Intra-Golgi transport: escalator or bucket brigade?

Andreas Nebenführ, University of Tennessee, Department of Botany, Knoxville, TN, 37996-1100

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1. The Problem

One of the great debates of current cell biology is centered around a puzzling dilemma that lies at the very heart of the Golgi apparatus: How can the structural and functional integrity of this organelle be maintained in face of a constant flux of secretory products through its midst? This question relates to the different roles that the Golgi plays in the secretory pathway. It is not only a way station *en route* to the cell surface and vacuoles/lysosomes, it also serves as an important biosynthetic organelle that harbors a large number of enzymatic activities. These two functions impose two conflicting requirements upon the Golgi. On the one hand, the flow of secreted proteins, lipids and carbohydrates has to proceed as smoothly as possible. On the other hand, the enzymes that operate on these molecules have to be retained within its cisternae. It is clear that these two processes can occur simultaneously only if there is a continuous and efficient interplay between mixing and sorting of substrates and enzymes. Mixing is necessary to ensure contact between enzymes and substrates, while sorting allows the substrates to move on and the enzymes to stay behind.

Cells are apparently able to solve this problem: several stable biochemical gradients across Golgi stacks have been described. A large number of enzymes have been shown to localize preferentially to subgroups of cisternae. Most of these studies were carried out in animals (e.g. Rabouille et al., 1995), but a few published reports suggest that this is also true for plants. For example, mammalian sialyltransferase is targeted to the same plant Golgi sub-compartment (*trans*) as in animal cells (Wee et al., 1998). Similarly, the only plant Golgi enzyme that has been localized at the EM level, soybean α-1,2 mannosidase I, also targets to the same sub-compartment (*cis*) as its mammalian homologs (Nebenführ et al., 1999). Indirect evidence for specific intra-Golgi localization of Golgi enzymes also comes from immuno-detection of specific epitopes created in the Golgi stack (e.g. Zhang and Staehelin, 1992). In addition, the membrane composition changes from *cis* to *trans*, with the *cis*-most cisternae being more like the ER and the *trans*-most cisternae resembling the composition of the plasma membrane (van Meer, 1998). How cells achieve this remarkable feat has been a matter of contentious debate for some time. In principle, several conceptual models can be proposed to solve this problem.

2. The Models

In order to facilitate the discussion of the different models it is helpful to define some of the terms used. The proteins that are carried in vesicles are referred to as "cargo" since they are thought to perform no specific function during transport. The two classes of macromolecules that are discussed as potential cargo molecules are the Golgi enzymes and the secretory proteins. In contrast, the proteins involved in mediating the shuttling of vesicles between cisternae are often called the "trafficking machinery". They include the coat proteins (COPI, see Aniendo et al., this volume) and targeting receptors (SNAREs, see Moore, this volume). In recent years an additional group of proteins that associate with the cytoplasmic side of Golgi membranes has been characterized, these are usually referred to as "Golgi matrix". "Anterograde transport" signifies the typical forward movement of secretory cargo from the ER via *cis*, medial, and *trans* Golgi to their final destinations. "Retrograde transport", likewise, is transport in the opposite direction.

2.1. The cisternal progression/maturation model

The cisternal progression model is historically the oldest model of intra-Golgi transport (Grassé, 1957). It is based on the premise that secretory cargo never leaves individual cisternae. According to this model, new cisternae continuously form from ER-to-Golgi transport vesicles at the *cis* side of the Golgi. This addition of new cisternae at the forming face gradually displaces the older cisternae through the stack until they reach the *trans* side where they are consumed by packaging of secretory cargo into post-Golgi transport vesicles. Over time, it has become obvious that this simple model cannot explain the apparently stable distribution of Golgi enzymes in the stack. Therefore a countercurrent of recycling vesicles has been postulated that would move resident

Golgi enzymes from the older to the younger cisternae (Fig. 1a; Schnepf, 1993, Bannykh and Balch, 1997, Mironov et al., 1997, Glick et al., 1997). At steady state, the rate of anterograde cisternal progression is predicted to match the rate of retrograde vesicle transport and the distribution of Golgi enzymes would consequently be stable. This modified model has been termed “cisternal progression/maturation” to indicate the evolving composition of the cisternae as they progress through the Golgi stacks. An interesting aspect of this updated form of the model is that *cis* cisternae form by fusion of anterograde ER-Golgi vesicles with retrograde intra-Golgi vesicles.

2.2. The vesicular shuttles model

The simplest way to explain the stable distribution of Golgi enzymes within a stack is to assume that the cisternae are stable, long-lived structures that house the various enzymatic activities. This implies that secretory products have to leave a cisterna in order to get to the next. In the vesicular shuttle model, this is accomplished by transport vesicles that move cargo in the anterograde direction (Farquhar and Palade, 1981). In order to accommodate emerging data that was not compatible with this strict form of the model, a second class of vesicles has been postulated that would recycle escaped ER residents or Golgi enzymes back to their proper location in an earlier compartment of the secretory pathway (Farquhar and Palade, 1981). However, this retrograde transport would be expected to be a minor component of the total vesicular transport, since the majority of vesicles likely carries cargo in the anterograde direction (Fig. 1b).

2.3. The percolation tower model

Interestingly, the cisternal progression/maturation model and the vesicular shuttle model are not mutually exclusive. It is conceivable that cisternae move slowly through the stack while vesicles rapidly shuttle cargo *and* enzymes in both anterograde and retrograde directions (Fig.1c; Pelham and Rothman, 2000). In this scenario the distribution of Golgi enzymes is ensured by their differential affinity for the different “age classes” of cisternae and their resulting differential propensity to enter transport vesicles. Similarly, the anterograde transport of secretory products follows simply from the continual addition of new cargo at the *cis* side and simultaneous removal from the *trans* side. This model offers two distinct advantages: First, it does not require the sorting step necessary for the formation of separate anterograde and retrograde vesicles. Second, it does not impose any directionality on the intra-Golgi vesicles, thus requiring fewer specific recognition steps, which is favored by the lack of Golgi-specific SNAREs (Pelham and Rothman, 2000).

2.4. The tubular connections model

An alternative model for anterograde cargo transport through stable cisternae assumes that Golgi cisternae can, on occasion, form direct tubular connections with cisternae at different levels in the stack (Fig. 1d; Ayala, 1994, Weidman, 1995). These direct connections would eliminate the need for transport vesicles since cargo can move directly from cisterna to cisterna. This scenario obviously requires additional assumptions that would ensure the directionality of cargo transport while at the same time maintaining the biochemical identities of the connected cisternae (Mironov et al., 1997).

3. The Data

3.1. Rise and fall of cisternal progression

The oldest speculations on intra-Golgi transport date back almost half a century (Grassé, 1957) to a time when the involvement of this organelle in the secretory pathway was still an unproven hypothesis. The model was based solely on EM observations and represents the original cisternal progression model. Subsequent experiments with radioactive tracers confirmed the direction of cargo transport within the Golgi from *cis* to *trans* and were generally viewed as supporting the model (reviewed in Farquhar and Palade, 1981), although alternative interpretations were usually not pursued.

The strongest evidence in favor of a cisternal progression model came from work in scale-producing algae (reviewed in Melkonian et al., 1991). These organisms synthesize large scales in the lumen of their Golgi cisternae from where they are delivered to the cell surface in membrane bounded transport carriers. The scales typically are too large to fit into the small intra-Golgi vesicle shuttles and consequently are found only within the cisternae. An extreme example of this is provided by the unicellular alga *Pleurochrysis scherfellii* where a single scale of up to 2 μm diameter is assembled per cisterna (Brown Jr. and Romanivicz, 1976). The dramatic size of these scales has even allowed the direct observation of their transport to the cell surface in living cells (Brown Jr., 1971).

During the 1970s, more sophisticated experimental protocols began to reveal

shortcomings of this simplistic cisternal progression model. In particular, the apparently stable differential composition of the cisternae within a stack seemed incompatible with a maturation of cisternae as they changed from *cis* to medial to *trans*. These concerns led to the formulation of an alternative model of intra-Golgi transport that assumed stable cisternae and shuttling transport vesicles (Farquhar and Palade, 1981). This model received strong support when it became possible to reconstitute elements of intra-Golgi transport in the test-tube (Balch et al., 1984). These experiments, took advantage of a series of new developments that allowed researchers to follow the progress through the Golgi by biochemical means. In particular, incomplete glycosylation of a foreign protein (the G-protein of vesicular stomatitis virus, or VSV-G) in a cell line that lacked N-acetyl glumosaminyl transferase I could rapidly be restored by fusion with a wild-type cell line, suggesting transfer of the VSV-G protein from one Golgi system to the other (Rothman et al., 1984b). Importantly, this effect could be recapitulated with isolated membranes from the two cell lines, which was interpreted as dissociative (i.e. vesicle-mediated) transport between independent Golgi stacks (Rothman et al., 1984a).

This biochemical approach resulted in the identification of a number of proteins necessary for vesicle formation and fusion, such as COPI coats, Arf1 GTPase, and NSF (N-ethyl maleimide-sensitive factor), thus greatly enhancing our understanding of the mechanisms of vesicular transport (Rothman and Wieland, 1996). At the same time, however, it has to be cautioned that the cell free assays could not formally distinguish between anterograde movement of secretory cargo and retrograde movement of modifying enzymes. While the data clearly were suggestive of anterograde vesicular shuttles, they could in principle also be interpreted in terms of recycling Golgi enzymes. A first indication that COPI vesicles indeed may play a role in retrograde transport came from work in yeast that demonstrated that COPI proteins interacted with the recycling signal on ER membrane proteins and were necessary for their return to the ER (Letourneur et al., 1994).

3.2. Renaissance of progression

A renewed interest in cisternal progression was sparked by new data that demonstrated (again) that bulky secretory products can traverse the Golgi stack without leaving the cisterna they reside in. The first such report in was a detailed analysis of the unicellular green alga *Scherffelia dubia* (Becker et al., 1995). This organism can produce large numbers of scales in a short time in response to experimental deflagellation (McFadden and Melkonian, 1986). Of the more than 10000 scales observed in 90 Golgi stacks of *Scherffelia*, not a single one was found in the abundant peri-Golgi vesicles (Becker et al., 1995). Similar observations were later made in mammalian fibroblasts that produce large collagen complexes. In this case the large electron-dense aggregates of procollagen were also seen only within the confines of cisternae and never in detached vesicles or tubules (Bonfanti et al., 1998). These results argued strongly in favor of a cisternal progression model and the data from mammalian cells helped to convince animal cell biologists.

At around this time it also became apparent that the vesicles that surround Golgi cisternae could recycle resident Golgi enzymes back to their proper location on the conveyor belt of cisternae (reviewed in Glick and Malhorta, 1998). This concept can be simulated mathematically by assuming differential affinity of Golgi residents for a single type of recycling vesicles. Using an iterative computational approach it is thus possible to generate a stable gradient of enzyme distribution (Glick et al., 1997). This simple model has been modified recently to include a variable affinity of Golgi enzymes for recycling vesicles. In particular, it has been proposed that an enzyme’s propensity to enter recycling vesicles increases dramatically when certain parameters within the cisterna reach a critical value during maturation (e.g. pH or membrane composition). This “triggered recycling” makes the model much more robust and able to tolerate (simulated) overexpression of individual Golgi enzymes (Weiss and Nilsson, 2000). While this theoretical model better matches the situation found *in vivo* it remains to be seen whether its basic assumptions can be confirmed in living cells. Taken together, these theoretical papers provided a hypothetical mechanism that allowed the dynamic maintenance of biochemical gradients in the Golgi and thereby removed one of the major objections to the original progression model.

3.3 What’s in a vesicle?

A central difference between the cisternal progression/maturation and vesicular shuttle models is the composition of the intra-Golgi vesicles (compare Figs. 1a and b). The former model assumes that these vesicles predominantly contain Golgi enzymes that are recycled to younger cisternae. In contrast, the latter model predicts that most vesicles would contain cargo

travelling in the anterograde direction. The percolation tower model, on the other hand, implies about similar numbers of molecules for anterograde and retrograde cargo. While the directionality of vesicle movement is very difficult to determine experimentally, it is possible to examine the contents of intra-Golgi vesicles using several techniques. Over the last few years, a number of publications have addressed this issue and provided a wealth of data that allows us to draw tentative conclusions.

It is generally assumed that vesicle formation at the Golgi is driven by the COPI coat (see Aniendo et al, this volume). Consequently, one group of experiments used a biochemical approach to determine the composition of purified COPI vesicles. In order to facilitate isolation of these vesicles the coat was often stabilized by the addition of GTPγS which prevents uncoating. These GTPγS-stabilized vesicles contained few Golgi enzymes, but also little anterograde cargo (Sönnichsen et al., 1996). This dilemma was solved when it was discovered that GTP hydrolysis is required for proper selection of cargo by the coat proteins (Pepperkok et al., 2000). In fact, COPI vesicles generated under these conditions did contain significant amounts of retrograde cargo, but still very little anterograde cargo (Lanoix et al., 2001). Interestingly, using this experimental approach it is possible to distinguish two different classes of COPI vesicles that are enriched in either *cis*-Golgi proteins or medial Golgi proteins, suggesting that they formed at different levels within the stacks (Lanoix et al., 2001). These results corroborate previous data from yeast that show that COPI is necessary for retrograde transport to the ER (Letourneur et al., 1994). Thus, biochemical as well as genetic data favors the idea that COPI vesicles are involved in retrograde but not anterograde transport.

Another approach to assessing the composition of intra-Golgi vesicles is by immuno-electron microscopy. In this case, the presence of specific proteins in peri-Golgi vesicles is tested with cross-reacting antibodies in thin sections prepared for electron microscopy. Initial studies using this approach seemed to confirm the vesicular shuttle model. Two populations of COPI-positive vesicles were described that contained either anterograde (VSV-G and proinsulin) or retrograde cargo (KDEL receptor) (Orci et al., 1997) while Golgi enzymes were found in lower concentrations (Orci et al., 2000a). It has been suggested that these two types of vesicles may perform distinct functions in intra-Golgi and Golgi-ER retrograde transport (Orci et al., 2000b). However, a more recent detailed analysis of Golgi stacks and their surrounding vesicles suggests that this initial conclusion may be wrong. A careful double-labeling study revealed that Golgi enzymes (mannosidase II) are more likely than anterograde cargo (VSV-G) to be present in peri-Golgi vesicles, although the latter were not completely excluded (Martínez-Menárguez et al., 2001). Similarly, COPI vesicles that form at the ER-Golgi intermediate compartment found in mammalian cells exclude anterograde cargo and instead preferentially harbor proteins that are recycled to the ER (Martínez-Menárguez et al., 1999).

The apparent discrepancy found in these morphological studies is difficult to resolve and may be a result of the enormous complexity of the Golgi apparatus. Complete three-dimensional (3D) tomographic reconstruction of large parts of Golgi complexes in mammalian cells reveals a bewildering multitude of membrane compartments of complex morphology and diverse, often unpredicted interactions (Ladinsky et al., 1999, Marsh et al., 2001). Given this complicated topology it is clearly difficult to unequivocally identify the compartment that is labeled (or not labeled) by the antibody. Double labeling for separate markers as well as careful interpretation of unlabeled membranes appears to be essential. Although one of the studies described (Martínez-Menárguez et al., 2001) follows this recipe, more experiments will be necessary to resolve this dispute. For example, to address the question of cargo enrichment in COPI vesicles it will be necessary to identify not only the vesicles but also their donor compartment since the composition of COPI vesicles can be expected to vary depending on the compartment on which they form (compare Lanoix et al., 2001).

3.4. Where's the anterograde cargo?

Further strong evidence in favor of the cisternal progression model comes from a comparison of large and small cargo molecules that are expressed in the same cell (Mironov et al., 2001). By combining temperature-sensitive mutants of VSV-G (tagged with green fluorescent protein, GFP) and an elaborate scheme of temperature shifts in a cell type that can be stimulated to produce procollagen it is possible to create synchronized waves and pulses of both types of secretory cargo that enter the Golgi stack in a finely controllable way. The central findings of this study are that both large and small cargo molecules traverse the stack at the same rapid rate and that neither of them enters peri-Golgi vesicles (Mironov et al., 2001). These results

clearly establish that cisternal progression is fast enough to account for the secretory transport rates reported in the past. They also discount peri-Golgi vesicles as playing a significant role in anterograde transport of secretory products (at least for some types of molecules), thus also putting in doubt the percolation model of intra-Golgi transport. Intriguingly, one set of experiments employing very small pulses of VSV-G-GFP protein revealed no lateral mobility of secretory cargo within the Golgi complex (Mironov et al., 2001). This is in striking contrast to the unrestricted mobility of Golgi enzymes within the interconnected Golgi apparatus of these cells (Cole et al., 1996). Thus, it appears that secretory products are confined to their individual stack within the mammalian Golgi ribbon and are excluded not only from the peri-Golgi vesicles but also the tubular connections between corresponding cisternae of adjacent stacks of the Golgi ribbon.

3.5. Plants do it too

While these recent studies highlight the high degree of sophistication in experimental approaches possible today in mammalian cell systems, it should not be overlooked that plant researchers have continued to provide indirect evidence in favor of the cisternal progression/maturation model. For example, in studying the biogenesis of dense vesicles that mediate transport to the protein storage vacuole in pea cotyledons it was found that protein aggregates form already at the *cis* side of the Golgi (Hillmer et al., 2001). As these aggregates move though the Golgi stack, they are always contained within membranes that are continuous with the Golgi cisternae, suggesting that these "dense buds" never dissociate and reach the *trans* Golgi by cisternal progression (Hillmer et al., 2001). These data are in apparent disagreement with similar observations of artificial protein aggregates in animal cells. In this case no continuities between the membranes of the Golgi and those surrounding the aggregates, the so-called "megavesicles", were found (Volchuk et al., 2000). A possible solution to this dilemma may lie in a 3D tomographic reconstruction of the megavesicles since it is possible that narrow connections to adjacent cisternae may not have been visible in the serial thin sections.

Another line of evidence comes from studies of the effects of the drug brefeldin A (BFA) on tobacco BY-2 suspension culture cells (Ritzenthaler et al., 2002). Treatment of BY-2 cells with BFA leads to a rapid loss of *cis* cisternae from Golgi stacks. Interestingly, these medial-*trans* Golgi remnants are still labeled by the *cis*-Golgi marker GmMan1-GFP (soybean mannosidase I-green fluorescent protein, Nebenführ et al., 1999). The disappearance of fluorescently labeled Golgi stacks instead coincides with the loss of Golgi cisternae of *trans*-like appearance. The simplest interpretation of this phenomenon is that the *cis*-Golgi marker protein cannot recycle back to younger cisternae due to the BFA-induced block in COPI formation (see Ritzenthaler and Robinson, this volume) and therefore progresses within the (former) *cis* cisternae as they mature into *trans*-like appearance (Ritzenthaler et al., 2002).

3.6. Tubule or not tubule – that is the question

A fairly recent concept in intra-Golgi transport is the idea of having direct membrane continuities between different levels of adjacent Golgi stacks (Fig. 1d; Weidman, 1995). These tubular connections could, in principle, allow (small) anterograde cargo to move through the Golgi without entering transport vesicles. So far, evidence for these connections has been sketchy and recent detailed 3D tomographic reconstructions of Golgi apparatus in high-pressure frozen/freeze-substituted mammalian cells revealed no evidence for them (Ladinsky et al., 1999, Marsh et al., 2001). However, a most recent 3D analysis of pancreatic beta cells stimulated for maximal insulin production suggests that such inter-cisternal connections can exist at least under certain conditions (B. Marsh, personal communication). The proposed tubules between neighboring stacks probably cannot exist in plant cells where Golgi stacks travel as individual units and do not form the ribbon found in mammalian cells (Boevink et al., 1998, Nebenführ et al., 1999). In this case, the connections would have to form within a single stack (Weidman, 1995), but there is no evidence for such tubules in plants to date.

One prediction of the tubular connections model is that secretory cargo uses these membrane continuities to travel from cisterna to cisterna (Weidman, 1995). However, given the high mobility of the Golgi enzymes within the membrane (Cole et al., 1996) it is difficult to envision how the distinct biochemical composition of adjacent cisternae could be maintained if these direct membrane continuities existed. This problem is exacerbated by the recent finding that anterograde cargo exhibits very little lateral mobility within the Golgi complex (Mironov et al., 2001). Larger secretory products most likely are also excluded from narrow tubules. Thus, given the dearth of direct evidence in favor of frequent inter-cisternal connections

and the overwhelming amount of data that is difficult to reconcile with this model, it seems doubtful whether tubular connections can play a significant role in intra-Golgi transport.

4. The Solution?

Given the large number of publications which have accumulated in recent years that provide experimental evidence for various aspects of the cisternal progression/maturation model, it is now an inescapable conclusion that cisternal progression does occur (Pelham and Rothman, 2000, Pelham, 2001, Barr, 2002). A direct consequence of this conclusion is that Golgi enzymes have to be recycled back to younger cisternae, a feat most likely accomplished by COPI vesicles. While this appears to be a full vindication of the earliest ideas of intra-Golgi traffic (Grassé, 1957) it has to be cautioned that the alternative modes of transport cannot be conclusively ruled out. Indeed, it is possible that COPI vesicles can also move in the anterograde direction to deliver certain kinds of secretory products to the next cisterna. Only a better understanding of the molecular mechanisms involved in vesicle targeting within the Golgi can be expected to provide a definitive answer. The new-found consensus that Golgi cisternae are not stable entities, however, provides a unifying conceptual framework with which we can interpret new results.

5. The Implications

One line of research that has been pursued for a number of years is aimed at identifying the targeting signals and mechanisms that allow modifying enzymes to maintain their specific positions within the Golgi (Opat et al., 2001). An interesting aspect of Golgi enzyme recycling is that it does not appear to rely on any sorting receptors. In other words, Golgi enzymes seem to be able to interact directly with the machinery necessary for vesicle formation to ensure efficient recycling. The two major hypotheses that have influenced our thinking in this matter are the kin-recognition model and the membrane thickness model. The kin-recognition model postulates that enzymes that reside in the same cisterna can interact with each other and therefore are sorted together (Nilsson et al., 1993). The membrane thickness model assumes that the thickness of the lipid bilayer, which increases in the Golgi from *cis* to *trans*, determines the positioning of the proteins since *cis*-Golgi enzymes tend to have shorter transmembrane domains than *trans*-Golgi enzymes (Bretscher and Munro, 1993). Both of these models were formulated within the context of the vesicular shuttle model, i.e. assuming stable cisternae which retain their enzymes, but they can be modified to fit the recycling requirement of the cisternal progression/maturation model (Füllekrug and Nilsson, 1998).

However, recent publications suggest that proteins of the Golgi matrix may also play a role in targeting Golgi enzymes. In particular, members of the p24 family of membrane proteins that are implicated in ER-Golgi traffic were found to form complexes with the Golgi matrix proteins GRASP55 and GRASP65 (Barr et al., 2001). GRASP55 can interact with the small GTPase Rab2 and appears to be necessary for the maintenance of both Golgi structure and membrane traffic (Short et al., 2001). A similar dual role has been proposed for GRASP65 (Nakamura et al., 1997, Barr et al., 1997). These data are suggestive of an intimate link between stacking of Golgi cisternae and membrane traffic through the stack. It will be interesting to see whether this connection can be confirmed and what its role in targeting of Golgi enzymes is.

One consequence of the cisternal progression/maturation model is that the *cis*-most cisterna of a Golgi stack is formed from both anterograde ER-to-Golgi transport vesicles and retrograde intra-Golgi recycling vesicles. This implies that new cisternae are “growing” until they reach a certain size and the next cisterna becomes initiated. It is not known what determines when a cisterna is “large enough”, but it seems reasonable to speculate that the next older cisterna can act as a template. Another problem arises in plant cells where Golgi stacks can travel through the cytoplasm (Boevink et al., 1998, Nebenführ et al., 1999) and the arrival of ER-to-Golgi vesicles may not represent a uniform, continuous stream. Do stacks under these conditions sometimes form smaller cisternae, or are there feedback mechanisms that ensure that always a complete cisterna is formed (Nebenführ and Staehelin, 2001)? This question relates to the way the membrane exchange between ER and Golgi is organized in cells with a high level of intracellular motility (see Hawes, this volume). Nothing is currently known about the spatio-temporal relationship of ER export sites and Golgi stacks in plants, but data from yeast suggests that there may be a functional interaction between the two organelles. The Golgi apparatus in the budding yeast *Saccharomyces cerevisiae* is dispersed into individual cisternae that do not form stacks. In contrast, the closely related species *Pichia pastoris* forms Golgi stacks like most other eukaryotes. Interestingly, *P. pastoris* contains

discrete ER export sites adjacent to Golgi stacks whereas transport vesicle formation occurs all over the ER in *S. cerevisiae* (Rossanese et al., 1999). This correlation has been interpreted as representing a functional constraint on the places of Golgi formation at least in yeast cells (Rossanese et al., 1999), but possibly also in mammalian cells (Hammond and Glick, 2000). It remains to be seen whether this also holds true for plant cells with their mobile Golgi stacks.

An even more fundamental question raised by the cisternal progression/maturation model is whether the Golgi can be considered a stable organelle at all. Given the continuous flux of its major distinguishing feature, the stacked cisternae, the question arises of what makes the Golgi the Golgi? How can the structural and functional integrity of this organelle be maintained when all its components appear to be just transport intermediates? Should the Golgi be considered a dynamic outgrowth of the ER, or does it contain structural elements that define its identity and that never become associated with another organelle? This also relates to questions of how the number of Golgi stacks in a cell can increase (only by fission, or also by *de novo* formation?), and to the fate of the Golgi during mitosis and cytokinesis (where it essentially disappears from mammalian, but not plant cells) or during experiments that disrupt normal membrane flow (such as BFA, where Golgi membranes fuse with the ER) (Warren and Wickner, 1996, Lippincott-Schwartz et al., 2000, Rossanese and Glick, 2001). The new data supporting the cisternal progression/maturation model seem to favor a dynamic definition of the Golgi that essentially postulates a self-organizing principle of balanced anterograde and retrograde transport, thus leading to a stable steady state (Mistelli, 2001). This interpretation appears to be supported by experiments that demonstrate cycling of Golgi enzymes through the ER (e.g. Zaal et al., 1999). However, data on Golgi matrix proteins seem to contradict this view and instead argue for a constant scaffold that acts as an anchor for Golgi membranes (e.g. Seemann et al., 2000). This issue clearly is the ‘next great debate’ that surrounds the Golgi apparatus and, given the history of Golgi research, we can expect many unexpected findings.

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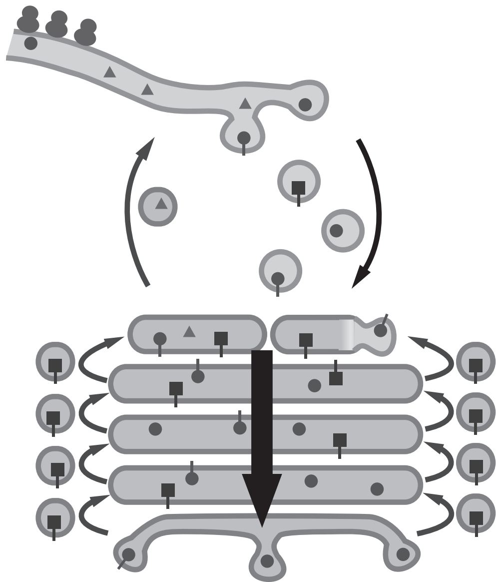
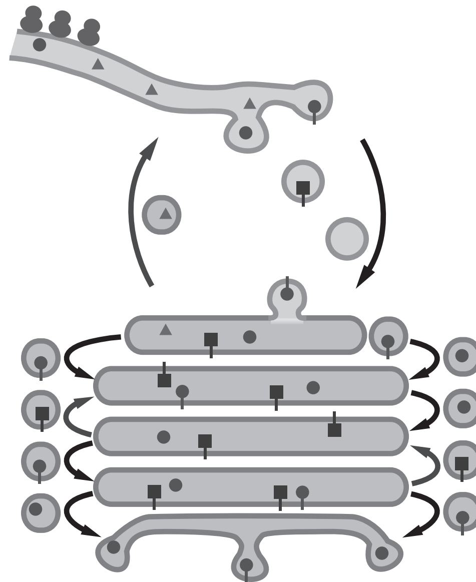
Figure Legend

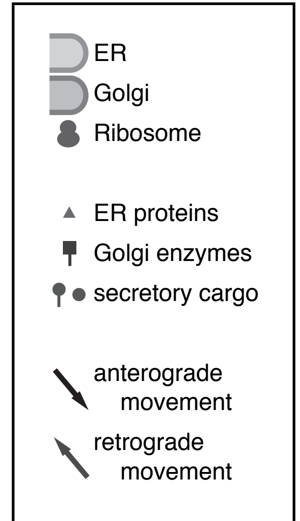
Figure 1: Schematic representation of the different models of intra-Golgi transport discussed in the text.

(a) Cisternal progression/maturation

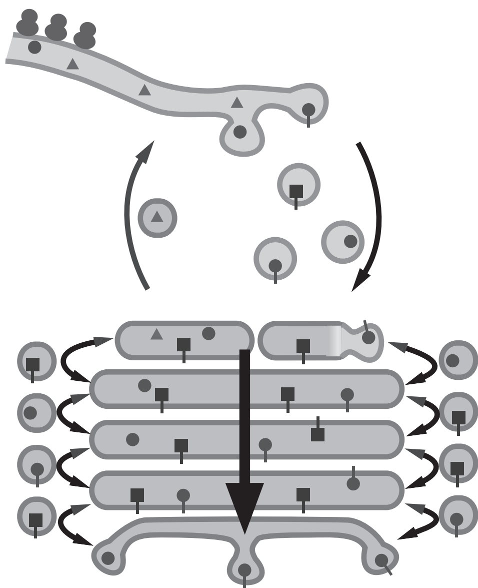


(b) Vesicular shuttles





(c) Percolation tower



(d) Tubular connections