thematic review

Regulation of ganglioside biosynthesis in the nervous system

Robert K. Yu,[1] Erhard Bieberich,[2] Tian Xia, and Guichao Zeng[2]

Institute of Molecular Medicine and Genetics, School of Medicine, Medical College of Georgia, Augusta, GA 30912

Abstract **Ganglioside biosynthesis is strictly regulated by the activities of glycosyltransferases and is necessarily controlled at the levels of gene transcription and posttranslational modification. Cells can switch between expressing simple and complex gangliosides or between different series within these two groups during brain development. The sequential biosynthesis of gangliosides in parallel enzymatic pathways, however, requires fine-tuned subcellular sequestration and orchestration of glycosyltransferases. A popular model predicts that this regulation is achieved by the vectorial organization of ganglioside biosynthesis: sequential biosynthetic steps occur with the traffic of ganglioside intermediates through subsequent subcellular compartments. Here, we review current models for the subcellular distribution of glycosyltransferases and discuss results that suggest a critical role of *N*-glycosylation for the processing, transport, and complex formation of these enzymes. In this context, we attempt to illustrate the regulation of ganglioside biosynthesis as well as the biological significance of *N*-glycosylation as a posttranslational regulatory mechanism. We also review the results of analyses of the 5′ regulatory sequences of several glycosyltransferases in ganglioside biosynthesis and provide insights into how their synthesis can be regulated at the level of transcription.**—Yu, R. K., E. Bieberich, T. Xia, and G. Zeng. **Regulation of ganglioside biosynthesis in the nervous system.** ***J. Lipid Res.*** **2004.** 45: **783–793.**

Supplementary key words ceramide • glycosyltransferase • posttranslational modification • *N*-glycosylation • transcription • transcription factor • Golgi • endoplasmic reticulum

Gangliosides are important constituents of cells and play a variety of biological functions, including cellular recognition and adhesion as well as signaling. The expression of gangliosides is not only cell specific and developmentally regulated but also closely related to the differentiation state of the cell (1, 2). Numerous studies have indicated that ganglioside changes during cellular differentiation are closely related to their metabolism, particularly in their biosynthesis. Thus, it is possible to control the expression of gangliosides by regulating glycosyltransferase (GT) activities that are responsible for ganglioside biosynthesis. The activities of GTs can be regulated at several levels, including transcriptional and posttranslational controls. At least two basic mechanisms of posttranslational control can be affected, phosphorylation and *N*-glycosylation. Phosphorylation/dephosphorylation offers an important "fast" regulatory mechanism in response to physiological demands, and its implications have recently been reviewed (3). Here, we focus on the *N*-glycosylation of GTs, an important step in regulating their activities, half-life, and intracellular transport and localization. In addition, in recent years, many cDNAs encoding GTs for ganglioside biosynthesis have been cloned by several investigators, including us. The genes of these enzymes can also be regulated at the transcriptional level during brain development. Analysis of the 5′ flanking region of the GT genes revealed a number of common features and potential sites for known transcription factors. Here, we review studies of the promotor region to gain insights into these regulatory mechanisms. An understanding of the regulation of GTs should provide powerful means for investigating the functional roles of gangliosides in cellular differentiation and proliferation.

N-GLYCOSYLATION OF GTS

Vectorial organization of ganglioside biosynthesis

The use of radiolabeled sphingolipid precursors has shown that in ganglioside biosynthesis, subsequent reactions are catalyzed by GTs that act in parallel pathways. As shown in **Fig. 1**, these pathways give rise to different series of simple and complex gangliosides. In general, ganglioside biosynthesis starts with ceramide, the common precursor for acidic and nonacidic glycosphingolipids. Ceramide is first converted to glucosylceremide by UDP-

Manuscript received 1 December 2003 and in revised form 23 February 2004.

Published, JLR Papers in Press, March 1, 2004.
DOI 10.1194/jlr.R300020-JLR200

[1] To whom correspondence should be addressed.
e-mail: ryu@mcg.edu
[2] E. Bieberich and G. Zeng contributed equally to this review.

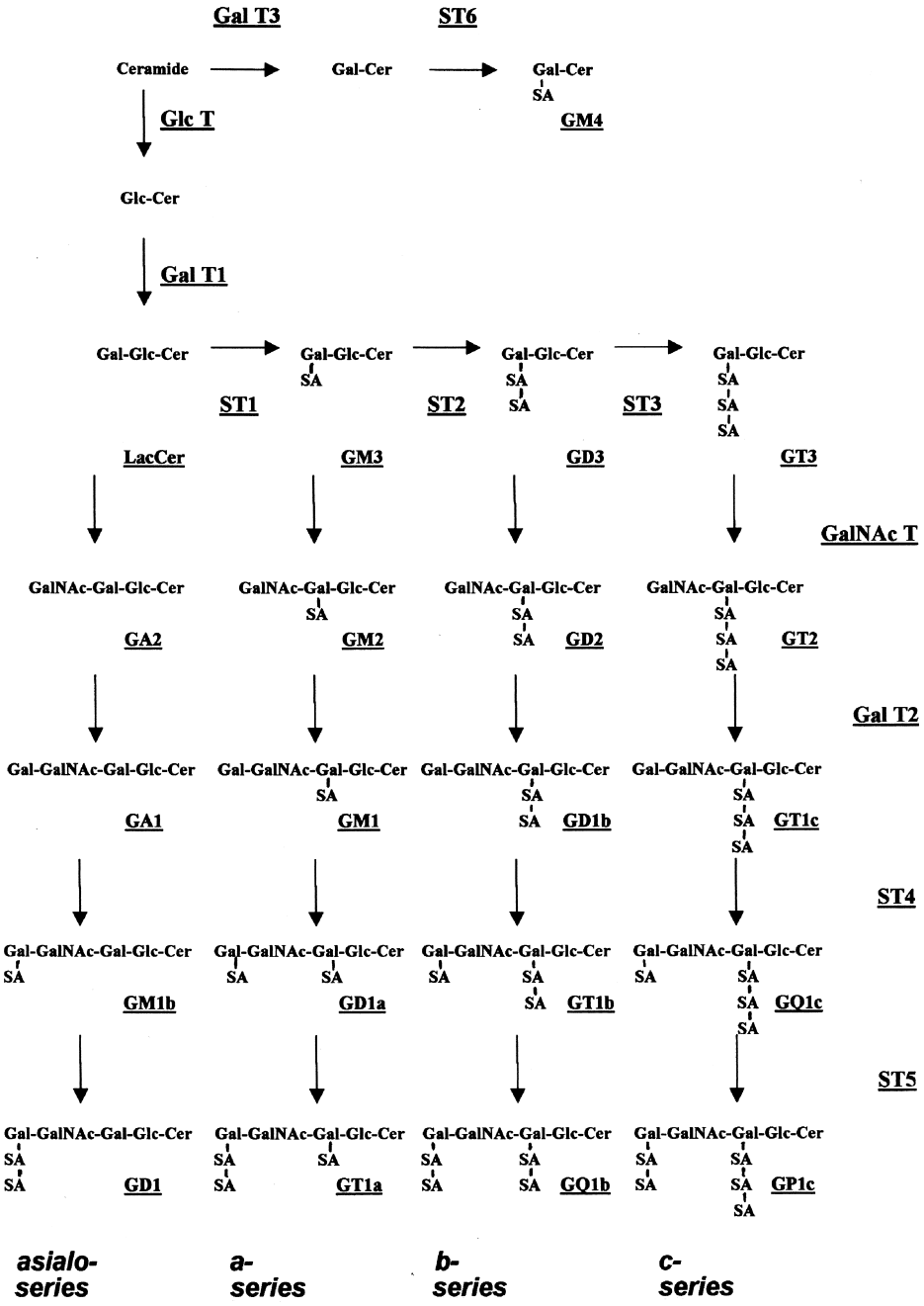

Fig. 1. Pathways of ganglioside biosynthesis. Cer, ceramide; SA, sialic acid; GalNAcT, *N*-acetylgalactosaminyltransferase I or GM2/GD2/GT2-synthase; GalT1, galactosyltransferase I; GalT2, galactosyltransferase II or GM1-synthase; GalT3, galactosyltransferase III; GlcT, glucosyltransferase; ST1, sialyltransferase I or GM3-synthase; ST2, sialyltransferase II or GD3-synthase; ST3, sialyltransferase III or GT3-synthase; ST4; sialyltransferase IV or GD1a-synthase; ST5, sialyltransferase V or GT1a-synthase; ST6, sialyltransferase VI or GM4-synthase. Ganglioside names are abbreviated according to Svennerholm (96).

glucose:ceramide β-glucosyltransferase, which is then followed by the addition of galactose, yielding lactosylceramide. The addition of the first sialic acid residue converts lactosylceramide to GM3, the precursor of most of the complex brain gangliosides, which is catalyzed by sialyltransferase I (ST1) or GM3-synthase. Further addition of sialic acid residues generates GD3 (catalyzed by ST2 or GD3-synthase) and GT3 (catalyzed by ST3 or GT3-synthase). ST1, ST2, and ST3 are all distinct enzyme entities arising from different genes. GM3, GD3, and GT3 represent the entry substrates for the biosynthesis of complex-type gangliosides in the a-, b-, and c-series pathways, respectively (Fig. 1). Further synthesis of the complex hexosamine-containing gangliosides is unique in that several identical GTs may participate in catalyzing the addition of various sugar residues to different ganglioside acceptor substrates. These enzymes include *N*-acetylgalactosaminyltransferase I or GM2/GD2-synthase, galactosyltransferase

II (GalT2) or GM1/GD1b-synthase, ST4 or GD1a/GT1b-synthase, and ST5 or GT1a/GQ1b-synthase. Most of the basic steps have been characterized using rather crude cell-free systems (4–9). The development of parallel pathways was achieved by Yu and Ando (10, 11) after the structural characterization of the c-series gangliosides, including GT3, GT2, GT1c, and GQ1c (Fig. 1). They also proposed the c-series pathway of ganglioside biosynthesis. The addition of the a- and b-series pathways is a logical extension of the proposed pathways based on the original work in Roseman's laboratory (4, 5). Subsequently, the so-called α-series pathway (12) was added. Evidence has been presented that some of the GTs may also catalyze the biosynthesis of the asialo-series of gangliosides, indicating a low degree of substrate specificity (13). The validity and completion of most of the steps have also been established by many investigators, including one of us (9–17).

In 1988, Sandhoff's group (18) demonstrated that equivalent steps in the parallel pathways are catalyzed by identical enzymes and that the biosynthesis of complex gangliosides is controlled by the entry reaction for each pathway. The validity of this observation was confirmed by us (17). We further demonstrated that GT3-synthase is the rate-limiting step for the c-series ganglioside biosynthesis (17). There is evidence that deviation from strict substrate specificity may be contributed, at least in part, by the cell- or tissue-specific expression of isoenzymes (19, 20).

Although glycosidases are clearly involved in the catabolism of gangliosides, the expression of cellular gangliosides has long been believed to rely to a large extent on the biosynthetic steps that provide the product gangliosides. In other words, to increase a particular ganglioside, the cell has to increase the activity of the enzyme that catalyzes its biosynthesis. Although this conclusion comes naturally, it is clear that the mechanism by which cells regulate the composition of gangliosides is not that simple. Other factors must also be considered. For this reason, we have proposed an enzyme kinetic model that predicts a steady-state concentration or continuous "flow" of gangliosides in each of the pathways (21). Assuming that the concentration of the other substrate, the sugar nucleotide, is in a constant supply, the steady state is dependent on the enzyme that consumes a ganglioside intermediate and not on the enzyme that catalyzes its biosynthesis. In other words, to increase a particular ganglioside, the cell has to decrease the activity of the enzyme that uses this ganglioside as its substrate. This model implies two consequences that distinguish it from the first assumption of regulating the biosynthesis of a product ganglioside. As the first consequence, our model allows for the simultaneous calculation of all enzyme activities from the ganglioside composition in any of the parallel pathways. This has led to a simplified kinetic analysis [multienzyme kinetic analysis (MEKA)] of ganglioside biosynthesis by matrix calculation, which shows a remarkable consistency with the actual measured concentrations and enzyme activities (21). As a second consequence, our model predicts the existence of distinct subcellular ganglioside pools that can be distinguished by the concrete values of the kinetic analysis. The existence of distinct subcellular ganglioside pools directing the biosynthetic flow and traffic of ganglioside intermediates has been suggested in several previous studies (22–24). However, only MEKA has been able to correlate quantitatively the ganglioside concentration in each pool with the localization of GTs that are required for its biosynthesis.

In a model of vectorial ganglioside biosynthesis, the traffic of ganglioside intermediates moves along with the subcellular localization and/or transport of GTs throughout the endoplasmic reticulum (ER)-to-Golgi axis. In cases in which the transport of the product ganglioside is slow, it will be enriched in a common pool with the enzyme that catalyzes its biosynthesis. However, if the transport of the product ganglioside is fast but its consumption is slow, the ganglioside will become enriched in the subcellular compartment where the enzyme that uses this ganglioside as its substrate is located (21). In the last two decades, tremendous progress has been achieved in localizing gangliosides and their respective GTs. Initial efforts used exclusively subcellular fractionation, probably because of the lack of antibodies that can be used for localization studies by immunofluorescence or electron microscopy (25–28). Meanwhile, specific antibodies against different gangliosides and epitope-tagged or fluorescent protein-linked GTs are available that have contributed significantly to the analysis of subcellular ganglioside and GT transport and localization (29–33). Most of these reports agree that the initial steps of ceramide and neutral glycosphingolipid biosynthesis occur in the ER (34). Interestingly, ceramide is first synthesized on the cytosolic face of the ER membrane and then glucosylated. Glucosylceramide flips to the luminal side and is converted to lactosylceramide by Golgi-resident galactosyltransferase I (Fig. 1). There is an ongoing debate, however, about the site of GM3 biosynthesis. Early studies reported that GM3 is synthesized in the Golgi apparatus, most likely in its *cis* subcompartment (34–36). Later studies, in particular from our laboratory, have shown that GM3 may already be present in the ER (33). These results were based on subcellular localization studies with GM3-synthase that was tagged with a fluorescent protein tag. At present, it is fair to conclude that the localization of GM3-synthase is most likely cell or tissue specific and may encompass the ER as well as Golgi compartments. There has also been considerable discussion of the subcellular localization of other GTs, in particular GM2/GD2-synthase and GD3-synthase. Subcellular localization studies reported in the literature indicate a Golgi localization for both enzymes (29, 31). Our own studies, however, could only confirm a strict Golgi localization for GD3-synthase, whereas GM2/GD2-synthase appeared to be broadly distributed in the ER and Golgi (30, 33). To date, we can only conclude from these results that GM3- and GM2/GD2-synthases are localized in the ER and/or Golgi, whereas GD3-synthase is distributed to the Golgi. These results are not trivial, because the subcellular localization of GTs may determine the sequence in which gangliosides are synthesized. For example, if GM3 encounters GM2/GD2-synthase before GD3-synthase,

it will most likely be converted to a-series complex gangliosides; in the opposite case, b-series complex gangliosides will prevail. At present, however, the possibility cannot be excluded that some of the results obtained with epitope- or fluorescent protein-tagged GTs are flawed by overexpression or tagging of the recombinant protein. Hence, we will have to wait for the availability of antibodies against the natural enzymes to clearly determine the subcellular localization of GTs under physiological conditions.

Ganglioside-specific GTs are *N*-glycosylated type II transmembrane proteins

Within the last decade, most of the cDNAs for GTs in the ganglioside biosynthetic pathways have been cloned from and expressed in various mammalian tissues and cells (37–41). Common to all of the GTs is the presence of three or four *N*-glycosylation sites and a transmembrane II topology that predicts a short cytoplasmic N-terminal tail, a single hydrophobic transmembrane domain, and a larger C-terminal, catalytically active domain that is sequestered in the ER lumen (42, 43). Maccioni's group and our group (29–33, 44) have clearly shown that GD3-synthase, GM2/GD2-synthase, and also GM3-synthase are expressed as *N*-glycosylated proteins with transmembrane II topology. Our group has shown that these three proteins undergo *N*-glycoprotein processing catalyzed by ER- and Golgi-resident glycosidases but do not acquire a complex *N*-glycan or *N*-linked oligosaccharide structure (30). At this point, it is necessary to briefly recapitulate the sequence of reactions that occur during *N*-glycosylation and *N*-glycoprotein processing (45, 46). Interestingly, *N*-glycosylation begins with an ER membrane-bound carrier lipid termed dolichol pyrophosphate. At the cytosolic face of the ER membrane, a mannose(9)-*N*-acetylglucosamine(2) (Man9GlcNAc2) oligosaccharide is synthesized by the sequential addition of sugar residues onto dolichol pyrophosphate. This oligosaccharide flips into the ER lumen, where it is further glucosylated to a Glc3Man9GlcNAc2 precursor. The dolichol-linked precursor oligosaccharide serves as a donor substrate for the *N*-glycosylation of a particular acceptor sequence of the type Asn-X-Ser/Thr within the nascent polypeptide of the presumptive *N*-glycoprotein. This reaction is catalyzed by oligosaccharyltransferase, which transfers the entire Glc3Man9GlcNAc2 precursor from dolichol pyrophosphate onto the Asn residue within the acceptor sequence (47, 48). It is important to note that this en bloc transfer occurs cotranslationally, before the *N*-glycoprotein is completely folded and has acquired its native conformation. After the en bloc transfer of the precursor oligosaccharide, ER- and Golgi-resident glycosidases trim down the Glc3Man9GlcNAc2 structure by sequential hydrolytic removal of terminal sugar residues from the *N*-linked oligosaccharide (49, 50). In the *trans*-Golgi subcompartment, the residual core structure of Man3–5GlcNAc2 will then be reglycosylated by the addition of GalNAc, galactose, and sialic acid residues, giving rise to complex *N*-glycoproteins (47). We have analyzed the structure of the *N*-linked oligosaccharides of GD3-synthase and GM2/GD2-synthase using enzymes that cleave off specific *N*-glycans from the *N*-glycoprotein (30). We found that in murine neuroblastoma (F-11) cells, ER- and Golgi-resident glycosidases trim down the precursor to a Man3–5 core structure that is not subsequently reglycosylated to form complex *N*-linked oligosaccharides. Based on this observation, we conclude that GD3- and GM2/GD2-synthases are transported from the ER to the Golgi. However, the lack of reglycosylation suggests that GD3- and GM2/GD2-synthases are not transported to the late Golgi or further. Again, this may vary in different cells and tissues, which will certainly provide incentives for future investigations.

N-glycosylation determines the stability and fate of GD3-synthase

Until 10 years ago, very little was known about the physiological significance of *N*-linked oligosaccharides. It was very well established that *N*-glycosylated viral surface proteins are needed to bind to cell membrane receptors (51), and it was known that the clearance of aged glycoproteins from the serum is mediated via the recognition of partially degraded *N*-glycans (52). It was also well established that the phosphorylation of mannose residues is the prerequisite for trafficking of soluble *N*-glycoproteins to the lysosomes mediated by Man-6-phosphate receptors (53). However, the significance of the majority of *N*-linked oligosaccharides remained persistently enigmatic. This situation changed when specific inhibitors for *N*-glycoprotein processing became available and most of the processing enzymes were cloned and extensively analyzed. Studies by Helenius's group (46, 54, 55) and Parodi (56) presented evidence for the significance of *N*-linked oligosaccharides and early *N*-glycoprotein processing for protein folding and maturation. Because *N*-glycosylation is a cotranslational process, in many cases, folding of the protein has only reached an immature state when the precursor glycan is attached to the polypeptide. The *N*-linked Glc3Man9GlcNAc2 oligosaccharide precursor is subject to hydrolytic cleavage of the first two terminal glucose residues, which is catalyzed in the ER by glucosidase I (46, 49). The next two glucose residues are removed by glucosidase II, which initiates further trimming by ER- and Golgi-resident mannosidases. The removal of mannose residues, however, only proceeds if the protein is properly folded. If the protein is not correctly folded, it will be reglucosylated, and a sensor/chaperone complex consisting of calnexin/calreticulin or calnexin/grp78 recognizes the *N*-linked Glc1Man9GlcNAc2 oligosaccharide and initiates refolding of the protein (47, 56). This cycle of deglucosylation and reglucosylation is repeated until the protein has been refolded by the sensor/chaperone complex into its correct conformation and the *N*-glycan can be processed further. In cases in which the correct folding cannot be achieved, the *N*-glycoprotein accumulates in the ER and will eventually be degraded by ER-resident proteases (46).

Although the validity of this model has been clearly demonstrated with viral glycoproteins, it was only recently

discovered that other *N*-glycoproteins are processed by a similar sensor/chaperone-driven mechanism. When we determined the turnover of GD3-synthase after incubation of murine neuroblastoma (F-11) cells with castanospermine, a specific inhibitor for the processing of glucosidase I, we found that the half-life of this sialyltransferase was reduced by 5-fold (30). Furthermore, we showed that nonprocessed GD3-synthase was not bound by calnexin, whereas the processed enzyme expressed in F-11 cells was. In addition, nonprocessed GD3-synthase was not transported to the Golgi but remained in the ER. This line of evidence suggested that GD3-synthase is one of the proteins that undergoes the deglucosylation and reglucosylation cycle for protein folding. Moreover, failure to completely process the *N*-linked oligosaccharides will interfere with the transport of GD3-synthase to the Golgi. Our results on the trafficking of GD3-synthase were consistent with those reported in the literature (29, 31, 32). However, we found that our model for the processing and transport of GD3-synthase did not apply to all GTs in ganglioside biosynthesis. We showed that GM2/GD2-synthase was not subject to enhanced turnover or impaired transport when *N*-glycoprotein processing was inhibited (30). We also did not find that GM2/GD2-synthase was bound by calnexin. These observations suggest that the processing and transport mechanisms for GTs are likely enzyme specific. However, most of the questions pertaining to these mechanisms remain unanswered. For example, what exactly happens during the deglucosylation and reglucosylation of GD3-synthase, and how is the nonprocessed and improperly folded form degraded? Is the Golgi transport of GD3-synthase just a default mechanism for enzyme that is released from the reglucosylation and deglucosylation cycle, or are there specific sorting receptors? What makes GD3-synthase different from GM2/GD2-synthase with respect to *N*-glycoprotein processing and trafficking? Although we cannot answer all of these questions at this time, most recent results from our group suggest that the processing and transport of GD3-synthase and GM2/GD2-synthase is intimately linked to their function in ganglioside biosynthesis (E. Bieberich and R. K. Yu, unpublished data).

Formation of a complex of GD3- and GM2/GD2-synthases promotes the biosynthesis of b-series complex gangliosides

The overexpression of GD3-synthase or GM2/GD2-synthase in exclusively GM3-expressing cells resulted in an unexpected observation (33): although the occurrence of either GD3 or GM2 was predicted, we found that b-series complex gangliosides were synthesized in both cases (Fig. 1). This puzzling result remained unanswered until recent evidence showed that GTs may form enzyme complexes (30, 31). The concept of accelerating or controlling metabolic pathways by enzyme complexes is not new, for it is well known that bacterial fatty acid synthase complexes exist. In fact, the concept of the formation of enzyme complexes in glycoconjugate biosynthesis was initially described by Roseman in 1970 (57). Experimental evidence for the presence of the GT complexes in ganglioside biosynthesis has only recently been provided (30, 33). To date, two binary complexes are known in the Golgi: GM2/GD2-synthase associates with either GM1/GD1b- or GD3-synthase. No evidence has been presented for the formation of a ternary complex. These two binary complexes of GM2/GD2-synthase have been determined by coimmunoprecipitation and fluorescence resonance energy transfer (FRET) experiments (58). The use of different variants of fluorescent protein tags has made it possible to visualize protein interaction in real time and in living cells, in particular when using multiple-photon confocal laser microscopy and spectral analyzers for the emitted light.

We have shown, for the first time, that the complex formation between GD3-synthase and GM2/GD2-synthase channels ganglioside biosynthesis toward b-series complex gangliosides (33). It should be noted that only one of the two enzymes was overexpressed by stable transfection, whereas the other partner in the complex was an endogenous enzyme. **Figure 2** shows that the physical association of the two enzymes results in their mutual activation and in the rapid conversion of GM3 to GD2. Before the complex with GM2/GD2-synthase, GD3-synthase forms a homodimer that is stabilized by disulfide linkages (29, 33). Presumably, the subsequent complex between GM2/GD2-

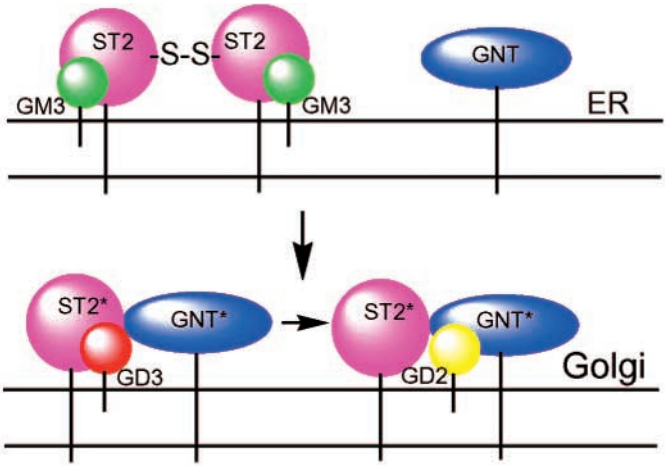

Fig. 2. Regulation of ganglioside biosynthesis by complex formation of ST2 and GalNAcT (GNT). In the endoplasmic reticulum, ST2 is dimerized by disulfide linkage, which may stabilize complex formation with GM3 and promote the transport of a GM3-ST2 complex to the Golgi. In the Golgi, ST2 and GalNAcT form a heterodimer with mutual enzyme activation. In this heterodimer, ST2 converts GM3 to GD3 and channels it to the active site of GalNAcT. GalNAcT converts GD3 to GD2; further glycosylation depends on the activity, subcellular localization, or complex formation with other glycosyltransferases. Asterisks indicate activated enzymes.

and GM1/GD1a-synthases converts GD2 to GD1b, which is followed by the biosynthesis of other b-series complex gangliosides (Fig. 1). In contrast to the GD3-synthase dimer, the other heterodimeric enzyme complexes (GM2/GD2-synthase with GM1/GD1b-synthase or GM2/GD2-synthase with GD3-synthase) appear not to be stabilized by disulfide linkage (31, 33). These experiments confirm our model of multienzyme kinetics in ganglioside biosynthesis, in that the ganglioside expression profile relies on the fast transport of intermediates to the next converting enzyme (21). It follows naturally that fast transport will result from enzyme complexes that are localized in one subcellular compartment, the Golgi. It was unexpected, however, that this complex formation also results in mutual enzyme activation (25). At this point, it should be noted that in several studies with COS cells or other cell types, overexpression of GD3-synthase or GM2/GD2-synthase does not necessarily promote the biosynthesis of b-series complex gangliosides; rather, it results in the expected increase of GD3, GM2/GD2, or a-series complex ganglioside levels (29, 59). Regulation of ganglioside biosynthesis is thus subject to various control mechanisms that may rely on the cell-specific formation of enzyme complexes. It is not known, however, which mechanism controls the specificity of this formation. Below, we discuss experimental evidence for a model that attempts to integrate posttranslational processing, transport of GTs, and complex formation with vectorial biosynthesis and trafficking of gangliosides.

Lipid chaperones and autoregulation of ganglioside biosynthesis

In previous studies, we showed that GD3-synthase binds tightly to GM3 (33, 60, 61). In fact, we used GM3 as a ligand for affinity purification of GD3-synthase and incorporated GM3 into a ribosome display to stabilize active GD3-synthase during in vitro translation (60, 61). Thus, it is likely that GM3 will also bind tightly to GD3-synthase that has been translated in vivo. We have tested this hypothesis by incubating F-11 cells with pyrene-labeled GM3 (33). F-11 cells were transfected with green fluorescent protein (GFP)-tagged GD3-synthase to monitor a potential pyrene-to-GFP FRET signal indicating binding of pyrene-GM3 to the GD3-synthase-GFP fusion protein. To our surprise, the predominant FRET signal was not localized in the Golgi but in the ER. Moreover, prolonged incubation with higher concentrations of pyrene-GM3 resulted in partial relocalization of the GD3-synthase fusion protein into the ER (33).

The observation that the inhibition of *N*-glycoprotein processing as well as incubation with GM3 delayed the distribution of GD3-synthase to the Golgi suggests that both the binding of GM3 and *N*-glycosylation participate in protein folding or transport (30, 31, 33). The observation that the addition of GM3 is required to stabilize GD3-synthase during in vitro translation indicates that GM3 may act as a "lipid chaperone" for assistance in protein folding (33, 61). GM3 may be bound by GD3-synthase very early during translation and assist in the maturation of this enzyme in the ER. This may go in hand with calnexin binding and early *N*-glycoprotein processing. Correctly folded and processed GD3-synthase would then dimerize by disulfide linkage and be transported to the Golgi. The homodimer would be split, and the enzyme would form a heterodimeric complex with GM2/GD2-synthase in the Golgi. GM3 would then be converted to GD3 and then to GD2 as a result of the mutual activation of GD3- and GM2/GD2-synthases (Fig. 2). Subsequently, a variety of other enzyme complexes (e.g., GM2/GD2-synthase and GM1/GD1b-synthase) may facilitate the biosynthesis of other b-series complex gangliosides.

Our model predicts that ganglioside biosynthesis is not just for the generation of gangliosides but that gangliosides may even regulate their own biosynthesis by binding to GTs and assisting their maturation, folding, and/or transport. However, in the case of GD3-synthase, this autoregulation will work only if GM3 is present in the ER. As discussed above, there is still an ongoing debate about where GM3 is synthesized. In F-11 cells, we found that GM3-synthase is predominantly localized in the ER, whereas other groups reported a distribution of this enzyme to the Golgi (33, 35). However, even if GM3-synthase is localized in the Golgi in particular cell types, this does not exclude the retrograde distribution of GM3 to the ER. This assumption is supported by our observation that exogenously added pyrene-labeled GM3 is transported to the ER (33). Cell fractionation studies pioneered by Forman and Ledeen (62) in 1972 reported a rapid transport of gangliosides from the cell body to the axon of neuronal cells. Subsequent studies showed that simple gangliosides are ubiquitously distributed to the ER, Golgi, plasma membrane, lysosomes, and mitochondria, suggesting anterograde and retrograde transport routes for GM3 and GD3 (22–25, 63). Complex gangliosides, however, appear to be predominantly enriched in the plasma membrane (24, 63). To determine the spatial relationship between ganglioside synthesis and transport, it will be crucial to develop new imaging methods for the subcellular distribution and complex formation of gangliosides and GTs. These methods will certainly rely on the generation of antibodies against endogenous gangliosides and GTs as well as on the synthesis of novel fluorescence-labeled analogs of gangliosides and fluorescent protein-tagged GTs.

With respect to the mechanism for the rapid regulation of ganglioside biosynthesis, the observation that the ganglioside composition rapidly changes during embryonic brain development has been intensively investigated for more than 30 years (64–66). We first demonstrated that the ganglioside composition during embryonic rat brain development undergoes a global change from simple to b-series complex gangliosides within less than 48 h between embryonic days E16 and E18 (2, 13). We applied MEKA to these data and found that the rapid switch from simple to b-series complex gangliosides is not reflected by the rates at which the expression of GD3-synthase and GM2/GD2-synthase are upregulated in E16–E18 embryonic rat brain tissue (21, 33, 67). Recently, we repeated the analysis of gangliosides and GT expression levels in embryonic

mouse brain and found it consistent with the data previously published for rat brain (67). Other studies in our laboratory on the neuronal differentiation of P19 cells showed that the emergence of b-series complex gangliosides appears to precede the robust induction of gene expression of the pertinent GTs (68). It is likely that a rapid switch from simple to complex gangliosides is achieved by a mechanism such as the one we propose, which involves posttranslational processing and complex formation of GTs. This may also be accelerated by the presence of GM3 as a potential lipid chaperone or emerging a- and b-series gangliosides as receptor modulators. It has been shown that a-series complex gangliosides modulate growth factor receptor function, which, in turn, may assist brain development and growth factor-dependent (auto)regulation of ganglioside biosynthesis (69–72). We have reported that b-series complex gangliosides attenuate apoptosis in neuronal cells, which is significant for selective neuronal differentiation during brain development (67). Rapid regulation of ganglioside biosynthesis by lipid (e.g., GM3) chaperone-assisted protein folding, *N*-glycoprotein processing-controlled protein turnover and transport, and enzyme complex formation may thus be intimately linked to neuronal differentiation. Cotranslational and posttranslational modification of GTs is an emerging field of ganglioside research that will certainly surprise us with new insights regarding the function of gangliosides and the significance of protein modification, in particular *N*-glycosylation, for enzyme regulation.

TRANSCRIPTIONAL REGULATION OF GANGLIOSIDE SYNTHASE GENES

During brain development, cell differentiation, and malignant cell transformation, the expression patterns of gangliosides undergo dramatic changes. Although the compartmentation of substrates and GTs as well as posttranslational modifications of the activity of GTs play key roles as indicated above, there is strong evidence that ganglioside biosynthesis is also intimately regulated at the transcriptional level. The promoters for six ganglioside synthase genes have been cloned to date (**Table 1**). The common feature is that all of these promoters are TATA-less and contain no CCAAT box but have several Sp1 binding sites in proximity to the transcription start site (73–81). This presents some features of housekeeping genes: no TATA box and the presence of GC boxes. The term housekeeping implies permanent expression with little regulation. However, these ganglioside synthase genes are tightly regulated during brain development, cell proliferation/differentiation, and malignant cellular transformation. In fact, even highly tissue-specific regulation has been shown to occur with this type of promoter (82, 83). Moreover, transcription complexes assembled in TATA-less promoters contain the same subunits, including transcription factor IID and TATA-binding proteins, as do complexes formed at TATA-containing promoters (84, 85). Although many of the components of the basal transcription machinery have been identified, cloned, and studied in varying detail, much less is known about regulatory transcription factor complexes. Recently, several initial experiments to transcription factor complexes on the promoters of ganglioside synthase genes have been performed (78, 79), and useful information has been obtained.

GM2/GD2-synthase gene promoter

GM2/GD2-synthase is a key enzyme controlling the expression of the a-series gangliosides and is highly expressed in almost all neuroblastoma cells (86) and in mouse brain late in development (87). The 5′ flanking fragment of the human GM2/GD2-synthase gene was the first cloned promoter for a ganglioside synthase gene (73). Studies of the promoter and genomic organization showed that this gene has three transcription start sites and three alternative exons (exons 1a, 1b, and 1c). Three promoters (P1, P2, and P3, respectively) in this gene have

TABLE 1. Promoters of ganglioside synthase genes

Gene	Species	Proximate Promoter[a]	Transcription Initiation Site	Remark	Reference
GM2/GD2-synthase	Human	<700 bp	3	At least three initiation sites corresponding to three alternative transcripts were determined; an enhancer sequence and a suppressive sequence were identified	(73)
GD3-synthase	Human, mouse, rat	<700 bp	1 for mouse and rat Multiple for human	A negative control region was defied; a GT-CG repeat sequence is present in the negative regulatory region	(74–76)
GM3-synthase	Human	<300 bp	Multiple	Four mRNA species may result from different initiation sites or alternative splicing; transcription of the gene is tissue specific	(77, 78)
GM1/GD1b-synthase	Mouse	<550 bp	1	A negative regulatory region was identified	(79)
Glucosylceramide synthase	Mouse	<600 bp	Not available	An enhancer and a suppressive sequence were identified	(80)
Galactosylceramide synthase	Human	<350 bp	1	Three positive *cis*-acting regulatory regions and one negative regulatory region were identified	(81)

All of the promoters listed are TATA-less and have no CCAAT box but have Sp1 consensus sites at the proximate regions.
[a] The base pairs of proximate promoters represent the sequences upstream of the translation start codon.

been defined for the individual transcription start sites (73). There are consensus binding sites for the transcription factors EGR-1, HNF-5, Sp-1, and PEA3 in the 5′ flanking region of exon 1a, three binding sites for Sp-1 and one for AP-2 and PEA3 in the 5′ flanking region of exon 1b, and one site for AP-2 and E2F and two S1 HS sites in the 5′ flanking region of exon 1c. The activities of the promoters P1 and P2 were strongly enhanced by a sequence (enhancer) located in exon 1. Furukawa et al. (73) also proposed a suppressive sequence (silencer) of 42 bp located upstream of exon 1c, which suppressed the transcription activity of the P3 promoter in a melanoma cell line in which no promoter activity was detected. These results indicate that cell type-specific expression of the GM2/GD2/GT2-synthase gene may be regulated by alterative promoters and/or the enhancer or silencer. It is not surprising that the mechanism for the transcriptional regulation of the GM2/GD2/GT2-synthase gene is complex, because GM2/GD2/GT2-synthase is one of the key enzymes in ganglioside biosynthesis, although this promoter is TATA-less and has GC-rich boxes.

GD3-synthase gene promoter

GD3-synthase initiates the synthesis of the b- and c-series gangliosides. Because the expression of GD3 and the b-series gangliosides is strictly regulated during brain development and in many types of cancer cells, the transcriptional regulation of the GD3-synthase gene has attracted much more attention than other promoters for ganglioside synthase genes. The promoter of the GD3-synthase gene has been cloned from rat and is the first promoter of several sialyltransferase genes involved in ganglioside biosynthesis (74). Its mouse and human counterparts were cloned afterward (75, 76). The rat promoter is 93% homologous to the mouse promoter, with no significant similarity to the human counterpart. There are no common binding sequences among the species except for Sp1 sites. In spite of the sequence differences in the rodent and human promoters, the promoters of all three species exhibit similar properties. They are TATA-less and contain GC-rich binding sites. The rat and mouse promoters have a single transcription start site, whereas the human promoter possesses multiple initiation sites. The proximal promoter region was defined within 500 or 700 bp upstream the ATG codon in rat and mouse or human promoter, respectively. Several Sp1 binding sites are present in this region, and their deletion results in a dramatic loss of the proximal promoter activities of the mouse (75) and rat (our unpublished data), suggesting that Sp1 plays a significant role in the transcriptional regulation of this gene. A negative control mechanism is present in the upstream region of the promoters. In addition, the promoters contain a GT-CG repeat sequence, although the lengths of the repeat sequences are slightly different among the species. These unique GT/CG repeats are present in the regions having suppressive activities, presenting a structure of Z-DNA in the promoters. The significance of the GT/CG repeats in the transcriptional regulation of the GD3-synthase gene remains to be elucidated. It would be very interesting to investigate how the expression of the GD3-synthase gene is regulated by different promoter sequences in human and rodents.

GM3-synthase gene promoter

Ganglioside GM3 has been suggested to play important roles in various cellular functions, and the GM3-synthase gene is regulated in brain development (88) in a tissue-specific manner (89). The 5′ flanking fragments of the human GM3-synthase gene were cloned (77, 78). Kim et al. (77) reported a single major transcription initiation site, whereas we (78) identified two initiation sites more than 100 bp upstream of the one reported by Kim and colleagues, suggesting that multiple initiation sites may be present. Different sizes of exon 1 have been reported (90, 91); there are four isoforms of GM3-synthase mRNA in fetal brain, which differ in the 5′ untranslated region. Only one of these isoforms can be detected in adult brain (92). These data indicate that the regulation of GM3-synthase expression may be developmentally dependent, and alterative splicing of the human GM3-synthase RNA may occur in a tissue-specific manner. A minimum promoter of 177 bp lies upstream of the initiation site and displays the highest promoter activity, as reported by Kim et al. (77). The promoter activities of the longer promoter fragments, from 300 to 1,600 bp, show no dramatic changes, as determined by both groups (77, 78). The proximal region contains no TATA or CCAAT boxes but contains three Sp1 sites. Deletion experiments to determine whether these Sp1 sites are actually functional in the regulation of GM3-synthase gene expression remain to be performed. Alternatively, DNA pulldown experiments using the proximal promoter fragment followed by transcription factor microarray analysis to identify the pulled down proteins have been performed (78). Eight transcription factors have been identified in this manner. Surprisingly, Sp1 was not pulled down by the fragment, although the promotor sequence contains three Sp1 sites; interestingly, only one of the eight factors has the consensus sequence on the fragment (78). Similar pulldown experiments have also been performed using promoter fragments of the mouse GM3- and GM1/GD1b-synthase genes. Thus, using a mouse GM3-synthase promoter fragment, 10 factors were identified, 7 of which are the same as those using the human promoter fragment (93). The results using the mouse GM1/GD1b-synthase promoter are quite different (see below). Therefore, the proteins pulled down by the promoter fragments cannot be attributable to nonspecificity of the procedure. The possibility exists that the seven factors are recruited as components of the transcription factor complexes and/or that the in vitro binding environments do not represent the in vivo conditions.

GM1/GD1b-synthase gene promoter

GM1 is one of the most widely investigated gangliosides. It plays important roles in the development and functions of the neural system (94). GM1-synthase is responsible for the synthesis of GM1/GD1b from GM2/GD2 in the ganglioside biosynthetic pathway. A 1,448 bp 5′ flanking frag-

ment of the mouse GM1/GD1b-synthase gene has been cloned (79). This promoter is again TATA-less, has no CCAAT box, and has a single transcription start site. The proximal 550 bp fragment shows the highest transcriptional activity. One negative regulatory region has also been identified. The binding proteins on the promoter fragment have been analyzed, and 27 transcription factors have been characterized as binding to consensus sites in the promoter region, whereas 4 other factors without consensus binding sites in this region have also been recruited, suggesting the possibility of complex formation of these transcriptional factors. How the synthesis of GM1 is regulated during neuronal differentiation in coordination with the regulation of the expression of the GM1-synthase gene remains to be elucidated.

Glucosylceramide synthase and galactosylceramide synthase gene promoters

Glucosylceramide synthase catalyzes the first step in glycosphingolipid synthesis, the transfer of glucose from UDP-glucose to ceramide. The product, glucosylceramide, serves as a core structure for more than 300 species of glycosphingolipids. The enzyme is a key regulatory factor controlling intracellular levels of ceramide and glycosphingolipids. The mouse ceramide glucosylceramide synthase gene promoter has been cloned (80). The promoter is TATA-less and contains no CCAAT box, but several Sp1 binding sites are found in the proximal region, which is similar to the other gene promoters described above. In addition to these general transcription factor binding sites, the motifs for AhR, NF-κB, AP-2, and GATA-1 binding sites are also present.

Galactosylceramide synthase is a key enzyme in the biosynthesis of galactocerebroside, the most abundant glycosphingolipid in the myelin sheath. Galactosylceramide serves as the precursor for the biosynthesis of GM4, the simplest ganglioside that is present, among other tissues, in myelin of the central nervous system (9). The human galactosylceramide synthase gene promoter has been cloned (81, 95). This promoter is TATA-less and has a single transcription initiation site. However, the promoter region contains a high GC content and multiple putative regulatory elements. Cell type-specific activities of those promoters have been observed. Three positive *cis*-acting regulatory regions were identified: a proximal region at 3,292/3,256, which contains the potential binding sites for known transcription factors such as Ets and Sp1 (GC box); a distal region at 3,747/3,688, comprising a number of binding sites such as the ERE half-site, NF1-like, TGGCA-BP, and CRE; and a positive *cis*-acting region distally localized at 31,325/31,083, consisting of binding sites for transcription factors such as nitrogen regulatory, TCF-1, TGGCA-BP, NF-IL6, CF1, bHLH, NF1-like, GATA, and Q-IRE. A negative *cis*-acting domain localized in a far distal region at 31,594/31,326 was also identified. These results suggest the presence of both positive and negative *cis*-regulatory regions as essential for the cell-specific expression in the TATA-less promoter of the human galactosylceramide synthase gene.

CONCLUSION

The temporal and spatial expression of nervous system gangliosides is tightly regulated by their biosynthesis during development. The regulation of ganglioside metabolism by posttranslational modification, subcellular localization, and complex formation of GTs among enzymes in sequence and/or with the substrates clearly represents a critical and essential step. Evidence has been provided that *N*-glycosylation affects the activity, topology in the Golgi/ER complexes, complex formation, and/or subcellular localization of GTs during neural development. On the other hand, ganglioside expression in the developing brain is also likely controlled to a large degree at the level of gene expression. Because the mechanisms that govern the expression of various GT genes during development are still poorly understood, it is crucial to characterize the promoter structures and the transcription factors or transcription factor complexes involved in the regulation of GT gene expression. Further studies should be directed in these directions.

The authors are indebted to Dr. Stacey Kraemer (Medical College of Georgia) for critically reading the manuscript and valuable discussions. The participation of Drs. Susumu Ando, Herb Yohe, Toshio Ariga, Bettina Freischultz, Luoyi Gao, Xinbin Gu, Dmitri Kapitonov, Ute Preuss, and Tewin Tencomnao during the various phases of the experimental work is gratefully acknowledged. This work was supported by National Institutes of Health Grant NS-11853 to R.K.Y.

REFERENCES

1. Yu, R. K., L. J. Macala, T. Taki, H. M. Weinfeld, and F. S. Yu. 1988. Developmental changes in ganglioside composition and synthesis in embryonic rat brain. *J. Neurochem.* **50:** 1825–1829.
2. Yu, R. K. 1994. Developmental regulation of ganglioside metabolism. *Prog. Brain Res.* **101:** 31–44.
3. Yu, R. K., and E. Bieberich. 2001. Regulation of glycosyltransferases in ganglioside biosynthesis by phosphorylation and dephosphorylation. *Mol. Cell. Endocrinol.* **177:** 19–24.
4. Basu, S., B. Kauman, and S. Roseman. 1965. Conversion of Tay-Sachs ganglioside to monosialoganglioside by brain uridine diphosphate D-galactose:glycolipid galactosyltransferase. *J. Biol. Chem.* **240:** 4115–4117.
5. Kaufman, B., S. Basu, and S. Roseman. 1968. Enzymatic synthesis of disialogangliosides from monosialogangliosides by sialyltransferases from embryonic chicken brain. *J. Biol. Chem.* **243:** 5804–5807.
6. Panzetta, P., H. J. F. Maccioni, and R. Caputto. 1970. Synthesis of retinal gangliosides during chick embryonic development. *J. Neurochem.* **35:** 100–108.
7. Maccioni, H. J. F., A. Arce, and R. Caputto. 1971. The biosynthesis of gangliosides, labelling of rat brain gangliosides in vivo. *Biochem. J.* **125:** 1131–1137.
8. Fishman, P. H., and R. O. Brady. 1976. Biosynthesis and function of gangliosides. *Science.* **194:** 906–914.
9. Yu, R. K., and S. H. Lee. 1976. In vitro biosynthesis of sialosylgalactosylceramide (G7) by mouse brain microsomes. *J. Biol. Chem.* **251:** 198–203.
10. Yu, R. K., and S. Ando. 1980. Structures of new complex gangliosides of fish brain. *Adv. Exp. Med. Biol.* **125:** 33–45.
11. Ando, S., and R. K. Yu. 1979. Isolation and characterization of two isomers of brain tetrasialogangliosides. *J. Biol. Chem.* **254:** 12224–12229.

12. Irie, F., K. I-P. J. Hidari, T. Tai, Y-T. Li, Y. Seyama, and Y. Hirabayashi. 1994. Biosynthetic pathway for a new series of gangliosides, GT1aα and GQ1aα. *FEBS Lett.* **351:** 291–294.
13. Seyfried, T. N., A. M. Novikov, R. A. Irwin, and J. V. Brigande. 1994. Ganglioside biosynthesis in mouse embryos: sialyltransferase IV and the asialo pathway. *J. Lipid Res.* **35:** 993–1001.
14. Yohe, H. C., and R. K. Yu. 1980. In vitro biosynthesis of an isomer of trisialoganglioside, GT1a. *J. Biol. Chem.* **255:** 608–613.
15. Yohe, H. C., L. J. Macala, and R. K. Yu. 1982. In vitro biosynthesis of a tetrasialoganglioside. *J. Biol. Chem.* **257:** 249–252.
16. Freischütz, B., M. Saito, H. Rahmann, and R. K. Yu. 1994. Activities of five different sialyltransferases in fish and rat brains. *J. Neurochem.* **62:** 1965–1973.
17. Freischütz, B., M. Saito, H. Rahmann, and R. K. Yu. 1995. Characterization of sialyltransferase-IV activity and its involvement in the c-pathway of brain ganglioside metabolism. *J. Neurochem.* **64:** 385–393.
18. Pohlentz, G., D. Klein, G. Schwarzmann, D. Schmitz, and K. Sandhoff. 1988. Both GA2, GM2, and GD2 synthases and GM1b, GD1a, and GT1b synthases are single enzymes in Golgi vesicles from rat liver. *Proc. Natl. Acad. Sci. USA.* **85:** 7044–7048.
19. Kono, M., Y. Yoshida, N. Kojima, and S. Tsuji. 1996. Molecular cloning and expression of a fifth type of α2,8-sialyltransferase (ST8Sia V). Its substrate specificity is similar to that of SAT-V/III, which synthesize GD1c, GT1a, GQ1b, and GT3. *J. Biol. Chem.* **271:** 29366–29371.
20. Nara, K., Y. Watanabe, I. Kawashima, T. Kai, Y. Nagai, and Y. Sanai. 1996. Acceptor substrate specificity of a cloned GD3 synthase that catalyzes the biosynthesis of both GD3 and GD1c/GT1a/GQ1b. *Eur. J. Biochem.* **238:** 647–652.
21. Bieberich, E., and R. K. Yu. 1999. Multi-enzyme kinetic analysis of glycolipid biosynthesis. *Biochim. Biophys. Acta.* **1432:** 113–124.
22. Miller-Podraza, H., and P. H. Fishman. 1982. Translocation of newly synthesized gangliosides to the cell surface. *Biochemistry.* **21:** 3265–3270.
23. Caputto, R., H. J. Maccioni, A. Arce, and B. L. Cumar. 1976. Biosynthesis of brain gangliosides. *Adv. Exp. Med. Biol.* **71:** 27–44.
24. Maccioni, H. J., C. Landa, R. Arce, and R. Caputto. 1977. The biosynthesis of brain gangliosides—evidence for a "transient pool" and an "end product" pool of gangliosides. *Adv. Exp. Med. Biol.* **83:** 267–281.
25. Trinchera, M., B. Pirovano, and R. Ghidoni. 1990. Sub-Golgi distribution in rat liver of CMP-NeuAc:GM3 and CMP-NeuAc:GT1b alpha 2-8sialyltransferases and comparison with the distribution of the other glycosyltransferase activities involved in ganglioside biosynthesis. *J. Biol. Chem.* **265:** 18242–18247.
26. Maccioni, H. J. F., J. L. Daniotti, and J. A. Martina. 1999. Organization of ganglioside synthesis in the Golgi apparatus. *Biochim. Biophys. Acta.* **1437:** 101–118.
27. Trinchera, M., and R. Ghidoni. 1989. Two glycosphingolipid sialyltransferases are localized in different sub-Golgi compartments in rat liver. *J. Biol. Chem.* **264:** 15766–15769.
28. Iber, H., G. van Echten, and K. Sandhoff. 1992. Fractionation of primary cultured cerebellar neurons: distribution of sialyltransferases involved in ganglioside biosynthesis. *J. Neurochem.* **58:** 1533–1537.
29. Martina, J. A., J. L. Daniotti, and H. J. Maccioni. 1998. Influence of N-glycosylation and N-glycan trimming on the activity and intracellular traffic of GD3 synthase. *J. Biol. Chem.* **273:** 3725–3731.
30. Bieberich, E., T. Tencomnao, D. Kapitonov, and R. K. Yu. 2000. Effect of N-glycosylation on turnover and subcellular distribution of N-acetylgalactosaminyltransferase I and sialyltransferase II in neuroblastoma cells. *J. Neurochem.* **74:** 2359–2364.
31. Giraudo, C. G., J. L. Daniotti, and H. J. Maccioni. 2001. Physical and functional association of glycolipid N-acetyl-galactosaminyl and galactosyl transferase in the Golgi apparatus. *Proc. Natl. Acad. Sci. USA.* **98:** 1625–1630.
32. Giraudo, C. G., and H. J. F. Maccioni. 2003. Ganglioside glycosyltransferases organize in distinct multienzyme complexes in CHO-K1 cells. *J. Biol. Chem.* **278:** 40262–40271.
33. Bieberich, E., S. MacKinnon, J. Silva, D. D. Li, T. Tencomnao, L. Irwin, D. Kapitonov, and R. K. Yu. 2002. Regulation of ganglioside biosynthesis by enzyme complex formation of glycosyltansferases. *Biochemistry.* **41:** 11479–11487.
34. Maccioni, H. J. F., C. G. Giraudo, and J. L. Daniotti. 2002. Understanding the stepwise synthesis of glycolipids. *Neurochem. Res.* **27:** 629–636.
35. Stern, C. A., T. R. Braverman, and M. Tiemeyer. 2000. Molecular identification, tissue distribution and subcellular localization of mST3GalV/GM3 synthase. *Glycobiology.* **10:** 365–374.
36. Kapitonov, D., E. Bieberich, and R. K. Yu. 1999. Combinatorial PCR approach to homology-based cloning: cloning and expression of mouse and human GM3 synthase. *Glycoconj. J.* **16:** 337–350.
37. Tsuji, S. 1996. Molecular cloning and functional analysis of sialyltransferases. *J. Biochem.* **120:** 1–13.
38. Nagata, Y., S. Yamahiro, J. Yodoi, K. O. Lloyd, H. Shiku, and K. Furukawa. 1992. Expression cloning of β1,4-N-acetylgalactosaminyltransferase cDNAs that determine the expression of GM2 and GD2 gangliosides. *J. Biol. Chem.* **267:** 12058–12089.
39. Haraguchi, M., S. Yamashiro, A. Yamamoto, K. Furukawa, K. Takamiya, K. O. Lloyd, H. Shiku, and K. Furukawa. 1994. Isolation of GD3 synthase gene by expression cloning of GM3 α-2,8-sialyltransferase cDNA using anti-GD2 monoclonal antibody. *Proc. Natl. Acad. Sci. USA.* **91:** 10455–10459.
40. Sasaki, K., K. Kurata, N. Kojima, N. Kurosawa, S. Ohta, N. Hanai, S. Tsuji, and T. Nishi. 1994. Expression cloning of a GM3-specific α2,8-sialyltransferase (GD3 synthase). *J. Biol. Chem.* **269:** 15950–15956.
41. Zeng, G., L. Gao, T. Ariga, and R. K. Yu. 1996. Molecular cloning of cDNA for rat brain GD3-synthase. *Biochem. Biophys. Res. Commun.* **226:** 319–323.
42. Kapitonov, D., and R. K. Yu. 1999. Conserved domains of glycosyltransferases. *Glycobiology.* **9:** 961–978.
43. Paulson, J. C., and K. J. Colley. 1989. Glycosyltransferases. Structure, localization, and control of cell type-specific glycosylation. *J. Biol. Chem.* **264:** 17615–17618.
44. Martina, J. A., J. L. Daniotti, and H. J. Maccioni. 2000. GM1 synthase depends on N-glycosylation for enzyme activity and trafficking to the Golgi complex. *Neurochem. Res.* **25:** 725–731.
45. Kornfeld, R., and S. Kornfeld. 1985. Assembly of asparagine-linked oligosaccharides. *Annu. Rev. Biochem.* **54:** 631–664.
46. Helenius, A., and M. Aebi. 2001. Intracellular functions of N-linked glycans. *Science.* **291:** 2364–2369.
47. Geetha-Habib, M., R. Noiva, H. A. Kaplan, and W. J. Lennarz. 1988. Glycosylation site binding protein, a component of oligosaccharyl transferase, is highly similar to three other 57 kD luminal proteins of the ER. *Cell.* **54:** 1053–1060.
48. Breuer, W., and E. Bause. 1995. Oligosaccharyl transferase is a constitutive component of an oligomeric protein complex from pig liver endoplasmic reticulum. *Eur. J. Biochem.* **228:** 689–696.
49. Kalz-Füller, B., E. Bieberich, and E. Bause. 1995. Cloning and expression of glucosidase I from human hippocampus. *Eur. J. Biochem.* **231:** 344–351.
50. Bieberich, E., and E. Bause. 1995. Man9-mannosidase from human kidney is expressed in COS-cells as a Golgi resident type II transmembrane N-glycoprotein. *Eur. J. Biochem.* **233:** 644–649.
51. Klenk, H. D., and R. Rott. 1980. Cotranslational and posttranslational processing of viral glycoproteins. *Curr. Top. Microbiol. Immunol.* **90:** 19–48.
52. Kawasaki, T., and G. Ashwell. 1976. Chemical and physical properties of an hepatic membrane protein that specifically binds asialoglycoproteins. *J. Biol. Chem.* **251:** 1296–1302.
53. Waheed, A., A. Hasilik, and K. von Figura. 1981. Processing of the phosphorylated recognition marker in lysosomal enzymes. Characterization and partial purification of a microsomal α-N-acetylglucosaminyl phosphodiesterase. *J. Biol. Chem.* **256:** 5717–5721.
54. Chen, W., J. Helenius, I. Braakman, and A. Helenius. 1995. Cotranslational folding and calnexin binding during glycoprotein synthesis. *Proc. Natl. Acad. Sci. USA.* **92:** 6229–6233.
55. Cannon, K. S., and A. Helenius. 1999. Trimming and readdition of glucose to N-oligosaccharides determines calnexin association of a substrate glycoprotein in living cells. *J. Biol. Chem.* **274:** 7537–7544.
56. Parodi, A. J. 1999. Reglucosylation of glycoproteins and quality control of glycoprotein folding in the endoplasmic reticulum of yeast cells. *Biochim. Biophys. Acta.* **1426:** 287–295.
57. Roseman, S. 1970. The synthesis of complex carbohydrates by multiglycosyltransferase systems and their potential function in intercellular adhesion. *Chem. Phys. Lipids.* **5:** 270–297.
58. Sekar, R. B., and A. Periasamy. 2003. Fluorescence resonance energy transfer (FRET) microscopy imaging of live cell protein localizations. *J. Cell Biol.* **160:** 629–633.
59. Rosales Fritz, V. M., J. L. Daniotti, and H. J. Maccioni. 1997. Chinese hamster ovary cells lacking GM1 and GD1a synthesize gangliosides upon transfection with human GM2 synthase. *Biochim. Biophys. Acta.* **1354:** 153–158.

60. Gu, X. B., T. J. Gu, and R. K. Yu. 1990. Purification to homogeneity of GD3 synthase and partial purification of GM3 synthase from rat brain. *Biochem. Biophys. Res. Commun.* **166:** 387–393.
61. Bieberich, E., D. Kapitonov, T. Tencomnao, and R. K. Yu. 2000. Protein-ribosome-mRNA display: affinity isolation of enzyme-ribosome-mRNA complexes and cDNA cloning in a single-tube reaction. *Anal. Biochem.* **287:** 294–298.
62. Forman, D. S., and R. W. Ledeen. 1972. Axonal transport of gangliosides in the goldfish optic nerve. *Science.* **177:** 630–633.
63. Riboni, L., R. Bassi, and G. Tettamanti. 1994. Effect of brefeldin A on ganglioside metabolism in cultured neurons: implications for the intracellular traffic of gangliosides. *J. Biochem.* **116:** 140–146.
64. Suzuki, K. 1965. The pattern of mammalian brain gangliosides. III. Regional and developmental differences. *J. Neurochem.* **12:** 969–979.
65. Vanier, M-T., M. Holm, R. Ohman, and L. Svennerholm. 1971. Developmental profiles of gangliosides in human and rat brain. *J. Neurochem.* **18:** 581–592.
66. Rosner, H. 1977. Gangliosides, sialoglycoproteins, and acetylcholinesterase of the developing mouse brain. *Roux's Arch. Dev. Biol.* **183:** 325–335.
67. Bieberich, E., S. MacKinnon, J. Silva, and R. K. Yu. 2001. Regulation of apoptosis during neuronal differentiation by ceramide and b-series complex gangliosides. *J. Biol. Chem.* **276:** 44396–44404.
68. Liour, S. S., D. Kapitonov, and R. K. Yu. 2000. Expression of gangliosides in neuronal development of P19 embryonal carcinoma cells. *J. Neurosci. Res.* **62:** 363–373.
69. Mutoh, T., A. Tokuda, T. Miyadai, M. Hamaguchi, and N. Fujiki. 1995. Ganglioside GM1 binds to Trk protein and regulates receptor function. *Proc. Natl. Acad. Sci. USA.* **92:** 5087–5089.
70. Yates, A. J., and A. Rampersaud. 1998. Sphingolipids as receptor modulators. An overview. *Ann. N. Y. Acad. Sci.* **845:** 57–71.
71. Hakomori, S. 2002. Glycosylation defining cancer malignancy: new wine in an old bottle. *Proc. Natl. Acad. Sci. USA.* **99:** 10231–10233.
72. Allende, M. L., and R. Proia. 2002. Lubricating cell signaling pathways with gangliosides. *Curr. Opin. Struct. Biol.* **12:** 587–592.
73. Furukawa, K., H. Soejima, N. Niikawa, and H. Shiku. 1996. Genomic organization and chromosomal assignment of the human beta1,4-N-acetylgalactosaminyltransferase gene. Identification of multiple transcription units. *J. Biol. Chem.* **271:** 20836–20844.
74. Zeng, G., L. Gao, and R. K. Yu. 1998. Isolation and functional analysis of the promoter of the rat CMP-NeuAc:GM3 alpha2,8 sialyltransferase gene 1. *Biochim. Biophys. Acta.* **1397:** 126–130.
75. Takashima, S., M. Kono, N. Kurosawa, Y. Yoshida, Y. Tachida, M. Inoue, T. Kanematsu, and S. Tsuji. 2000. Genomic organization and transcriptional regulation of the mouse GD3 synthase gene (ST8Sia I): comparison of genomic organization of the mouse sialyltransferase genes. *J. Biochem. (Tokyo).* **128:** 1033–1043.
76. Furukawa, K., M. Horie, K. Okutomi, and S. Sugano. 2003. Isolation and functional analysis of the melanoma specific promoter region of human GD3 synthase gene. *Biochim. Biophys. Acta.* **1627:** 71–78.
77. Kim, S. W., S. H. Lee, K. S. Kim, C. H. Kim, Y. K. Choo, and Y. C. Lee. 2002. Isolation and characterization of the promoter region of the human GM3 synthase gene. *Biochim. Biophys. Acta.* **1578:** 84–89.
78. Zeng, G., L. Gao, T. Xia, T. Tencomnao, and R. K. Yu. 2003. Characterization of the 5′-flanking fragment of the human GM3-synthase gene. *Biochim. Biophys. Acta.* **1625:** 30–35.
79. Xia, T., L. Gao, R. K. Yu, and G. Zeng. 2003. Characterization of the promoter and the transcription factors for the mouse UDP-Gal:betaGlcNAc beta1,3-galactosyltransferase gene. *Gene.* **309:** 117–123.
80. Ichikawa, S., K. Ozawa, and Y. Hirabayashi. 1998. Molecular cloning and characterization of the mouse ceramide glucosyltransferase gene. *Biochem. Biophys. Res. Commun.* **253:** 707–711.
81. Tencomnao, T., R. K. Yu, and D. Kapitonov. 2001. Characterization of the human UDP-galactose:ceramide galactosyltransferase gene promoter. *Biochim. Biophys. Acta.* **1517:** 416–423.
82. Boehm, S. K., J. R. Gum, R. H. Erickson, J. W. Hicks, and Y. S. Kim. 1995. Human dipeptidyl peptidase IV gene promoter: tissue-specific regulation from a TATA-less GC-rich sequence characteristic of a housekeeping gene promoter. *Biochem. J.* **311:** 835–843.
83. Gumina, R. J., N. E. Kirschbaum, K. Piotrowski, and P. J. Newman. 1997. Characterization of the human platelet/endothelial cell adhesion molecule-1 promoter: identification of a GATA-2 binding element required for optimal transcriptional activity. *Blood.* **89:** 1260–1269.
84. Carcamo, J., L. Buckbinder, and D. Reinberg. 1991. The initiator directs the assembly of a transcription factor IID-dependent transcription complex. *Proc. Natl. Acad. Sci. USA.* **88:** 8052–8056.
85. Pugh, B. F., and R. Tjian. 1991. Transcription from a TATA-less promoter requires a multisubunit TFIID complex. *Genes Dev.* **5:** 1935–1945.
86. Cheung, N. K., U. M. Saarinen, J. E. Neely, B. Landmeier, D. Donovan, and P. F. Coccia. 1985. Monoclonal antibodies to a glycolipid antigen on human neuroblastoma cells. *Cancer Res.* **45:** 2642–2649.
87. Yamamoto, A., M. Haraguchi, S. Yamashiro, S. Fukumoto, K. Furukawa, K. Takamiya, M. Atsuta, H. Shiku, and K. J. Furukawa. 1996. Heterogeneity in the expression pattern of two ganglioside synthase genes during mouse brain development. *Neurochemistry.* **66:** 26–34.
88. Kono, M., S. Takashima, H. Liu, M. Inoue, N. Kojima, Y. C. Lee, T. Hamamoto, and S. Tsuji. 1998. Molecular cloning and functional expression of a fifth-type alpha 2,3-sialyltransferase (mST3Gal V: GM3 synthase). *Biochem. Biophys. Res. Commun.* **253:** 170–175.
89. Fukumoto, S., H. Miyazaki, G. Goto, T. Urano, K. Furukawa, and K. Furukawa. 1999. Expression cloning of mouse cDNA of CMP-NeuAc:lactosylceramide alpha2,3-sialyltransferase, an enzyme that initiates the synthesis of gangliosides. *J. Biol. Chem.* **274:** 9271–9276.
90. Ishii, A., M. Ohta, Y. Watanabe, K. Matsuda, K. Ishiyama, K. Sakoe, M. Nakamura, J. Inokuchi, Y. Sanai, and M. Saito. 1998. Expression cloning and functional characterization of human cDNA for ganglioside GM3 synthase. *J. Biol. Chem.* **273:** 31652–31655.
91. Kapitonov, D., E. Bieberich, and R. K. Yu. 1999. Combinatorial PCR approach to homology-based cloning: cloning and expression of mouse and human GM3-synthase. *Glycoconj. J.* **16:** 337–350.
92. Kim, K. W., S. W. Kim, K. S. Min, C. H. Kim, and Y. C. Lee. 2001. Genomic structure of human GM3 synthase gene (hST3Gal V) and identification of mRNA isoforms in the 5′-untranslated region. *Gene.* **273:** 163–171.
93. Zeng, G., L. Gao, T. Xia, T. Tencomnao, and R. K. Yu. 2003. Characterization of the 5′-fragments of the human GM3-synthase gene. *Biochem. Biophys. Acta.* **1625:** 30–35.
94. Ledeen, R. W., G. Wu, Z. H. Lu, D. Kozireski-Chuback, and Y. Fang. 1998. The role of GM1 and other gangliosides in neuronal differentiation. Overview and new finding. *Ann. N. Y. Acad. Sci.* **845:** 161–175.
95. Tencomnao, T., D. Kapitonov, E. Bieberich, and R. K. Yu. 2004. Transcriptional regulation of the human UDP-galactose:ceramide galactosyltransferase (hCGT) gene expression: functional role of GC-box and CRE. *Glycoconj. J.* In press.
96. Svennerholm, S. 1964. The gangliosides. *J. Lipid Res.* **5:** 145–154.

ERRATA

In the article "Substrate reduction reduces gangliosides in postnatal cerebrum-brainstem and cerebellum in GM1 gangliosidosis mice" by Kasperzyk et al., published in the April 2004 issue of the *Journal of Lipid Research* (Volume **46**, pages 744–751), the affiliations should read as follows:

J. L. Kasperzyk,* A. d'Azzo,† F. M. Platt,§ J. Alroy,** and T. N. Seyfried*,[1]

Department of Biology,* Boston College, Chestnut Hill, MA; Department of Genetics,† St. Jude Children's Research Hospital, Memphis, TN; Glycobiology Institute,§ Department of Biochemistry, University of Oxford, Oxford, United Kingdom; and Department of Pathology,** Tufts University School of Medicine and Veterinary Medicine, New England Medical Center, Boston, MA

[1]To whom correspondence should be addressed.
e-mail: thomas.seyfried@bc.edu

*****

In the article "Prediction of PPAR-α ligand-mediated physiological changes using gene expression profiles" by Frederiksen et al., published in the March 2004 issue of the *Journal of Lipid Research* (Volume **45**, pages 592–601), the digital object identifier (DOI) should read: DOI 10.1194/jlr.M300239-JLR200.

*****

In the article "Regulation of ganglioside biosynthesis in the nervous system" by Yu et al., published in the May 2004 issue of the *Journal of Lipid Research* (Volume **45**, pages 783–793), the digital object identifier (DOI) should read: DOI 10.1194/jlr.R300020-JLR200.

*****

In the article "Cholesterol metabolism in the central nervous system during early development and in the mature animal" by Dietschy and Turley, published in the August 2004 issue of the *Journal of Lipid Research* (Volume **45**, pages 1375–1397), the digital object identifier (DOI) should read: DOI 10.1194/jlr.R400004-JLR200.