What sugar next? Dimerization of sphingolipid glycosyltransferases

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One of the great riddles of glycobiology is the function of the glycosphingolipids. Their vital role is clear from the fact that the lack only of subsets of glycosphingolipids results in premature death (1). Hundreds of glycosphingolipids populate the surface of mammalian cells. These may serve a general function in membrane structure as their physical properties are different from those of the bulk glycerophospholipids (2). In contrast, the mammalian glycolipids are only a small subset of the millions of theoretical structures that can be formed from, for example, five monosaccharides. The large amount of structural information in their carbohydrate backbone makes them exquisitely suited for mediating specific interactions. Indeed, only a limited number of specific glycosphingolipids occur on a particular cell, and a defined set of enzymes is responsible for their ordered synthesis and degradation. Although some information is available about the transcriptional regulation of the various glycosyltransferases, most glycolipid synthesis occurs within a single cellular compartment (the late Golgi), and it is unclear how the relative amounts of the various glycosphingolipids are controlled. The paper by Giraudo *et al.* (3) reports the finding that two glycosyltransferases responsible for sequential steps in glycolipid assembly form a molecular complex. This result predicts channeling of substrates whereby the product of the first enzyme is preferentially used by the second and not by a competing transferase. Independent findings on glycosylation of lipids in the ER and proteins in the trans Golgi show that both homo- and heterodimerization are common themes in the organization of glycosylation events.

Mammalian glycosphingolipids are synthesized by the stepwise addition of monosaccharides to ceramide (Fig. 1). Ceramide is synthesized in the ER, and the last glycosylation events occur in the late Golgi. An attractive possibility would be that the processing of the complex glycosphingolipids is organized as an assembly line in the Golgi similarly to that of glycoproteins, with the first steps occurring in the cis Golgi, subsequent steps in the medial Golgi, and terminal glycosylation in the trans Golgi and trans Golgi network (TGN). In support of this idea, after interference with intra-Golgi transport by drugs like monensin and brefeldin A or by a biological process like mitosis, cell fractionation studies localized the enzymes for the synthesis of LacCer, Lc_3, and GM3 (but also GD3, disialo-LacCer) to early Golgi and those synthesizing all higher glycolipids (but also Gb_3) to late Golgi (see refs. 4 and 5). However, later studies suggested that the bulk of each of these enzymes is located in the trans Golgi and TGN (6–8).

According to the present picture of glycolipid synthesis, ER-synthesized ceramide reaches the Golgi probably by two different mechanisms (9). On the cytosolic surface of the Golgi, ceramide is glucosylated to GlcCer (10). GlcCer translocates across the Golgi membrane and is converted to LacCer. Translocation is energy-independent (11, 12) and may not occur in the cisterna, where GlcCer is synthesized (13). Other evidence has suggested that translocation into the cisterna of Gb_3 synthesis was mediated by the multidrug transporter MDR1 P-glycoprotein (14). Our own unpublished work in fibroblasts (R. J. Raggers, D. Sillence, J. Wijnholds, N. Zelcer, R. Klingenstein, K. Sandhoff, and G.v.M.) shows that natural GlcCer was translocated to the Golgi lumen and quantitatively converted to LacCer and GM3 independent of MDR1. Part of the GlcCer bypassed this mechanism, reached the cytosolic surface of the plasma membrane, and was subsequently translocated across the plasma membrane by MDR1. It is unclear whether the latter pool of GlcCer was synthesized at a different location (15, 16) or escaped translocation from a common site of synthesis

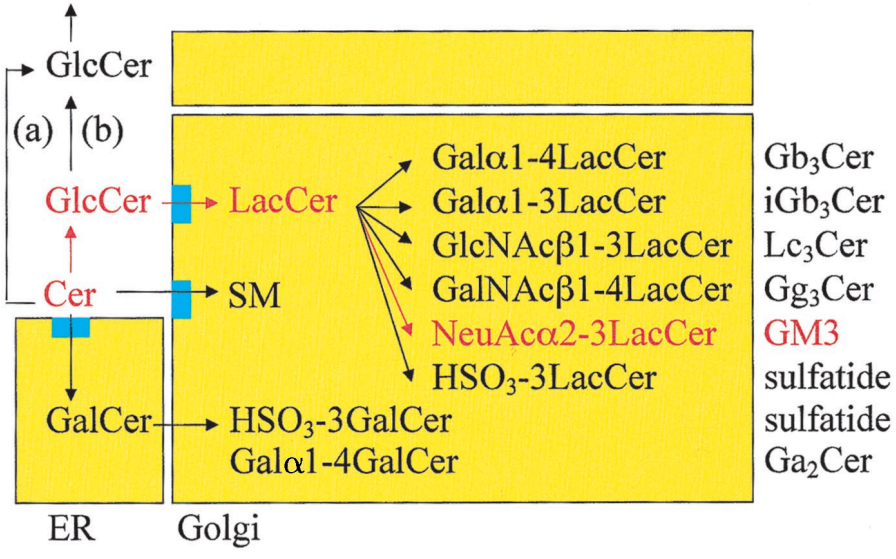

Fig. 1. Early glycosphingolipid biosynthesis in ER and Golgi complex. Lipids outside the yellow organelles are synthesized on the cytosolic surface. Blue boxes indicate the translocation of a lipid across the membrane. Cer, ceramide; Ga, gala; Gal, galactose; GalNAc, *N*-acetylgalactosamine; Gb, globo; Gg, ganglio; Glc, glucose; GlcNAc, *N*-acetylglucosamine; iGb, isoglobo; NeuAc, neuraminic/sialic acid; SM, sphingomyelin. For nomenclature of glycolipids, see ref. 26. The synthesis of the ganglioside GM3 (3) is indicated in red. (*a*) GlcCer is synthesized at two locations, or (*b*) transport competes with translocation.

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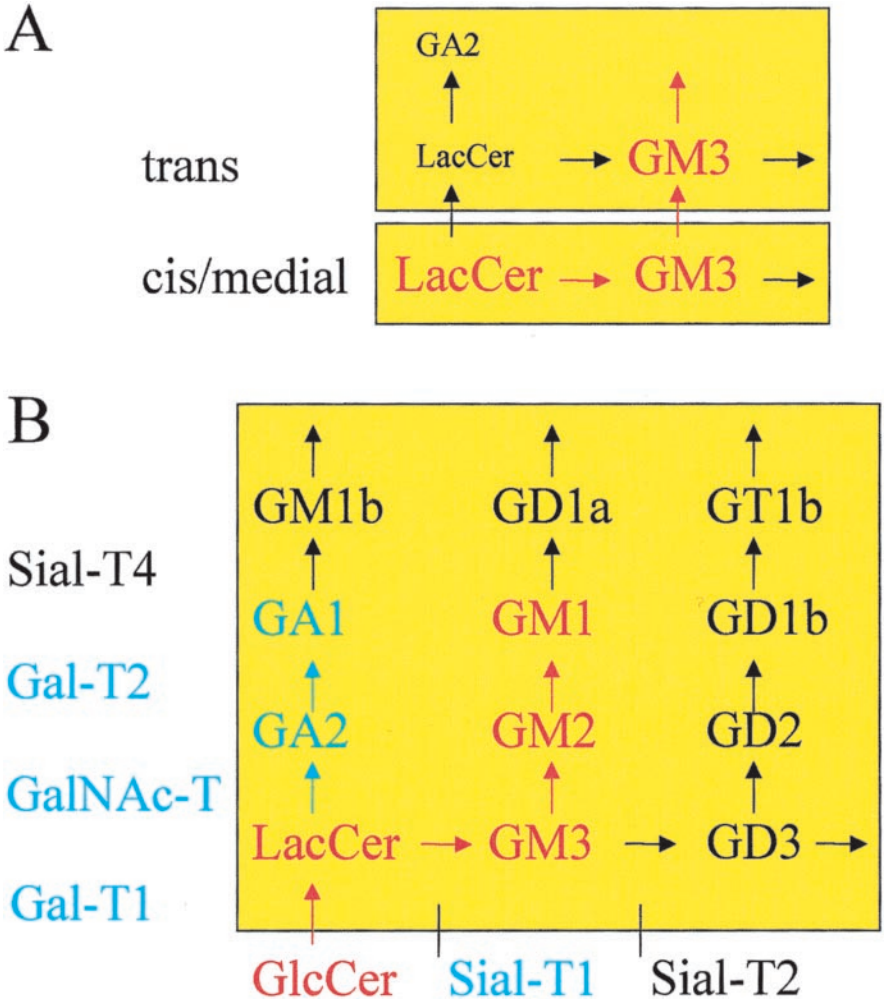

Fig. 2. Biosynthesis of the first gangliosides in the Golgi. (*A*) Conversion of LacCer to GM3 in an earlier Golgi cisterna than conversion to GA2. (*B*) Conversions to GM3 and GA2 in the same trans-Golgi cisterna. For enzyme nomenclature, see ref. 5; for alternatives for Gal-T1 and Gal-T2: GalT-2 and GalT-3, respectively; see ref. 27. GalT-1 is the ceramide galactosyltransferase synthesizing GalCer. All of these enzymes have now been cloned. Enzymes and gangliosides discussed in the text are indicated in blue and red.

by diffusing into a pathway toward the plasma membrane. GlcCer slowly recycles from the plasma membrane to the Golgi lumen. LacCer in the Golgi is converted to higher glycolipids, as indicated in Figs. 1 and 2.

In the presence of two enzymes that use LacCer as a substrate, synthesis of GM3 had priority over that of GA2 (17). Potentially, GM3 was synthesized in an earlier compartment than GA2 (Figure 2*A*), as suggested by brefeldin experiments (4), not inconsistent with localization experiments (6–8). If, on the contrary, both enzymes are in the same trans-Golgi cisterna (Fig. 2*B*), they possess very different K_Ms for LacCer, or LacCer is channeled from Gal-T1 to Sial-T1. Channeling could occur if the two enzymes formed a functional complex, as proposed 30 years ago by Roseman (18). Giraudo *et al.* (3) now report a complex for the next two enzymes in ganglioside biosynthesis, GalNAc-T and Gal-T2 (3). The enzyme complex may channel GM3 straight into GM1, which may explain why the brain contains ample GM1 and GD1a but hardly any GM2. These type II glycosyltransferases interact via their N-terminal domains: the cytosolic tail and the transmembrane region. When cotransfected, the truncated N-terminal domain of Gal-T2 competed with full length Gal-T2 in coimmunoprecipitations and *in vitro* inhibited the two-step conversion of GM3 to GM1. This result argues in favor of a specific interaction between the two transferases rather than common residence in glycolipid rafts (2). In contrast to this noncovalent interaction, GalNAc-T forms disulfide-bonded homodimers (19). It will be interesting to learn whether the full complex is a heterotetramer *in vivo* (Fig. 3), or whether it forms a multicomponent complex, as has been identified in the yeast cis Golgi (20), and whether any of these interactions are involved in localizing these enzymes to the TGN, as has been proposed in kin-recognition models of protein glycosyltransferases and glycosidases (see ref. 21).

Whereas most of the sphingolipid glycosyltransferases structurally resemble the protein glycosyltransferases, there are two clear exceptions. The ceramide glucosyltransferase that synthesizes GlcCer on the cytosolic surface of the Golgi is a type III protein, with its C-terminal mass in the cytosol (10). This protein forms noncovalent dimers or oligomers (22). Wouldn't it be nice if its partner turned out to be the elusive Golgi GlcCer translocator? The second atypical glycosyltransferase is the ceramide galactosyltransferase, a type I protein with a C-terminal ER-retention signal that synthesizes GalCer on the luminal side of the ER (23, 24). A long-standing question has been how this protein gets access to its UDP-galactose in the ER lumen in the absence of an ER UDP-galactose transporter. Our recent

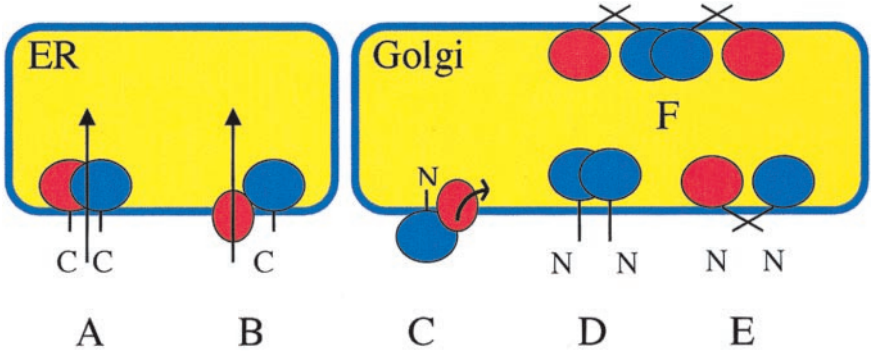

Fig. 3. Various possibilities for functional association of transferases and transporters in glycosphingolipid biosynthesis. For details see text: (*A*) heterodimer of glucuronosyltransferases forming a UDP-glucuronic acid pore; (*B*) ceramide galactosyltransferase complexed to the Golgi UDP-galactose transporter; (*C*) ceramide glucosyltransferase complexed with, potentially, the Golgi GlcCer translocator; (*D*) homodimer of the GalNAcT; (*E*) heterodimer of GalNAcT and Gal-T2; (*F*) combination of *D* and *E*.

work has now demonstrated that the ceramide galactosyltransferase retains the Golgi UDP-galactose transporter UGT1 in the ER by forming a complex (H. Sprong, H. Segawa, S. Degroote, T. Nilsson, M. Kawakita, P. van der Sluijs and G.v.M., unpublished data). This transferase is a member of the family of ER glucuronosyltransferases. Other members have been proposed to form noncovalently linked heterodimers (ref. 25; S.-i. Ikushiro, personal communication). In a great number of respects, dimerization correlated with UDP-glucuronic acid uptake, suggesting that the transferase dimer itself forms the actual pore.

Besides knowledge at the level of gene regulation of the biosynthetic enzymes and translocators, insight into their topological organization will uncover mechanisms by which the relative amounts and locations of the various glycosphingolipids are regulated. Surely knowing where and under which conditions each glycosphingolipid is present will greatly contribute to understanding its unique biological function.

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