


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. 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. 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, 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. 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. 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 GD1a gangloside to the plasma membrane. Only low temperature effectively blocked the translocation. It appears that vesicular transport is the most likely mechanism for the transport of glycosphingolipids from the site of synthesis to the plasma membrane. 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\(^16\) (Lec2 and clone 1021) have a 90% reduction in the sialylation of both glycoproteins and glycolipids compared with wild-type cells. Sialosylactosylceramide (GM\(_3\) ganglioside) is the major glycolipid in parent cells, while Lec2 and clone 1021 cells have lactosylceramide as the predominant glycolipid.\(^8\) 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 synthases 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’-disothiocyanato-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.