Membrane Dynamics at the Endoplasmic Reticulum–Golgi Interface

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Movement of cargo through the secretory pathway of eukaryotic cells occurs as a discontinuous process involving the activity of 50–80-nm vesicles (28). Vesicles forming on the endoplasmic reticulum (ER) by the COPII coat machinery (35) were first detected as coated elevations on transitional elements directly facing the Golgi apparatus. Subsequently, intermediates harboring ER-derived cargo were found in regions distant from the Golgi stack (16, 33). These structures contained a different coat complex, COPI or coatomer, recognized to be involved in vesicle-mediated recycling (20). It is now apparent that ER export and recycling are tightly coupled. We review recent evidence regarding the organization and role of ER to Golgi intermediates in these events.

Pleomorphic Elements Function in ER to Golgi Transport

Stereological analysis of serial thin sections revealed the general morphological organization and distribution of ER export/recycling sites (4). Export from the ER was found to be associated with one or more COPII-coated budding ER cisternae that face towards a central cavity (Fig. 1). The central cavity is filled with a collection of closely opposed vesicles and convoluted tubules containing COPI coats (4). We have termed these central compact clusters of pleomorphic elements as vesicular-tubular clusters (VTCs) for their morphological appearance (3), but they have also been referred to as ERGIC (ER–Golgi intermediate compartment) (16). The juxtaposition of ER-derived buds and a central VTC composes a morphological unit of organization termed an export complex (4) (Fig. 1). ER-derived buds cannot be detected outside export complexes, suggesting a local specialization of the cytoplasm that is likely to be enriched in transport components. While VTC-containing export complexes are scattered throughout the cytoplasm, VTCs found in the perinuclear region of the cell are believed to form a more extensive array of tubulo-cisternal elements referred to as the cis-Golgi network (CGN) (24).

Biochemical Composition of VTCs

COPII coats direct the sorting and concentration of cargo during export from the ER (1, 3, 5). The dissociation of a COPII vesicular carrier from ER budding elements demarcates the first boundary between the ER and downstream organelles of the secretory pathway. The formation of this boundary is consistent with morphological observations that VTCs lack continuity with the ER (4, 32, 40). Although there have been reports of apparent connections between VTCs and the ER, they appear only when selected viral glycoproteins have been overexpressed (18) or in cells infected with viruses that mature in the early secretory pathway (21). The structures generated under these conditions are likely to be tubular elaborations of ER or ER exit sites in response to the presence of viral proteins. In general, a large body of biochemical and morphological evidence is consistent with the conclusion that VTCs are the first distinct compartment downstream from the ER.

Although VTCs define a unique compartment, their composition remains to be firmly established. During export from the ER, ribophorin and components of the protein translocation machinery such as Sec61, as well as resident ER proteins including the folding chaperones calnexin and BiP, are efficiently excluded from COPI vesicles (5, 31). Therefore, they are unlikely to be functional components of VTCs. An important contribution to the identification of ER to Golgi intermediates came with the discovery of the closely related (90% identity) VTC marker proteins p53 and p58 (16). p53/p58 are type 1 transmembrane proteins that continuously recycle between the ER and VTCs but are concentrated in VTCs at steady state. Other proteins enriched in VTCs are the small GTPases Rab1 (29) and Rab2 (7), p24 family members that are potentially involved in cargo selection during export from the ER (10, 36), and other membrane components involved in the targeting/fusion of COP II vesicles (11). To date, there are no

1. Abbreviations used in this paper: CGN, cis-Golgi network; VTC, vesicular-tubular cluster.

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markers that can be defined as resident proteins, emphasizing that VTCs are highly dynamic structures.

**VTCs Undergo Maturation in a COPI-dependent Fashion**

After release from the ER, COPII vesicles rapidly lose their coats (2) and become associated with VTCs containing COPI coats. COPI is involved in the formation of vesicles that promote the retrograde transport of proteins containing terminal KXXX motifs (13, 22) and Phe residues (12, 37). The mechanism by which VTCs initiate the recruitment of COPI coats has been investigated. Using an assay that reconstitutes budding from ER microsomes in vitro (31), it was found that uncoated COPII vesicles can bind COPI components before their fusion to VTCs. This coupled exchange between COPII and COPI coats was proposed to serve as a tagging mechanism to mark components for rapid retrieval to the ER from VTCs (31). More recent studies have demonstrated that p53/p58, possibly in conjunction with p24 family members, is a component of the recruitment machinery (39). Therefore, segregation of retrograde- and anterograde-transported proteins is an important activity of the tubular elements comprising VTCs.

Although COPI vesicles are principally associated with VTCs and early Golgi compartments, they have recently also been proposed to participate in ER export. They can form on yeast ER membranes in vitro (6), and COPI components are closely associated with unusual ER cisternae in mammalian cells in vivo (27). However, the COPI vesicles formed in vitro from yeast ER membranes lack cargo molecules typically found in COPII vesicles, and the COPII-enriched region adjacent to the ER elements in mammalian cells does not contain buds. Therefore, it is presently more likely that COPII performs an exclusive role in sorting and concentration of cargo during ER export, while COPI functions in retrograde retrieval from VTCs and Golgi compartments.

**VTCs Can Be Mobilized to the Golgi Region Along Microtubules**

Morphological evidence has now provided clear support for a role for microtubules in ER to Golgi transport (34). When cells are incubated at reduced temperature (15°C), they accumulate VTCs at peripheral sites. After transfer to 37°C, these VTCs redistribute to the central Golgi region in a microtubule-dependent fashion. Live cell imaging using a green-fluorescence protein–cargo chimera (Presley, J.F., N.B. Cole, and J. Lippincott-Schwartz. 1996. Mol. Biol. Cell. 7:74a) revealed that VTCs migrated towards the Golgi in a saltatory fashion at ~1 μm/s. At the cis face of the Golgi, they appeared to fuse to form the CGN. Combined with the fact that ER buds are localized to the re-
region surrounding VTCs at steady state (4), it is apparent that the entire export complex serves as a mobile collecting/recycling device promoting acquisition and delivery of cargo to the Golgi apparatus.

Formation and Consumption of VTCs

At least two opposing models can be envisioned for the formation and consumption of VTCs. One model (Fig. 2A) suggests that the tubular elements of VTC/CGN are unique compartments that contain a core of nonrecycling, resident proteins distinct from those found in either the ER or the Golgi. In this model, ER-derived COPII vesicles undergo heterotypic fusion with these tubular elements. As described above, retrograde recycling would be mediated by COPI vesicles. Anterograde transport from these tubular elements to subsequent Golgi compartments would most likely require a second round of budding to retain compartment identity. However, COPII function is complete after export from the ER (2, 5, 31), and genetic experiments in yeast (13, 22) argue against the involvement of known COPI components in anterograde transport. Therefore, vesicle budding will require either currently unrecognized components of the COPI machinery that distinguish between anterograde or retrograde transport, or a new coat machinery.

In the second model (Fig. 2B), tubular elements of VTCs could form de novo from the homotypic fusion of ER-derived COPII vesicles. Homotypic fusion between like compartments is a common feature of both the exocytic and endocytic pathways. In this model, the tubular elements of VTCs would then move en bloc to the central Golgi region, where they could undergo further homotypic fusion with elements derived from other peripheral sites to form the CGN (3). During transit to the central Golgi region, COPI vesicles would direct recycling components back to the ER, thereby maintaining a steady-state balance between input and output of membrane. Although this model is attractive, key evidence that COPII vesicles or VTCs undergo homotypic fusion is missing.

Movement of Cargo through the Golgi Stack

The method by which VTCs are formed and consumed may provide critical insight into the ensuing mechanism(s) involved in the movement of anterograde-transported cargo through compartments of the Golgi stack. Transport will necessarily involve vesicular carriers since morphological and biochemical evidence favors the view that individual compartments initially formed by the fusion of COPII vesicles remain compartmentalized. Selective recruitment of these enzymes would necessarily be coupled to specificity determinants directing vesicle targeting and fusion. In this model, a compartment derived from the fusion of VTCs (lacking processing enzymes) would first become selectively enriched with cis processing enzymes (Fig. 2B). As the composition of the maturing compartment changes, so does the competition for the COPI machinery by processing enzymes within the compartment for recycling, thereby ensuring “self-directed” maturation to the medial- and trans-Golgi states (Fig. 2B). The apparent polarized organization of the stack would be a direct consequence of VTCs continuously contributing to the formation of new, cis-most elements (Fig. 2B). The TGN, where both COPI-mediated recycling and clathrin-mediated anterograde transport would be occurring, may be maintained through additional input of membrane from the endocytic pathway. The concept of directed maturation was proposed as early as 1957 by Grasse (14) and later refined by Morre and co-workers (25) as cisternal progression. New knowledge of the importance of COPI in retrograde traffic provides a potential mechanistic basis for this model.

Evidence for the Directed Maturation Model

Considerable evidence supports directed maturation. First, it is consistent with a major role for COPI in retrograde transport. Second, it accounts for the striking dependence of both retrograde and anterograde transport of cargo on the COPI machinery because processing of anterograde-directed cargo will not occur when COPI-mediated retrieval is blocked (2, 13, 31). Third, after mitotic disassembly of the Golgi, COPI vesicles appear to be markedly enriched in Golgi processing enzymes relative to anterograde cargo (38). Thus, COPI vesicles may sort and concentrate these enzymes, consistent with their high diffusional mobility in the membrane (9). Indeed, the lack of precise compartmental localization of various processing enzymes is compatible with the predicted variable affinity of COPI for different Golgi enzymes. Fourth, retrograde transport is required to maintain the localization of Golgi processing enzymes (15, 19) and for Golgi enzymes to maintain the structure of the stack (26). Fifth, all molecules migrate through the Golgi stack at a uniform rate, as opposed to variable rates of exit from the ER because of differences in sorting and concentration via COPII vesicles (3). Moreover,
assembled structures found in early Golgi compartments that are too large to enter carrier vesicles, such as ApoE containing lipoprotein particles, procollagen, casein submicelles, and scale plates in algae and virus particles, are able to undergo normal processing by Golgi enzymes during their delivery to the surface. Sixth, numerous observations have established that either under- or overexpression of a wide variety of Golgi “marker” proteins leads to dramatic changes in normal Golgi structure, reflecting a dynamic basis for organization of the stack. Finally, directed maturation could account for the observation that the regeneration of Golgi stacks occurs at export complexes after treatment with brefeldin A (a reagent that collapses Golgi compartments to the ER) (8) and during recovery from mitosis (23).

The Future

We have focused on recent results that now clearly establish the importance of VTCs as key intermediates in the secretory pathway. New approaches that provide insight into the mechanism(s) of protein retrieval and anterograde flow should help us understand the molecular basis for the function of the VTCs in transport of cargo from the ER to and through the Golgi apparatus.

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