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Traffic through the Golgi apparatus

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The role of vesicles in cargo transport through the Golgi apparatus has been controversial. Large forms of cargo such as protein aggregates are thought to progress through the Golgi stack by a process of cisternal maturation, balanced by a return flow of Golgi resident proteins in COPI-coated vesicles. However, whether this is the primary role of vesicles, or whether they also serve to transport small cargo molecules in a forward direction has been debated. Two papers (Martínez-Menárguez et al., 2001; Mironov et al., 2001, this issue) use sophisticated light and electron microscopy to provide evidence that the vesicular stomatitis virus membrane glycoprotein (VSV G)* is largely excluded from vesicles in vivo, and does not move between cisternae, whereas resident Golgi enzymes freely enter vesicles as predicted by the cisternal maturation model. Both papers conclude that vesicles are likely to play only a minor role in the anterograde transport of cargo through the Golgi apparatus in mammalian tissue culture cells.

The mammalian Golgi typically consists of a ribbon-like structure in which stacks of cisternae alternate with regions rich in COPI-coated vesicles and tubules (Fig. 1). Early studies in a variety of systems suggested that large structures, such as algal scales and collagen aggregates, are restricted to cisternal membranes and proceed through Golgi stacks by a process of maturation, in which new cisternae form on the cis side of the stack, whereas mature ones fragment and peel off the trans side. With the realization that the Golgi contains many resident proteins such as glycosyltransferases, a retrograde transport role was invoked for the vesicles to explain how these enzymes managed to stay in place on the escalator system (Martínez-Menárguez et al., 2001, this issue).

There is now general agreement that this constitutes one form of transport. However, in vitro studies using Golgi membranes from VSV-infected cells have clearly shown that VSV G protein can enter COPI coated vesicles (Ostermann et al., 1993). Furthermore, in pancreatic cells, proinsulin could be detected in a subset of vesicles, as revealed by immuno-EM (Orci et al., 1997). Thus, the possibility that vesicles could also carry anterograde traffic between cisternae, and indeed might do so efficiently, has long also been considered.

Two factors have made it difficult to decide whether vesicles make a major or a minor contribution to forward transport, relative to cisternal maturation. One is that there have been relatively few detailed studies of vesicle content in vivo, and conclusions concerning the presence of anterograde cargo or resident Golgi enzymes have been contradictory. The other is that a previous study of collagen transport from Luini and colleagues appeared to show much slower transport of aggregates than had previously been found for VSV G protein: when exit of collagen from the ER was blocked with a drug that prevented its folding, aggregates were still visible in the cis-Golgi an hour later (Bonfanti et al., 1998). This suggested a slow movement of cisternae, and thus that a vesicular shuttle might be required to provide rapid transit of VSV G and other proteins (Pelham and Rothman, 2000).

In the present work, the Luini group uses cells expressing both collagen and VSV G, and more stringent conditions to block collagen exit from the ER (Mironov et al., 2001). They conclude that their previous results can be explained not by slow cisternal movement, but by residual entry of collagen into the Golgi stack. Under the new conditions, VSV G and collagen transit the Golgi at essentially identical rates, removing the need to postulate a rapid bypass of the cisternae. This does not prove that VSV G never enters vesicles, but does suggest that cisternal maturation can be rapid enough to explain the secretion rates commonly measured. In this respect, the data fit a study of artificial protein aggregates, which were shown to pass rapidly from cis- to trans-Golgi (Volchuk et al., 2000).

Most remarkably, Mironov et al. (2001) use conditions where a short pulse of GFP-tagged VSV G is delivered from ER to Golgi, and find that only a subset of the stacks in the Golgi ribbon become labelled. Both light and electron microscopy reveal that at least some cisternae then travel through the stacks without any noticeable spreading of VSV G either into earlier or later cisternae, or into adjacent stacked regions, or even into vesicles. In some cases, the entire cisterna seems then to form the carrier from Golgi to plasma membrane. Thus, the conclusion is that VSV G not only does not need to enter vesicles to pass rapidly through the Golgi, but may actually be largely excluded from them under these conditions. In contrast, Golgi enzymes are

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*Abbreviation used in this paper: VSV G, vesicular stomatitis virus membrane glycoprotein.

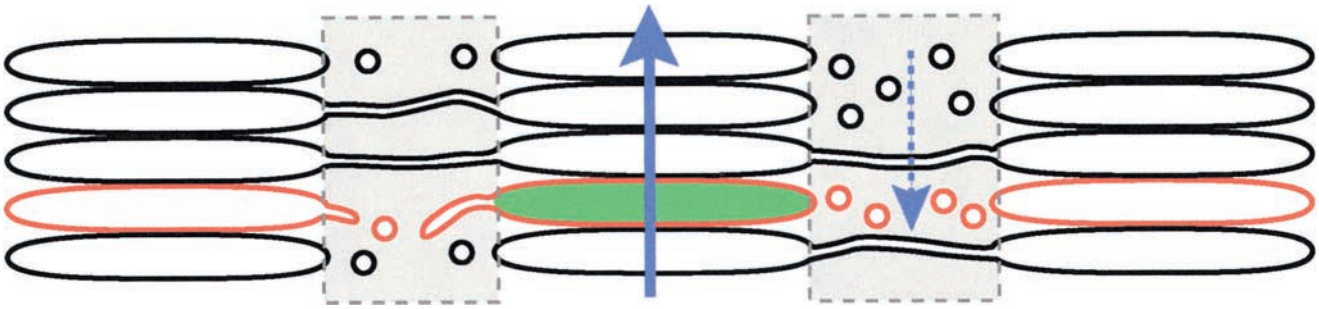

Figure 1. **Schematic structure of the Golgi.** The Golgi ribbon consists of cisternal stacks separated by tubulovesicular domains (gray boxes). Tubular connections between equivalent cisternae are well documented (Ladinsky et al., 1999); whether cisternae at different levels are also sometimes connected by tubules is less clear. Individual glycosyltransferases tend to be found at a characteristic level of the stack (red). They enter vesicles and seem to be able to move along the ribbon. When VSV G is delivered to the Golgi in a short pulse, it enters only a subset of the stacks (Mironov et al., 2001). Single cisternae containing VSV G (green) can then move through the stack; exclusion of VSV G from the tubulovesicular regions prevents its transfer both to adjacent cisternae in the same stack and to other stacks in the ribbon. Blue arrows indicate forward movement of cisternae and presumed net retrograde movement in the tubulovesicular regions.

present throughout the Golgi ribbon, and appear to be freely exchangeable between the stacks that make it up (Cole et al., 1996; Mironov et al., 2001).

The work of Martínez-Menárguez et al. (2001) provides independent evidence that entry of VSV G into vesicles is inefficient compared to that of Golgi enzymes. Using double label cryoimmuno-EM, they show that COPI-coated peri-Golgi buds and vesicles contain the enzyme mannosidase II, but are substantially depleted of VSV G relative to the cisternae. These data are in agreement with earlier data showing that not only large structures but also soluble secretory proteins such as albumin seem to be excluded from vesicles in vivo (Dahan et al., 1994), as shown also for a secreted form of horseradish peroxidase by Mironov et al. (2001). However, they appear to conflict with a previous study which concluded that Golgi enzymes are relatively depleted from COPI vesicles in pancreatic cells (Orci et al., 2000). Martínez-Menárguez et al. (2001) suggest that this may in part reflect the difficulty, in single label studies, of identifying the compartment from which a given COPI vesicle has budded. Certainly, there is room for further investigation of these discrepancies, but the preferential sorting of enzymes into vesicles, as measured by double label, seems inescapable.

These studies do not address some of the more esoteric questions raised by theoretical models of the Golgi, such as whether different cisternae in the same stack are connected by tubules or only by shuttling vesicles, how many classes of such vesicles exist, and whether they travel one way only or are at least partly bidirectional. Nor do they rule out the possibility that vesicles contribute to cargo transport in some circumstances. However, they do emphasize one very important point, namely that segregation of cargo and enzymes occurs within contiguous stretches of Golgi membrane. It seems that the Golgi can be considered as a two-phase system. The flat cisternal membranes are accessible to both enzymes and cargo, whereas the curved ends, together with the vesicles and/or tubules derived from them, represent a selective barrier to the diffusion of cargo, preventing the spread of VSV G even between adjacent stacks in the Golgi ribbon (Fig. 1). A possibly analogous morphology has been described for yeast Golgi membranes, which seem to form a network consisting of swollen nodules which eventually give rise to cargo-containing post-Golgi carriers, connected by thinner tubules into which resident Golgi proteins presumably segregate (Morin-Ganet et al., 2000).

The reluctance of cargo to enter tubulovesicular regions of the Golgi contrasts with the eagerness of even large molecules such as collagen to enter such structures during ER exit. Presumably, it is the COPII coat that dictates entry, but by analogy with the Golgi one can consider ER exit sites as a phase distinct from ER cisternae; in this case, it is one into which cargo preferentially partitions.

It should come as no surprise that proteins get sorted in the Golgi, but how this is achieved remains quite mysterious. The key sorting processes seem to be subtle, breaking down under some conditions in vitro, and perhaps also in vivo. However, sorting of Golgi enzymes into vesicles has been observed in vitro, and the latest investigation of this is also reported by Lanoix et al. (2001, this issue). This study, together with earlier ones (see Lanoix et al., 2001, for references), concludes that sorting of membrane proteins into one class of COPI vesicle is mediated by direct interaction of their cytoplasmic tails with either COPI subunits or the activator protein (GAP) for the associated Arf1 GTPase, thus priming coat formation. Such positive selection can exclude other proteins by competition, and results in rapid recycling of the selected proteins to the ER.

What is less clear is why Golgi enzymes should be preferentially incorporated into a second class of COPI vesicles that are thought to mediate intra-Golgi traffic (Lanoix et al., 2001; Martínez-Menárguez et al., 2001). Such enzymes are prevented from reaching the cell surface not by their cytoplasmic tails but by their short transmembrane spans. These may be incompatible with sterol and sphingomyelin-rich membrane domains, which could in turn be excluded from vesicles. Hence, enzymes would tend to partition into phospholipid-rich vesicles rather than sterol-rich cisternae (Munro, 1995). However, even if this explanation is correct, it does not seem intuitively obvious that the longer transmembrane domain of VSV G should be excluded from vesicles. Can lipid sorting explain the observed segregation? Or do Golgi enzymes have some affinity for COPI or ArfGAP,

but not enough for them to enter ER-bound COPI vesicles? Could the VSV G tail have some affinity for other peripheral Golgi proteins, thus keeping it out of vesicles? Would partitioning of membrane proteins into vesicles be sufficient to exclude soluble secretory proteins?

These questions are not easy to answer, but at least the methods are available to address them. It would be interesting to know, for example, whether changes to transmembrane domains or cytoplasmic tails affect entry into vesicles in vivo, how this affects progress through and between Golgi stacks, and whether this correlates with sorting in vitro. It is a sign of the advances in this field that such precise questions can now be addressed.

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