Proc. Natl. Acad. Sci. USA
Vol. 91, pp. 2708–2712, March 1994
Cell Biology

Transport of newly synthesized glucosylceramide to the plasma membrane by a non-Golgi pathway

DALE E. WARNOCK*, MALLORY S. LUTZ†, WENDY A. BLACKBURN†, WILLIAM W. YOUNG, JR.†, AND JACQUES U. BAENZIGER*

*Department of Pathology, Washington University School of Medicine, St. Louis, MO 63110; and †Departments of Biological and Biophysical Sciences and Biochemistry, Health Sciences Center, University of Louisville, Louisville, KY 40292

Communicated by Gilbert Ashwell, December 7, 1993

ABSTRACT **High-gradient magnetic affinity chromatography (HIMAC) has been used to obtain highly enriched plasma membranes, free of intracellular membrane contaminants, from cultured Chinese hamster ovary (CHO) cells in yields of ≥80%. Using this procedure we have characterized the transport of glucosylceramide (GlcCer) and the ganglioside GM3 to the plasma membrane. Newly synthesized GlcCer reaches the plasma membrane in 7.2 min, whereas GM3 requires 21.5 min to reach the plasma membrane. Brefeldin A prevents transport of newly synthesized GM3 and sphingomyelin to the plasma membrane but has no effect on the transport of GlcCer. Similarly, incubation of CHO cells at 15°C blocks transport of GM3 and sphingomyelin to the plasma membrane but has no effect on GlcCer movement. We propose that carrier-mediated transport accounts for a major fraction of the plasma membrane GlcCer. Pulse–chase studies with either [^{3}H]glucose or [^{3}H]palmitate indicate that newly synthesized GlcCer which has reached the plasma membrane is not utilized for the synthesis of GM3 but is instead rapidly either degraded or converted into an as yet unidentified product. Our results indicate that in addition to serving as a precursor for higher glycosylation in the Golgi, a major fraction of newly synthesized GlcCer is rapidly transported to the plasma membrane by a non-Golgi pathway and then rapidly turned over.**

Three distinct mechanisms for the movement of lipids from their sites of synthesis to the plasma membrane have been described in animal cells (reviewed in ref. 1). (*i*) The vesicular protein secretory pathway has been shown to target endogenous sphingomyelin (2) and ganglioside GM3 (3) as well as short-chain derivatives of sphingomyelin and glucosylceramide (GlcCer) (4–6) to the plasma membrane. (*ii*) Soluble carriers such as lipid-transfer proteins account for a major portion of the movement of the phospholipids phosphatidylcholine (7) and phosphatidylethanolamine (8, 9) to the plasma membrane. (*iii*) A vesicular transport pathway which is distinct from the secretory pathway accounts for the transport of newly synthesized cholesterol (10) to the plasma membrane. Transfer of glucose to ceramide by UDPglucose–ceramide glucosyltransferase has been localized to the cytosolic surface of the Golgi apparatus (11–14), indicating that GlcCer must be translocated to the luminal leaflet for higher glycosylation to occur. We have determined that 45% of GlcCer in Chinese hamster ovary (CHO) cells is located in the plasma membrane (15). Since GlcCer is synthesized on the cytosolic surface of the Golgi membrane and translocated to the luminal surface, GlcCer could be transported to the plasma membrane through the vesicular protein secretory pathway and/or by an alternative pathway through the cytosol, perhaps by lipid-transfer proteins as proposed by Sasaki (16). We have addressed this possibility in CHO cells, which have a simple glycolipid pattern consisting of GlcCer, lactosylceramide (LacCer), and GM3. Our results indicate that newly synthesized GlcCer is transported to the plasma membrane through the cytosol by a mechanism which is independent of the vesicular pathways used for the transport of proteins and cholesterol.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

EXPERIMENTAL PROCEDURES

Materials. Unless otherwise specified, chemicals and chromatography reagents were purchased from Sigma. Sulfosuccinimidyl-3-(4-hydroxyphenyl)propionate (sulfo-SHPP) was from Pierce. Brefeldin A (BFA) was from Epicentre Technologies (Madison, WI). Trans^{35}S-label ([^{35}S]methionine/[^{35}S]cysteine, 1 mCi/mmol; 1 Ci = 37 GBq) was from ICN. [^{3}H]Palmitate and [^{3}H]glucose were from DuPont/NEN.

Cell Culture. CHO and ldlD cells were grown in α minimal essential medium (α-MEM) supplemented with 10% (vol/vol) heat-inactivated fetal bovine serum. CHO K1 cells were maintained in Dulbecco's modified Eagle's medium (DMEM) containing 10% iron-supplemented heat-inactivated fetal bovine serum, 2 mM glutamine, 1× nonessential amino acids, 1 mM proline, and 1× penicillin/streptomycin. Cells were released by trypsin/EDTA treatment and plated on fresh culture dishes 48 hr prior to experimental manipulation.

Metabolic Labeling. For pulse–chase experiments, cells were rinsed three times in Dulbecco's phosphate-buffered saline (PBS) and incubated for 1 hr at 37°C in methionine- and cysteine-free medium. The medium was replaced with fresh methionine- and cysteine-free medium containing Tran^{35}S-label at 150 μCi/ml. After 20 min of incubation, incorporation was terminated by washing twice with PBS and the chase was initiated by addition of complete medium. For metabolic labeling of lipids [^{3}H]palmitate [10 μCi/ml in α-MEM with 0.01% (wt/vol) defatted bovine serum albumin (dialyzed against α-MEM)] was added to cells. After 3 hr, the cells were rinsed with ice-cold binding buffer (0.14 M NaCl/5.4 mM KCl/0.33 mM Na_2HPO_4/0.34 mM KH_2PO_4/0.8 mM $MgSO_4$/2.7 mM $CaCl_2$/20 mM Hepes, pH 7.4) and membranes were prepared for isolation by high-gradient magnetic affinity chromatography (HIMAC). For pulse–chase experiments with ldlD cells, the plates were rinsed twice in glucose-free α-MEM and incubated for 1 hr at 37°C in glucose-free α-MEM. The medium was replaced with fresh, temperature-equilibrated, glucose-free α-MEM containing [^{3}H]glucose (25 μCi/ml) and the cells were allowed to incorporate label for the indicated times. Incorporation was terminated by washing the cells twice with α-MEM and the chase was initiated by addition of complete α-MEM containing 10% fetal bovine serum, 20 μM galactose, and 200 μM *N*-acetylgalactosamine.

Abbreviations: BFA, brefeldin A; GlcCer, glucosylceramide; LacCer, lactosylceramide; HIMAC, high-gradient magnetic affinity chromatography; FeDex, dextran-coated iron particles; WGA, wheat germ agglutinin.

HIMAC. HIMAC was performed as described (17) with wheat germ agglutinin (WGA) coupled to superparamagnetic colloidal iron particles coated with dextran (WGA/FeDex) (17).

Enzyme and Protein Assays. Protein determinations and enzyme assays were performed as described (15).

Immunoprecipitations and Western Blots. Fibronectin subunits were immunoprecipitated from cell homogenates with monoclonal antibody 16, specific for human integrin $\alpha 5$ and analyzed by SDS/PAGE as described (18).

Lipid Analysis. Membrane pellets were extracted twice on ice with at least 20 volumes of $CHCl_3$/MeOH, 2:1 (vol/vol), followed by $CHCl_3$/MeOH, 1:2 (vol/vol), twice in an Omni-Mixer (Omni International, Norwalk, CT). The phospholipids of crude lipid extracts were analyzed by two-dimensional thin-layer chromatography (TLC) (15). The glycosphingolipids from a portion of [^{3}H]palmitate-labeled lipid extracts were purified by an acetylation procedure (15). Samples were analyzed on high-performance TLC plates (Merck) in the solvent $CHCl_3$/MeOH/H_2O, 62:30:6 (vol/vol). The solvent Et_2O/MeOH, 99:1 (vol/vol), was used for ceramide quantitation of crude lipid extracts (19). Plasma membrane GM3 was identified by cell surface periodate oxidation followed by derivatization of the lipid extract with dinitrophenylhydrazine (3). Radiolabeled TLC spots were located with a linear analyzer (EG & G Berthold, Salem, MA) and quantitated by scraping into scintillation vials and assaying in the presence of 10 ml of BudgetSolv scintillation fluid. Incorporation of [^{3}H]palmitate into LacCer was not sufficient to allow for its quantitation in these studies. To determine the time intervals between addition of [^{3}H]palmitate to the cells and appearance of labeled GlcCer and GM3 in the plasma membrane, these data were analyzed by linear regression analysis using INPLOT software (GraphPad, San Diego).

RESULTS

Newly Synthesized GlcCer and GM3 Are Transported to the Plasma Membrane with Different Kinetics. WGA/FeDex can be used in conjunction with HIMAC to prepare highly enriched plasma membranes from cultured cells with yields of 80–90%. Previously, we used HIMAC to determine the distribution of glycosphingolipids in CHO cell plasma membranes (15). We have now examined the kinetics of GlcCer and GM3 transport to the plasma membrane, using the same method. Subconfluent CHO cells were incubated with [^{3}H]palmitate, and plasma membranes were isolated by HIMAC using WGA/FeDex. Lipid extracts of the plasma membrane fractions were analyzed by TLC to determine the incorporation of [^{3}H]palmitate into GlcCer and GM3 at each time point (Fig. 1). Extrapolation of the results from three separate experiments, including the one shown in Fig. 1, indicated that GM3 first appeared in the plasma membrane at 21.5 ± 2.3 min (mean ± SE), whereas GlcCer appeared at 7.2 ± 1.0 min. After their initial appearance in the plasma membrane, the amounts of labeled GlcCer and GM3 continue to increase linearly for at least 6 hr at 37°C. The ratio of GlcCer to GM3 in the plasma membrane after 90 min of labeling was ≈1.7, whereas we had previously reported a ratio of ≈4.0 for whole cells (3). This difference in ratios is consistent with the greater enrichment of GM3 in the plasma membrane as compared with GlcCer (Table 1).

Since we previously observed that incorporation of [^{3}H]palmitate into GlcCer began immediately following addition of label to CHO cells (3), the 7-min interval required for GlcCer to reach the plasma membrane must reflect the time required for transport. The kinetics for transport of newly synthesized GM3 to the cell surface are consistent with our previous results, which indicated that GM3 reaches the plasma membrane through the same vesicular transport system used by newly synthesized glycoproteins (3). The more rapid transport of GlcCer to the plasma membrane indicates that GlcCer either must reach the plasma membrane by a different mechanism or must traverse the Golgi more rapidly than GM3. The GlcCer present in the plasma membrane fraction is not likely to reflect contamination with intracellular membranes, as we previously demonstrated that <5% of endoplasmic reticulum markers and <13% of Golgi markers were present in these plasma membrane fractions. Further, at steady state the proportion of GlcCer in the retained fraction was considerably less than the proportion of GM3: 40% and 58%, respectively (Table 1).

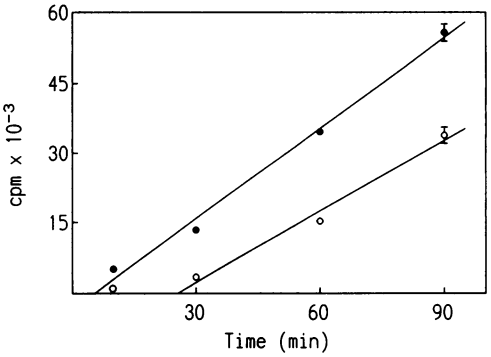

FIG. 1. Time course for transport of newly synthesized GlcCer and GM3 to the plasma membrane. CHO cells were incubated with [^{3}H]palmitate (10 μCi/ml) in α-MEM/10% fetal bovine serum at 37°C for the times indicated. One 150-mm culture dish was used for each time point. Incorporation was terminated by washing twice with binding buffer at 4°C and incubating with WGA/FeDex (1 μg/ml) for 1 hr at 4°C. Unbound WGA/FeDex was removed by washing the cells twice in ice-cold binding buffer and plasma membrane was isolated by HIMAC. The amount of GlcCer (●) and GM3 (○) in the retained, plasma membrane fractions was analyzed by TLC. Each point indicates the cpm in the retained fraction and represents the mean ± SEM of duplicate TLC analyses.

Transport of GM3 and GlcCer to the Plasma Membrane Is Differentially Affected by BFA and Reduced Temperature. We examined the mechanism by which GlcCer is transported to

Table 1. Distribution of lipids in the plasma membrane in the presence of BFA and at 15°C

Lipid†	% retained		
	Control	+ BFA	15°C
GM3	58.1 ± 1.0	23.3 ± 2.5**	ND
GlcCer	40.4 ± 4.1	36.0 ± 2.7	45.7 ± 2.4
Cer	22.7 ± 3.8	20.6 ± 1.3	28.6 ± 3.9
SM	62.0 ± 2.4	29.0 ± 2.7**	36.4 ± 5.2**
PC	36.9 ± 4.0	27.3 ± 1.7*	33.0 ± 4.5
PI	31.1 ± 3.0	26.2 ± 1.3	32.5 ± 3.6
PS	64.7 ± 3.7	48.5 ± 4.3*	46.2 ± 4.1**
PE	42.7 ± 3.5	31.6 ± 1.7*	35.7 ± 6.4

Subconfluent CHO cells were labeled for 3 hr with [^{3}H]palmitate (10 μCi/ml) at either 15°C or at 37°C in the presence or absence of BFA (1 μg/ml). Incorporation of label was terminated by washing twice with ice-cold binding buffer. Cells were incubated with WGA/FeDex (1 μg/ml) at 4°C and plasma membranes were isolated by HIMAC. The amounts of [^{3}H]palmitate-labeled phospho- and glycolipids in retained (plasma membrane) and nonretained (intracellular membrane) fractions were determined; % retained = [retained/nonretained + retained)] × 100. Data are presented as mean ± SEM for four separate control experiments, five BFA experiments, and three 15°C experiments. ND, not detectable. *, $P < 0.05$ versus control value; **, $P < 0.01$ versus control value.

†Cer, ceramide; SM, sphingomyelin; PC, phosphatidylcholine; PI, phosphatidylinositol; PS, phosphatidylserine; PE, phosphatidylethanolamine.

the plasma membrane by using two methods known to disrupt vesicular traffic in the protein secretory pathway. BFA blocks the transport of newly synthesized proteins to the plasma membrane (reviewed in ref. 20). We determined the distribution of [^{3}H]palmitate-labeled lipids in plasma membranes (retained) and intracellular membranes (nonretained) isolated from CHO cells after 3 hr of [^{3}H]palmitate incorporation in the presence of BFA at 1 μg/ml (Table 1). BFA markedly reduced the proportion of GM3 and sphingomyelin reaching the plasma membrane and caused a more modest decrease in the proportion of phosphatidylcholine, phosphatidylserine, and phosphatidylethanolamine reaching the plasma membrane. In contrast, BFA had no effect on the distribution of GlcCer, ceramide, or phosphatidylinositol to the plasma membrane.

We confirmed the change in distribution of newly synthesized GM3 following BFA treatment by using the periodate cell surface labeling technique (3). CHO cell monolayers were labeled with [^{3}H]palmitate for 3 hr in the presence or absence of BFA at 1 μg/ml, and sialic acid moieties exposed at the cell surface were selectively oxidized with sodium metaperiodate. After lipid extraction, oxidized GM3 (GM3 aldehyde) was derivatized with dinitrophenylhydrazine, and the product was quantitated by TLC (3). The proportion of newly synthesized GM3 accessible to periodate oxidation at the cell surface was reduced from 46.2 ± 1.8% (mean ± SE for duplicate analyses) in control cells to 10.5 ± 0.2% in BFA-treated cells. This indicated that BFA had reduced the transport of newly synthesized GM3 to the plasma membrane by ≈80%, a figure consistent with the extent to which BFA blocks protein secretion (9).

The effect of BFA on incorporation of [^{3}H]palmitate into lipids is shown in Table 2. Labeling of GM3 increased 1.7-fold compared with controls, even though the proportion of GM3 reaching the plasma membrane was reduced from 58% to 23% (Table 1). Thus, BFA prevents transport but not synthesis of GM3. BFA increased the incorporation of [^{3}H]palmitate into sphingomyelin 3.6-fold (Table 2) and blocked the transport of sphingomyelin to the plasma membrane (Table 1), in agreement with previous reports (2, 21, 22). The effect of BFA on sphingomyelin synthesis and transport is consistent with evidence that sphingomyelin synthesis occurs primarily in the Golgi compartment (23). BFA also increased incorporation of [^{3}H]palmitate into GlcCer >5-fold (Table 2 and refs. 2 and 24). Despite the large increase in GlcCer labeling, the distribution of GlcCer to the plasma membrane is not altered by BFA (Table 1), suggesting a major difference in the trafficking of GlcCer as compared with its final biosynthetic product, GM3, in CHO cells.

Table 2. Incorporation of [^{3}H]palmitate by CHO cells treated with BFA or incubated at 15°C

Lipid*	Control, cpm × 10^{-5}	+ BFA cpm × 10^{-5}	+ BFA % of control	15°C cpm × 10^{-5}	15°C % of control
GM3	2.6	4.3	165	ND	—
GlcCer	2.2	12.4	564	0.8	36
Cer	1.5	4.1	273	1.6	107
SM	25.7	92.6	360	1.2	5
PC	42.5	50.0	118	11.2	26
PI	4.0	7.2	180	2.6	65
PS	2.5	2.1	84	0.3	12
PE	9.2	10.9	118	2.5	27

Incorporation of [^{3}H]palmitate into phospholipids and glycolipids of CHO cells was determined as described in Table 1. Values are expressed as incorporation into total membranes, which was the sum of the retained and nonretained fractions. Data are from the mean of duplicate analyses for one representative experiment. ND, not detectable.

*See footnote to Table 1 for abbreviations.

BFA-mediated disruption of transport through the vesicular transport pathway utilized by proteins destined for the plasma membrane or secretion was confirmed by examining the transport of a newly synthesized integral membrane protein, the recombinant human α5 integrin subunit, to the plasma membrane in the presence and absence of BFA (Fig. 2). When CHO K1 cells expressing recombinant human α5 integrin subunit were labeled with [^{35}S]methionine/cysteine for 20 min and "chased" for 100 min, 75% of the cell-associated integrin α5 subunit was found in the plasma membrane (retained) fraction (Fig. 2*A*). When the same experiment was performed in the presence of BFA at 1 μg/ml, only 13% of the integrin α5 was recovered in the plasma membrane fraction (Fig. 2*B*). Integrin α5 found in the retained fraction was incompletely processed, indicating that this represents contamination from intracellular sources. Thus, BFA effectively prevents transport of integrin α5 to the plasma membrane.

We also examined the effect of reduced temperature on the transport of newly synthesized GM3 and GlcCer to the plasma membrane. At 15°C, vesicular transport of proteins (25) and cholesterol (26) is blocked whereas the nonvesicular movement of newly synthesized phosphatidylcholine to the plasma membrane is not affected (7). Incubation of CHO cells at 15°C reduced the incorporation of [^{3}H]palmitate into GlcCer to 36% of the amount seen at 37°C (Table 2) but did not effect the distribution of GlcCer (Table 1). Newly synthesized GlcCer is, therefore, transported to the plasma membrane at both 15°C and 37°C. In contrast, [^{3}H]palmitate incorporation into sphingomyelin and phosphatidylserine was reduced to <12% of that obtained at 37°C (Table 2). The transport of both phospholipids to the plasma membrane was also reduced significantly (Table 1). The distributions of ceramide, phosphatidylcholine, phosphatidylinositol, and phosphatidylethanolamine were not affected by incubation at 15°C (Table 1). The synthesis of GM3 was inhibited at 15°C, making it impossible to evaluate its distribution at that temperature.

Plasma Membrane GlcCer Is Rapidly Turned Over But Is Not Converted into GM3. We next examined the fate of newly synthesized GlcCer which had reached the plasma membrane. Since a major fraction of GlcCer is converted to GM3 by the addition of galactose and sialic acid as it passes through the Golgi compartment, we wished to determine whether plasma membrane GlcCer could return to the Golgi compartment and be converted to GM3. For these studies we used ldlD cells, deficient in UDP-galactose/UDP-*N*-

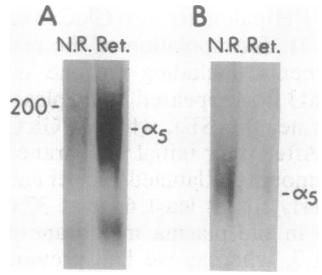

FIG. 2. BFA prevents transport of newly synthesized integrin α5 to the plasma membrane. Subconfluent CHO K1 cells expressing recombinant human integrin α5 were labeled with Trans^{35}S-label for 20 min and then returned to complete medium for 100 min. The incorporation of ^{35}S and the 100-min chase were performed in the absence (*A*) or the presence (*B*) of BFA (1 μg/ml). Plasma membranes were then isolated by HIMAC. Aliquots of nonretained (N.R.) and retained (Ret.) fractions from each time point were pelleted for 2 hr at 190,000 × g_{ave}. The membrane pellets were solubilized and integrin α5 was immunoprecipitated as described (18).

acetylgalactosamine-4-epimerase (27). GlcCer was the only glycolipid detected in ldlD cells which had been labeled at 15°C for 2 hr with [^{3}H]glucose in the absence of galactose. Over half of the [^{3}H]GlcCer in these cells was distributed to the plasma membrane (Fig. 3, zero chase time). The cells were then chased at either 15°C or 37°C in the presence of galactose to allow conversion of GlcCer to GM3. The plasma membrane pool of [^{3}H]GlcCer decreased rapidly at 37°C (Fig. 3), while the [^{3}H]GlcCer in intracellular membranes decreased more slowly (Fig. 3). No reciprocal increase in [^{3}H]GM3 was observed during the chase—i.e., ≤12% of the [^{3}H]GlcCer present at the beginning of the chase was recovered as GM3 (data not shown). In contrast, 40% of labeled GlcCer was converted to GM3 when ldlD cells were labeled with [^{3}H]glucose in the presence of galactose for 3 hr at 37°C (data not shown). Only a small fraction of the [^{3}H]GlcCer present in the plasma membrane after labeling at 15°C was available for conversion into GM3. Instead, plasma membrane GlcCer was degraded, either in the plasma membrane itself or after passage to internal membranes. The $t_{1/2}$ of total GlcCer disappearance was 75 min at 37°C. Although the decrease in GlcCer occurred at both 37°C and 15°C, it was more rapid at 37°C. The turnover of GlcCer is more rapid than the estimated rates of bulk plasma membrane turnover (28), indicating that a selective mechanism not directly related to bulk plasma membrane turnover may be responsible for removing [^{3}H]GlcCer from the cell.

DISCUSSION

Even though lipids are the major components of membranes, understanding the mechanisms by which their distribution and transport are regulated has remained a challenging area of investigation. Plasma membrane isolation using WGA/FeDex and HIMAC is well suited for characterizing the trafficking of lipid components to and from the plasma membrane. While cell surface labeling techniques such as galactose oxidation (29) and periodate oxidation (3) can be used to assess the plasma membrane distribution of individual glycolipid species, WGA/FeDex in conjunction with HIMAC allows the simultaneous analysis of the distribution and trafficking of multiple plasma membrane components.

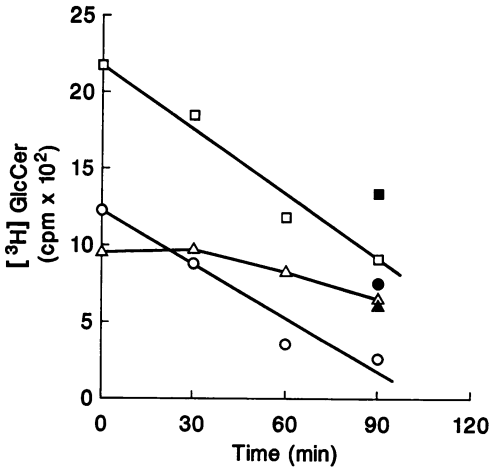

FIG. 3. Plasma membrane GlcCer turns over rapidly but is not converted into GM3. Subconfluent ldlD CHO cells were washed twice in glucose-free DMEM (GF-DMEM) and then incubated for 1 hr at 37°C in GF-DMEM to deplete the cells of glucose. The cells were then equilibrated to 15°C and labeled at 15°C for 2 h in GF-DMEM containing [^{3}H]glucose (25 μCi/ml). [^{3}H]Glucose incorporation was terminated by washing twice with α-MEM/10% fetal bovine serum. Cells were then incubated at 37°C (open symbols) or 15°C (filled symbols) in α-MEM/10% fetal bovine serum/20 μM galactose/200 μM *N*-acetylgalactosamine for the indicated times. Chases were terminated by washing cells twice in ice-cold binding buffer and plasma membranes were isolated by HIMAC. The ^{3}H-labeled glycolipids, including [^{3}H]glucosylceramide, in a 5% aliquot of each sample were quantitated by TLC: total GlcCer (□, ■); GlcCer in the retained fraction (○, ●); and GlcCer in the nonretained fraction (△, ▲).

Under steady-state conditions 60–70% of GM3 distributes to the plasma membrane of CHO cells (15). The kinetics of transport to the plasma membrane we have obtained for GM3 coincide with those reported previously (3) and support the view that GM3 reaches the plasma membrane constitutively by bulk flow through the secretory pathway (30–32). Disruption of the vesicular transport pathway by exposure to BFA does not inhibit the synthesis of GM3 but does block the transport of GM3 and glycoproteins such as the integrin α5 subunit to the plasma membrane.

GlcCer, the precursor to GM3, is synthesized on the cytosolic surface of the Golgi membrane (11–14). Trinchera *et al.* (33) have reported that galactose is transferred to GlcCer on the cytosolic surface of rat liver Golgi membrane. However, mutants of CHO cells which are not able to transport UDP-galactose into the Golgi lumen have markedly reduced levels of LacCer (34, 35). Therefore in CHO cells GlcCer must be translocated to the luminal surface of the Golgi prior to addition of galactose and sialic acid to form LacCer and GM3, respectively. Under steady-state conditions 40% of GlcCer is found in the plasma membranes of CHO cells, indicating either that at least a fraction of GlcCer can pass through the Golgi compartment without addition of galactose and sialic acid or that GlcCer can reach the plasma membrane by an alternative route. The rapid kinetics of GlcCer transport to the plasma membrane (Fig. 1) indicate that the vast majority of GlcCer which reaches the plasma membrane in CHO cells does not traffic through the Golgi secretory pathway. In support of this conclusion, disruption of vesicular transport by BFA or incubation at 15°C does not prevent transport of newly synthesized GlcCer to the plasma membrane. Alternative mechanisms for reaching the plasma membrane are known to exist.

Cholesterol reaches the plasma membrane by a vesicular route which is distinct from that of the Golgi apparatus (10). GlcCer transport shares two features with cholesterol transport: (*i*) more rapid kinetics than estimates for movement through the Golgi compartment and (*ii*) continued transport in the presence of BFA. GlcCer transport clearly differs from cholesterol transport in that GlcCer transport to the membrane continues at 15°C whereas cholesterol transport is blocked at this temperature (10).

The characteristics of GlcCer transport more closely resemble carrier-mediated transport of phosphoglycerides such as phosphatidylcholine to the plasma membrane (16). The kinetics of GlcCer transport, while not as rapid as those of phosphatidylcholine (7), are significantly more rapid than that of GM3 or estimates of bulk flow through the Golgi compartment (Fig. 1). Furthermore, the distribution of GlcCer is predominantly to intracellular membranes as is seen for the phosphoglycerides with the exception of phosphatidylserine (Table 1). GlcCer, like phosphatidylcholine (7), is transported to the plasma membrane at 15°C. Finally, movement of GlcCer to the plasma membrane is not inhibited by BFA. The properties of GlcCer transport make it likely that cytosolic glycolipid-transfer proteins, which have been isolated from a number of tissues and shown to transfer glycosphingolipids between membranes (reviewed in ref. 16), account for the transport of GlcCer to the plasma membrane.

Based on the data we have obtained we propose that GlcCer trafficking in CHO cells has the following features. GlcCer, which is synthesized on the cytosolic leaflet of Golgi membranes, has two fates. It can be translocated to the luminal surface of the Golgi membranes, where it is effi-

ciently converted to LacCer and GM3 as it proceeds to the plasma membrane. Alternatively, GlcCer may be transported directly to the cytosolic leaflet of the plasma membrane by a cytosolic carrier protein. Incubation at 15°C may prevent translocation of GlcCer to the luminal surface of the Golgi membranes, thereby preventing LacCer and GM3 synthesis and increasing plasma membrane accumulation of GlcCer. Newly synthesized GlcCer which has reached the plasma membrane, whether labeled with [^{3}H]Glc or [^{3}H]palmitate (data not shown), is not converted to LacCer or GM3. Instead the plasma membrane GlcCer is either rapidly degraded or converted into an as yet unidentified product at a site(s) which also remains to be identified.

Kobayashi and Pagano (6) reported that transport of fluorescent GlcCer to the plasma membrane is inhibited in mitotic cells and concluded that fluorescent GlcCer most likely reaches the plasma membrane via a vesicular transport pathway. The presence of GlcCer in the plasma membrane was determined by back-exchange with albumin in the medium. Since only GlcCer in the outer leaflet of the plasma membrane is sensitive to back-exchange and the rate of transbilayer movement of GlcCer is very low, this assay cannot detect GlcCer on the inner leaflet of the plasma membrane. In contrast, preparation of plasma membranes by HIMAC allows the analysis of all endogenous lipids in the plasma membrane.

The presence of GlcCer on the cytosolic surface of the plasma membrane has a number of potential implications. Thompson and Tillack (36) have suggested that glycolipid aggregation in phospholipid matrices would result in the formation of microdomains with special biochemical characteristics. Whether these domains form under physiological conditions and what their effect on membrane physiology may be are not known. GlcCer is the precursor for many complex glycolipid species. Regulation of the amount of GlcCer available for conversion to complex glycolipids could be affected by its trafficking. The rapid rate of GlcCer degradation is notable in light of studies examining activation of signal transduction pathways by GlcCer (37) and ceramide (38, 39). Should the site and rate of GlcCer degradation be regulated, it could contribute to regulation of such signal transduction pathways.

We thank Tina Roberts for help with the fibronectin receptor immunoprecipitations. This work was supported by National Institutes of Health Grant R37-CA21923 (J.U.B.), by National Institutes of Health Grant GM42698, by a Monsanto–Washington University Biomedical Research Grant, and by American Cancer Society Grant FRA-358 (W.W.Y.).

1. Voelker, D. R. (1990) *Experientia* **46**, 569–579.
2. Kallen, K. J., Quinn, P. & Allan, D. (1993) *Biochem. J.* **289**, 307–312.
3. Young, W. W., Jr., Lutz, M. S. & Blackburn, W. A. (1992) *J. Biol. Chem.* **267**, 12011–12015.
4. Lipsky, N. G. & Pagano, R. E. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 2608–2612.
5. Lipsky, N. G. & Pagano, R. E. (1985) *J. Cell Biol.* **100**, 27–34.
6. Kobayashi, T. & Pagano, R. E. (1989) *J. Biol. Chem.* **264**, 5966–5973.
7. Kaplan, M. R. & Simoni, R. D. (1985) *J. Cell Biol.* **101**, 441–445.
8. Sleight, R. G. & Pagano, R. E. (1983) *J. Biol. Chem.* **258**, 9050–9058.
9. Vance, J. E., Aasman, E. J. & Szarka, R. (1991) *J. Biol. Chem.* **266**, 8241–8247.
10. Urbani, L. & Simoni, R. D. (1990) *J. Biol. Chem.* **265**, 1919–1923.
11. Coste, H., Martel, M. B. & Got, R. (1986) *Biochim. Biophys. Acta* **858**, 6–12.
12. Futerman, A. H. & Pagano, R. E. (1991) *Biochem. J.* **280**, 295–302.
13. Trinchera, M., Fabbri, M. & Ghidoni, R. (1991) *J. Biol. Chem.* **266**, 20907–20912.
14. Jeckel, D., Karrenbauer, A., Burger, K. N. J., Van Meer, G. & Wieland, F. (1992) *J. Cell Biol.* **117**, 259–267.
15. Warnock, D. E., Roberts, C., Lutz, M. S., Blackburn, W. A., Young, W. W., Jr., & Baenziger, J. U. (1993) *J. Biol. Chem.* **268**, 10145–10153.
16. Sasaki, T. (1990) *Experientia* **46**, 611–616.
17. Becich, M. J. & Baenziger, J. U. (1991) *Eur. J. Cell Biol.* **55**, 71–82.
18. Roberts, C. J., Birkenmeier, T. M., McQuillan, J. J., Akiyama, S. K., Yamada, S. S., Chen, W., Yamada, K. M. & McDonald, J. A. (1988) *J. Biol. Chem.* **263**, 4586–4592.
19. Merrill, A. H. J. & Wang, E. (1986) *J. Biol. Chem.* **261**, 3764–3769.
20. Klausner, R. D., Donaldson, J. G. & Lippincott-Schwartz, J. (1992) *J. Cell Biol.* **116**, 1071–1080.
21. Hatch, G. M. & Vance, D. E. (1992) *J. Biol. Chem.* **267**, 12443–12451.
22. Bruning, A., Karrenbauer, A., Schnabel, E. & Wieland, F. T. (1992) *J. Biol. Chem.* **267**, 5052–5055.
23. Futerman, A. H., Stieger, B., Hubbard, A. L. & Pagano, R. E. (1990) *J. Biol. Chem.* **265**, 8650–8657.
24. Van Echten, G., Iber, H., Stotz, H., Takatsuki, A. & Sandhoff, K. (1990) *Eur. J. Cell Biol.* **51**, 135–139.
25. Saraste, J. & Kuismanen, E. (1984) *Cell* **38**, 535–549.
26. Kaplan, M. R. & Simoni, R. D. (1985) *J. Cell Biol.* **101**, 446–453.
27. Kingsley, D. M., Kozarsky, K. F., Hobbie, L. & Krieger, M. (1986) *Cell* **44**, 749–759.
28. Steinman, R. M., Mellman, I., Muller, W. A. & Cohn, Z. A. (1983) *J. Cell Biol.* **96**, 1–27.
29. Gahmberg, C. G. & Hakomori, S. (1973) *J. Biol. Chem.* **248**, 4311–4317.
30. Karrenbauer, A., Jeckel, D., Just, W., Birk, R., Schmidt, R. R., Rothman, J. E. & Wieland, F. T. (1990) *Cell* **63**, 259–267.
31. Klausner, R. D. (1989) *Cell* **57**, 703–706.
32. Wieland, F. T., Gleason, M. L., Serafini, T. A. & Rothman, J. E. (1987) *Cell* **50**, 289–300.
33. Trinchera, M., Fiorilli, A. & Ghidoni, R. (1991) *Biochemistry* **30**, 2719–2724.
34. Briles, E. B., Li, E. & Kornfeld, S. (1977) *J. Biol. Chem.* **252**, 1107–1116.
35. Deutscher, S. L. & Hirschberg, C. B. (1986) *J. Biol. Chem.* **261**, 96–100.
36. Thompson, T. E. & Tillack, T. W. (1985) *Annu. Rev. Biophys. Biophys. Chem.* **14**, 361–386.
37. Shayman, J. A., Deshmukh, G. D., Mahdiyoun, S., Thomas, T. P., Wu, D., Barcelon, F. S. & Radin, N. S. (1991) *J. Biol. Chem.* **266**, 22968–22974.
38. Bielawska, A., Linardic, C. M. & Hannun, Y. A. (1992) *J. Biol. Chem.* **267**, 18493–18497.
39. Dressler, K. A., Mathias, S. & Kolesnick, R. N. (1992) *Science* **255**, 1715–1718.