

# Compartmentation of the Golgi Complex: Brefeldin-A Distinguishes *trans*-Golgi Cisternae from the *trans*-Golgi Network

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**Abstract.** The Golgi complex is composed of at least four distinct compartments, termed the *cis*-, medial, and *trans*-Golgi cisternae and the *trans*-Golgi network (TGN). It has recently been reported that the organization of the Golgi complex is disrupted in cells treated with the fungal metabolite, brefeldin-A. Under these conditions, it was shown that resident enzymes of the *cis*-, medial, and *trans*-Golgi return to the ER. We report here that 300-kD mannose 6-phosphate receptors, when pulse-labeled within the ER of brefeldin-A-treated cells, acquired numerous N-linked galactose residues with a half time of approximately 2 h, as measured by their ability to bind to RCA-I lectin affinity columns. In contrast, *Limax flavus* lectin chromatography revealed that <10% of these receptors acquired sialic acid after 8 h in brefeldin-A. Two lines of evidence suggested that proteins within and beyond

the TGN did not return to the ER in the presence of brefeldin-A. First, the majority of 300-kD mannose 6-phosphate receptors present in the TGN and endosomes did not return to the ER after up to 6 h in brefeldin-A, as determined by their failure to contact galactosyltransferase that had relocated there. Moreover, although mannose 6-phosphate receptors did not acquire sialic acid when present in the ER of brefeldin-A-treated cells, they were readily sialylated when labeled at the cell surface and transported to the TGN. These experiments indicate that galactosyltransferase, a *trans*-Golgi enzyme, returns to the endoplasmic reticulum in the presence of brefeldin-A, while the bulk of sialyltransferase, a resident of the TGN, does not. Our findings support the proposal that the TGN is a distinct, fourth compartment of the Golgi apparatus that is insensitive to brefeldin-A.

**B**IOCHEMICAL fractionation experiments and the immunocytochemical localization of specific glycosyltransferases and their products have led to the notion that the Golgi complex is divided into at least three distinct subcompartments (11, 21, 23, 27). The first compartment, termed the *cis*-Golgi, is thought to house N-acetylglucosamine (GlcNAc)<sup>1</sup>-1-phosphodiester  $\alpha$ -N-acetylglucosaminidase (18), an enzyme involved in the construction of mannose 6-phosphate (man6P) residues on lysosomal enzyme oligosaccharide side chains. The medial Golgi houses GlcNAc transferase I (12), and galactosyltransferase is located predominantly in *trans*-Golgi cisternae (37). Since proteins pass through the Golgi by a series of vesicular transfers (39) from the *cis*- to the medial to the *trans*-Golgi (3, 42), the assembly of N-linked oligosaccharide chains is regulated by the order in which proteins gain access to particular mannosidases and glycosyltransferases (27). Beyond oligosaccharide assembly, the significance of Golgi compartmentation is not fully understood.

It has recently been reported that the organization of the Golgi stack is severely altered in cells treated with the fungal metabolite, brefeldin-A (BFA). Individual Golgi cisternae become difficult to identify by immunocytochemistry (13, 28, 45), and the export of proteins from the ER is blocked (13, 31, 32). Remarkably, enzymes that normally reside in the Golgi complex are relocated to the ER, where they act upon proteins accumulated there (9, 28, 29). Furthermore, the redistribution of Golgi enzymes to the ER is entirely reversible; after removal of BFA, the Golgi stack reassembles and transport through the secretory pathway resumes (9, 13, 28, 29, 32).

Lippincott-Schwartz et al. (28) and Doms et al. (9) noted that proteins retained within the ER of BFA-treated cells appear not to acquire sialic acid residues, despite the conversion of their N-linked oligosaccharides from high-mannose to complex-type structures. The lack of sialic acid addition suggested that enzymes in the *trans*-Golgi and/or *trans*-Golgi network (TGN) might not relocate to the ER, unlike enzymes that reside in earlier Golgi compartments. To further investigate this potential distinction between early and late Golgi subcompartments, we set up a sensitive assay to test whether galactosyltransferase and sialyltransferase return to the ER in BFA-treated CHO cells. Consistent with analyses of other glycoproteins (9, 28), we found that mannose 6-phos-

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1. *Abbreviations used in this paper:* BFA, brefeldin A; dMM, deoxymannojirimycin; GlcNAc, N-acetylglucosamine; man6P, mannose 6-phosphate; TGN, *trans*-Golgi network.

phate receptors, when pulse-labeled in the ER of BFA-treated cells, readily acquired N-linked galactose, yet only rarely acquired sialic acid residues. We show here that the failure of proteins to acquire sialic acid is most likely due to a failure of sialyltransferase to relocate to the ER, rather than an inability of the enzyme to act within the ER. Our results indicate that unlike the *cis*-, medial, and *trans*-Golgi cisternae, the TGN is insensitive to BFA action.

## Materials and Methods

### Materials

Brefeldin-A was either the generous gift of Dr. R. Klausner (National Institutes of Health) or purchased from Epicenter Biotechnology (Madison, WI). RCA-I agarose, 3-[3-(cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS), galactosyltransferase,  $\beta$ -galactosidase, and deoxymanojirimycin (dMM) were from Sigma Chemical Co. (St. Louis, MO). Pentamannosyl phosphate-Sepharose and *Limax flavus* slug lectin-Affigel were prepared as described (17, 34).

### Cells

Chinese hamster ovary (CHO) clone 13, clone 15B, and clone 1021 cells were originally obtained from Dr. S. Kornfeld by Dr. J. Rothman, who provided them to our laboratory. CHO clone 13 cells cannot translocate UDP-galactose into the Golgi and display an apparent defect in galactosyltransferase (6); CHO 15B cells lack GlcNAc transferase I activity (20); clone 1021 cells lack CMP-sialic acid translocase activity, and thus cannot add sialic acid to oligosaccharide chains (6, 8). CHO clone 15B and clone 1021 cell lines were grown as monolayers in  $\alpha$ MEM containing 7.5% fetal calf serum and antibiotics. CHO wild-type and clone 13 cells were grown in suspension culture in the same media.

### Metabolic Labeling

Cells were labeled with [<sup>35</sup>S]methionine and cysteine (Translabel; Amersham Corp., Arlington Heights, IL) at 0.1 mCi/ml in  $\alpha$ MEM lacking cysteine and methionine but containing 10% dialyzed FCS. Chase periods were initiated by washing cells twice in TD (25 mM Tris Cl, pH 7.4, 5.4 mM KCl, 137 mM NaCl, 0.3 mM Na<sub>2</sub>HPO<sub>4</sub>), followed by addition of complete media. After the times indicated, cells were washed once in ice-cold TD and lysed with 1.0 ml RIPA buffer (50 mM Tris HCl pH 7.2, 0.15 M NaCl, 1% Triton X-100, 1% deoxycholate, 0.1% SDS, and 0.1% gelatin). Lysates were centrifuged at 330,000 g for 10 min in a centrifuge (model TL-100; Beckman Instruments, Fullerton, CA). Suspension cells were labeled at a density of  $\sim 2 \times 10^5$ /ml.

### Lectin Chromatography

300-kD mannose 6-phosphate receptors were isolated from cell extracts by pentamannosyl-phosphate Sepharose chromatography (34). Receptors isolated from the equivalent of  $\sim 125 \mu$ g cell extract were then incubated with 100  $\mu$ l RCA-I agarose in a total volume of 1 ml, 50 mM Hepes pH 7.5, 150 mM NaCl, 0.5% CHAPS, 5 mM  $\beta$ -glycerophosphate for 30 min at room temperature. The slurry was poured into a column, washed with 3 ml of the same buffer (except that it contained 0.05% Triton X-100 instead of CHAPS), and eluted with 1 ml of the former buffer containing 0.1 M galactose. Slug lectin chromatography was carried out according to Goda and Pfeffer (17). Samples were then precipitated in TCA, electrophoresed in 6% SDS polyacrylamide gels, dried, and autoradiographed as previously described (34).

### Cycloheximide Treatment

CHO clone 1021 cells were metabolically labeled for 60 min in the presence of 10  $\mu$ g/ml BFA, and then chased for 4 h in the presence or absence of 100  $\mu$ g/ml cycloheximide, shown previously to block protein synthesis by 98% (34). Samples were then analyzed as described in Fig. 1 and above.

## Surface Labeling of CHO Clone 13 Cells and Glycopeptide Analysis

Cell surface man6P receptor oligosaccharides were labeled with UDP-[<sup>3</sup>H]Gal (Amersham Corp.) and galactosyltransferase according to Duncan and Kornfeld (10). Cells were then incubated at 37°C (in 100 ml suspension culture) for 0 or 6 h in the presence or absence of 10  $\mu$ g/ml BFA. Man6P receptors were isolated by pentamannosyl phosphate-Sepharose chromatography (34). Purified receptors were digested with pronase (CalBiochem-Behring Corp., La Jolla, CA), and the resulting glycopeptides were subjected to jack bean  $\beta$ -galactosidase digestion and Sephadex G-25 chromatography to assess sialic acid acquisition (10).

### Other Techniques

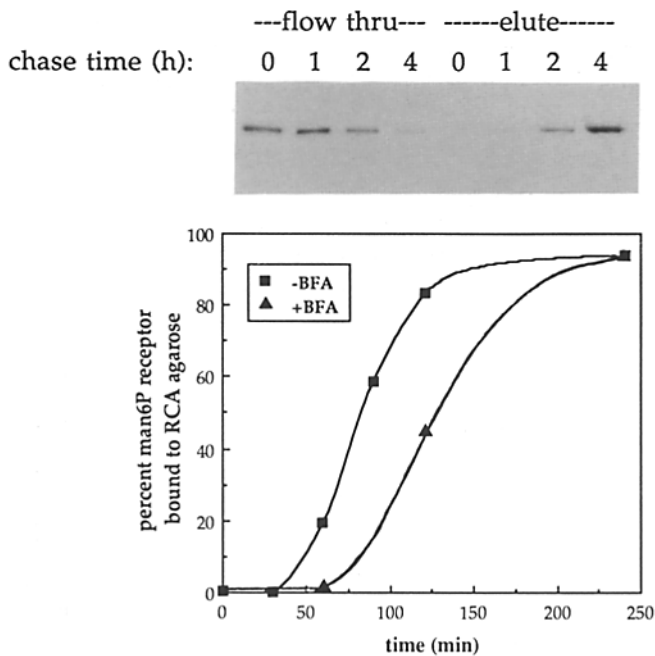
Protein was determined according to the method of Bradford (5) using BSA as standard. Autoradiograms were quantified using a densitometer (model 300A; Molecular Dynamics, Sunnyvale, CA). Neuraminidase treatment of man6P receptors was carried out using *Arthrobacter ureafaciens* neuraminidase as described (17).

### Results

If cells are treated with 10  $\mu$ g/ml BFA, proteins within the ER acquire endoglycosidase H-resistant oligosaccharides (9, 28). Since resistance to endoglycosidase H reflects the concerted action of the medial Golgi enzymes, GlcNAc transferase I and Golgi mannosidase II (27), these data suggest that resident enzymes of the medial Golgi return to the ER upon BFA treatment. Indeed, the redistribution of mannosidase II has been shown directly (28). We tested whether galactosyltransferase, a *trans*-Golgi enzyme (37), and sialyltransferase, an enzyme localized to both the *trans*-Golgi and the TGN (38; see also reference 2), also return to the ER in the presence of BFA.

The 300-kD man6P receptor was used as a marker for the action of galactosyl- and sialyltransferases. This receptor provides a sensitive means to detect glycosyltransferase action, since it contains 19 potential N-linked oligosaccharide addition sites (30) and is highly glycosylated. The experiment was carried out as follows. CHO cells were labeled with [<sup>35</sup>S]methionine and cysteine for 60 min to label newly synthesized man6P receptors. At this time, labeled man6P receptors reside in the ER, since these receptors require up to 3 h to fold and be completely exported from this compartment (19, 40). Cells were then chased for various times in the presence of BFA, and the potential acquisition of galactose residues was determined by affinity chromatography of isolated man6P receptors on columns of the galactose-specific lectin, RCA-I (1). To further increase the sensitivity of the assay, we used CHO clone 1021 cells that lack apparent sialyltransferase activity (6, 8). In these cells, any added galactose residues will be present at the termini of N-linked oligosaccharides and fully accessible for optimal lectin binding (1).

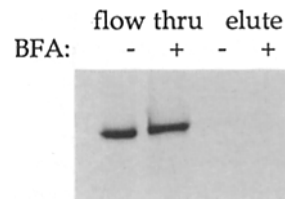
Fig. 1 (*top*) shows the results obtained from such an experiment. Man6P receptors isolated from cells immediately after the labeling period should not have contained galactose. As expected, none of the man6P receptors bound to RCA-I agarose; all were recovered in the flowthrough fraction. In contrast, with increasing times of BFA treatment, man6P receptors acquired the ability to be retained on an RCA-I column, and could be eluted from such columns with galactose. Quantitative analysis of this experiment (Fig. 1, *bottom*) showed that half of the man6P receptors gained the



**Figure 1.** Man6P receptors acquire galactose within the ER of BFA-treated cells. (Top) CHO clone 1021 cells were labeled with [<sup>35</sup>S]methionine and cysteine for 60 min in the presence of 10 μg/ml BFA, and then chased in growth media containing BFA for the indicated times. Man6P receptors were then isolated by affinity chromatography and applied to RCA-I agarose. Fractions that flowed through the column (left) or were eluted with 0.1 M galactose (right) were subjected to SDS-PAGE and autoradiography. (Bottom) Kinetics of galactose addition in the presence or absence of BFA. Data points were obtained by densitometric scanning of the autoradiogram shown or from another obtained in an experiment in which BFA was omitted from the labeling and chase media. Triangles, +BFA; squares, -BFA.

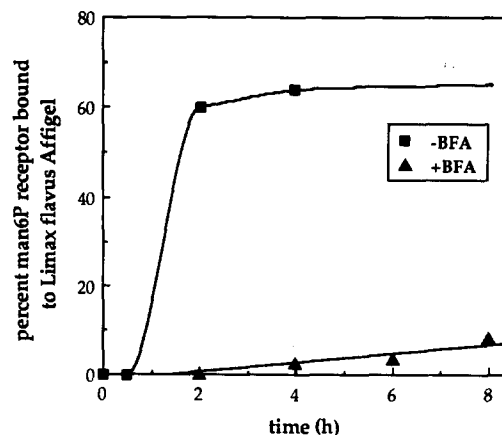
capacity to bind RCA-I agarose within ~2 h. This was somewhat slower than the rate observed in the absence of BFA ( $t_{1/2} = 80$  min). A control experiment showed that galactose addition was not due to the action of newly synthesized galactosyltransferase, also accumulated in the ER, since cycloheximide had essentially no effect on the extent of galactose addition (not shown).

RCA-I lectin binds to galactose residues present on both N- and O-linked oligosaccharides (1). To determine if the binding observed was due to the addition of N-linked galactose residues, we carried out a parallel experiment using CHO clone 15B cells that lack the activity of the medial Golgi enzyme, GlcNAc transferase I (20). In these cells,



**Figure 2.** Man6P receptors fail to acquire galactose in cells lacking GlcNAc transferase I. CHO clone 15B cells were metabolically labeled for 60 min in the presence or absence of 10 μg/ml BFA and then chased in growth media in the presence or absence of BFA

for 3 h. Man6P receptors were then isolated by affinity chromatography and analyzed by RCA-I chromatography as in Fig. 1.

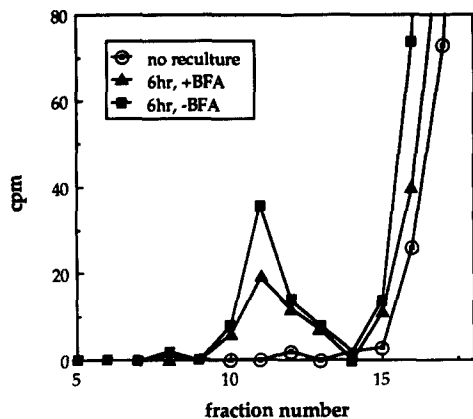


**Figure 3.** Man6P receptors acquire limited sialic acid within the ER of BFA-treated cells. CHO wild type cells were metabolically labeled for 60 min in the presence or absence of 10 μg/ml BFA and then chased in the presence or absence of BFA for the indicated times. Fresh BFA was added after 4 h to ensure its continued action (13). Man6P receptors were then isolated by affinity chromatography and applied to slug lectin-Affigel columns. The percentage of man6P receptors eluted from such columns was determined by densitometric scanning of autoradiograms as in Fig. 1.

galactose cannot be added to N-linked oligosaccharides because the oligosaccharides lack penultimate GlcNAc. O-linked sugar assembly is unaffected, since the O-linked sugar acceptor would be GalNAc, rather than GlcNAc. As shown in Fig. 2, man6P receptors isolated from CHO clone 15B cells, incubated in the presence or absence of BFA for 3 h, did not bind to RCA-I columns. Failure of these receptors to bind to RCA-I columns was not due to the presence of sialic acid-blocked, O-linked galactose, because neuraminidase treatment did not increase the RCA-I binding capacity of the isolated receptors (not shown). We conclude that man6P receptors within the ER acquire N-linked galactose in BFA-treated CHO cells. This is consistent with recent results of Lippincott-Schwartz et al. (29) who used indirect immunofluorescence to show the essentially quantitative redistribution of galactosyltransferase to the ER in BFA-treated cells.

#### The Fate of Sialyltransferase in the Presence of BFA

Sialic acid addition was monitored in BFA-treated, CHO wild-type cells, using the sialic acid-specific lectin from the slug, *Limax flavus*. Fig. 3 shows the kinetics with which man6P receptors acquired the capacity to bind to slug lectin columns and be eluted by excess sialic acid. In the absence of BFA, man6P receptors acquired sialic acid during their transit through the Golgi complex. Sialic acid addition was extensive after 2 h of chase, and by 4 h, 66% of newly synthesized man6P receptors had acquired the ability to bind to slug lectin-Affigel, as we have previously shown (17). In the presence of BFA, <10% of total man6P receptor molecules bound to slug lectin-Affigel after 8 h of incubation (Fig. 3). This small amount of sialic acid addition was not due to newly synthesized sialyltransferase because it was also observed in the presence of cycloheximide (not shown). The electrophoretic mobility of the sialic acid-containing man6P



**Figure 4.** Gel filtration analysis of man6P receptor resialylation. CHO clone 13 cells were surface labeled with UDP-[<sup>3</sup>H]galactose and galactosyltransferase and then harvested immediately (*open circles*) or recultured for 6 h in the presence (*triangles*) or absence (*squares*) of 10  $\mu$ g/ml BFA. Man6P receptors were then isolated, digested with pronase to prepare glycopeptides, and then further digested with  $\beta$ -galactosidase to cleave [<sup>3</sup>H]galactose from oligosaccharides that had not received sialic acid. Cleavage products were resolved by Sephadex G25 chromatography; fractions eluting at the void volume (which resisted  $\beta$ -galactosidase cleavage and thus contain sialic acid) are shown. The counts per minute in the excluded peak of the control reaction (-BFA) represents 4% of the total glycopeptide radioactivity recovered. In other experiments, as much as 6% resialylation was observed.

receptors was faster in BFA-treated cells (not shown), indicating that these receptors contained significantly fewer sialic acid residues than native man6P receptors (17). These experiments indicate that either a very limited amount of sialyltransferase activity was present in the ER after 8 h with BFA, or alternatively, that the bulk of sialyltransferase activity was not redistributed to the ER after 8 h. It is important to note that sialyltransferase could have been redistributed to the ER in BFA, but might not have been able to function there.

Under normal conditions, man6P receptors recycle from the cell surface to the TGN, where they come into contact with sialyltransferase (10, 17, 26, 35), but not galactosyltransferase (10). We reasoned that if sialyltransferase failed to return to the ER in BFA, this enzyme should remain in the TGN, and thus be able to act upon man6P receptors arriving in that compartment. To test this, we used an experimental scheme devised by Duncan and Kornfeld (10) to measure the transport of man6P receptors from the cell surface back to the TGN. The approach uses CHO clone 13 cells, in which glycoproteins lack galactose residues and thus can be labeled at the plasma membrane using galactosyltransferase and UDP-[<sup>3</sup>H]galactose. The transport of man6P receptors from the surface to the TGN is then monitored by the addition of sialic acid residues to their <sup>3</sup>H-labeled oligosaccharide side chains (10).

CHO clone 13 cells were surface labeled at 4°C and warmed for either 0 or 6 h in the presence or absence of BFA. At the end of the incubation, man6P receptors were isolated and incubated with pronase to obtain glycopeptides. Sialic acid acquisition was then determined by digestion with  $\beta$ -galactosidase and gel filtration chromatography. If an oligosac-

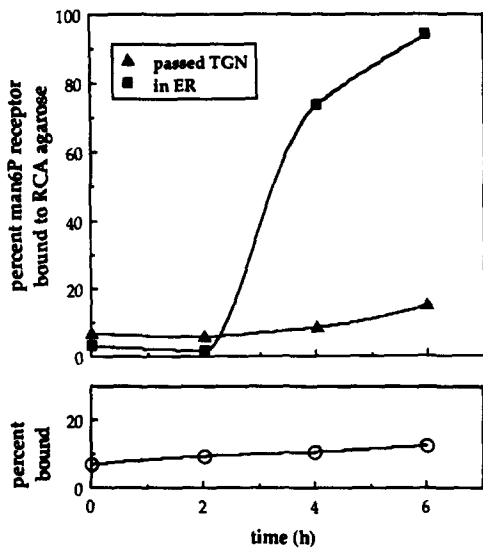
charide received sialic acid, the <sup>3</sup>H-labeled glycopeptide would resist  $\beta$ -galactosidase cleavage and be excluded from Sephadex G25. In contrast, digestion of galactose-terminating oligosaccharides would yield [<sup>3</sup>H]galactose, which would be included in the gel filtration column (10).

When cells were harvested immediately after surface labeling, essentially no radioactivity was found in excluded column fractions (Fig. 4, *open circles*), as would be expected, since at this time, man6P receptor glycopeptides should not contain sialic acid. In contrast, sialic acid addition was readily observed when cells were recultured for 6 h in either the presence or the absence of BFA, as monitored by the presence of <sup>3</sup>H-glycopeptides in the void volume fraction (Fig. 4, *solid symbols*). Although the extent of sialylation was reproducibly lower (~65%) in the presence of BFA than in its absence, significant addition of sialic acid was detected under both conditions. The lower level of sialylation observed in BFA may be explained, in part, by a 20% decrease in the rate of endocytosis observed in the presence of 10  $\mu$ g/ml BFA (32). In summary, this experiment demonstrates that at least a large proportion of sialyltransferase activity was present in a compartment reached by cell surface man6P receptors in BFA-treated cells. This compartment cannot represent the ER, since man6P receptors pulse-labeled there were not modified by sialyltransferase (Fig. 3).

We also used the man6P receptor as an additional marker to test whether transient occupants of the TGN return to the ER in BFA. CHO cells were metabolically labeled and chased for 4 h to permit newly synthesized man6P receptors to achieve their steady-state distribution, primarily in endosomes, the plasma membrane, and the TGN (7, 15, 16, 22). The reversible inhibitor of Golgi  $\alpha$ -mannosidase I, dMM (4), was included in the labeling and chase media, so that man6P receptors within and beyond the TGN would bear high-mannose oligosaccharides. Next, dMM was washed away, and cells were incubated for various lengths of time in the presence or absence of BFA. We then monitored the potential galactosylation of man6P receptors that would occur if they returned to the ER.

In the absence of BFA, high mannose oligosaccharide-containing man6P receptors within and beyond the TGN would not be expected to gain access to the mannosidases and glycosyltransferases that lie proximal to the TGN (10). This was verified by isolating man6P receptors after various chase intervals and subjecting them to RCA-I chromatography. Fig. 5 (*bottom*) presents the results of such an experiment. In the absence of BFA, ~7% of the man6P receptors bound to RCA-I at time zero, showing that the dMM was >90% effective in inhibiting  $\alpha$ -mannosidase I. After a chase period of up to 6 h, there was only a very slight increase (~5%) in the fraction of man6P receptors that bound to an RCA-I column. These results confirm that the majority of CHO cell man6P receptors do not appear to return to the site of Golgi  $\alpha$ -mannosidase I (*cis*/medial Golgi) after transport to the TGN (10).

Fig. 5 (*top*) shows the results obtained if man6P receptors were pulse-labeled either in the ER (*squares*) or chased to compartments beyond the TGN (*triangles*) in the presence of dMM, and then further chased for various times without dMM, but in the presence of BFA. If the receptors were present in the ER, they were efficiently modified by galactosyltransferase. The rate of galactose addition was slightly



**Figure 5.** The majority of man6P receptors within and beyond the TGN do not return to the ER upon BFA addition. CHO clone 1021 cells were pretreated with 1 mM dMM for 30 min and metabolically labeled for 60 min in the presence of dMM. Cells were then chased in growth media for 3 h without dMM to allow man6P receptors to move out of the ER and into the TGN and late endosomes. Cells were then chased further in the presence (*top*, triangles) or absence (*bottom*, open circles) of 10  $\mu$ g/ml BFA. Alternatively, cells were not chased before BFA addition to determine the fate of ER-localized man6P receptors (*squares*). Galactose acquisition was determined as in Fig. 1.

slower than that observed in the absence of dMM pretreatment (Fig. 1), probably due to the time required to reverse the effect of dMM (4, 10, 33). On the other hand, if the receptors were permitted to pass through the TGN prior to the addition of BFA, only a very small increase was detected in the amount of man6P receptors containing galactose, even after 6 h of incubation (Fig. 5, triangles). The small increase observed ( $\sim 8\%$ ) was very close to that observed in the absence of BFA (Fig. 5, bottom).

Duncan and Kornfeld have shown that only  $\sim 10\%$  of man6P receptors labeled at the surface of CHO cells can be detected to acquire sialic acid upon their return to the TGN after 6 h of reculture (10). In our hands, only  $\sim 4\text{--}6\%$  of the surface-labeled receptor acquired sialic acid after 6 hours (Fig. 4). If this value represents the total amount of man6P receptor transiting through the TGN in 6 h, it would be difficult to detect the potential return of such a small quantity of man6P receptors from the TGN to the ER, especially with the  $\sim 2\text{-h}$  lag for galactose addition in that compartment (Fig. 5). However, results identical to those shown in Fig. 5 were obtained using BW5147 cells, in which 40% of surface-labeled man6P receptors return to the TGN in 3 h (10). This strongly supports the conclusion that the majority of man6P receptors within and beyond the TGN do not return to the ER in BFA.

## Discussion

We have shown that the 300-kD man6P receptor, when pulse-labeled within the ER of BFA-treated cells, acquires numerous N-linked galactose residues and many fewer sialic

acid residues. These data confirm the findings of Lippincott-Schwartz et al. (29) who have used immunofluorescence to show that the *trans*-Golgi enzyme, galactosyltransferase, returns to the ER in the presence of BFA. In addition, our experiments suggest that sialyltransferase has a different fate in BFA than galactosyltransferase, since the half time for sialic acid addition to proteins within the ER ( $\sim 60$  h) was 30 times greater than that measured for galactose addition. The simplest explanation for these findings would be that sialyltransferase, a component of the TGN, is not redistributed to the ER in BFA, while the *trans*-Golgi enzyme, galactosyltransferase, is. If true, this supports the proposal that the *trans*-Golgi and TGN are distinct compartments (21).

The strongest line of evidence supporting the idea that the TGN is insensitive to BFA comes from an experiment in which we monitored the transport of surface-labeled man6P receptors from the surface back to the TGN. Man6P receptors were transported to a compartment housing active sialyltransferase, whether or not the culture media contained BFA. Moreover, this compartment was not the ER, since man6P receptors pulse-labeled within the ER did not receive sialic acid. Unfortunately, our attempts to localize sialyltransferase by indirect immunofluorescence under these conditions were unsuccessful, probably due to the particularly low abundance of this enzyme in cultured cells. Nevertheless, although not shown directly, it is most likely that the sialyltransferase-containing compartment represents the TGN.

Ulmer and Palade have recently shown that the protein, glycoporphin, acquired O-linked sialic acid when present in the ER of BFA-treated erythroleukemia cells (45). However, these workers noted that sialylation was not complete, even after 6 h. The O-linked oligosaccharides of glycoporphin contain sialic acid both in  $\alpha\text{-}2,3$  linkage to galactose, as well as in  $\alpha\text{-}2,6$  linkage to GalNAc (43). If the GalNAc-specific sialyltransferase is located in the *trans*-Golgi along with galactosyltransferase, whereas the Gal-specific sialyltransferase is located primarily in the TGN, only the GalNAc-specific enzyme would redistribute to the ER in BFA. Under these conditions, O-linked oligosaccharides would become partially sialylated, while N-linked sugars would not. If this is correct, the findings of Ulmer and Palade (45) would be entirely consistent with those reported here and elsewhere (9, 28).

Several models have been proposed to explain BFA action. One possibility is that the target of BFA is associated with the ER. By blocking ER export, BFA might be uncovering a vesicular recycling pathway that functions between the Golgi and ER (9, 28, 29). This model could easily explain why most Golgi enzymes are redistributed to the ER, if one assumes that each cisterna of the Golgi complex communicates with its proximal neighbor by shuttling transport vesicles. This model does not explain why TGN components do not return to the ER, because putative shuttling vesicles might be expected to move between the TGN and *trans*-Golgi as frequently as they traverse between *trans*- and medial Golgi cisternae. If the target of BFA is indeed the ER, our data would suggest that transport between the TGN and *trans*-Golgi cisternae differs significantly from transport between more proximal Golgi cisternae.

Another possibility is that BFA acts on the Golgi complex and interferes with the mechanism whereby Golgi compartmentation is maintained (9, 28, 29). This model would re-

quire that *cis*-, medial and *trans*-Golgi cisternae be related by a common compartmentation machinery that would not apply to the TGN. Whatever mechanism BFA uses to block ER export and disrupt Golgi organization, the apparent insensitivity of the TGN to BFA action highlights a fundamental difference between this compartment and more proximal Golgi cisternae.

Unlike the *cis*-, medial, and *trans*-Golgi cisternae, the TGN functions to sort proteins into distinct classes of transport vesicles, bound for prelysosomes (10), secretory storage granules (44), and specific domains of the plasma membrane (14, 36). The TGN is the site to which man6P receptors recycle (10, 17, 26, 35), and where viral glycoproteins accumulate at 20°C (24, 41). The TGN is also a much more extended and tubular structure than the other Golgi cisternae, and thus, it is distinguished by its unique morphological characteristics. Despite numerous distinctions between the TGN and other Golgi cisternae, it is likely that the TGN is nevertheless, an integral part of the Golgi complex. Griffiths et al. (25) have shown that the TGN and more proximal Golgi cisternae are highly interrelated structures. At 20°C, the TGN increases in size, concomitant with a decrease in size of preceding Golgi compartments (25). In addition, these workers have calculated that 12% of the TGN surface area is comprised of flattened cisternae which are morphologically indistinguishable from, and located immediately adjacent to, the other cisternae of the Golgi stack. Thus, although the TGN is functionally distinct from other Golgi cisternae (21), it is appropriate to consider it as a specialized subcompartment of the Golgi complex. An important future challenge will be to elucidate the mechanism by which the TGN gains (and maintains) its identity.

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