Mini-Review

Variations on the Intracellular Transport Theme: Maturing Cisternae and Trafficking Tubules

Alexander A. Mironov,* Peggy Weidman,§ and Alberto Luini‡

*Unit of Morphology, ‡Laboratory of Molecular Neurobiology, Department of Cell Biology and Oncology, Istituto di Ricerche Farmacologiche Mario Negri, 66030 S. Maria Imbaro (Chieti), Italy; and §Department of Biochemistry and Molecular Biology, School of Medicine, St. Louis University Health Scientific Center, St. Louis, Missouri 63104

Technological breakthroughs of the last decade have advanced our understanding of vesicular trafficking from a largely descriptive approach to a molecular science. An impressive conservation of the molecular transport machinery across phyla has been revealed (30). Although the current consensus is that intracellular protein transport is mediated by transport vesicles, evidence suggestive of nonvesicular transport mechanisms continues to accumulate. As a consequence, two formerly rejected models, namely transport by cisternal progression (13) and traffic via membranous tubules (24, 37), are attracting renewed interest. Here, we provide updated versions of these models, review both new and old evidence relevant to their application to transport pathways, and discuss the implications as well as the difficulties posed by these alternative mechanisms.

Cisternal Progression–Maturation

The model of transport by cisternal progression has been largely based on morphological observations of scale-covered green algae. The best characterized case is that of *Pleurochrysis scheffelii*; however, many related organisms also display the same secretory phenomena (for a recent review see reference 2). The scales of *P. scheffelii* are much too large to be packaged into vesicles and appear to be transported by the progression of Golgi cisternae towards the plasmalemma, where the cisternae fuse to release the secretory product. This progression model was discounted as a universal mechanism of intra-Golgi transport because its original formulation, in which each cisterna contains all of the enzymes necessary for scale assembly and moves to the plasma membrane as a unit, seemed inapplicable to most other cell types (12). It was then thought that biosynthetic Golgi enzymes do not move through the stack. Indeed, in all animal cells that have been studied, different cisternae exhibit a distinct and stable enzymatic composition.

The transport of supramolecular structures through the Golgi complex is not, however, restricted to algae. Over the years, it has been observed in plants (for review see reference 31) and also in several animal cell types. For instance, casein submicelles in lactating mammary gland cells (4), apolipoprotein E in liver cells (9), procollagen in fibroblasts (17), and large proteinaceous membrane thickenings in urothelial cells (32) are detected throughout the Golgi cisternae but are consistently absent from Golgi vesicles. Many of these secretory products are simply too large to be packaged into transport vesicles. The movement of such large particles through Golgi stacks suggests that some sort of progression mechanism occurs in many different cell types. If this is the case, however, the progression model must be revised to account for the observed constancy of the cisternal enzyme distribution.

One way to overcome this difficulty is to assume that the anterograde shift of each cisterna is coupled with retrograde relocation of its enzymes into the next proximal cisterna. The cisternal progression scenario could be as follows (Fig. 1 *A*): The material exiting from the ER converges towards the Golgi complex and forms a new *cis*-cisterna. The nonsecretory material (e.g., ER-resident proteins, soluble *N*-ethyl-maleimide–sensitive factor attachment protein [SNAP] receptors) is retrieved from the *cis*-Golgi into the ER, while at the same time the defining components of the medial compartment (e.g., medial Golgi enzymes) flow backward into the *cis*-elements. The *cis*-compartment thus acquires medial Golgi features and, in effect, becomes a medial compartment. The process repeats itself (the medial compartment becoming *trans*) until the cargo reaches the TGN. Finally, the secretory material in the TGN is progressively released through the formation of secretory granules, vesicles, or via other mechanisms, consuming the organelle. TGN resident proteins then recycle back to the *trans*-Golgi, thereby transforming it into the next TGN ready for secretion. The essential characteristic of this mechanism is the maturation of the cisternae, with the consequence that cargo progresses across the stack. From this point of view, cisternal maturation–progression would be a more proper definition of the model. The model also implies that the intercisternal protein matrix (5) should be dynamic, forming at the *cis*-side, where it may act to organize incoming intermediate compartment membrane into a new *cis*-cisterna, and then dis-

Address all correspondence to Alexander Mironov, Consorzio Mario Negri Sud, Via Nazionale, 66030 S. Maria Imbaro (Chieti), Italy. Tel.: 39-872-507-344. Fax: 39-872-578-240. E-mail: mironov@cmns.mnegri.it

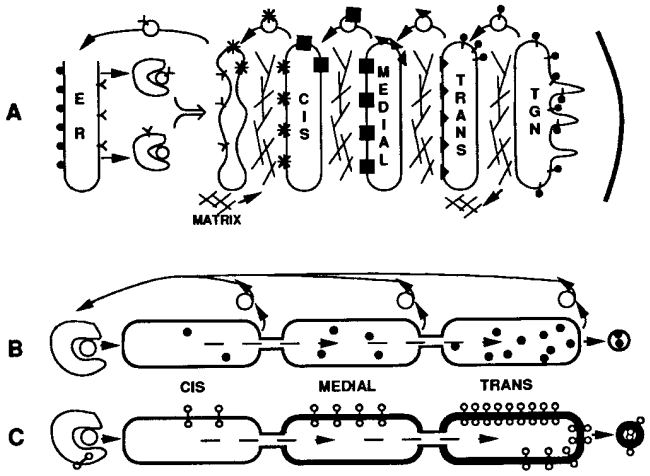

Figure 1. (*A*) Cisternal maturation–progression. According to an alternative view, the TGN consists of tubules emanating from the *trans*-compartment (16). (*B* and *C*) Possible mechanisms of vectorial cargo flow along tubular continuities; see text for details.

assembling at the *trans*-side when the TGN is consumed. Attractive candidates for matrix components are the cytoskeletal proteins spectrin and ankyrin (11), which reversibly associate with the Golgi complex and the *cis*-Golgi matrix protein GM130 (25).

Is the maturation mechanism compatible with our current knowledge about the location of Golgi enzymes? It is consistent with the observed lack of sharp separation between *cis*-, medial, and *trans*-Golgi enzymes in different cisternae (27 and references therein), while it is seemingly in contrast with the lack of reports of such enzymes outside the Golgi stacks. It is possible, however, that the concentrations of the enzymes transiting in such carriers might be too low to be detected by morphological approaches. Indeed, low concentrations of Golgi-resident enzymes have been observed in isolated Golgi-derived vesicles (33). Moreover, evidence is accumulating that resident Golgi enzymes and proteins are not stationary but can move through the Golgi stack (14 and references therein), as required by the model.

In summary, the available evidence is consistent with the possibility that cisternal progression–maturation occurs in at least some mammalian cells. The main features of the model now need to be tested in a systematic and integrated fashion. A key advancement, we believe, will be the development of suitable systems for analysis of retrograde transport of Golgi enzymes.

Tubules in Intracellular Traffic

Tubules are a prominent feature of both endocytic and exocytic transport pathways. They have been implicated as carriers or vesicle precursors in "dissociative" transport from endosomes (21) to the plasma membrane, from the TGN to plasma membrane (16), and from the intermediate compartment to the Golgi complex (20). It has also been proposed that they form direct linkages between different compartments (23). Here, we will focus on their possible role as membranous bridges.

ER and the Golgi Complex. Tubular continuities between the ER and the Golgi have been described many times in thin-sectioned specimens from a variety of tissues and cell types. Because they are not always found, it has been suggested that they might be artifactual (1). However, when techniques specifically designed for revealing complex structures in tridimensional space have been used, tubular connections have generally been observed (19 and references therein). Thus, their existence is very likely; however, their abundance, the regulation of their formation (which might be affected by temperature; 34), and their function remain unclear.

Tubules are also a well-characterized feature of the Golgi complex. Both the *cis*- and the *trans*-most Golgi elements are largely tubular. The central part of the complex consists of a continuous ribbon-like structure comprising several stacks of cisternae interlinked by tubular–reticular networks (28). Connectivity between adjacent stacks is also indicated by the rapid diffusion of enzymes within the intact Golgi of living cells (8). Golgi tubules are dynamic and form rapidly both in vivo and in vitro (6, 37; Lippincott-Schwartz, J., personal communication). Given that the evidence for anterograde vesicular intra-Golgi traffic remains ambiguous (18, 36, 37), while that for participation of the coat protein (COP I) machinery in retrograde vesicular traffic is quite strong (18), it has been proposed that anterograde transport occurs by transient tubular connections between heterotypic cisternae (37).

Such a model for transport by tubular networks connecting adjacent Golgi stacks has interesting implications, as well as difficulties. Since intrastack cisternal connections are almost never seen, tubular transport might occur via interstack connections in a direction tangential, rather than perpendicular, to the stacks (35). Thus, a functional Golgi unit would consist of a linear *cis–trans* series of cisternae located in different stacks and joined by tubules. Although it is generally assumed that these tubules link only homologous cisternae, this has not been rigorously tested because of the extreme complexity of the interstack tubular–reticular zone, and the possibility of heterologous connections cannot be excluded. A seeming difficulty of such a model is that it may appear inconsistent with the fact that transport is not disrupted when the Golgi complex is dispersed into ministacks after microtubule depolymerization (7 and references therein). Moreover, dispersed stacks are a uniform feature of the Golgi in plants and unicellular organisms. Nevertheless, even the noninterconnected Golgi stacks of plants (dictiosomes) exhibit abundant tubular networks at the periphery that may interconnect cisternae within the same stack (31 and references therein). Tridimensional views of isolated in vitro mammalian Golgi stacks have also revealed cisternae, apparently within the same stack, that are interconnected by peripheral tubular networks (38). In addition, a recent tridimensional reconstruction of nocodazole-induced ministacks also suggested that a tubular network connects apparently heterotypic cisternae within a single ministack (Polishchuk, R., A. Fusella, A. Luini, and A. Mironov. 1996. *Mol. Biol. Cell.* 7[Suppl.]:598a). Thus, disruption of the Golgi complex might give rise to other spatial arrangements of cisternae and tubules (26). The conserved nature of tubular networks in Golgi structure suggests they play an important role in its function. Defining this function

will require identification of the molecules that control Golgi tubule dynamics, and the development of methods to specifically disrupt intra-Golgi tubular connections.

TGN and Endocytic System. In many cells, early endosomes consist mostly of separate tubular networks connected with classical cisternal–vesicular endosomes (22 and references therein). Although the specific function of these networks is not understood, time-lapse video recordings show that endocytosed material enters discrete swellings of these networks and moves at rapid rates through the endocytic tubules (15). Evidence for near-normal sorting and transport in an apparently continuous interconnected tubular structure is found in many brefeldin A–treated cells, where early endosomes and the TGN fuse into an extensive tubular network with no detectable effect on transferrin recycling and iron uptake (39). These examples of cargo movement along tubules may represent a transport paradigm applicable to other traffic pathways.

Mechanistic Questions Raised by the Tubular Traffic Model. The model requires that distinct membrane domains and vectorial traffic be maintained within connected tubular networks. The general problem of membrane differentiation in a continuous network is exemplified by the rough and smooth domains of the ER. Here, it is clear that the domain localization of resident proteins involves both retention and signal-based retrieval. Although the mechanism of retention is unknown, by analogy with plasma membrane domains it is possible that retention involves association of resident proteins with elements of the cytoskeleton, each other, and/or a luminal matrix. A more dynamic retention mechanism might involve lipid-based sorting. For example, matching of *trans*-membrane domain length and membrane thickness (a function of membrane lipid composition) might serve as a sorting mechanism (3). A variation on this theme would be protein segregation by matching of protein shape (wedge, cone, or cylinder) with membrane curvature (29). Various combinations of protein–protein and protein–lipid interactions could be sufficient to account for retention of both membrane and soluble constituents. An attraction of lipid-based sorting is that the lipid composition of membranes varies throughout the secretory pathway as a consequence of lipid biosynthetic activities and cholesterol uptake (3).

In vesicular transport models, the problem of vectorial flow is solved by the dissociation of vesicles from the donor membrane and by vesicle-targeting molecules that impart directionality. In tubular traffic, directionality would have to depend on different principles. For example, a bidirectional traffic system in which continuous membrane addition at the proximal end of a tubular network (via the arrival of recycling vesicular intermediates) and membrane removal from the distal end (via formation of transport vesicles) could support a directional flow through an extended membrane system (Fig. 1 *B*). This would be analogous (on a different scale) to the mechanism proposed to drive plasma membrane flow towards the cell body of growing neurons, which depends, at least in part, on insertion of membrane at the growth cone during secretion (10). Directional flow might also occur if the membrane is differentiated along its length such that the affinity of transported proteins for the local environment increases from the entry to the exit sites (Fig. 1 *C*). A gradual increase in membrane thickness or substrate–enzyme affinity along the direction of the flow could, for example, provide such an affinity gradient for transiting proteins (3). These mechanisms might be coordinated with transient fission and reformation of tubular connections (37) to further control cargo backflow.

Concluding Remarks

The vesicular transport paradigm has reached a stage of maturity where a satisfactory understanding of the underlying molecular mechanisms is coming within our reach. At the same time, the ever-evolving view of organelle structure and function seems to question the usefulness of embracing a single mechanistic model. Our view is that vesicular traffic and the models discussed in this paper are not mutually exclusive and may, in fact, be part of a spectrum of interrelated mechanisms. For instance, the retrograde traffic of Golgi enzymes, necessary for cisternal maturation, may occur via vesicles or tubules. Intermittent tubular continuities might mediate the fast anterograde transport of most cargo, and supramolecular structures could progress by a slower cisternal maturation process. While the strongest evidence for these alternative mechanisms may be limited to certain cell types or situations, these might simply represent the regulatory extremes of the system. It is hoped that since key aspects of these models are testable, they will be subjected to a more systematic investigation.

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