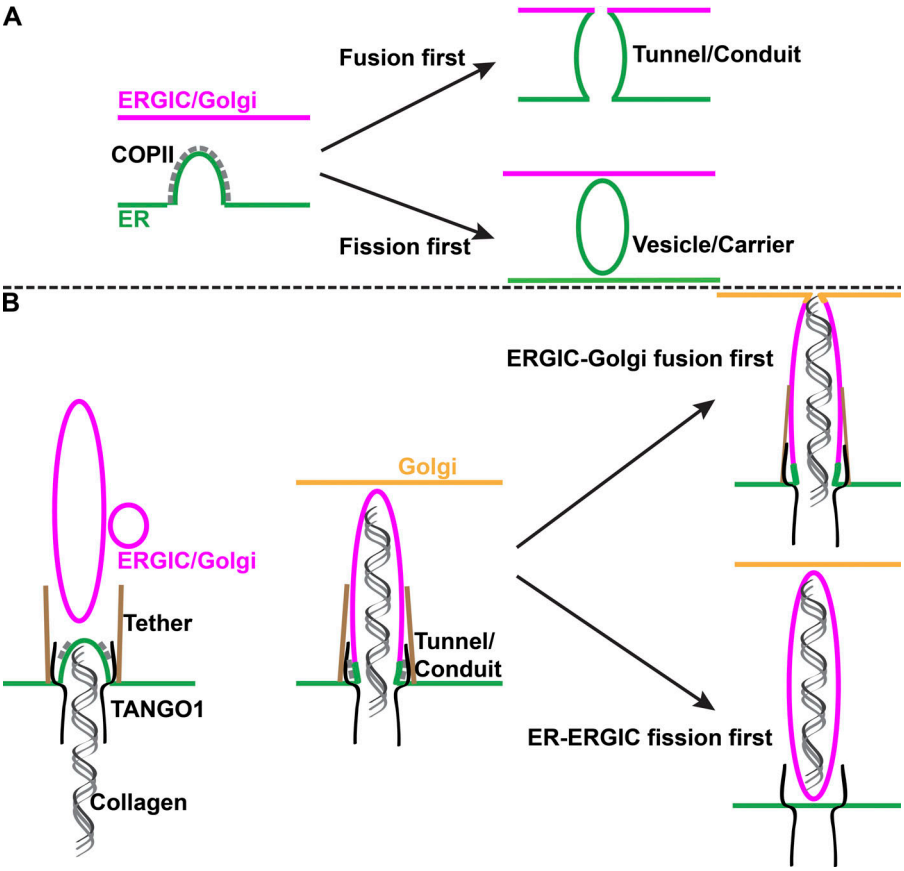

Figure 1. **Cargo, the timing of fission, and spatial compartmental organization dictates the nature of transport intermediates. (A)** Budding vesicles can fuse to the target membrane, creating a tunnel between donor and acceptor. However, if fission precedes fusion, this will generate a stand-alone vesicle. **(B)** Recruitment of a downstream compartment to the budding site creates the exit route via a tunnel. For cargoes like procollagens and lipoprotein particles, TANGO1 family proteins capture cargo and tether post-ER membranes. Tethered membranes fuse with each other and to the nascent bud at the ER. In this case, the "transporting intermediate" is, in fact, the downstream compartment of the secretory pathway. In *Drosophila melanogaster*, the tethered membrane is likely Golgi, while in mammalian cells the membrane would be the ERGIC. At this point, a further fusion of the ERGIC to the Golgi could even result in a long ER–ERGIC–Golgi tube.

The obvious challenge is to ask how two compartments joined by a tunnel are kept biochemically distinct. How do COP coats, conventional cargo receptors, cargo proteins, and TANGO1 function as gatekeepers? COPII could serve as collars to regulate the recruitment and passage of transmembrane proteins including cargo receptors. Cargo receptors concentrated at an ERES could form an active or physical barrier with their bulky luminal mass. Filling an open tunnel with cargo could also prevent unwanted proteins from leaking through. Finally, the assembly of TANGO1 at an ERES into a fence extending into the ER lumen could create a partitioned area dedicated to cargo organization and selection.

Acknowledgments

We thank James Rothman and Ben Glick for valuable discussions. We apologize for not citing the work of many because of space limitations.

We acknowledge support from the Spanish Ministry of Economy and Competitiveness through the Program "Centro de Excelencia Severo Ochoa 2013-2017" (SEV-2012-0208), support from the CERCA Program/Generalitat de Catalunya and fellowship IJCI-2017-34751 (to I. Raote). V. Malhotra is an Institució Catalana de Recerca i Estudis Avançats professor at the Centre for Genomic Regulation and the work in his laboratory is funded by grants from MINECO's Plan Nacional (BFU2013-44188-P) and Consolider (CSD2009-00016). The project has received research funding from the European Union. This paper reflects only the authors' views. The European Union is not liable for any use that may be made of the information contained therein.

The authors declare no competing financial interests.

1. Morré, D.J., et al. 1971. *Adv. Cytopharmacol.* 1:159–182.
2. Claude, A. 1970. *J. Cell Biol.* 47:745–766.
3. Caro, L.G., and G.E. Palade. 1964. *J. Cell Biol.* 20:473–495.
4. Jensen, D., and R. Schekman. 2011. *J. Cell Sci.* 124:1–4.
5. Saito, K., et al. 2009. *Cell.* 136:891–902.
6. Ma, W., and J. Goldberg. 2016. *Proc. Natl. Acad. Sci. USA.* 113:10061–10066.
7. Raote, I., et al. 2018. *eLife.* https://doi.org/10.7554/eLife.32723
8. McCaughey, J., et al. 2018. *J. Cell Biol.* https://doi.org/10.1083/jcb.201806035
9. Malhotra, V., and P. Erlmann. 2015. *Annu. Rev. Cell Dev. Biol.* 31:109–124.
10. Beznoussenko, G.V., et al. 2014. *eLife.* https://doi.org/10.7554/eLife.02009
11. Itzhak, D.N., et al. 2016. *eLife.* 5:e16950. https://doi.org/10.7554/eLife.16950
12. Hammond, A.T., and B.S. Glick. 2000. *Mol. Biol. Cell.* 11:3013–3030.
13. Mironov, A.A., et al. 2003. *Dev. Cell.* 5:583–594.
14. Gorur, A., et al. 2017. *J. Cell Biol.* 216:1745–1759.
15. Omari, S., et al. 2018. *Proc. Natl. Acad. Sci. USA.* 115:E10099–E10108.
16. Robinson, D.G., et al. 2015. *Plant Physiol.* 168:393–406.
17. Mironov, A.A., and G.V. Beznoussenko. 2012. *Int. J. Mol. Sci.* 13:6800–6819.
18. Kurokawa, K., et al. 2014. *Nat. Commun.* 5:3653.
19. Valm, A.M., et al. 2017. *Nature.* 546:162–167.
20. Guo, Y., et al. 2018. *Cell.* 175:1430–1442.e17.