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Review

The Debate about Transport in the Golgi—Two Sides of the Same Coin?

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Historical precedent in biology suggests that when divergent views are held on the basis of divergent observations that do not actually contradict each other, then the essence of both views is probably correct and the truth lies somewhere in between. The Golgi appears to be no exception.

A vigorous controversy has evolved over the past five years concerning the nature of transport of secretory and other cargo across the Golgi stack: is it carried out by transport vesicles budding and fusing among static cisternae, or do the cisternae themselves move with no vesicles being involved? For various reasons, each of these mechanisms had proven difficult to establish or to rule out in simple and compelling ways. As a result there has been a considerable polarization of the field. Now, it now seems that the essence of both views is likely to be correct.

Here, we review recent data that bring the two previous extreme views into accord while resolving the difficulties associated with each of them. While cisternal maturation can nicely explain rapid protein secretion in budding yeast, it operates too slowly in the tightly stacked Golgi of animal cells to explain the transport of most proteins, yet it is important for certain large macromolecular aggregates that may not be accommodated in vesicles. Transport vesicles provide the "fast track" taken across the Golgi by most proteins in animal cells. But, rather than moving uniquely forward (or backward), as originally envisioned by proponents of vesicle (or cisternal maturation) models, it now appears that transport vesicles may "percolate" up-and-down the stack in a kind of bidirectional random walk.

Background

The controversy about Golgi transport occurs against a background of well-established core molecular principles that explain how transport vesicles bud and fuse to carry out the fundamental cellular activity of intercompartmental transport (Rothman and Wieland, 1996; Mellman and Warren, 2000). Neither these molecular mechanisms nor, in particular, the physiological importance of vesicles for Golgi function are at issue.

Indeed these vesicles are central to any discussion of the Golgi. Typically 70–90 nm in diameter, they bud from each of the 4–6 cisternae that comprise the Golgi stack in a typical mammalian cell, ranging from the *cis* face (where proteins enter the stack from the ER) to the opposite *trans* face of the stack (where proteins can exit for later compartments) (Orci et al., 1986, 1997). They are named COPI vesicles because their budding is driven by the assembly of the COPI coat from cytoplasmically derived subunits (coatomers). The coatomers attach to Golgi membranes via the GTPase ARF1 (when it is in its GTP-bound form) and then polymerize into a COPI coat, forming an approximately spherical template that shapes the adherent membrane into a vesicle. When ARF hydrolyzes its bound GTP, the coatomers dissociate and the now uncoated vesicle can fuse when its now exposed v-SNAREs (packaged during budding) partner cognate t-SNAREs on the target membrane (Golgi or ER) (Rothman and Wieland, 1996; Barlowe, 2000).

Transport into the Golgi from the ER is carried out by a compositionally distinct coated vesicle, termed COPII, which also employs a GTP-switch mechanism for coating and uncoating (Barlowe et al., 1994; Springer et al., 1999; Barlowe, 2000). COPII coats bud (or possibly tubulate) membranes containing secretory cargo from transitional zones of ER. The ER-derived vesicles uncoat and join to form or fuse with vesicular-tubular clusters (VTCs), also termed intermediate compartments (ICs). These large carriers then deliver their cargo to the *cis* face of the stack, either by maturing into or fusing with preexisting *cis*-Golgi membranes (Bannykh and Balch, 1997; Presley et al., 1997; Scales et al., 1997; Wooding and Pelham, 1998; Ladinsky et al., 1999; Martinez-Menarguez et al., 1999).

Cargo destined for post-Golgi locations (plasma membrane, storage vesicles, endosomes, lysosomes, and so on; when we use the unmodified term "cargo" we refer to these and related classes of proteins) thus enters the stack at the *cis* face (Pelham, 1998). These same cargo depart the stack from the *trans* face, now sorted into a series of distinct carriers for delivery to one or another target membrane. Within the stack the cargo exists as an unfractionated mixture, present in every cisterna at essentially the same concentration.

Resident proteins of the ER constitute a special class of protein that can also enter the Golgi along with cargo, but when they do they have a different fate. Residents of the lumen of the ER are invariably marked via a common signal peptide (KDEL) (Munro and Pelham, 1987). This insight led to the discovery of retrograde transport, the process by which these escaped ER resident proteins are returned from the Golgi to the ER (Lewis et al., 1990; Semenza et al., 1990). A KDEL-specific receptor awaits its ligand in the Golgi where it sequesters it into transport vesicles targeted to the ER. The ER and Golgi are marked with distinct t-SNAREs, containing Ufe1p (Syntaxin 18 in animals; Lewis et al., 1997; Hatsuzawa et al., 2000) and Sed5p (Syntaxin 5 in animals; Hardwick and Pelham, 1992; Bennett et al., 1993), respectively, to allow anterograde and retrograde pathways to operate between the ER and the Golgi without interference.

Roots of the Controversy

Two related questions lie at the center of the current debate (Rothman and Wieland, 1996; Glick and Malhotra, 1998; Pelham, 1998) about how proteins are transported in the Golgi: (1) Do COPI vesicles mediate *anterograde* (i.e., *cis*-to-*trans*) transport? (it is clear that COPI

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vesicles mediate retrograde transport from Golgi to ER); Or (2), is anterograde transport instead mainly due to en bloc movement of entire cisternae (i.e., cisternal progression) up the stack?

When this controversy arose in 1994, it had been widely accepted for about a decade that vesicles (Balch et al., 1984b; Farquhar, 1985)—and in particular COPI vesicles (Orci et al., 1986)—mediate anterograde transport. Even in the earliest studies of COPI vesicles (Orci et al., 1986) it was noted that about half of the COPI vesicles budding from Golgi cisternae contain the VSV G protein, a viral-encoded cargo protein that is targeted to the plasma membrane, suggesting that these vesicles carry the cargo across the Golgi stack.

The event that precipitated the current controversy was the unexpected result from yeast genetics that COPI vesicles mediate retrograde transport from the Golgi to the ER (Cosson and Letourneur, 1994; Letourneur et al., 1994). As COPI vesicles are the only vesicles observed to bud within the confines of the Golgi stack, how could the same vesicle (assuming all COPI vesicles are functionally equivalent) carry ER residents backward while also carrying different cargo forward (Pelham, 1994)?

A new possibility thereby emerged (really, an old possibility resurrected): there is no vesicle for anterograde (*cis*-to-*trans*) transport. The alternative, moving cisternae, is a model originally derived largely from electron microscopic studies of plants and algae (Beams and Kessel, 1968; Becker and Melkonian, 1996). Cisternal "progression" had been in favor from the 1960s up to the 1980s when COPI vesicles were identified and shown to contain cargo. Cisternal progression, or flow, requires that cisternae form de novo at the *cis* face and disassemble at the *trans* face.

More recently, it was recognized that for the Golgi to retain its complement of designated residents (including processing enzymes like glycosyltransferases) in the face of the continuous loss from the *trans* face that a cisternal progression would create, transport vesicles operating within the Golgi must be postulated to carry such proteins retrograde from later to earlier cisternae. As a result, new cisternae forming at the *cis* face from fusing COPII vesicles and/or VTCs would "mature" as they progress across the stack: gaining glycosyltransferases, losing ER residents by retrograde transport, and retaining cargo. This new and critical aspect of the cisternal progression model is encapsulated in the currently preferred term, cisternal progression/maturation (Glick et al., 1997; Glick and Malhotra, 1998; Pelham, 1998). The requirement for retrograde transport (both within the stack and from the stack to the ER) provides a compelling and complete role for COPI vesicles in this model.

But, what about the observation that many COPI vesicles budding from isolated Golgi contain cargo and can carry them between stacks (Orci et al., 1989; Ostermann et al., 1993)? Ardent "progressionists" took the view that this was either an in vitro artifact of some kind, or the result of the spilling of abundant anterograde-moving cargo into backward-moving vesicles, although the purpose of this was not apparent. Equally ardent vesicle "secessionists" regarded this as a COP-out; they insisted that there was no direct evidence that cisternae move at all.

This debate was focused recently by two principal observations. First, immunoelectron microscopy of intact cells (Orci et al., 1997) revealed that there are actually two distinct populations of COPI vesicles: one population contains the KDEL receptor but little if any cargo; the other contains cargo (VSV G protein or proinsulin) but little if any KDEL receptor. Both types bud throughout the Golgi stack. While it could still not be formally ruled out that both types of COPI vesicles move exclusively backward, it became very hard to see how cargo could accidentally spill-over into one but not the other vesicle population. And if the cargo-containing COPI vesicles move anterograde—even to a limited extent—then a cisternal progression would no longer be required to explain cargo transport (but could occur in parallel).

Second, in a quantitative pulse–chase experiment employing electron microscopy, it was definitively shown that biosynthetic aggregates (collagen precursors) much larger than COPI vesicles nonetheless traverse the Golgi stack in animal cells (Bonfanti et al., 1998). Since the aggregates could not fit in COPI vesicles, they must be carried in the anterograde direction by a different mechanism. Since serial sections indicated that the aggregates were retained within cisternae—as distinct from being enclosed in some sort of "megavesicle"—this result constituted compelling evidence for cisternal flow. However, the rate of aggregate movement was far slower than that of most proteins—and even bulk lipids—studied in other cells. These cross the stack in 5–15 min, while fully 20%–25% of the aggregates originally in the *cis* half of the stack remain there even after 1 hr, hinting that a COPI vesicle pathway may coexist and provide an even faster track for anterograde transport than cisternal flow.

In sum, two alternative models for anterograde transport were widely considered, each with its own apparent problems:

Cisternal progression/maturation naturally explained the transport of aggregates and the fixed concentration of cargo throughout the stack. While it provided a compelling role for COPI vesicles in retrograde transport, it did not provide a compelling explanation for the existence of a population of COPI vesicles carrying the cargo that exits the *trans* face of the stack. Moreover, the rate of transport of aggregates across the stack was substantially slower than that reported for most cargo proteins.

Vesicle transport by a specialized population of COPI vesicles provided a compelling explanation for the existence of two types of COPI vesicles, but did not explain how aggregates much larger than these vesicles could be transported. It was also mechanistically unclear how two sets of cargo could be packaged in separate COPI vesicles that bud using the same coat protein. Moreover, selective vesicles moving exclusively anterograde would be expected to generate a *cis* $<$ *trans* concentration gradient of their cargo, and this is not observed.

New Results

None of the data favoring cisternal progression/maturation explicitly exclude the possibility of simultaneous vesicle transport, and vice versa. In fact, during the last year or so, new information has emerged that constrains both models away from their previous extremes and suggests that both may operate.

With Respect to Cisternal Progression

In contrast to the natural procollagen aggregates, artificial protein aggregates (formed by tandem repeats of a

spontaneously dimerizing mutant of the FK506 binding protein), which accumulate in the *cis*-most cisternae during a 15 degree temperature block, transit the stack on a fast track when the block is released (Volchuk et al., 2000). And unlike the procollagen aggregates, the artificial aggregates actually become enveloped into megavesicles, possibly drastic size variants of COPI vesicles, which then mediate their anterograde transport (Volchuk et al., 2000). The studies of Bonfanti et al. (1998) and Volchuk et al. (2000) are complementary in that they suggest that when aggregates can enter megavesicles they will be on the same fast track as COPI vesicles, but when they cannot enter any vesicle they will transit at the slower pace of cisternal progression.

An initial comparison (presented in a preliminary form; Bonfanti et al., 1999) of the rate of transport of the VSV G protein with that of procollagen aggregates in the same Golgi confirms the expectation from the published work (Bonfanti et al., 1998) that procollagen aggregates traverse on a "slow track" as compared to other cargo, establishing the pace of cisternal progression. The more rapid rate of transport of most cargo must then be due to movement between cisternae, which we presume involves the COPI population containing these same cargo, which would then have to fuse in the anterograde direction at least some of the time.

Immunoelectron microscopy of intact cells (Orci et al., 2000a) and cell-free budding reactions (Sönnichsen et al., 1996) reveals that while steady-state Golgi residents like glycosyltransferases are present in COPI vesicles, they are at a lower concentration than in the cisternae from which the vesicles bud. If cisternal flow were the *only* means of anterograde transport, then the transferases and other residents would need to be substantially concentrated in the retrograde-moving COPI vesicles to allow cargo to remain with a nonrecycled portion of cisternal membrane which can depart the *trans* face for post-Golgi locations (Glick et al., 1997). This constraint relaxes when cisternal flow is slower than most anterograde vesicular transport. Therefore, the lack of enrichment of resident proteins independently suggests the existence of anterograde vesicular traffic. One study (Lanoix et al., 1999) has claimed up to 10-fold enrichment of glycosyltransferases in putative COPI vesicles produced in vitro, but this figure can be challenged because it depends critically on the nature and purity of the membranes from which the vesicles bud. Since at least 90% of the proteins in Golgi cisternae are residents (Quinn et al., 1984), it is hard to imagine a mechanism by which much further concentration would be possible.

With Respect to the Nature of the Two Classes of COPI Vesicles

There is mounting evidence that distinct biochemical mechanisms can be used to create COPI vesicles of different composition, even from the same membrane. On one hand, the KDEL receptor oligomerizes and binds the GTPase-activating protein ARFGAP (Aoe et al., 1997), which in turn could provide a foothold for both ARF and coatomer subunits. This active role of the KDEL receptor may contribute directly to Golgi function, explaining why it is needed for this (at least in yeast; Semenza et al., 1990). On the other hand, members of the p24 family of membrane proteins have cytoplasmic tails that can interact directly with coatomer (Harter and Wieland, 1998) and initiate vesicle formation (Bremser et al., 1999). A surprising finding is that the tails affect, in a sequence-specific manner, the conformation and properties of coatomer (Reinhard et al., 1999), including its ability to stimulate the GTPase activity of ARF (Goldberg, 1999, 2000). This suggests that the loading of membrane proteins into vesicles could be controlled by a kinetic proofreading mechanism (Goldberg, 2000). While other genetic (Springer et al., 2000) and biochemical evidence (Szafer et al., 2000) suggests that the picture will be complex and that much remains to be learned, the striking biochemical results noted above give clues as to how the sorting of proteins into two parallel sets of COPI vesicles—those destined ultimately for the ER and those restricted to intra-Golgi movement—might be achieved according to kinetic mechanisms.

COPI vesicles containing cargo have been found to possess their own unique SNARE, GOS28, which is undetectable in the other, KDEL receptor–containing population of vesicles (Orci et al., 2000b). GOS28 and the syntaxin with which it participates in a SNARE complex, Syntaxin5 (Sed5p in yeast), are both approximately evenly distributed throughout the entire stack (Hay et al., 1998; Orci et al., 2000b). In the absence of any additional constraints, this suggests that COPI vesicles, containing both GOS28 and a quantum of cargo, can bud at any level of the stack and potentially fuse at any other level.

However, COPI-coated vesicles and their uncoated progeny are highly constrained: these vesicles appear to be dynamically retained near their budding site by a carpet of flexible tethers that connects them to the Golgi cisternae at every level of the stack (Orci et al., 1998). One tethering system, located mainly at the *cis* face, has been well-characterized (Sönnichsen et al., 1998). As yet largely uncharacterized but molecularly distinct tethers are observed to link vesicles to the *medial* and *trans* portions of the stack. Tethering may physically constrain COPI vesicles to fuse close to where they bud, effectively limiting protein transfer to adjacent cisternae in the stack (Orci et al., 1998; Sönnichsen et al., 1998).

Since the stack has differing tethers in its *cis* versus *trans* portions, it is entirely possible that anterograde versus retrograde flow of the two distinct classes of COPI vesicles could be dictated by differential interactions with these molecularly distinct sets of tethers. But, we speculate that there is an even simpler and nonexclusive possibility.

Percolating Vesicles?

Complexes of SNARE proteins bridging membranes provide the underlying mechanism of intracellular membrane fusion (Weber et al., 1998) and the pattern of cognate pairing of SNARE proteins dictates the specificity of the fusion process (McNew et al., 2000). In light of this, what is known so far about the distribution of Golgi SNAREs (and much more needs to be learned) should dramatically change both the "progressionist" and the "secessionist" perspectives on the role of post-Golgi cargo containing COPI vesicles. A die-hard progressionist would now be hard-put to say that these vesicles *only* move backward when their t-SNAREs are located everywhere up and down the stack. A die-hard secessionist would be equally hard-put to say that these vesicles *only* move anterograde for the very same reason. In fact, since there are no proteins known to be restricted entirely to a single cisterna, it is hard to see how absolutely unidirectional movement could be achieved whatever molecules may contribute to vesicle targeting in addition to SNAREs.

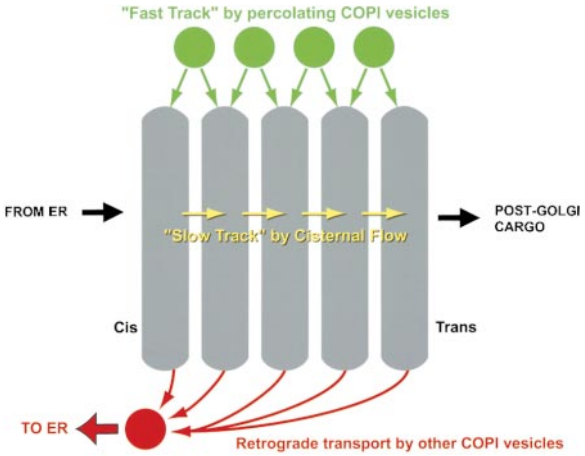

Figure 1. Model for Flow in the Golgi

COPI vesicles contain cargo (green) and Golgi resident proteins (not shown). They are proposed to move up and down the stack in a stochastic process, termed percolation, and to provide the fast track by which cargo flows across the stack. A slower track is generally provided by cisternal flow (yellow). A second population of COPI vesicles (red) exits the Golgi carrying the KDEL receptor, escaped ER resident proteins, and ER-based transport machinery back to the ER. They, too, may percolate or they may move directly from Golgi cisternae to the ER. Only the latter possibility is indicated to simplify the diagram.

Instead, we suggest, by way of hypothesis, that the cargo-containing COPI vesicles may "percolate" up and down the stack, limited by tethering mainly to transfers between adjacent cisternae in the stack, largely unable to distinguish up from down because the same SNAREs are present throughout. These vesicles would migrate in a stochastic process resembling a random walk, giving rise to the fast track of anterograde transport (Figure 1). Net flow in the *cis*-to-*trans* direction would result because entry and exit are restricted to opposite faces, but would of course be less efficient than for unidirectionally moving vesicles. But simulations indicate that percolating vesicles may require only twice the number of transfers, on the average, as unidirectional vesicles to allow their cargo to transit the stack (Glick et al., 1997). Distinct tethers at the *trans* face (Kooy et al., 1992) could explain why cargo (including that packaged in megavesicles; Volchuk et al., 2000) accumulates in the *trans*-Golgi network when exit from the stack is blocked (Griffiths and Simons, 1986). Whether the retrograde-transporting COPI vesicles also percolate or whether they go directly from Golgi cisternae to the ER, or both, can not yet be considered because the intra-Golgi distribution of their SNARE proteins is not yet known.

Percolating vesicles would nicely resolve what have been thorny issues for the "classical" vesicle model in which COPI vesicles are proposed to move unidirectionally across the stack in the anterograde direction. The classical model requires distinct sets of v- and t-SNAREs for each cisterna that do not appear to exist (Pelham, 1998). But percolating vesicles could potentially transport cargo all the way across the stack with just a single set of SNAREs, by using them over-and-over-again throughout. In the classical model, membrane must be recycled retrograde within the stack by a special mechanism. Percolating vesicles would automatically recycle since they would move bidirectionally. Percolating vesicles would not generate the *cis* < *trans* gradient of cargo predicted by the classic model. Rather, even if they concentrate these cargo, percolating vesicles would maintain the cargo at a fixed concentration throughout the stack by bidirectionally equilibrating them among neighboring cisternae at every level.

Percolating vesicles would also elegantly explain a variety of observations concerning transport in cell-free systems on a unified basis. In studies of transport in cell-free extracts in the 1980s, it was found that the VSV G glycoprotein (normally targeted to the cell surface) was transported from isolated "donor" Golgi stacks to an "acceptor" population added from uninfected cells (Balch et al., 1984a, 1984b). COPI vesicles were soon implicated in this process (Orci et al., 1986). These coated vesicles, containing the viral glycoprotein, bud from the donor stacks and transfer to the acceptor stacks (Orci et al., 1989), and were formally shown to be transport intermediates because the isolated vesicles fuse with the acceptor stack (Ostermann et al., 1993). Indeed, it is this body of work that gave rise to the "classical" model that COPI vesicles mediate anterograde transport. The vesicles, which are now understood to be dynamically retained on Golgi stacks by tethering, presumably transfer from the tethers of donor stacks to those of acceptor stacks during collisions between them.

The above notwithstanding, it has been found recently that glycosyltransferases and other Golgi residents are also included in COPI vesicles, albeit at low levels, and that these vesicles can be produced from "acceptor" stacks (lacking VSV G glycoprotein) and fuse with the "donor" stacks (containing VSV G protein but lacking glycosyltransferase due to mutation), resulting in glycosylation of the VSV G protein (Love et al., 1998; Lin et al., 1999). These kinds of results indicating the ability of resident proteins like glycosyltransferases to migrate in COPI vesicles had been interpreted as if they were necessarily contradictory to the earlier studies showing migration of cargo (VSV G protein) in the COPI vesicles.

But, the possibility of pecolation makes it apparent that the transport of cargo and resident proteins in COPI vesicles may simply be two aspects of a more general function of COPI vesicles: bidirectional movement up and down the Golgi stack. Cell-free reconstitution leads to COPI budding at every level of the stack, along with the full spectrum of the activities of those vesicles. Depending on how a given cell-free transport assay is designed, either cargo or resident protein transfer between cisternae can be measured, both due to the budding and fusion of COPI vesicles.

The ability of glycosyltransferases to enter percolating COPI vesicles, even at low levels, would also provide a natural mechanism to explain how these and presumably other resident Golgi proteins can systematically but transiently explore the entire stack (Harris and Waters, 1996; Linstedt et al., 1997; Storrie et al., 1998), while also maintaining asymmetric steady-state *cis–trans* distributions that resemble overlapping peaks in a separation by chromatography (Rabouille et al., 1995). Percolation allows resident proteins to sample all of the cisternae and partition according to the nature of their membrane anchors into those that have the most favorable lipid composition (Munro, 1998) or other property.

Note that the maintenance of Golgi asymmetry does place a constraint on percolating vesicles, namely that

they do not include all Golgi components with equal facility. Otherwise, rapid interchange would lead to complete uniformity. This requirement is simply met, at least in principle. For example, exclusion of sphingolipids from the vesicles (Brügger et al., 2000) which are synthesized in the Golgi complex and delivered to the plasma membrane, would both help to prevent backflow of these compounds and encourage their accumulation within cisternae. The resultant gradient of lipid composition could then provide a physical basis for the segregation of percolating Golgi residents (Bretscher and Munro, 1993). This is just one possibility—any mechanism that spatially regulates the partitioning of Golgi residents into vesicles would suffice.

Parallel Pathways of Cisternal and Vesicle Flow Subserving Iterative Sorting

In summary, COPI vesicles—most likely percolating up and down the stack—provide a straightforward and compelling explanation of the "fast track" (5–10 min) taken by most cargo across the Golgi stack. Protein aggregates that are too large to enter COPI vesicles and cannot effectively form megavesicles can still transit the stack via the "slow track" provided by cisternal progression. Progression on this slow track could also provide a mechanism to explain the gradual turnover of Golgi membranes.

However, we hasten to point out that this balance could be different in different cell types and might well be subject to dynamic regulation. For example, net decreases or increases in the amount of Golgi membrane may be required acutely at or following mitosis, or following rapid exocytosis. Control of the rate of formation of new cisternae could well underlie these and other events.

What about cells like budding yeast whose Golgi membranes are not usually stacked? In contrast to the stacked cisternae in animal cells, cisternal maturation provides the fast track for anterograde transport in budding yeast. Here new Golgi compartments form in an ongoing fashion by fusion of anterograde-moving COPII vesicles and their derived VTCs with retrograde moving vesicles (Morin-Ganet et al., 1998, 2000; Wooding and Pelham, 1998). These compartments (functionally equivalent to the cisternae in stacked Golgi) are themselves subject to gradual maturation by the ongoing, selective withdrawal of ER-based transport machinery and Golgi resident proteins in departing retrograde COPI vesicles (Letourneur et al., 1994; Lewis and Pelham, 1996; Wooding and Pelham, 1998). This mechanism is fundamentally similar to cisternal progression/maturation in well-stacked Golgi, since in both cases the oldest cisternae contain the fewest ER constituents. The difference is that in stacked Golgi, the age of a cisterna is marked by its position (like rings in a tree) while in dispersed Golgi position is largely independent of age. Thus if percolation occurs in yeast, the lack of spatial restriction will make it a less ordered process than in animal cells.

From a broader biological perspective, the distinction between the cisternal progression/maturation and the COPI pathways does not seem especially significant. Fundamentally, both are forms of vesicular transport—cisternae are vesicles after all, even though they are very large ones. Furthermore, it is immaterial for the overall function of the Golgi as an interative sorting device whether anterograde movement is due to vesicles or cisternal flow, or a mixture of the two. In all cases, an anterograde-moving current of cargo is created that allows repeated opportunities in successive cisternae for a retrograde-moving countercurrent (comprised of other COPI vesicles) to gradually and efficiently filter out the few ER residents that escape by accident and the many ER-based components of transport vesicles that must leave the ER by design.

That the Golgi operates as such an iterative sorting machine, analogous to a distillation tower, is clearly predicted from the selectivity of retrograde transport for ER-based components and the serial nature of transport across a stack (Rothman, 1981; Rothman and Wieland, 1996). Direct evidence confirming this prediction can now be found in the declining *cis*-to-*trans* gradients of escaped KDEL-tagged ER proteins and of an ER-based SNARE (membrin, the mammalian homolog of yeast Bos1p). Escaped proteins penetrate at most one or two cisternae (Dean and Pelham, 1990; Stinchcombe et al., 1995) implying that they are very efficiently recaptured by the retrograde current. By contrast, the SNARE membrin—a component of the transport machinery itself—penetrates deeply, requiring the entire stack for its gradual depletion (Hay et al., 1998), probably reflecting the fact that the major constituents of COPII vesicles are necessarily delivered to the Golgi in far greater numbers than occasionally escaping ER proteins.

The overall picture that emerges is that the Golgi functions as an intermediate compartment separating the ER from later compartments. It is formed of a stack of essentially identical compartments—rather than a single larger compartment—because the former strategy allows more efficient overall separation due to iterative sorting. Its sequential cisternae allow a countercurrent of retrograde- and anterograde-moving constituents to operate, gradually separating them into two classes for two principal fates, return to the endoplasmic reticulum or distribution elsewhere throughout the cell. The distinctions among the cisternae in an operating Golgi, including the steady-state retention of its own constituents, most likely result from its own activity as a countercurrent distributor.

Conclusions and Perspectives

The Golgi operates as an iterative sorting device. Its essential principle, the use of countercurrents of forward and backward moving constituents to successively purify cargo proteins for export away from the other proteins of the ER, can be accomplished by various closely related strategies.

In animals, and presumably other species that have well-formed Golgi stacks, this separation is reflected in steady-state concentration gradients across the stack. Here, net forward flow appears to be mainly due to COPI vesicles, which we speculate percolate up and down the stack, and less to cisternal progression; retrograde flow is mediated by a distinct population of COPI vesicles selective for ER residents and recycling transport machinery.

In budding yeast, which frequently lack a well-organized stack, forward flow is much less dependent on COPI vesicles. Here, the same forward- and backward-moving currents exist as in stacked Golgi, but they are now executed mainly as a function of cisternal age and not position, resulting from continuous maturation as ER-based components are successively withdrawn from the same compartment (maturing as it ages).

While we have emphasized what is known, much more is not. Future studies will need to address several key issues. Techniques are now available to study the detailed kinetics of transport of two or more markers simultaneously, cell by cell. By comparing different proteins, the existence of "fast" and "slow" tracks may be verified and their relative importance for different cargo and in different cell types and growth states determined. A major question concerns the precise composition and formation of COPI vesicles. Most models require at least two classes of these, whether anterograde/retrograde within the stack or intra-Golgi/Golgi→ER, and we need to better understand both what is in them and how they are formed. Are vesicles of different composition generated by spatial restriction of their components to separate budding sites, or are they created by distinct biochemical mechanisms? What controls the partitioning of proteins into them? These problems will probably require quantitative analysis at the level of single vesicles. Many other questions remain about the transport machinery and how it is harnessed to organize the architecture of the Golgi apparatus. What exactly are the roles of tethers, and how are these peripheral membrane proteins recruited to different regions of the Golgi stack? When we understand the details, the larger picture may snap into focus.

Students are frequently taught that the Golgi is built like a stack of coins. It now seems clear that taking sides in the recent dispute between vesicle transport and cisternal progression has been a lot like flipping coins—except the two sides turned out to be the same! We are hopeful that as a result of the kinds of developments featured in this review, the field can now emerge from its recent state of polarization and excessive focus on what has been by all appearances a diversion, to reorient to a more productive focus on the core issues. At the crossroads of membrane traffic, the Golgi offers an unsurpassed opportunity to build on a solid foundation of molecular mechanisms to understand the physiology and pathology of transport processes in cells.

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