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The Golgi Complex: In Vitro Veritas?

Review

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Understanding the structure and function of the Golgi complex has proved to be among the more challenging problems in cell biology. The last several years have turned out to be particularly exciting in this respect since they have yielded new insights and ideas at an increasingly rapid pace. This period of advance has largely been due to the development of powerful new biochemical, morphological, and genetic approaches to unraveling the complexities of this organelle. While much remains to be discovered, the problem now is how to integrate this wealth of information. To see if this is possible, we will first summarize how the Golgi is commonly believed to work and then evaluate the strength of the evidence that underlies these views.

Present View of the Golgi

The Golgi complex is essentially a carbohydrate factory. In animal cells, it is engaged in the biosynthesis of glycolipids and of oligosaccharide portions of glycoproteins and proteoglycans. In plant cells, it has the additional task of producing a wide range of extracellular polysaccharides. The factory receives its substrates for glycosylation from the endoplasmic reticulum (ER), transports its raw materials (monosaccharide precursors) in from the cytosol, and distributes its completed protein, lipid, and polysaccharide products to a variety of destinations.

Transport through the Golgi Is Directional

One of the most compelling features of the Golgi complex is its structure. In electron micrographs, the Golgi is seen as a set of flattened cisternae with dilated rims arranged like a stack. The cisternae are also associated with an array of small vesicles, as well as with a network of tubules emanating from both sides of the stack (Figure 1). Morphologically and functionally, the Golgi complex has a distinct polarity (Farquhar and Palade, 1981). Newly synthesized membrane and secretory proteins coming from the ER enter the Golgi through the cis face, traverse across the stack, and leave via the trans face. A reticulum of tubules emanating from the trans-most cisterna, collectively referred to as the trans-Golgi network (TGN) or trans-Golgi reticulum, probably reflects the actual sites of exit (Griffiths and Simons, 1986; Geuze and Morré, 1991). On route through the Golgi, newly synthesized glycoproteins are subjected to a series of posttranslational modifications, most notably the ordered remodeling of their N-linked oligosaccharide side chains and biosynthesis of O-linked glycans.

The Golgi Consists of Multiple Subcompartments

Given the directionality of transport and the sequential nature of the glycosylation events, it is commonly thought that the Golgi consists of a series of functionally distinct compartments corresponding to structurally distinct cisternae or sets of cisternae (Dunphy et al., 1985; Duden et al., 1991a). Accordingly, enzymes involved in the early Golgi events in terminal N-glycosylation—such as the trimming of the high mannose chain to its man_5 form by mannosidase I—have been placed in the cis Golgi (Kornfeld and Kornfeld, 1985; Pelham, 1989). Enzymes involved in intermediate steps, such as the transfer of N-acetylglucosamine to the man_5 sugars (GlcNAc transferase I), are thought to be restricted to medial cisternae (Dunphy et al., 1985). Finally, enzymes that catalyze the addition of terminal galactose and sialic acid moieties (such as galactosyltransferase and sialyltransferase) are placed in trans cisternae and/or the TGN (Roth and Berger, 1982; Roth et al., 1985; Griffiths and Simons, 1986; Duncan and Kornfeld, 1988; Taatjes et al., 1988). The scheme thus predicts that a series of sequential biochemical reactions occurs within separate compartments, through which glycoprotein substrates pass as if on an assembly line.

Transport Is Mediated by Nonselective Carrier Vesicles

A consequence of this multicompartment arrangement is that newly synthesized proteins must be transferred from one Golgi compartment to the next. The most widely held view is that transport vesicles bud from the dilated rims of cisternae and then fuse with the next. Such a mechanism is consistent with the existence of the numerous small vesicles found in the vicinity of the Golgi (Farquhar and Palade, 1981).

Since transport of proteins and small molecules through the secretory pathway does not appear to require any identifiable signals, it is also thought that transit through the Golgi occurs by "bulk flow" (Pfeffer and Rothman, 1987). In other words, the intercisternal transport vesicles may not selectively include components intended for transport. Rather, resident proteins of any one cisterna must be selectively excluded from entering these transport vesicles, most likely reflecting the selective retention of resident proteins in their appropriate cisternae. A few cellular and viral glycoproteins are known that appear to have such retention signals, resulting in their accumulation in the Golgi (Colley et al., 1989; Swift and Machamer, 1991; Nilsson et al., 1991; Munro, 1991).

Exit from the Golgi Can Be Selective or Nonselective

Finally, at the level of the TGN, terminally glycosylated proteins are sorted to their destinations (Griffiths and Simons, 1986). Proteins intended for transport to lysosomes

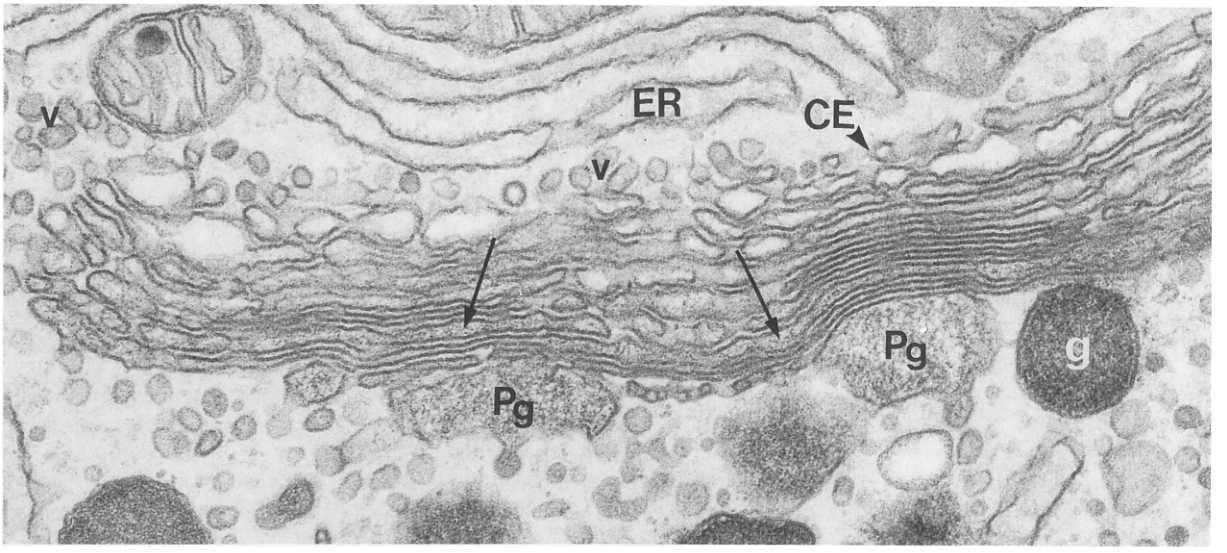

Figure 1. Transverse Section through a Golgi Complex of a Mucous Cell of the Brunner's Gland

Electron micrograph is taken from Rambourg and Clermont (1990) with permission. The trans side is recognizable by maturing (Pg) and mature (g) secretory granules. The discontinuous profile of the fenestrated network is seen on the cis side (CE). Small vesicles are marked V and the endoplasmic reticulum is marked ER. Also notice areas where the stack is disrupted by discontinuities (48,500×).

and endosomes are sequestered into clathrin-coated buds of the TGN, owing to specific recognition determinants (Kornfeld and Mellman, 1989; Pearse and Robinson, 1990). Proteins lacking these signals, including soluble proteins destined for secretion, are by default packaged into transport vesicles of the constitutive pathway that are targeted to the plasma membrane. In the TGN of polarized or secretory cells (endocrine and exocrine secretory cells, epithelial cells, and neurons), other routes of exit may occur, leading to inclusion either within storage granules (Kelly, 1985; Huttner and Tooze, 1989) or within transport vesicles targeted specifically to the apical or basolateral plasma membranes (Bennett et al., 1988; Wandinger-Ness et al., 1990) or to the dendritic and axonal cell surfaces (Dotti and Simons, 1990).

How Good Is the Evidence for the Current View of the Golgi?

As we have seen, there are three key elements underlying our general view of the Golgi: that the Golgi consists of several discrete compartments (cis, medial, trans, and TGN) corresponding to individual or groups of cisternae; that transit between these compartments occurs via vesicular carriers; and that the transport process is inherently nonselective with respect to the passenger proteins. Let us examine the strength of the evidence in favor of each of these propositions and interpret the data accordingly.

How Many Golgi Compartments Are There?

Although originally thought to be a "fixation artifact," the basic cisternal organization of the Golgi complex is now accepted as a certainty. Similarly, the cis to trans directionality of transport is quite clear from early morphological and autoradiographic analysis in exocrine cells where the sidedness of the Golgi is readily identifiable (Farquhar and Palade, 1981), as well as from immunoelectron microscopy studies using viral glycoproteins as markers (Bergmann and Singer, 1983). What is less than clear, however, is the extent to which the Golgi is divided into distinct compartments.

As mentioned earlier, some of the enzymes involved in early, intermediate, and late steps in the terminal glycosylation of N-linked sugar chains have been localized to one to three individual cisternae by electron microscopy using immunoperoxidase or immunogold cytochemistry (Roth and Berger, 1982; Slot and Geuze, 1983; Dunphy et al., 1985; Roth et al., 1985). However, a definitive demonstration that these enzymes exist in separate cisternae can only be provided by visualizing two or more enzymes simultaneously. The Golgi complex in the cell is not the idealized stack that we are accustomed to recognize in diagrams, but it may be subject to twists that make individual sections difficult to interpret. To date, simultaneous localization of two or more enzymes has not been accomplished. In addition, the localization of key enzymes such as mannosidase I remains to be established; this is a critical unknown, since mannosidase I is thought to be the first glycosidase to modify N-linked oligosaccharides upon their arrival in the Golgi.

A further complication is introduced by the fact that there appears to be considerable variation between cell types. For example, in goblet cells of the intestine, galactose α2,6-sialyltransferase (the enzyme responsible for adding

terminal sialic acid residues to N-linked sugars) has been localized to trans cisternae, as is the case in hepatocytes. In neighboring absorptive cells of the gut, however, almost the entire Golgi stack is labeled (except for the cis cisterna) (Roth et al., 1986). It has also recently become clear that Golgi glycosyltransferases may have multiple isoforms with either Golgi or plasma membrane distributions. For example, two isoforms of β1-4 galactosyltransferase have been found that differ by a 13 amino acid addition at the cytoplasmic N-terminus (Lopez et al., 1991). The short galactosyltransferase remains in the Golgi, while the long form is directed to the plasma membrane.

Cell fractionation experiments have also contributed to our view of the Golgi as a multicompartment organelle, since it has been possible to partly separate membranes containing several of the enzymes involved in Golgi processing events (Goldberg and Kornfeld, 1983; Dunphy and Rothman, 1983). However, interpretation of these data is limited on two accounts. First, in the absence of definitive immunocytochemical localization, it is impossible to know whether the different fractions correspond to different sets of cisternae. Second, the enzymes studied have been separated into only three fractions, the highest density fractions containing enzymes acting early in glycosylation (glucosidase, enzymes involved in generating mannose 6-phosphates on lysosomal enzymes) and the lightest density fractions containing terminally acting glycosyltransferases (galactosyl and sialyltransferases). The five enzymes that are involved in "intermediate" processing steps (mannosidase I and II, GlcNAc transferase I, II, and IV, and fucosyltransferase) were all found to cosediment in a single peak of intermediate density. Thus, although consistent with the existence of at least three physically and functionally distinct compartments, these observations also suggest that many of the carbohydrate processing steps may occur within a single compartment. Since the enzymes involved in N-glycosylation act sequentially (and specifically) (that is, mannosidase I creates the substrate for GlcNAc transferase, which creates the substrate for mannosidase II, which creates the substrate for galactosyltransferase, etc.), there is no a priori need for physical separation to ensure proper processing. Indeed, having GlcNAc transferase and galactosyltransferase in a common compartment would explain the existence of lactosamine repeats in the terminal chains of many glycoproteins (Cummings and Kornfeld, 1984; Howe et al., 1988).

A further reason for caution in interpreting existing immunocytochemistry and fractionation results is the growing appreciation of the fact that the individual Golgi cisternae—and perhaps even entire stacks—may not be as static and structurally distinct as we have come to believe. Examination of the Golgi using high voltage electron microscopy has suggested that adjacent stacks of cisternae are joined via extensive tubular interconnections (Rambourg and Clermont, 1990). Similarly, real-time imaging of living cells labeled with the fluorescent lipid analog NBD–ceramide has revealed that the Golgi is capable of forming a large number of tubules that may serve to connect otherwise "separate" stacks of cisternae (Cooper et al., 1990). Whether the tubules form connections between cisternae in register (i.e., cis to cis, medial to medial) or out of register (cis to medial) is unknown. The possibility must also be considered that even within a single stack, adjacent cisternae might at least transiently possess similar tubular interconnections.

What Is a Compartment Anyway?

A question that clearly needs discussion at this point is what exactly defines a cellular compartment. Classically, membrane-bounded compartments have been viewed as comprising physically distinct entities each with its own distinct protein compositions and, presumably, unique functions. Accordingly, all of any one type of organelle can be considered a compartment, but so can a distinct region (or "subcompartment") within an organelle. By this criterion, compartment boundaries should be easily delineated by immunoelectron microscopic localization of resident marker proteins. However, this simple view is complicated by several factors. For example, even the localization of two Golgi enzymes to different cisternae by double label immunoelectron microscopy might not unequivocally demonstrate that they are present in physically distinct compartments. Such a distribution of enzymes could reflect the existence of subdomains in an otherwise continuous membrane array, analogous to the lateral heterogeneity found between the rough and the smooth ER. Conversely, markers may be segregated into physically distinct membranes that nevertheless remain functionally continuous by repeated membrane fissions and fusions or transient tubular interconnections. The existence of such homotypic interactions—interactions among "like" elements—would play an important role in preserving continuity among equivalent but physically separated organelles. Thus, the simple test of localizing different markers to distinct membranes cannot by itself serve to identify distinct compartments.

The definition of a compartment is further confounded when considering organelles that are also involved in membrane traffic. Given that there is likely to be continuous transport and recycling of membrane components between such compartments, compartment identities are not static and therefore may not always be entirely biochemically distinct. The object, then, is to identify only those components that are endogenous, i.e., not involved in recycling. Conceivably, endogenous (or resident) components may be defined by their ability to interact with a compartment-specific structural framework, such as a cytosolic membrane coat or skeleton. It is clear that only by identifying the machinery responsible for entry into, retention within, or exit from a compartment will we be able to precisely and consistently determine compartment boundaries. In the absence of this information, compartments can only be tentatively defined as functionally equivalent and possibly physically continuous populations of membrane-bounded structures that are both functionally and physically distinct from all other compartments.

A Three Compartment View of the Golgi Complex

Since we do not possess the information required to de-

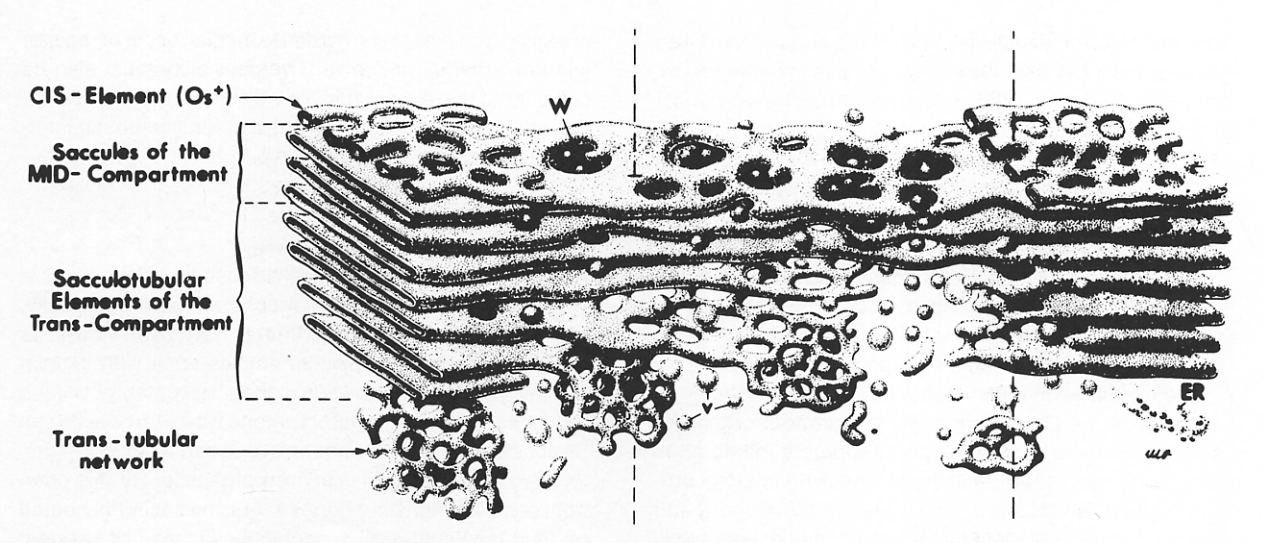

Figure 2. Three-Dimensional Reconstruction of the Golgi Complex from a Nonciliated Epithelial Cell of the Ductuli Efferentes of the Rat

Taken from Rambourg and Clermont (1990), with permission. The osmiophilic cis compartment, the saccules (cisternae) of the medial compartment, as well as the cisternae and tubules of the trans compartment are all indicated.

scribe definitively the compartmentalization of the Golgi complex, models for the organization of the Golgi should be kept as simple as possible, adding complexity only as it is necessitated by additional functional requirements. As we have discussed, there are three minimal functions that the Golgi must accomplish: first, receipt and sorting of membrane and soluble cargo arriving at the cis Golgi from the ER; second, glycosylation and processing of glycoproteins and glycolipids; and third, sorting of membrane and soluble cargo arriving and exiting at the trans face of the Golgi. Assuming that distinct compartments are required to accomplish these functions, at least three compartments are required, defined here as the cis-Golgi network (CGN), the medial Golgi, and the TGN, respectively. Although the details of Golgi morphology vary considerably between different cell types, such a three compartment subdivision is generally consistent with the overall structural organization of the mammalian Golgi from three-dimensional reconstructions (Rambourg and Clermont, 1990) (Figures 2 and 3). It is also consistent with the functional organization of the Golgi in Saccharomyces cerevisiae, which, on the basis of mutations affecting Golgi function, has recently been proposed to consist of at least three compartments (Graham and Emr, 1991).

The CGN

At a minimum, the cis side of the Golgi stack must function in the receipt of newly synthesized material from the ER. Structurally, however, the cis-most cisterna of the Golgi is typically associated with a continuous array of tubules that are selectively stained after prolonged osmication (Lindsey and Ellisman, 1985; Rambourg and Clermont, 1990) (Figure 2). Given this distinctive morphology, and by analogy to the TGN, it seems appropriate to refer to this region as a distinct compartment called the CGN (Huttner and Tooze, 1989; Hsu et al., 1991; Pelham, 1991). For several reasons, it also seems reasonable to suggest that the CGN also includes the poorly understood but possibly interconnected system of tubules between the ER and the Golgi, referred to as the "salvage compartment" (Warren, 1987; Pelham, 1991) (Figure 3).

In addition to serving as a site of entry into the Golgi, recent evidence suggests that the CGN functions in the recycling of protein and lipid components back to the ER, while having a relatively limited role in glycosylation. Using both animal and yeast cells, Pelham and colleagues have found that soluble ER proteins containing the KDEL (or HDEL) ER retention marker are delivered to a post-ER

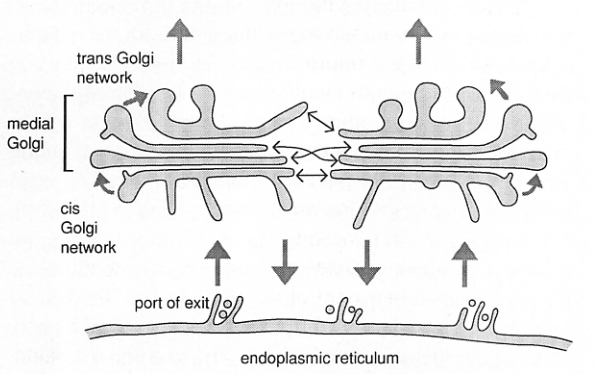

Figure 3. Schematic Representation of the Three Compartment Model of the Golgi Complex

The ports of exit are the sites from which proteins leave the ER. As discussed in the text, the "intermediate compartment" defined by the p53 and p58 antigens probably consists both of the CGN, the ports of exit from the ER, and the carrier elements responsible for bidirectional transport between the ER and the CGN. The lateral arrows would be sites where direct fusions would interconnect CGN–CGN, medial–medial, and TGN–TGN compartments of two adjacent stacks. Compare these sites to the discontinuities in Figure 1.

site, in which little "Golgi-like" oligosaccharide processing occurs and from which these proteins are returned to the ER (Pelham, 1988, 1989; Dean and Pelham, 1990). In animal cells, the lysosomal enzyme cathepsin D was given a C-terminal KDEL tag and found not to receive any terminal sugar modifications associated with Golgi-linked glycosylation. However, mannose residues on the enzyme did receive a GlcNAc-phosphate. Thus, although the KDEL construct reached a GlcNAc-phosphotransferase-containing compartment, it did not encounter mannosidase I, a glycosidase that would remove the phosphotransferase substrate. Although it may yet turn out that some KDEL proteins receive further oligosaccharide modifications, KDEL receptors can retrieve these proteins before they reach the bulk of the oligosaccharide transferases; thus, recycling is a function associated with a distinct "early" Golgi compartment.

Without immunocytochemical localization of these constructs (the KDEL receptor or the phosphotransferase), it is impossible to be certain that these events occur in the CGN. However, this appears likely, given that our model assumes that the tubules and vesicles defining the CGN are also associated with antigens such as p53, p58, and rab2 (Saraste et al., 1987; Schweizer et al., 1988; Chavrier et al., 1990), which have been used to define the so-called "intermediate compartment" (Schweizer et al., 1990). These antigens label structures that appear to serve as intermediates in transport from the ER to the Golgi as well as in the recycling of Golgi components back to the ER (Lippincott-Schwartz et al., 1990). The markers also label presumed sites of exit from the ER ("transitional elements," Palade, 1975) as well as the cis-most Golgi cisterna (Saraste and Svensson, 1991; Lotti et al., 1992), suggesting that they are not endogenous CGN components, but recycle between the ER and CGN. Thus, the intermediate compartment probably comprises the exit sites from both the ER and the CGN as well as the dynamic "pool" of vesicles and tubules that reflects the continuous recycling between the two sites (Figure 3). Although incubation at low temperature has also been used to delineate boundaries in this region, like all temperature blocks, forward transport is dependent on length of incubation, making precise interpretation even more difficult than usual.

We thus view the CGN as a unique Golgi compartment responsible for receiving and sorting components arriving from the ER. Structurally, we predict it will ordinarily (but perhaps not always) consist of the cis-most Golgi cisterna and the associated vesicles and tubules that include the intermediate compartment of Schweizer et al. (1990). In addition to the transport of KDEL-tagged proteins to and from the Golgi and the limited post-ER glycosylation events to which these proteins are subjected, the CGN might also be predicted to include a variety of other activities that have been relegated to the purgatory between the ER and the Golgi. These would include the addition of the first N-acetylgalactosamine residue to O-linked sugars, the assembly of coronavirus particles (Tooze et al., 1988; Machamer et al., 1990), and fatty acylation of membrane protein cytoplasmic domains (Rizzolo and Kornfeld, 1988; Bonatti et al., 1989). Experiments in yeast also give at least indirect support to the existence of lipid recycling to the ER from the CGN as well (Cleves et al., 1991).

The Medial Golgi

The medial Golgi would function primarily as a glycosylation device. Structurally, it corresponds to the cisternae and tubules in the middle of the Golgi stack (Figure 3), whose structures vary widely in different cells (Rambourg and Clermont, 1990). While distinct from the CGN or TGN, we assume that the medial cisternae represent a single functionally continuous compartment, irrespective of the actual number of physically separate cisternal elements in any one Golgi complex. Nevertheless, owing to the sidedness of the Golgi, the medial cisternae may appear structurally distinct. For example, since one face of a medial cisterna is apposed to the CGN while another face is apposed to the TGN, the two faces may have different compositions, owing to their interactions with structurally distinct compartments. Most of the glycosylation reactions (N-linked, O-linked, as well as glycolipid synthesis), as well as polysaccharide synthesis normally associated with the Golgi, are viewed as occurring in the medial compartment. This arrangement would account for cell type heterogeneity in cisternal number and enzyme distribution by placing them all in a single compartment. At present, there is insufficient data to know if this is really the case.

If the medial Golgi serves only to mediate glycosylation reactions, why the characteristic cisternal morphology? One possibility might be simply to enhance the efficiency of glycosylation. Although precise measurements are difficult to make, transport systems for sugar nucleotide precursors are kinetically inefficient (Deutscher et al., 1984). Moreover, while the terminal transferases also have relatively high K_ms for substrate (high micromolar range), they may work at rates that may exceed the rate of sugar nucleotide transport (Weinstein et al., 1982; C. Hirschberg, personal communication). Thus, the cisternal stack by its flattened structure may serve to enhance the efficiency of glycosylation by minimizing lumenal volume and increasing membrane surface area.

The TGN

The TGN is viewed as the compartment that mediates the sorting and final exit of material from the Golgi. Structurally, it is likely to be defined by the sacculotubular network located on the trans side of the Golgi that varies in structure in different cell types (Rambourg and Clermont, 1990) (Figures 2 and 3). The TGN can be visualized in the electron microscope after labeling cells with C_6–NBD–ceramide (Pagano et al., 1989), with C_5–DMB–ceramide (Pagano et al., 1991), or after accumulating vesicular stomatitis virus (VSV) G protein at 20°C (Griffiths et al., 1985). The TGN may also have a lower pH than preceding compartments in the pathway (Anderson and Pathak, 1985).

The TGN is known to undergo dynamic changes in size, depending on the amount of protein traffic through the compartment (Griffiths et al., 1989). In glandular cells producing secretion granules, several cisternae on the trans side form condensing vacuoles, presuming that all these cisternae are part of the TGN (Rambourg and Clermont,

1990). The extreme case is represented by the Golgi complex of algae where flakes are assembled in the lumen of the Golgi cisternae (Melkonian et al., 1991). Since these large flakes are first observed several cisternae away from the cis side, it seems reasonable to assume that the flakes assemble in saccular portions of the TGN and that entire TGN cisternae peel off and are transported to the cell surface.

The main function of the TGN is to sort proteins and lipids leaving for different post-Golgi destinations (Griffiths and Simons, 1986; Simons and van Meer, 1988). The TGN also receives membrane traffic from the endocytic pathway (Farquhar and Palade, 1981; Kornfeld and Mellman, 1989), and, unlike all other Golgi subcompartments, its degree of continuity with the plasma membrane is increased following treatment with brefeldin A (BFA) (Lippincott-Schwartz et al., 1991). The TGN is also the only Golgi compartment that fails to enter the ER following BFA (Chege and Pfeffer, 1990; Ulmer and Palade, 1991; Reaves and Banting, 1992). Certain "late" protein modification events also occur in the TGN, such as galactose α2,6 sialylation (Duncan and Kornfeld, 1988), tyrosine sulfation (Huttner and Baeuerle, 1988), and proteolytic cleavage at dibasic residues on viral glycoproteins and cellular precursor proteins (Sossin et al., 1990).

We propose that the TGN also operates as a directional valve to control the flow of membrane proteins and lipids in biosynthetic transport (Figure 3). Such a function would contribute to the efficiency of forward transport by preventing passenger proteins and lipids from returning to the medial Golgi after reaching the TGN. In practice, this would predict that there is no recycling from the TGN to the medial Golgi. The need for this function is best illustrated by considering the transport and distribution of membrane lipids through the Golgi. Several lipids such as cholesterol, glycolipids, and sphingomyelin are present in very low concentrations in the ER, although they are abundant in the Golgi (van Meer, 1989). If there were lipid recycling from each Golgi compartment, it would be difficult to account for these differences in lipid composition. Placing a valve in the pathway makes the lipid sorting problem easier to solve. Several studies have demonstrated directly that there is little backflow of glycoproteins from the TGN into proximal Golgi compartments (Duncan and Kornfeld, 1988; Neefjes et al., 1988; Chege and Pfeffer, 1990). Also, recycling of glycosphingolipids through the TGN (Schwartzmann and Sandhoff, 1990) would be easier to explain if these plasma membrane lipids would not encouter medial Golgi enzymes; otherwise, with time, only complex glycolipids would be present on the cell surface.

Does Transport through the Golgi Require Vesicular Carriers?

The second key element underlying our current view of the Golgi concerns the role of carrier vesicles in mediating transport through the Golgi complex. While transport vesicles may represent the most likely mechanism to accomplish transfer between compartments and/or individual cisternae, how strong is the evidence that they actually perform this function? In intact cells, perhaps the best argument comes from the early cell–cell fusion experiments of Rothman and colleagues (Rothman et al., 1984a, 1984b). Here, CHO cell glycosylation mutants were infected with VSV and then fused with uninfected cells. Transfer of the VSV G protein from Golgi membranes in the infected to the uninfected cells was monitored by a glycosylation event that could be carried out by only the uninfected recipient, which was chosen to complement the glycosylation defect of the infected cell. These observations indicated that efficient transfer of VSV G protein occurs between spatially distinct Golgi stacks. This is most easily explained by a mechanism involving VSV G protein–containing transport vesicles that form from the infected cell Golgi, diffuse through the heterokaryon's cytosol, and fuse with Golgi membranes in the uninfected cell. Conceivably, the same transport vesicles could mediate intercisternal transport within a single Golgi stack.

The major limitation of these data is that they are indirect. They do not identify the vesicles nor do they definitely establish their site of origin. One cannot exclude the possibility that the G protein moved by lateral diffusion through tubules connecting the heterologous Golgi stacks, which otherwise remain separated during the assay. Furthermore, even the existence of vesicles involved in inter-Golgi transport does not demonstrate that they have a similar role in intercisternal transport within a single stack, as is assumed by our current view of the Golgi.

In Vitro Transport through the Golgi and the Role of Carrier Vesicles

The most extensive series of considerations favoring the role of transport vesicles in intra-Golgi transport comes from experiments designed to reconstitute transport in vitro. That such transport can be achieved has been demonstrated over the past several years by the pioneering work of Rothman and coworkers. In their approach, Golgi fractions prepared from VSV-infected GlcNAc transferase–deficient 15B cells ("donor Golgi") are incubated with similar fractions from uninfected wild-type cells ("acceptor Golgi"). Upon the addition of ATP and cytosol-derived factors, there is a progressive appearance of [^{3}H]GlcNAc-labeled (Balch et al., 1984) and endo H–resistant (Fries and Rothman, 1981) VSV G protein, reflecting its transfer from mutant "donor" to wild-type "acceptor" membranes.

This transfer has also been dissected kinetically into two major stages: an early stage presumed to correlate with the budding of transport vesicles from the donor Golgi, and a late stage reflecting the docking and subsequent fusion of the vesicles with the acceptor Golgi. The early and late stages also differ in their requirements and inhibitor sensitivities (Balch et al., 1984; Rothman and Orci, 1990). Given that VSV G protein in early Golgi compartments was transported with the highest efficiency (Fries and Rothman, 1981), the transport event assayed was postulated to reflect the formation of vesicles from cis (early) cisternae and the fusion of these vesicles with medial cisternae (which contain the GlcNAc transferase) (Rothman and Orci, 1990) (Figure 4). However, the identi-

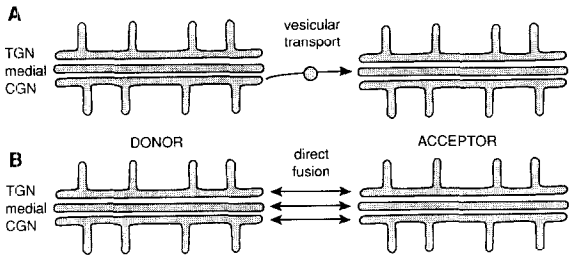

Figure 4. Two Models to Account for Donor–Acceptor Connections in Inter-Golgi Transport In Vitro

(A) Vesicular transport, as predicted by the current view of the Golgi and by the work of Rothman and colleagues (Rothman and Orci, 1990). (B) Vesicle-independent direct fusions between donor and acceptor compartments (CGN–CGN, medial–medial, and TGN–TGN), perhaps mediated by tubular extensions. While these tubules may be amplified by BFA, as described in the text, they may exist and function even in the absence of the drug.

ties of the donor and acceptor cisternae have not yet been established, nor can kinetic analysis alone provide a unique interpretation, especially when transport is monitored indirectly by linkage to glycosylation, which itself is a kinetically complex series of reactions (see above).

Analysis of this Golgi transport system has already led to the discovery of several discrete protein factors whose activities are required to mediate the assay (Rothman and Orci, 1990). Some of these represent the mammalian homologs of yeast genes known to be involved in intracellular transport in intact cells (Wilson et al., 1989; Clary et al., 1990). One important factor, designated NSF (NEM-sensitive fusion protein), is homologous to the yeast *sec18* gene product and is thought to act at a late stage in transport, at the time when transport vesicles fuse with the acceptor membranes (Malhotra et al., 1989; Orci et al., 1989; Rexach and Schekman, 1991) both in ER to Golgi and in Golgi as well as in post-Golgi transport (Graham and Emr, 1991). The precise function of NSF, however, remains unknown. Another factor, designated SNAP (for soluble NSF attachment protein), is homologous to the yeast *sec17* gene product and is thought to be involved in NSF binding to membranes (Clary et al., 1990).

The strongest evidence for the existence of a vesicular intermediate in the in vitro transport reaction has been provided by the identification and isolation of the presumed carrier vesicles themselves. During the early stages of the reaction, isolated Golgi membranes generate an array of coated tubules, buds, and vesicles (Orci et al., 1986). The vesicles have been isolated (after elution from their membrane-bound state by high salt), and their coats have been found to contain several unique components (Malhotra et al., 1989). One of these, β-COP, was previously identified by Kreis and coworkers as a 110 kd peripheral protein tightly associated with the Golgi (Allan and Kreis, 1986; Duden et al., 1991b; Serafini et al., 1991). These non-clathrin-coated vesicles are good candidates for carrier vesicles. First, they contain VSV G protein, the protein being transported (Orci et al., 1986, 1989; Serafini et al., 1991). Next, conditions that block the transport reaction in general result in the accumulation or lack of formation of vesicles. Most interestingly, treatment of cells or isolated membranes with the nonhydrolyzable GTP analog, GTPγS, both blocks glycosylation of the VSV G protein and results in the accumulation of VSV G protein–containing coated buds and vesicles (Orci et al., 1989). Similar results were obtained using AlF_4, an inhibitor of conventional GTP-binding "G proteins" (Orci et al., 1989; Kahn, 1991). The stage at which these inhibitors act remains unknown (Donaldson et al., 1991b).

In spite of this large amount of correlative evidence, additional data will be required before it is certain that the coated vesicles serve as unique and obligatory transport intermediates either in vitro or in intact cells. Perhaps the most important piece of missing information pertains to whether the vesicles have a composition consistent with their presumed transport function. For example, it will be important to confirm that the Golgi vesicles do not contain resident proteins, e.g., glycosyltransferases, that are not intended for transport. The analogous point is well established for plasma membrane clathrin-coated vesicles. Many examples are known where proteins destined for coated vesicle-mediated endocytosis accumulate at coated pits, while those that are not efficiently internalized do not enter coated pits (Pearse and Robinson, 1990).

Another critical unknown is a demonstration that the Golgi coated vesicles are functional in vitro, i.e., that VSV G protein–containing vesicle fractions can be used to reconstitute transport when added to acceptor Golgi. The analogous experiment has recently been accomplished for a less well-characterized vesicle fraction that mediates transport from the ER to the Golgi in yeast (Groesch et al., 1990; Rexach and Schekman, 1991). Thus, in principle, this approach should be possible. However, with all such experiments, it will be important to ensure that the coated vesicle fractions used are not contaminated with uncoated, fusion-competent membranes.

BFA Blocks Vesicle Formation without Blocking Transport

A reason for examining the features of the Golgi vesicles so carefully is indicated by a recent paper that raises the possibility that, at least under certain conditions, these vesicles may not be required for transport in vitro (Orci et al., 1991). In this work, the effect of BFA on the Golgi transport assay was determined. BFA is a macrocyclic fungal antibiotic that blocks the transport of membrane and secretory proteins through the Golgi (Takatsuki and Tamura, 1985; Misumi et al., 1986). In intact cells, the drug causes a dramatic and rapid retrograde transport of Golgi components back to the ER via a system of tubular extensions (Lippincott-Schwartz et al., 1990). The mechanism of BFA action is unknown, but one intriguing property is its ability to rapidly, and reversibly, trigger the dissociation of β-COP (Donaldson et al., 1991a; Klausner et al., 1992).

When isolated Golgi was treated with BFA, β-COP was no longer associated with the membranes nor were any coated buds or vesicles detected (Orci et al., 1991). Nevertheless, the transfer of VSV G protein from donor to acceptor Golgi continued with similar kinetics and efficiency

as in untreated controls. The only difference was that transfer in BFA-treated Golgi lost its sensitivity to GTPγS inhibition.

There are two possible interpretations of these results. The first is that coated vesicles that form in the assay system are not obligatory intermediates in the transport event, even in the absence of the drug. Alternatively, BFA may induce an aberrant form of transport that nevertheless utilizes enzymatic machinery required for vesicular transport in the absence of the drug. It is at present impossible to distinguish between these two possibilities. However, BFA-treated Golgi in vitro appear to form tubular extensions that may serve to interconnect donor and acceptor Golgi stacks, thus resulting in transfer to the acceptor across these tubular bridges (Orci et al., 1991). This possibility is appealing since it could reflect the BFA-induced tubulation of the Golgi seen in intact cells. However, additional electron microscope immunocytochemistry will be required to establish continuous interconnections between antigenically distinct Golgi elements.

Vesicular Transport or Golgi Fusion?

The fact that "transport" (i.e., G protein glycosylation) can occur in the absence of vesicle formation raises a number of important questions: What transport step(s) is actually being measured in vitro? What is the relationship of the events reconstituted in vitro to those found in intact cells? Is transport in vitro ever completely dependent on vesicle formation, even in the absence of BFA? If vesicles are not involved, is transfer from donor to acceptor Golgi mediated by direct fusion, perhaps via tubular extensions? As mentioned above, it is unclear whether the G protein is transferred across a predicted compartment boundary (donor CGN to acceptor medial) or within a single compartment (donor medial to acceptor medial).

It is becoming increasingly clear that all membrane-bound organelles have a propensity to form tubules in intact cells and in vitro, with or without BFA treatment. For example, the ER (Lee and Chen, 1988), mitochondria (Johnson et al., 1980), peroxisomes (Yamamoto and Fahimi, 1987), endosomes (Hopkins et al., 1990; Tooze and Hollinshead, 1991; Hunziker et al., 1991b; Lippincott-Schwartz et al., 1991), and lysosomes (Swanson et al., 1987; Lippincott-Schwartz et al., 1991) are all capable of forming dynamic tubular membrane networks. One well-studied example is the early endosome, the structure of which varies in different cell types (Marsh et al., 1986; Tooze et al., 1991). Cell-free assays have documented that the early endosome elements can fuse avidly with each other (Gruenberg and Howell, 1989). Other examples of processes involving homotypic fusion in vitro are the assembly of the nuclear envelope (Burke and Gerace, 1986) and the formation of ER (Dabora and Sheetz, 1988).

The Golgi complex is also a dynamic structure that can be disassembled by microtubule depolymerization into fragments that remain functional despite being dispersed throughout the cytoplasm (Thyberg and Moskalewski, 1985). Upon microtubule reassembly, the Golgi fragments rapidly reassemble and assume their characteristic cisternal organization (Ho et al., 1989). A similar series of events must occur during mitosis when the Golgi fragments into populations of small vesicles and tubules (Lucocq et al., 1989). During telophase, these fragments reassemble.

Given that homotypic fusion occurs so commonly, it would be premature to dismiss the possibility that the intra-Golgi transport activity observed in vitro actually reflects a process of regulated tubule formation and/or direct fusion of donor and acceptor Golgi elements (Figure 4). Indeed, even in untreated Golgi, both in vitro and in intact cells, tubules can be found that are similar to those observed in BFA-treated Golgi (Hermo et al., 1980; Braell et al., 1984 [see Figure 8b in this reference]; Griffiths et al., 1985). That a direct fusion event is possible is also suggested by the fact that the predicted diffusion coefficient of intact Golgi stacks is ~10-fold less than that of the much smaller coated vesicles (N. Ktistakis, unpublished data). Thus, one might expect a decrease in the kinetics of VSV G protein transfer if BFA were to switch the signal generated in vitro from a vesicle-dependent phenomenon (as is presumed to occur in the absence of the drug) to a direct, tubule-mediated fusion event. However, G protein is transported from donor to acceptor with the same kinetics in the presence or absence of BFA (Orci et al., 1991). Finally, the fact that the two N-linked chains of VSV G protein are processed simultaneously in vitro as opposed to sequentially in permeabilized cells may also indicate that compartmental boundaries may not be strictly preserved, i.e., that direct fusion may occur, during the cell-free assay (Schwaninger et al., 1991).

The minimal model that we discussed (see Figure 3) predicts only two functional and presumably physical discontinuities in the Golgi: from the CGN to the medial Golgi, and from the medial Golgi to the TGN. Thus, according to this view, transport through the Golgi involves two intercompartmental transfers, reflecting transfer from the site of entry to the site of glycosylation, and from the site of glycosylation to the site of exit.

Considering available data, we consider it likely that the intercompartmental transfers between CGN/medial and medial/TGN boundaries occur in vivo via vesicular carriers. While the putative transporters remain to be identified, the β-COP-containing vesicles (Malhotra et al., 1989; Serafini et al., 1991) are obvious but unconfirmed candidates, whether or not they are ultimately found to function as carrier vesicles in in vitro Golgi assays. The tubular interconnections known to occur in the Golgi may also play a role in intercompartmental transfer, but we view these as probably being more important for establishing and maintaining intracompartmental links that serve to interconnect functionally identical Golgi compartments and to mix their contents. These tubular connections may break and fuse continuously, accounting for the dynamic behavior of the Golgi complex after microtubule disassembly and reassembly. However, if their formation is tightly controlled by a regulated process of assembly and disassembly of the β-COP-containing Golgi coat, even tubule-based intercompartmental transfer is not inconceivable. Moreover, whether cell-free assays of Golgi function reflect such an intercompartmental transfer, or intracompartmental transfer between wild-type and mutant Golgi, remains to be

solved. Only further characterization of the machinery responsible for intra-Golgi transport will decide between these two possibilities. Nevertheless, based on the evidence both from the in vitro assays and from yeast genetics, it is clear that the few proteins identified thus far, such as NSF and SNAP, are generally important elements, irrespective of the precise pathways or whether transport occurs via vesicles or tubules.

Is Transport through the Golgi Selective or Nonselective?

Having considered the evidence for the compartmental organization of the Golgi and for the role of carrier vesicles in transport, we come to the last of the three general elements underlying our view of Golgi function, namely, that transport of passenger proteins through the Golgi is inherently nonselective (Pfeffer and Rothman, 1987). The evidence for this view is largely indirect or negative, in that efforts to identify discrete signals on proteins that are required for forward transport have thus far been unsuccessful. On the other hand, as discussed above, there is now considerable evidence in favor of several distinct "retention signals" that effectively prevent forward transport of membrane or lumenal proteins after reaching their prescribed destinations in the ER or various Golgi compartments (Pelham, 1991). If this view is correct, selectivity in transport through the secretory pathway may occur by "default," i.e., transport of passenger proteins proceeds owing to the absence of a retention signal. This concept, however, is not necessarily incompatible with the existence of selective signals for forward transport. It is clear that such signals exist and play an important role in directing the traffic of proteins as they leave the TGN. Examples include the mannose 6-phosphate residues that specify transport of hydrolytic enzymes to lysosomes (Kornfeld and Mellman, 1989) and the cytoplasmic domain determinants that target newly synthesized membrane proteins to the basolateral surface of polarized cells (Hunziker et al., 1991a; Brewer and Roth, 1991). Since the transport of fluorescent lipids from the Golgi to the plasma membrane in nonpolarized cells occurs very rapidly, it is thought that "constitutive" transport from the Golgi to the cell surface may not require specific signals (Karrenbauer et al., 1990). Analogous results were obtained in experiments in which a tripeptide containing a cognate site for N-linked glycosylation presumably also involved transport from the ER (Wieland et al., 1987; Helms et al., 1990). While these experiments suggest that signals are not necessary for transport, the fact that they are released with rapid kinetics does not alone establish the absence of such signals.

Experiments showing that different secretory and membrane proteins are transported with different kinetics may indicate that signals or receptors are involved in forward transport (Lodish et al., 1983; Lodish, 1988). Similarly, the suggestion that newly synthesized viral spike glycoproteins are present in Golgi membranes at a density several-fold greater than in the ER is also consistent with the existence of signal-driven forward transport (Griffiths et al., 1984). However, both of these observations might also be reconciled with a nonselective mechanism of transport. Given recent evidence that exit from the ER is linked to the folding of newly synthesized proteins, differential folding rates among proteins may indirectly affect their transport kinetics. Moreover, if the intrinsic rate of transport of glycoproteins through the Golgi is slow relative to the rate of ER exit, then one might also expect a higher concentration of certain passenger proteins in the Golgi.

At present, most of the attention paid to the question of signals in transport concerns ER to Golgi or post-Golgi transport. Whether transport through the Golgi complex itself is selective or nonselective is still an open question.

Perspectives

After years of descriptive work, the Golgi complex is slowly starting to reveal its secrets. We have now entered an exciting period of research, during which it will become possible to define the molecular mechanisms responsible for generating and maintaining Golgi structure and function. The first phase is already well under way and has been characterized by a search for essential bits and pieces of the Golgi machinery, a number of which have already been found (NSF/sec18, αSNAP/sec17, β-COP, ARF [Stearns et al., 1990], rab6p [Goud et al., 1990], sec7p [Achstetter et al., 1988], and sec14p [Bankaitis et al., 1990]). As we have seen, however, it is at present difficult to know precisely what steps are controlled by each of these components. Nevertheless, the observed conservation of Golgi proteins between S. cerevisiae and mammals is most encouraging for our ability to confirm in living cells the function of components identified in vitro. The combination of cell-free analysis and genetics has proven its worth. The next phase will have to deal with the questions that have arisen. How many Golgi compartments are there? Are compartment boundaries defined by specific protein frameworks? If so, how do they function and how are they regulated? Does transport between Golgi compartments require vesicular carriers? What is the role of tubules? How does the machinery responsible for forward traffic relate to the machinery controlling homotypic fusion? How is specificity of forward and backward traffic regulated? How does lipid composition and organization affect transport? What function does the stack structure have? How do microtubules interact with the Golgi elements? The challenge will be to integrate the information we are now collecting in the context of how the Golgi complex works as a whole.

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