Cargo carriers from the Golgi to the cell surface

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In this issue, Malhotra and colleagues use biochemical approaches to identify a new class of secretory cargo carriers (CARTS) that do not contain the larger cargoes, collagen or Vesicular stomatitis virus (VSV)-G glycoprotein. CARTS appear to be basolateral membrane-directed carriers that use myosin for their motility but not for their formation.

Protein secretion involves the collection of proteins into transport carriers that form at the exit (or ‘trans’) face of the Golgi apparatus for delivery to the cell surface. Multiple classes of secretory carriers form at the trans Golgi (Anitei and Hoflack, 2011). Some deliver cargo continuously to the cell surface; others release cargo in response to a signal. Regulated and constitutive secretory cargoes traverse the Golgi complex together and are sorted just before their exit. Proteins destined for different domains of the plasma membrane are also packaged into different carriers that bud from the Golgi and are delivered to either the apical or basolateral surface, respectively. Also departing the Golgi are clathrin-coated vesicles that carry newly synthesized lysosomal enzymes to endocytic compartments.

Despite the importance of protein secretion, the carriers that transport cargo from the Golgi to the cell surface have not yet been isolated or characterized. When visualized in live cells expressing GFP-tagged cargo, Golgi-to-cell surface carriers appear as variably sized vesicles and tubules of 1–8 µm in length (Hirschberg *et al*, 1998; Toomre *et al*, 1999; Polishchuk *et al*, 2003; Anitei and Hoflack, 2011). Both actin- and microtubule-based motors participate in their formation, along with phosphatidylinositol 4-phosphate that is needed to recruit components that participate in membrane budding and scission.

In this issue, Wakana *et al* (2012) report the identification of transport carriers (CARTs from the *trans* Golgi network to the cell surface or CARTS) that mediate the Golgi-to-cell surface transport of a select set of cargo proteins. Unexpectedly, the authors report that collagen and VSV-G glycoprotein use a different carrier for their transport to the cell surface; CARTS also use myosin II for motility but not for vesicle scission (see Figure 1).

Wakana *et al* (2012) first characterize the vesicle formation process by monitoring TGN46. TGN46 is a protein of unknown function that localizes to the trans Golgi at steady state but cycles between the Golgi and the cell surface. Thus, TGN46 should be present in the Golgi and to a lesser extent, in secretory transport vesicles and endocytic and recycling

vesicles. The authors use digitonin to permeabilize HeLa cells and monitor vesicle budding that occurs upon addition of ATP and rat liver cytosol. They use differential centrifugation to remove large membranes and identify a population of putative carriers that only sediment upon centrifugation at high speed and form in the presence of ATP and cytosol. TGN46-vesicle formation requires protein kinase D, a kinase needed for secretory carrier formation in cells (Liljedahl *et al*, 2001). Next, the authors use antibodies that recognize the cytoplasmic domain of TGN46 to immuno-isolate intact vesicles; controls show that the isolated membranes do not represent lysosomes, endosomes or the Golgi itself. Satisfyingly, the isolated vesicles include a secretory cargo: exogenously expressed, signal sequence containing, horseradish peroxidase. This is good evidence that the isolated carriers represent exocytic vesicles.

Mass spectrometry was used to identify candidate transport vesicle proteins; low yields precluded the authors from

Figure 1 PAUF and collagen export from the Golgi require protein kinase D, which distinguishes these export events from the transport of proteins to the apical surface. Small cargoes like PAUF use myosin II for vesicle motility after carrier formation; large cargoes like collagen and VSV-G may use myosin for both carrier formation and motility.

carrying out a rigorous analysis. Nevertheless, pancreatic adenocarcinoma upregulated factor (PAUF or ZG16B) and lysozyme were identified and confirmed as endogenous, soluble cargo proteins, together with synaptotagmin II, Rab6A, Rab8A and myosin II. Expression of a protein kinase D mutant enabled the authors to accumulate PAUF in trans Golgi tubules; in cells, PAUF carriers were distinct from those coated with COPI, COPII and clathrin. By EM, the carriers were round to elongated, 100–250 nm diameter structures. The identification of an endogenous, constitutively secreted protein will be valuable to those studying secretion.

Myosin II has been reported to play a role in the formation of vesicles containing VSV-G glycoprotein (cf. Miserey-Lenkei *et al*, 2010). Wakana *et al* (2012) showed that PAUF secretion was inhibited in the presence of blebbistatin, a myosin II inhibitor. However, in the presence of blebbistatin, PAUF-containing punctate structures detected by light microscopy were unchanged in total number or distribution, suggesting that CARTS formation is myosin II independent.

Many studies of protein secretion have monitored the trafficking of VSV-G glycoprotein (Hirschberg *et al*, 1998; Toomre *et al*, 1999; Polishchuk *et al*, 2003). G protein is convenient and well studied but an important property that is often overlooked is the tendency of viral glycoproteins to form crystalline arrays within the secretory pathway, especially if proteins are accumulated in the trans Golgi by incubation of cells at 20°C (Griffiths *et al*, 1985). Under these conditions, cryoelectron microscopy has documented the oligomerization of viral glycoproteins. Large protein assemblies like these and like collagen may require modification of the vesicle formation process to accommodate the larger proteins (Malhotra and Erlmann, 2011; Jin *et al*, 2012). Thus, it was especially interesting that collagen and VSV-G protein are not detected in PAUF-containing vesicles en route to the cell surface. This may explain why PAUF carriers were not dependent upon myosin

II (Wakana *et al*, 2012) while VSV-G carriers were (Miserey-Lenkei *et al*, 2010)—perhaps the larger carriers of VSV-G and collagen have a greater need for myosin II in their formation.

Several models can explain the formation of the two transport vesicle classes detected. A trivial explanation would be that the carriers are distinct because they are destined for different plasma membrane domains—apical versus basolateral. However, only basolateral transport requires protein kinase D (Yeaman *et al*, 2004) and protein kinase D is important for all the cargoes studied here—suggesting that both carrier types are basolaterally directed. Simply by default, collection of large assemblies into a nascent vesicle may physically exclude soluble PAUF protein. Alternatively, larger cargoes may use a molecularly distinct class of transport carrier. Yet to be identified are the protein constituents that define CARTS—proteins that collect cargoes together with the vesicle targeting and fusion machinery that must be included in all functional, newly formed transport vesicles. Once these markers are identified, it will become possible to distinguish between these two models and to isolate CARTS in larger quantities for full mass spec analysis. For now, the findings confirm the segregation of small and large cargoes into different vesicles that traverse the path from the Golgi to the cell surface and clarify the role of myosin in transporting these vesicles, but not necessarily pinching them off from the trans Golgi.

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Conflict of interest

The author declares that she has no conflict of interest.

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