A role for glycosphingolipids in protein sorting
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Summarizing discussion

All mammalian cells synthesize glycosphingolipids and high levels are present in myelinating cells, and in epithelia of the intestine and kidney. Glycosphingolipids are indispensable for the development of multicellular organisms and for proper differentiation of tissues, particularly the nervous system (111, 222-224). However, little is known about the functions of glycosphingolipids in individual cells.

Galactosylceramide is synthesized in the lumen of the endoplasmic reticulum

The biochemical pathways of glycosphingolipid biosynthesis are relatively well-understood and many enzymes involved have now been cloned. A next challenge is to resolve how the synthesis of glycosphingolipids is organized and controlled in the cell. We determined the cellular localization of UDP-galactose:ceramide galactosyltransferase (GalT-1). GalT-1 catalyzes the transfer of galactose from UDP-galactose to ceramide, yielding galactosylceramide and UDP. We have shown that the enzyme is exclusively localized to the endoplasmic reticulum and nuclear envelope by immunogold electron microscopy on ultrathin cryosections. Knock-out mice lacking GalT-1 do not make GalCer, galactodiglyceride and their derivatives, demonstrating that there is only one GalT-1 (111, 112, 126). The GalT-1 activity previously observed in Golgi membrane fractions is an in vitro artefact, and could be attributed to the UDP-glucose:ceramide glucosyltransferase (CGlcT). Based on the primary structure of GalT-1 and on protease protection assays, we conclude that GalT-1 is a type I membrane protein with the largest part in the lumen of the endoplasmic reticulum. Furthermore, GalT-1 enzyme activity required import of UDP-galactose into the lumen of the endoplasmic reticulum by a UDP-galactose transporter activity that is present in CHO cells but absent from CHOlec8 cells.

UDP-galactose import in the ER requires a UDP-galactose transporter

Galactosylation of glycolipids also occurs in the Golgi. For example, GlcCer is converted to lactosylceramide by the UDP-galactose:glucosylceramide β-1,4-galactosyltransferase in the lumen of the Golgi (122, 239, 240, 244). UDP-galactose is synthesized in the cytosol (226), and its translocation into the lumen of the Golgi is facilitated by an antiporter, that transports UMP in the opposite direction (227, 228). The cDNA of the UDP-galactose translocator (UGT) has been cloned and the expression product localized to the Golgi (235). Some cell types possess a UDP-galactose transporter activity in the ER, and this activity is affected in cell lines with a mutation in the Golgi UGT (236). We have transfected CHOlec8 cells with GalT-1, and showed that co-transfection with the UGT greatly stimulates both the synthesis of lactosylceramide in the Golgi and the synthesis of galactosylceramide in the ER, in vivo as well as in vitro. Using immunofluorescence microscopy and subcellular fractionation, we found that UGT in UGT-CHOlec8 cells is located to the Golgi, but that in cells co-transfected with GalT-1 a significant fraction of UGT located to the ER. Furthermore, UGT could be co-immunoprecipitated by anti-GalT-1 antibodies from cells transfected with GalT-1. We conclude that GalT-1 ensures a supply of UDP-galactose in the ER lumen by retaining UGT in the ER, probably as a molecular complex. Oligomerization is a common theme in the organization of glycosylation events. It allows proper co-localization of proteins involved in the same biosynthetic process, and the channeling of substrates to increase the efficiency and specificity of enzymatic reactions.

Glycosphingolipids are required for the sorting of melanosomal proteins

Individual cells survive without glycolipids, indicating that glycosphingolipids are not essential for cell survival and growth (263). We found a dramatic phenotype for glycosphingolipid-
deficient GM95 melanoma cells: whereas the parental MEB4 mouse melanoma cell line is black, GM95 cells are white. GM95 cells failed to synthesize melanin pigment, because the tyrosinase, the first and rate-limiting enzyme in melanin formation, was not transported to melanosomes, but accumulated in the Golgi. Tyrosinase with a lengthened transmembrane domain, and tyrosinase-related protein 1 (TRP-1) reached melanosomal structures via the plasma membrane instead of the direct intracellular route from the Golgi complex. Pigmentation and intracellular transport of tyrosinase and TRP-1 were restored upon transfection with CGlcT. Two direct pathways from the Golgi complex to endosomes are known. They select their cargo through interactions between an adaptor-protein complex (AP-1 and AP-3) and a sorting signal in the cytoplasmic tails of cargo proteins. Both tyrosinase and TRP-1 contain an AP-3 signal and are missorted in GM95 cells, indicating a defect in AP-3 mediated sorting. Biosynthetic transport of lysosomal enzymes was unaffected in GM95 cells, showing that the AP-1 pathway was not affected by the absence of glycolipids.

Sphingolipids, and in particular glycosphingolipids, are able to form lateral microdomains in an environment of glycerolipids (61). These microdomains are believed to serve as platforms for various cellular events such as protein sorting in the Golgi (103, 116). The cellular localization, biosynthetic transport and microdomain association of a glycosylphosphatidylinositol-anchored protein was normal in GM95 cells (293), indicating that glycosphingolipids are not essential for the formation and the proper functioning of these type of microdomains.

Taken together, glycosphingolipids are required for sorting of melanosomal proteins in the Golgi. An attractive scenario would be that glucosylceramide on the cytosolic surface of the Golgi is involved in a comparable fashion as phosphoinositides are involved in the regulation of AP-1 and AP-2 activities (284, 294). Alternatively, glycosphingolipids may laterally aggregate into a microdomain, possibly in the cytosolic leaflet of the Golgi, that could act as a scaffold for AP-3 mediated sorting. New studies are on their way to elucidate how (and which) glycosphingolipids carry out this role at the molecular level.