body against the non-specific lipid transfer protein (sterol carrier protein 2). Biochim. biophys. Acta *812* (1985) 387–392.
119 Van Meer, G., Plasma membrane cholesterol pools. TIBS *12* (1987) 375–376.
120 Verner, K., and Schatz, G., Protein translocation across membranes. Science *241* (1988) 1307–1313.
121 Von Heijne, G., and Blomberg, C., Trans-membrane translocation of proteins. The direct transfer model. Eur. J. Biochem. *97* (1979) 175–181.
122 Wattenberg, B. W., and Silbert, D. F., Sterol partitioning among intracellular membranes-testing a model for cellular sterol distribution. J. biol. Chem. *258* (1983) 2284–2289.
123 Westerman, J., and Wirtz, K. W. A., The primary structure of the nonspecific lipid transfer protein. Biochem. biophys. Res. Commun. *127* (1985) 333–338.
124 Wickner, W. T., and Lodish, H. F., Multiple mechanisms of protein insertion into and across membranes. Science *230* (1985) 400–407.
125 Wieland, F. T., Gleason, M. L., Serafini, T. A., and Rothman, J. E., The rate of bulk flow from the endoplasmic reticulum to the cell surface. Cell. *50* (1987) 289–300.
126 Yeagle, P. L., Cholesterol and the cell membrane. Biochim. biophys. Acta *822* (1985) 267–287.

0014-4754/90/060599-13$1.50 + 0.20/0

Glycolipid transfer protein and intracellular traffic of glucosylceramide

T. Sasaki

Department of Biochemistry, Cancer Research Institute, Sapporo Medical College, South-1, West-17, Sapporo 060 (Japan)

Summary. Glycolipid transfer protein (GL-TP), a nonglycosylated protein with a molecular weight of 22,000 K, has been purified from pig brain. The protein transfers, by a carrier mechanism, glycolipids with a β-glucosyl or β-galactosyl residue directly linked to either ceramide or diacylglycerol. GL-TP appears to be present in most animal cells, and evidence has been obtained which indicates that it is a cytoplasmic protein. Little is known about the function of GL-TP. Current evidence indicates that glycosphingolipid glycosylation occurs at the luminal side of the Golgi apparatus, except for the glucosylation of ceramide, which has been shown to occur at the cytoplasmic side of the Golgi or endoplasmic membrane. It appears most likely that GL-TP participates in the intracellular traffic of glucosylceramide.
Key words. Glycosphingolipid; topography of glycolipid glycosylation; the Golgi apparatus; glucosylceramide; monensin; glycolipid transfer protein.

Intracellular location of glycosphingolipids and of enzymes of glycosphingolipid biosynthesis

Glycosphingolipids are localized predominantly, if not exclusively, in the outer leaflet of the plasma membrane[31]. It is now known that the glycosylation of glycosphingolipids occurs by the sequential addition of monosaccharides from sugar nucleotides to an acceptor, and is catalyzed by glycosyltransferases located in the Golgi membrane[20, 25, 35, 37, 42, 50]. Although the localization of glycolipid glycosyltransferases in specific cisternae in discrete parts of the Golgi apparatus has not been investigated experimentally one might expect, by analogy with glycoprotein glycosyltransferases[18, 38], that such a localization does occur. The sequential glycosylation of one glycosphingolipid to the next higher homologue involves a very small pool of intermediates which does not mix with the main pool of cellular glycosphingolipids[31]. Current evidence indicates that the small pool of intermediates is located in the Golgi apparatus. It is assumed that the endoplasmic reticulum is the site of ceramide biosynthesis, since fatty acid incorporation generally occurs in the endoplasmic reticulum.

Transport of glycosphingolipids from the Golgi apparatus to the plasma membranes

Very little is known about the mechanism of glycosphingolipid transport from the site of synthesis to the plasma membrane. Dower et al.[17] examined the kinetics of ganglioside transport from an intracellular site of synthesis to the plasma membrane in cultured cells. These authors distinguished surface and intracellular gangliosides by oxidizing cell surface gangliosides with sodium periodate and reacting the oxidized gangliosides with dinitrophenylhydrazine. It was found that the transfer of gangliosides from the site of synthesis to the cell surface required approximately 20 min. A variety of drugs, including inhibitors of protein synthesis and energy metabolism, modulators of cytoskeleton, and monensin, had no effect on the transport of newly synthesized GD_{1a} ganglioside to the plasma membrane[32]. Only low temperature effectively blocked the translocation. It appears that vesicular transport[21, 33] is the most likely mechanism for the transport of glycosphingolipids from the site of synthesis to the plasma membrane[32]. According to the vesicular transport model, glycosphingolipids are trans-

ported to the cell surface via shuttling vesicles which bud off from one membrane and fuse with another.

Topography of glycolipid glycosylation in the Golgi apparatus

Our understanding of the topography of glycolipid glycosylation in the Golgi apparatus is limited. Yusuf et al.[57–59] showed that tunicamycin inhibits the synthesis of GM_1 and GM_2 gangliosides in isolated Golgi vesicles; this inhibition was found to be due to a block in carrier-mediated transport of nucleotide sugars across the Golgi vesicles, consistent with the luminal orientation of glycosyltransferases involved in GM_1 and GM_2 synthesis.

Evidence strongly suggesting that the glycosylation of glycolipids occurs in the lumen of the Golgi apparatus has been obtained through analyses of glycosylation mutants of Chinese hamster ovary cells. Mutant cell lines of one complementation group[46] (Lec2 and clone 1021) have a 90 % reduction in the sialylation of both glycoproteins and glycolipids compared with wild-type cells. Sialosyllactosylceramide (GM_3 ganglioside) is the major glycolipid in parent cells, while Lec2 and clone 1021 cells have lactosylceramide as the predominant glycolipid[8,45]. The Lec2 cells were found to be deficient in the mechanism needed to translocate CMP-sialic acid across Golgi vesicle membranes from an external compartment[16]. Other biochemical analyses strongly suggest that this translocation deficiency is the primary defect responsible for the mutant phenotype[8,16].

Mutant cells of the second complementation group[46] (Lec8 and clone 13) have an 80–90 % reduction in both galactosylation and sialylation of their glycoproteins and glycolipids when compared to wild-type cells[8,45,47]. Clone 13 cells have glucosylceramide as the major glycolipid[8]. The primary biochemical defect in Lec8 and clone 13 cells was found to be their inability to translocate UDP-galactose into the lumen of the Golgi apparatus[15]. Mutant cells of the first and second complementation groups were found to possess the appropriate nucleotide sugars, glycoprotein and glycolipid acceptors, and glycosyltransferases[8].

These results provided strong evidence for the orientation of the catalytic sites of glucosylceramide galactosyltransferase and lactosylceramide sialyltransferase toward the lumen of the Golgi, since mutants that are unable to translocate UDP-galactose and CMP-sialic acid into the lumen of the Golgi apparatus are defective in the syntheses of lactosylceramide and GM_3 ganglioside.

Topography of glucosylceramide synthesis in the Golgi apparatus

The topography of ceramide glucosyltransferase and de novo synthesized glucosylceramide has been examined by Got and coworkers with Golgi vesicles from porcine submaxillary glands[14]. In this tissue, the UDP-glucose-ceramide glucosyltransferase is associated with membranes of the Golgi apparatus[13]. Two lines of evidence indicate that ceramide glucosyltransferase is an enzyme whose catalytic site faces the cytoplasmic side of the Golgi membrane. Firstly, ceramide glucosyltransferase was found to have protease-sensitive sites facing the cytoplasmic side of the Golgi membrane. Vesicles isolated from the Golgi apparatus that are sealed and have 'right-side-out' orientation (cytoplasmic side out) were treated with either pronase or trypsin. Ceramide glucosyltransferase was inactivated by nearly 100 % following treatment of the Golgi vesicles with these proteases. Under the same conditions, ovomucoid-β-D-galactosyltransferase, a protein known to face the lumen of the Golgi, was found to be protease-resistant in intact vesicles and protease-sensitive in disrupted vesicles. Secondly, the synthesis of glucosylceramide was blocked when intact vesicles were treated with the stilbene derivative DIDS (4,4′-diisothiocyano-2,2′-stilbenedisulfonic acid), which seems to interact with the anion-binding sites of glycosyltransferases[44]. Under the same conditions, ovomucoid-β-D-galactosyltransferase was resistant to DIDS in intact vesicles and sensitive to DIDS in disrupted vesicles. Since DIDS has been shown to be a membrane-impermeable agent[10,11,51], it is likely that a key part of the catalytic site of ceramide glucosyltransferase faces the cytoplasmic side of the Golgi membrane.

In addition, evidence has been obtained by the same authors[14] in support of the cytoplasmic orientation of the newly synthesized glucosylceramide. Using glucosylceramidase from human placenta to probe the orientation of this compound, they found that the glucosylceramide synthesized in the isolated Golgi vesicles is equally sensitive both in intact and in disrupted vesicles to hydrolysis by the enzyme, which suggested that the newly synthesized glucosylceramide is oriented towards the cytoplasmic face of the Golgi membrane.

Suzuki et al.[48] showed that glucosylceramide is synthesized by BHK-21 cell microsomes from dolichol-phosphate-glucose (Dol-P-Glc) and ceramide. Their results suggest that glucosylation of ceramides may be achieved by two separate enzyme reactions using either UDP-Glc or Dol-P-Glc as glucose donors. The enzyme that synthesizes Dol-P-Glc has cytoplasmic protease-sensitive sites[43]. The flip-flop of monoglycosylated dolichol-phosphate intermediates in endoplasmic membranes has been postulated[24].

From these results, it appears that cytoplasmically oriented glucosylceramide is somehow utilized as the acceptor by glucosylceramide galactosyltransferase, which seems to add galactose to the acceptor from a luminal pool of UDP-galactose. How the transmembrane movement of glucosylceramide occurs is not known. The movement could be part of the reaction of glucosylceramide galactosyltransferase or be due to protein-mediated glucosylceramide flip-flop.

Site of glucosylation of ceramide in the Golgi apparatus: studies using monensin

Through the study of the effect of monensin on glycosphingolipid metabolism in cultured cells, a great deal of evidence has been obtained in support of the theory that the initial glucosylation of ceramide and the subsequent elongation of the oligosaccharide chain of glycosphingolipids occur at different sites within cells. Saito et al.[39] found that exposure of human fibroblasts to monensin, an ionophore for monovalent cations, caused a marked accumulation of glucosylceramide and, to a much smaller extent, lactosylceramide. The cellular content of more highly glycosylated neutral glycosphingolipids and gangliosides remained the same in the presence or absence of monensin. Monensin greatly increased the incorporation of labeled glucose and galactose into glucosylceramide and lactosylceramide and, at the same time, reduced the incorporation into higher glycosylated glycosphingolipids. Since Gaucher-disease fibroblasts, which have a genetically impaired lysosomal glucocerebrosidase activity, accumulated even more glucosylceramide and lactosylceramide than normal cells upon exposure to monensin, these authors concluded that monensin affects biosynthesis rather than merely disrupting lysosomal glycosphingolipid catabolism.

Miller-Prodraza and Fishman[32] also reported the effect of monensin on the biosynthesis and levels of glycosphingolipids in rat glioma cells and in mouse neuroblastoma cells. In monensin-treated cells, the labeling of glucosylceramide increased several-fold, whereas the labeling of more complex neutral glycosphingolipids and gangliosides decreased; progressively more inhibition of the synthesis was found in more highly glycosylated lipids.

It has been shown that monensin inhibits intra-Golgi transport of glycoproteins, which results in the accumulation of membrane and secretary protein precursors in middle or trans Golgi cisternae and in the blocking of terminal glycosylation by preventing exposure of glycoproteins to glycosyltransferases located in trans regions of the Golgi[19,21]. Lipsky and Pagano[28] showed that monensin markedly inhibits the translocation of glucosylceramide from the Golgi apparatus to the plasma membrane. These authors used a fluorescent analogue of ceramide to study the intracellular synthesis and transport of sphingomyelin and glucosylceramide in Chinese hamster fibroblasts by fluorescence microscopy[27]. Treatment of the cells with monensin inhibited the appearance of intracellularly synthesized glucosylceramide analogue at the plasma membrane, resulting in a marked accumulation of the analogue in the Golgi apparatus.

Taken together, these results suggest that glucosylation of ceramide occurs on the proximal face of the Golgi, while higher glycosylation of glycosphingolipids occurs on the distal face of the Golgi.

Glycolipid transfer protein (GL-TP)

Metz and Radin[29] found and characterized glucosylceramide transfer activity in bovine spleen cytosol. The activity facilitates the transfer of glucosylceramide from liposomes to erythrocytes or to erythrocyte ghosts. These authors partially purified the active protein[30,36]. A protein probably identical to the one described by Metz and Radin[29,30] was purified to homogeneity from pig brain by Abe et al.[4] and Abe and Sasaki[1]; 2.6 mg of the purified protein (GL-TP) was obtained from 4 kg of pig brain at a yield of 19%[41]. The final preparation was about 6000-fold purified as compared with the membrane-free supernatant and had a specific activity of 40.6 nmol of galactosylceramide (GalCer) transferred/min/mg protein in a [^{3}H]GalCer transfer assay[1,53], and of 140 nmol of pyrene-labeled GalCer (PyrGalCer) transferred/min/mg protein in a PyrGalCer transfer assay[5]. The homogeneity of the purified protein was indicated by several lines of evidence. The protein has an isoelectric point of about 8.3. A molecular weight of 22,000 has been estimated from its behavior on SDS polyacrylamide gel electrophoresis (PAGE). A molecular weight of 15,000 was calculated from AcA-54 gel filtration. It appears that the elution of GL-TP from an AcA-54 column is delayed owing to a hydrophobic interaction of the protein with the gel matrix. Studies on the primary structure have revealed that GL-TP consists of a single chain of 208 amino acids with the blocked NH_2 terminus (A. Abe, unpublished observations). The protein has a calculated molecular weight of 23,761. Gly, Ala, Val, Leu, Ile, Met, Phe, Try, and Pro residues constitute 51.9% of the amino acid residues in GL-TP. The protein has no apparent homology with phosphatidylcholine transfer protein from bovine liver[6], nonspecific lipid transfer protein from bovine liver[49], fatty acid binding protein from rat liver[23] and small intestinal epithelium[7], sphingolipid activator protein-1[34], or co-β-glucosidase (SAP-2)[34]. The amino acid composition of GL-TP[1] is obviously different from that of GM_2-activator from human liver[26]. GL-TP is not glycosylated. The purified GL-TP is a mixture of two forms of a protein; one of them (about 15%) with an intramolecular disulfide bond, and a reduced form (about 80%) without one[2,3]. Results were obtained which indicated that GL-TP with an intramolecular disulfide bond has twice as much activity as the reduced form, and formed more GL-TP-glycolipid complex. The protein has one additional free sulfhydryl group, which is reactive with 5,5′-dithiobis(2-nitrobenzoic acid) and N-ethylmaleimide under nondenaturing conditions[3]. Its activity is inhibited partly (about 60%) by 10 mM N-ethylmaleimide and completely by 2.5 mM mercuric chloride. GL-TP has also been partially purified from bovine brain[9,22,52], rat brain[53], rat liver[54], human spleen, and from several cell lines of rat, mouse, pig, and human origin.

Intracellular location of GL-TP in PK(15) cells, a pig kidney cell line, was studied by immunochemical method (F. Roerink, A. Abe and T. Sasaki, unpublished observations). Cultured PK(15) cells were disrupted by N_2-cavitation and subcellular fractions were obtained by differential pelleting. Acid N-acetyl-β-hexosaminidase, a lysosomal marker, remained latent after the N_2-cavitation. GL-TP in each subcellular fraction was released by sonication. Almost all activity of GL-TP was recovered in the cytosol fraction. GL-TP in each fraction was concentrated by phosphocellulose chromatography and separated by SDS-PAGE. Bands of GL-TP on the gel were transferred to a nitrocellulose membrane and detected by immunochemical staining using an affinity-purified anti-GL-TP antibody. Most of the protein reactive with the anti-GL-TP was found in the cytosol fraction, which indicated a cytoplasmic location of GL-TP.

The pH dependence of glycolipid transfer facilitated by GL-TP from pig brain was compared with that facilitated by GM_2-activator from human liver, a cofactor of lysosomal N-acetyl-β-hexosaminidase A, in an assay of PyrGalCer transfer from donor to acceptor vesicles. GL-TP facilitated PyrGalCer transfer over a pH range from 5 to 8 but did not facilitate the transfer at or below pH 4. GM_2-activator facilitated PyrGalCer transfer at pH 4; the transfer activity at neutral pH was lower than the activity at pH 4.5–5. These results are consistent with those reported by Conzelmann et al.[12], who showed that the rate of GM_2-activator-facilitated ganglioside transfer at pH 7.1 was 15 % of the rate of that found at pH 4.2. GL-TP from pig brain facilitates the transfer of all glycosphingolipids examined so far[55]. These include glucosylceramide, galactosylceramide, lactosylceramide, sulfatide, lactosylceramide-II^3-sulfate, globotriaosylceramide, globotetraosylceramide, globopentaosylceramide, sialosyllactosylceramide, and GM_1 ganglioside. The transfer of Manβ1 → 4Glcβ1 → ceramide and Manα1 → 4Manβ1 → 4Glcβ1 → ceramide is also facilitated by GL-TP from pig brain. In addition to glycosphingolipids, GL-TP from pig brain also facilitates the transfer of 3-[Galβ1]-*sn*-1,2-diacylglycerol, 3-[Galα1 → 6Galβ1]-*sn*-1,2-diacylglycerol, and 3-[Glcβ1]-*rac*-1,2-dipalmitylglycerol. The protein does not facilitate the transfer of 3-[Manα1 → 3Manα1]-*sn*-1,2-diacylglycerol, 3-[Glcα1]-*sn*-1,2-diacylglycerol, phosphatidylcholine, phosphatidylinositol, phosphatidylethanolamine, cholesterol, or cholesteryl oleate. GL-TP from pig brain stimulates the transfer of pyrene-labeled sphingomyelin to a very small but significant extent. These results were interpreted as an indication that GL-TP from pig brain transfers glycolipids with a β-glucosyl or β-galactosyl residue directly linked to either ceramide or diacylglycerol[55,56]. It is obvious that the specificity of GL-TP is not directed to the nonreducing part of glycolipids and that GL-TP has a low specificity for the nonpolar portion of glycolipids, because the protein facilitates the transfer of both glycosphingolipids and glyceroglycolipids.

It has been shown that GL-TP facilitates the transfer of glycolipids only when both the donor and acceptor membranes are in the liquid-crystalline state[52]. If either donor or acceptor membranes are in the gel state, GL-TP-mediated transfer is markedly reduced. Furthermore, the rate of glycolipid transfer facilitated by GL-TP is markedly affected by the curvature of the donor and acceptor membranes. In GL-TP-mediated transfer, a strong preference was found for sonicated unilamellar vesicles over multilamellar liposomes[9], which indicated that the interaction of GL-TP with membranes is severely affected by the packing of lipids in the membranes.

Binding of a glycolipid to GL-TP has been shown by three different methods. In the first method, formation of a complex between [^{3}H]GalCer and GL-TP was shown by PAGE at pH 4.3[1]. About 0.13 mol of [^{3}H]GalCer was bound per mol of GL-TP under the conditions used. Addition of 4.2 mM N-ethylmaleimide to the incubation mixture reduced the binding of [^{3}H]GalCer to GL-TP to 65 % of the control level. In the second method, the formation of a GL-TP-glycolipid complex was shown by the binding of PyrGalCer to GL-TP[5]. The binding was measured by an increase in pyrene monomer fluorescence upon addition of GL-TP to phospholipid vesicles containing PyrGalCer. In a non-quenched vesicle assay, an immediate drop in the intensity ratio of eximer to excited monomer was observed upon addition of GL-TP to the vesicles. In a quenched vesicle assay, where the phospholipid vesicles contained N-trinitrophenylphosphatidylethanolamine in addition to PyrGalCer to quench the fluorescence due to PyrGalCer present in the vesicles, an increase in excited monomer fluorescence was observed upon addition of GL-TP to the vesicles. These results indicate that GL-TP enhances a release of PyrGalCer monomer from the vesicles, which can be interpreted as being due to the binding of PyrGalCer monomer to GL-TP. In the third method, the GL-TP-[^{3}H]GalCer complex was isolated by Sephadex G-75 gel filtration[1].

The GL-TP-glycolipid complex is formed as a result of removal of a glycolipid molecule from a membrane and binding to a GL-TP molecule. It appears that local perturbation of the structure of the lipid layer is induced by the protein, and that this perturbation is an essential step in the extraction of the glycolipid by the protein. The specificity of GL-TP is consistent with a simple mechanism for glycolipid extraction by GL-TP, because the specificity suggests that GL-TP recognizes and binds the sugar residue directly linked to either ceramide or diacylglycerol in addition to the nonpolar portion of glycolipids.

The complex formed between GL-TP and [^{3}H]GalCer was isolated by Sephadex G-75 gel filtration from an incubation mixture containing the protein and liposomes containing [^{3}H]GalCer[1]. The isolated [^{3}H]GalCer-GL-TP complex contained about 0.12 mol of [^{3}H]GalCer per mol of GL-TP. An incubation of the GL-TP-[^{3}H]GalCer complex with liposomes resulted in the transfer of

[^{3}H]GalCer from the complex to the acceptor liposomes[1]. This result indicates that the GL-TP-glycolipid complex functions as the intermediate in the glycolipid transfer.

The net mass transfer of [^{14}C]GalCer facilitated by GL-TP was investigated by the use of monomolecular lipid film spread at an air-water interface[40]. It appears that GL-TP binds one mol of glycolipid per mol of the protein. However, it has not been possible to saturate the purified GL-TP with glycolipids under any of the several different conditions examined. During net mass transfer of a glycolipid molecule from donor to acceptor membranes, GL-TP carrying a glycolipid monomer unloads the bound glycolipid onto an acceptor membrane. Therefore, it appears that the GL-TP without a bound lipid represents the state of the protein after net mass transfer of a glycolipid molecule to an acceptor membrane. It appears to be a property of GL-TP distinct from other lipid transfer proteins that the protein exists both with and without bound glycolipid.

As has been discussed in the earlier sections of this review, vesicular transport is the most probable mechanism for the transport of glycosphingolipids from the site of synthesis to the plasma membranes. The glycosylation of glycolipids, except for the glucosylation of ceramide, occurs in the lumen of the Golgi apparatus. Therefore, glycosphingolipids always have a luminal orientation in transport vesicles, and are always inaccessible from the cytosol. Available data indicate that GL-TP is a cytosolic protein. Glucosylceramide is the only glycosphingolipid that seems to be exposed on the cytoplasmic face of the cellular membranes. Therefore, it is most likely that GL-TP may participate in the intracellular transport of glucosylceramide.

1 Abe, A., and Sasaki, T., Purification and some properties of the glycolipid transfer protein from pig brain. J. biol. Chem. *260* (1985) 11231–11239.

2 Abe, A., and Sasaki, T., Formation of an intramolecular disulfide bond of glycolipid transfer protein. Biochim. biophys. Acta *985* (1989) 45–50.

3 Abe, A., and Sasaki, T., Sulfhydryl groups in glycolipid transfer protein: formation of an intramolecular disulfide bond and oligomers by Cu^{2+}-catalyzed oxidation. Biochim. biophys. Acta, *985* (1989) 38–44.

4 Abe, A., Yamada, K., and Sasaki, T., A protein purified from pig brain accelerates the intermembranous translocation of mono- and dihexosylceramides, but not the translocation of phospholipids. Biochem. biophys. Res. Commun. *104* (1982) 1386–1393.

5 Abe, A., Yamada, K., Sakagami, T., and Sasaki, T., A fluorimetric determination of the activity of glycolipid transfer protein and some properties of the protein purified from pig brain. Biochim. biophys. Acta *778* (1984) 239–244.

6 Akeroyd, R., Moonen, P., Westerman, J., Puyk, W. C., and Wirtz, K. W. A., The complete primary structure of the phosphatidylcholine-transfer protein from bovine liver: isolation and characterization of the cyanogen bromide peptides. Eur. J. Biochem. *114* (1981) 385–391.

7 Alpers, D. H., Strauss, A. W., Ockner, R. K., Bass, N. M., and Gordon, J. I., Cloning of a cDNA encoding rat intestinal fatty acid binding protein. Proc. natl Acad. Sci. USA *81* (1984) 313–317.

8 Briles, E. B., Li, E., and Kornfeld, S., Isolation of wheat germ agglutinin-resistant clones of Chinese hamster ovary cells deficient in membrane sialic acid and galactose. J. biol. Chem. *252* (1977) 1107–1116.

9 Brown, R. E., Stephenson, F. A., Markello, T., Barenholz, Y., and Thompson, T. E., Properties of a specific glycolipid transfer protein from bovine brain. Chem. Phys. Lipids *38* (1985) 79–93.

10 Cabantchik, Z. I., Knauf, P. A., and Rothstein, A., The anion transport system of the red blood cell; the role of membrane protein evaluated by the use of 'probes'. Biochim. biophys. Acta *515* (1978) 239–302.

11 Capasso, J. M., and Hirschberg, C. B., Effect of atractylosides, palmitoyl coenzyme A, and anion transport inhibitors on translocation of nucleotide sugars and nucleotide sulfate into Golgi vesicles. J. biol. Chem. *259* (1984) 4263–4266.

12 Conzelmann, E., Burg, J., Stephan, G., and Sandhoff, K., Complexing of glycolipids and their transfer between membranes by the activator protein for degradation of lysosomal ganglioside GM_2. Eur. J. Biochem. *123* (1982) 455–464.

13 Coste, H., Martel, M. B., Azzar, G., and Got, R., UDP-glucoseceramide glucosyltransferase from porcine submaxillary glands is associated with the Golgi apparatus. Biochim. biophys. Acta *814* (1985) 1–7.

14 Coste, H., Martel, M. B., and Got, R., Topology of glucosylceramide synthesis in Golgi membranes from porcine submaxillary glands. Biochim. biophys. Acta *858* (1986) 6–12.

15 Deutscher, S. L., and Hirschberg, C. B., Mechanism of galactosylation in the Golgi apparatus: a Chinese hamster ovary cell mutant deficient in translocation of UDP-galactose across Golgi vesicle membranes. J. biol. Chem. *261* (1986) 96–100.

16 Deutscher, S. L., Nuwayhid, N., Stanley, P., Briles, E. I. B., and Hirschberg, C. B., Translocation across Golgi vesicle membranes: a CHO glycosylation mutant deficient in CMP-sialic acid transport. Cell *39* (1984) 295–299.

17 Dower, S., Miller-Podraza, H., and Fishman, P. H., Translocation of newly synthesized gangliosides to the cell surface. Biochemistry *21* (1982) 3265–3270.

18 Dunphy, W. G., Brands, R., Rothman, J. E., Attachment of terminal N-acetylglucosamine to asparagine-linked oligosaccharides occurs in central cisternae of the Golgi stack. Cell *40* (1985) 463–472.

19 Elbein, A. D., Inhibitors of the biosynthesis and processing of N-linked oligosaccharide chains. A. Rev. Biochem. *56* (1987) 497–534.

20 Eppler, C. M., Morré, D. J., and Keenan, T. W., Ganglioside biosynthesis in rat liver: characterization of cytidine-5′-monophospho-*N*-acetylneuraminic acid:hematoside (GM_3) sialyl transferase. Biochim. biophys. Acta *619* (1980) 318–331.

21 Farquhar, M. G., Progress in unraveling pathways of Golgi traffic. A. Rev. Cell Biol. *1* (1985) 447–488.

22 Gammon, C. M., Vaswani, K. K., and Ledeen, R. W., Isolation of two glycolipid transfer proteins from bovine brain: reactivity toward gangliosides and neutral glycosphingolipids. Biochemistry *26* (1987) 6239–6243.

23 Gordon, J. I., Alpers, D. H., Ockner, R. K., and Strauss, A. W., The nucleotide sequence of rat liver fatty acid binding protein mRNA. J. biol. Chem. *258* (1983) 3356–3363.

24 Haselbeck, A., and Tanner, W., Dolichyl phosphate-mediated mannosyl transfer through liposomal membranes. Proc. natl Acad. Sci. USA *79* (1982) 1520–1524.

25 Keenan, T. W., Morre, D. J., and Basu, S., Ganglioside biosynthesis: concentration of glycosphingolipid glycosyltransferases in Golgi apparatus from rat liver. J. biol. Chem. *249* (1974) 310–315.

26 Li, S.-C., Hirabayashi, Y., and Li, Y.-T., A protein activator for the enzymic hydrolysis of GM_2 ganglioside. J. biol. Chem. *256* (1981) 6234–6240.

27 Lipsky, N. G., and Pagano, R. E., Sphingolipid metabolism in cultured fibroblasts: microscopic and biochemical studies employing a fluorescent ceramide analogue. Proc. natl Acad. Sci. USA *80* (1983) 2608–2612.

28 Lipsky, N. G., and Pagano, R. E., Intracellular translocation of fluorescent sphingolipids in cultured fibroblasts: endogenously synthesized sphingomyelin and glucocerebroside analogues pass through the Golgi apparatus en route to the plasma membrane. J. Cell Biol. *100* (1985) 27–34.

29 Metz, R. J., and Radin, N. S., Glucosylceramide uptake protein from spleen cytosol. J. biol. Chem. *255* (1980) 4463–4467.

30 Metz, R. J., and Radin, N. S., Purification and properties of a cerebroside transfer protein. J. biol. Chem. *257* (1982) 12901–12907.

31 Miller-Podraza, H., Bradley, R. M., and Fishman, P. H., Biosynthesis and localization of gangliosides in cultured cells. Biochemistry *21* (1982) 3260–3265.

32 Miller-Podraza, H., and Fishman, P. H., Effect of drugs and temperature on biosynthesis and transport of glycosphingolipids in cultured neurotumor cells. Biochim. biophys. Acta *804* (1984) 44–51.

33 Morré, D. J., Kartenbeck, J., and Franke, W. W., Membrane flow and interconversions among endomembranes. Biochim. biophys. Acta *559* (1979) 71–152.
34 Nakano, T., Sandhoff, K., Stümper, J., Christomanou, H., and Suzuki, K., Structure of full-length cDNA coding for sulfatide activator, a co-β-glucosidase and two other homologous proteins: two alternate forms of the sulfatide activator. J. Biochem. *105* (1989) 152–154.
35 Pacuszka, T., Duffard, R. O., Nishimura, R. N., Brady, R. O., and Fishman, P. H., Biosynthesis of bovine thyroid gangliosides. J. biol. Chem. *253* (1978) 5839–5846.
36 Radin, N. S., and Metz, R. J., Cerebroside transfer protein. Meth. Enzymol. *98* (1983) 613–622.
37 Richardson, C. L., Keenan, T. W., and Morré, D. J., Ganglioside biosynthesis: characterization of CMP-*N*-acetylneuraminic acid: lactosylceramide sialyltransferase in Golgi apparatus from rat liver. Biochim. biophys. Acta *488* (1977) 88–96.
38 Roth, J., and Berger, E. G., Immunocytochemical localization of galactosyltransferase in HeLa cells: codistribution with thiamine pyrophosphatase in trans-Golgi cisternae. J. Cell Biol. *93* (1982) 223–229.
39 Saito, M., Saito, M., and Rosenberg, A., Action of monensin, a monovalent cationophore, on cultured human fibroblasts: evidence that it induces high cellular accumulation of glucosyl- and lactosylceramide (gluco- and lactocerebroside). Biochemistry *23* (1984) 1043–1046.
40 Sasaki, T., and Demel, R. A., Net mass transfer of galactosylceramide facilitated by glycolipid transfer protein from pig brain: a monolayer study. Biochemistry *24* (1985) 1079–1083.
41 Sasaki, T., and Abe, A., Glycolipid transfer protein from pig brain. Meth. Enzymol. *179* (1990) in press.
42 Siegrist, H. P., Burkart, T., Wiesmann, U. N., Herschkowitz, N. N., and Spycher, M. A., Ceramide-galactosyltransferase and cerebroside-sulphotransferase localization in Golgi membranes isolated by a continuous sucrose gradient of mouse brain microsomes. J. Neurochem. *33* (1979) 497–504.
43 Snider, M. D., Sultzman, L. A., and Robbins, P. W., Transmembrane location of oligosaccharide-lipid synthesis in microsomal vesicles. Cell *21* (1980) 385–392.
44 Spiro, M. J., and Spiro, R. G., Effect of anion-specific inhibitors on the utilization of sugar nucleotides for *N*-linked carbohydrate unit assembly by thyroid endoplasmic reticulum vesicles. J. biol. Chem. *260* (1985) 5808–5815.
45 Stanley, P., Altered glycolipids of CHO cells resistant to wheat germ agglutinin. ACS Symp. Ser. *128* (1980) 213–221.
46 Stanley, P., Selection of lectin-resistant mutants of animal cells. Meth. Enzymol. *96* (1983) 157–184.
47 Stanley, P., Membrane mutants of animal cells: rapid identification of those with a primary defect in glycosylation. Molec. cell. Biol. *5* (1985) 923–929.
48 Suzuki, Y., Ecker, C. P., and Blough, H. A., Enzymatic glucosylation of dolichol monophosphate and transfer of glucose from isolated dolichyl-D-glucosyl phosphate to ceramides by BHK-21 cell microsomes. Eur. J. Biochem. *143* (1984) 447–453.
49 Westerman, J., and Wirtz, K. W. A., The primary structure of the nonspecific lipid transfer protein (sterol carrier protein 2) from bovine liver. Biochem. biophys. Res. Commun. *127* (1985) 333–338.
50 Wilkinson, F. E., Morré, D. J., and Keenan, T. W., Ganglioside biosynthesis: characterization of uridine diphosphate galactose: GM_2 galactosyltransferase in Golgi apparatus from rat liver. J. Lipid Res. *17* (1976) 146–153.
51 Wolosin, J. M., A procedure for membrane-protein reconstitution and the functional reconstitution of the anion transport system of the human-erythrocyte membrane. Biochem. J. *189* (1980) 35–44.
52 Wong, M., Brown, R. E., Barenholz, Y., and Thompson, T. E., Glycolipid transfer protein from bovine brain. Biochemistry *23* (1984) 6498–6505.
53 Yamada, K., and Sasaki, T., A rat brain cytosol protein which accelerates the translocation of galactosylceramide, lactosylceramide and glucosylceramide between membranes. Biochim. biophys. Acta *687* (1982) 195–203.
54 Yamada, K., and Sasaki, T., Rat liver glycolipid transfer protein: a protein which facilitates the translocation of mono- and dihexosylceramides from donor to acceptor liposomes. J. Biochem. *92* (1982) 457–464.
55 Yamada, K., Abe, A., and Sasaki, T., Specificity of the glycolipid transfer protein from pig brain. J. biol. Chem. *260* (1985) 4615–4621.
56 Yamada, K., Abe, A., and Sasaki, T., Glycolipid transfer protein from pig brain transfers glycolipids with β-linked sugars but not with α-linked sugars at the sugar-lipid linkage. Biochim. biophys. Acta *879* (1986) 345–349.
57 Yusuf, H. K. M., Pohlentz, G., and Sandhoff, K., Tunicamycin inhibits ganglioside biosynthesis in rat liver Golgi apparatus by blocking sugar nucleotide transport across the membrane vesicles. Proc. natl Acad. Sci. USA *80* (1983a) 7075–7079.
58 Yusuf, H. K. M., Pohlentz, G., Schwarzmann, G., and Sandhoff, K., Ganglioside biosynthesis in Golgi apparatus of rat liver: stimulation by phosphatidylglycerol and inhibition by tunicamycin. Eur. J. Biochem. *134* (1983b) 47–54.
59 Yusuf, H. K. M., Pohlentz, G., and Sandhoff, K., Ganglioside biosynthesis in Golgi apparatus: new perspectives on its mechanism. J. Neurosci. Res. *12* (1984) 161–178.

0014-4754/90/060611-06$1.50 + 0.20/0