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REVIEW ARTICLE

Emerging concepts of ganglioside metabolism*

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*Dedicated to Prof. Wilhelm Stoffel on the occasion of his 90th birthday.

(Received 12 April 2018, revised 15 May 2018, accepted 17 May 2018, available online 16 June 2018)

doi:10.1002/1873-3468.13114

Edited by: Sandro Sonnino

Gangliosides (GGs) are sialic acid-containing glycosphingolipids (GSLs) and major membrane components enriched on cellular surfaces. Biosynthesis of mammalian GGs starts at the cytosolic leaflet of endoplasmic reticulum (ER) membranes with the formation of their hydrophobic ceramide anchors. After intracellular ceramide transfer to Golgi and trans-Golgi network (TGN) membranes, anabolism of GGs, as well as of other GSLs, is catalyzed by membrane-spanning glycosyltransferases (GTs) along the secretory pathway. Combined activity of only a few promiscuous GTs allows for the formation of cell-type-specific glycolipid patterns. Following an exocytotic vesicle flow to the cellular plasma membranes, GGs can be modified by metabolic reactions at or near the cellular surface. For degradation, GGs are endocytosed to reach late endosomes and lysosomes. Whereas membrane-spanning enzymes of the secretory pathway catalyze GSL and GG formation, a cooperation of soluble glycosidases, lipases and lipid-binding cofactors, namely the sphingolipid activator proteins (SAPs), act as the main players of GG and GSL catabolism at intralysosomal luminal vesicles (ILVs).

Keywords: catabolism; endocytosis; enzyme catalysis; gangliosides; lipid-binding proteins; membrane lipids; metabolic diseases; organellar membranes; sphingolipids; topology

Historical aspects of structure, cellular location, and functions of gangliosides

Sphingolipids (SLs) had been discovered by the German physician Johannes L. W. Thudichum in 1884, when he analyzed the molecular composition of alcoholic brain extracts and their components generated by acid hydrolysis [1]. He named a so far unknown nitrogen containing organic base sphingosine, which turned out to be the main backbone of most sphingolipids.

Gangliosides were first described by Ernst Klenk, when he isolated acidic glycolipids from postmortem brain tissue of infantile patients with amaurotic idiocy, a rare inherited disease. He named the N-acetylneuraminic acid containing glycosphingolipids (GSLs) gangliosides, as they appeared to be enriched in ganglion cells [2,3]. In aqueous solution, gangliosides form huge, quite stable water-soluble micelles with a size of more than a million Dalton as determined by ultracentrifugation. The first correct chemical structure of a complex brain ganglioside (GG), the ganglioside GM1a (Fig. 1B), was published in 1963 by Kuhn and Wiegand [4], allowing the deduction of other GG and GSL structures including those of accumulating glycolipids in ganglioside storage diseases [5–7]. Early

Abbreviations
ASM, acid sphingomyelinase; BMP, bis(monoacylglycero)phosphate; CerS, ceramide synthase; CERT, ceramide transfer protein; DHCer, dihydroceramide; ER, endoplasmic reticulum; GalCer, galactosylceramide; GG, ganglioside; GlcCer, glucosylceramide; GM2AP, GM2 activator protein; GSL, glycosphingolipid; GT, glycosyltransferase; HexA, hexosaminidase A; ILV, intralysosomal luminal vesicle; KDS, 3-keto-dihydrosphingosine; LacCer, lactosylceramide; PA, phosphatidic acid; PG, phosphatidylglycerol; PI, phosphatidylinositol; PM, plasma membrane; PPCA, protective protein/cathepsin A; SAP, sphingolipid activator protein; Sa, sphinganine; SL, sphingolipid; So, sphingosine; TGN, trans-Golgi network.

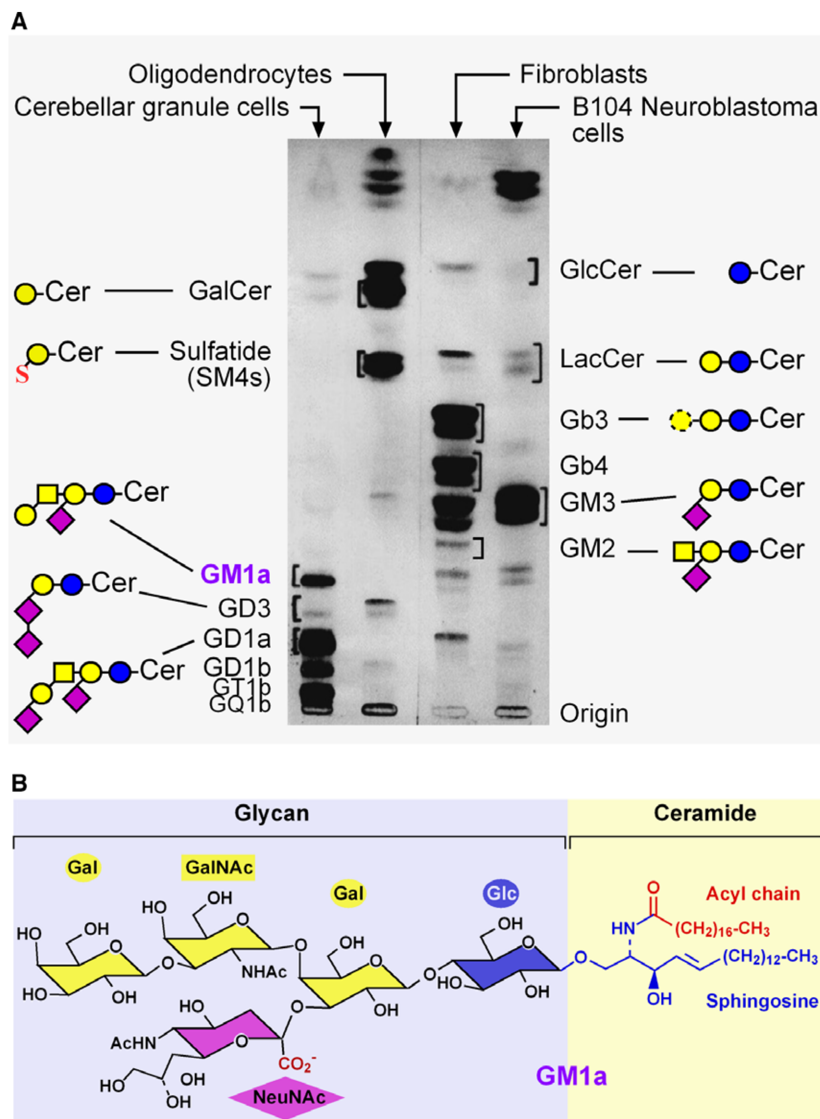

Fig. 1. (A) Separation of [14C]-galactose labeled glycosphingolipids by thin layer chromatography. Cell cultures were incubated with [14C]-galactose for 2 days, lipids were extracted and separated by thin layer chromatography (TLC), and visualized by fluorography [270]. Symbols for some GSLs are indicated (for structural code of the symbols see Fig. 2) and illustrate increasing retention on normal phase TLC of GSLs with a growing polar glycan moiety. (B) Chemical structure of the ganglioside GM1a, a major brain GGs of mammals and preferred ligand of cholera toxin. GM1a is composed of a defined polar glycan moiety and a variable hydrophobic ceramide residue, which anchors GM1a to lipid bilayers. In mammalian neurons the ceramide anchor is composed predominantly of C18-sphingosine and stearic acid as shown here, but both, the sphingoid base and the N-bound acyl chain may vary in length and structure.

metabolic studies with radiolabeled precursors showed a slow ganglioside turnover in rat brain with a half-life of about 10 days [8], which is in contrast to studies in cell culture that yielded a much faster turnover rate [9–11]. These discrepancies probably reflect differences in endocytosis and turn over found *in vivo* compared to cell culture conditions. The analysis of GG and SL storage diseases drove the elucidation of GG metabolism and its catabolic blocks in gangliosidoses [6]. It triggered the identification and characterization of enzymes and lipid-binding proteins essential in GSL and GG catabolism [12–14]. Comparative studies in the laboratories of Klenk, Yamakawa, and Hakomori developed the concept that pattern, distribution, and location of GGs and GSLs are species, cell type, and organelle specific. Mammalian neurons synthesize primarily GGs of the ganglio-series [7,15,16] (Fig. 1A), oligodendrocytes in the brain produce predominantly myelin forming GSLs, galactosylceramide, sulfatide (SM4s; Fig. 1A), and a minor amount of ganglioside GM4.

In humans, fibroblasts of the skin and many cells of visceral organs, however, generate mainly GSLs of the globoseries (Fig. 1A), whereas male murine germ cells, for example, switch between GGs of the a- and 0-series (Fig. 2) upon differentiation when they are crossing the blood–testis barrier [17–19]. A similar shift was observed in small intestinal epithelial cells during suckling-to-weanling transition [20]. The type of GGs/GSLs expressed may have a direct influence on the

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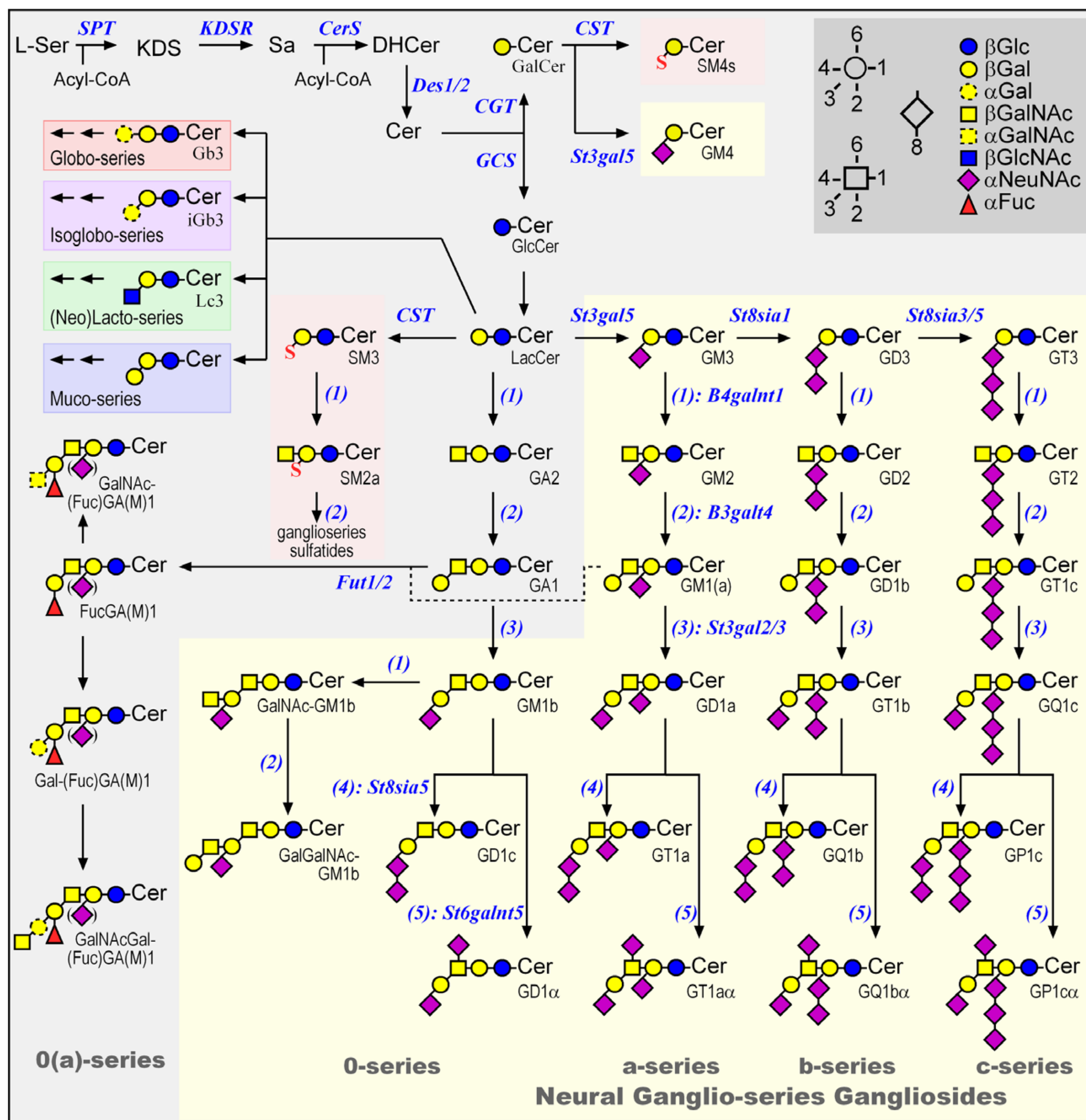

Fig. 2. Pathways of combinatorial ganglioside biosynthesis. The majority of GSL/GG structures are based on the initial attachment of glucose in β-anomeric linkage to the 1-O-position of ceramides. Lactosylceramide represents a fundamental branching point in GSL synthesis. It is substrate for at least six different glycosyltransferases and a sulfotransferase guiding GSL synthesis into different GSL series. GM3 synthase (*St3gal5*) is one of them guiding GSLs into a-, b-, and c-series of neuronal GGs. Cer, ceramide; DHCer, dihydroceramide; GalCer, β-galactosylceramide; GlcCer, β-glucosylceramide; KDS, 3-keto-dihydrosphingosine/3-keto-sphinganine; L-Ser, L-serine; Sa, sphinganine/dihydrosphingosine. Ganglioside names are abbreviated according to Svennerholm [271,272] and recommended by IUPAC [273].

(de)differentiation process itself and it was shown that different GSLs support the human myeloid HL-60 cell line to differentiate either into monocytes/macrophages or into granulocytes [21–23]. Furthermore, specific GGs/GSLs appear to support the activation of different types of T cells [24].

GGs are enriched in the nervous system [25], where they form cell-type-specific patterns on surfaces of

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neurons which change with differentiation [26–29]. As long-chain base they contain mostly C18-sphingosine, however, with cellular differentiation C20-sphingosine containing GGs appear and their content increases throughout the life span [30]. Together with high levels of cholesterol and sphingomyelin, GGs stabilize neuronal plasma membranes [31,32]. With their glycan moieties, they are involved in cell-to-cell adhesion processes [33,34].

Gangliosides (GGs) are sialic acid containing glycosphingolipids (GSLs) expressed in a cell-type-specific manner and abundant in neuronal plasma membranes (PMs) [35]. However, as components of plasma membranes they also cover surfaces of a variety of other mammalian cells [7].

The hydrophilic oligosaccharide head group of amphiphilic GSLs and GGs is anchored onto the anticytosolic leaflet of membranes and plasma membranes (PMs) with a hydrophobic ceramide moiety, composed of a sphingoid base, a long-chain amino alcohol. Thus the glycan moiety of GGs is facing the cellular surface, and being part of the glycocalix. GGs are GSLs containing one or more of about 50 different sialic residues, all being derivatives of the neuraminic acid [36]. GGs can serve as ligands of lectins and modulate the activity of membrane proteins such as EGF receptor, leptin, and insulin receptor [37–45]. These interactions depend on the structure of the oligosaccharide head group and that of the ceramide anchor, both of which may vary significantly with the cell type. Neuronal gangliosides of the A- and B- series are anchored to cellular membranes by a ceramide moiety containing almost exclusively stearic acid [46] (Fig. 1). GM3 and GGs of many other organs, like liver and intestine, prefer long-chain fatty acids and palmitic acid in their ceramide anchor [47]. Interestingly, the length of the acyl chain may affect the endosomal and intracellular trafficking of GM1 in cell culture as demonstrated by the incorporation of different acyl chains into synthetically prepared GM1 [48,49].

Mammalian neurons are enriched in gangliosides of the a- and b-series (e.g., GM1a, GD1a, GT1b, etc., Table 1) having a hydrophilic tetraosyl moiety with a varying number of sialic acids. The head groups of the axonal GGs, GD1a, and GT1b, stabilize the axon – myelin interaction efficiently by binding myelin-associated glycoprotein of the surrounding inner myelin sheet [33,50,51]. The physiological functions of GGs, as indicated by the analysis of mutant mice, are profound but still poorly understood [52].

GGs are also important as effective binding sites of lectin-type bacterial exotoxins like tetanus toxin, cholera, shiga, and botulinum toxins [53–60].

Intracellular pathways of ganglioside metabolism

Metabolic steps of GG metabolism are intimately connected with intracellular trafficking between organellar membranes in the forecourt of eukaryotic cells [14,61]. Biosynthesis and catabolism of GGs proceed in a stepwise manner through the same membrane-bound lipid intermediates, which occur, however, at different organellar membranes of different intracellular routes: mainly the secretory pathways in case of synthesis and the endocytotic pathways in case of degradation (Figs 2–4).

Biosynthesis starts with the formation of the hydrophobic ceramide anchor at the cytoplasmic leaflet of ER membranes followed by the action of glycosyltransferases (GTs) localized in Golgi and TGN membranes of the secretory pathway. Remodeling and trimming of complex GGs has been observed by sialidases and other hydrolases at membranes of the PM and endosomes, and by glycosyltransferases at the TGN and the PM, whereas the constitutional catabolism of GG and GSL takes place at intralysosomal luminal vesicles (ILVs) of late endosomes and lysosomes [62].

Emerging concepts of GG metabolism at organellar membranes

In eukaryotic cells, metabolism of GGs and GSLs is strictly associated with intracellular membrane trafficking. Their main biosynthetic pathways occur at membranes of the secretory pathway, the ER, Golgi, and TGN, and their constituent catabolic pathways take place at membranes of the endocytotic pathways, mainly at the surface of intraluminal vesicles of endosomes and lysosomes (Fig. 3).

The metabolism of amphiphilic, membrane-bound glycolipids is catalyzed at the membrane-water interphase (a) either by membrane-spanning enzyme proteins in case of biosynthetic pathways, or (b) by soluble catabolic hydrolases with the help of soluble lipid-binding protein cofactors at the surface of ILVs.

Concentrating lipid substrates and biosynthetic membrane-spanning enzymes or multienzyme complexes together within the same membrane increases the reaction rates and avoids needless molecular waste in the cellular environment. Lipid substrates and

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Table 1. Schematic formulas of gangliosides mainly from the mammalian nervous system[a]. Sialic acid containing glycosphingolipids are underlined in yellow.

	Ganglioside/GSL	Structure	Occurrence
	Cer	Ceramide, *N*-acylsphingosine	Ubiquitous
	GalCer	Galβ1→1Cer	Myelin sheaths, Kidney
	GM4	Neu5Acα2→3Galβ1→1Cer	Kidney[b], Myelin[c]
	GlcCer	Glcβ1→1Cer	Ubiquitous
	LacCer (GA3)	Galβ1→4Glcβ1→1Cer	Human brain with multiple sulfatase deficiency[d], mouse brain with GM3S- and GalNacT-deficiency[e], neutrophils (Intracellular granules)[f]
0-series	Gg_3Cer (GA2)	GalNAcβ1→4Galβ1→4Glcβ1→1Cer	Human brain variants of GM2-gangliosidosis[g]
	Gg_4Cer (GA1)	Galβ1→3GalNAcβ1→4Galβ1→4Glcβ1→1Cer	GM1 gangliosidosis brain[h] Digestive tract (mouse)[i]
	GM1b	Neu5Acα2→3Galβ1→3GalNAcβ1→4Galβ1→4Glcβ1→1Cer	Only trace amounts In adult mammalian Brain[b]
	GD1c	Neu5Acα2→8Neu5Acα2→3Galβ1→3GalNAcβ1→4Galβ1→4Glcβ1→1Cer	Only trace amounts In adult mammalian Brain[b]
	GD1α	Neu5Acα2→3Galβ1→3(Neu5Acα2→6) GalNAcβ1→4Galβ1→4Glcβ1→1Cer	Only trace amounts In adult mammalian Brain[b]
A-series	GM3	Neu5Acα2→3Galβ1→4Glcβ1→1Cer	Cerebellar Spongines[j] and GM2S-deficiencies[k,l,m] Astrocytes[n], Relatively broad distributed, prominent GG in muscle[o] and endothelial cells[p,q]
	GM2	GalNAcβ1→4(Neu5Acα2→3)Galβ1→4Glcβ1→1Cer	Neuronal enrichment in GM2-Gangliosidis[r]; Mouse liver[s] human tumors such as melanoma, neuroblastoma, glioblastoma, renal cell carcinoma
	GM1a	Galβ1→3GalNAcβ1→4(Neu5Acα2→3)Galβ1→4Glcβ1→1Cer	Major ganglioside in adult mammalian brain Mouse intestine, Testis
	GD1a	Neu5Acα2→3Galβ1→3GalNAcβ1→4(Neu5Acα2→3)Galβ1→4Glcβ1→1Cer	Major ganglioside in adult mammalian brain
	GT1a	Neu5Acα2→8Neu5Acα2→3Galβ1→3GalNAcβ1→4(Neu5Acα2→3)Galβ1→4Glcβ1→1Cer	Major ganglioside in adult mammalian brain
	GT1α	Neu5Acα2→3Galβ1→3(Neu5Acα2→6) GalNAcβ1→4(Neu5Acα2→3)Galβ1→4Glcβ1→1Cer	Erythrocytes, spleen bone marrow, testis, liver, adult brain[t]
B-series	GD3	Neu5Acα2→8Neu5Acα2→3Galβ1→4Glcβ1→1Cer	Embryonic neuronal tissue,[u,v], lymphocytes
	GD2	GalNAcβ1→4(Neu5Acα2→8Neu5Acα2→3) Galβ1→4Glcβ1→1Cer	Pediatric and adult solid tumors including neuroblastoma, glioma, retinoblastoma, . . .[w]
	GD1b	Galβ1→3GalNAcβ1→4(Neu5Acα2→8Neu5Acα2→3)Galβ1→4Glcβ1→1Cer	Major ganglioside in adult mammalian brain
	GT1b	Neu5Acα2→3Galβ1→3GalNAcβ1→4(Neu5Acα2→8Neu5Acα2→3)Galβ1→4Glcβ1→1Cer	Major ganglioside in adult mammalian brain
	GQ1b	Neu5Acα2→8Neu5Acα2→3Galβ1→3GalNAcβ1→4(Neu5Acα2→8Neu5Acα2→3)Galβ1→4Glcβ1→1Cer	Major ganglioside in adult mammalian brain

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Table 1. (Continued).

	Ganglioside/GSL	Structure	Occurrence
	GQ1bα	Neu5Acα2→3Galβ1→3(Neu5Acα2→6) GalNAcβ1→ 4(Neu5Acα2→8Neu5Acα2→3) Galβ1→4Glcβ1→1Cer	Human thoracic cord[x,y]
C-series	GT3	Neu5Acα2→8Neu5Acα2→8Neu5Acα2→3Galβ1→4Glcβ1→1Cer	
	GT2	GalNAcβ1→4(Neu5Acα2→8Neu5Acα2→8Neu5Acα2→3)Galβ1→4Glcβ1→1Cer	
	GT1c	Galβ1→3GalNAcβ1→4(Neu5Acα2→8Neu5Acα2→8Neu5Acα2→3)Galβ1→4Glcβ1→1Cer	Mammalian brain[z], chicken brain[aa], fish brain[ab]
	GQ1	Neu5Acα2→3Galβ1→3GalNAcβ1→4(Neu5Acα2→8Neu5Acα2→8Neu5Acα2→3) Galβ1→4Glcβ1→1Cer	Only trace amounts in adult mammalian brain Formed during mammalian brain development[b]
	GP1c	Neu5Acα2→8Neu5Acα2→3Galβ1→3GalNAcβ1→4(Neu5Acα2→8Neu5Acα2→8Neu5Acα2→3) Galβ1→4Glcβ1→1Cer	
	GP1cα	Neu5Acα2→3Galβ1→3(Neu5Acα2→6) GalNAcβ1→4(Neu5Acα2→8Neu5Acα2→8Neu5Acα2→3)Galβ1→4Glcβ1→1Cer	
Fucosylated	Fuc-GM1	Fucα1→2Galβ1→3GalNAcβ1→4(Neu5Acα2→3)Galβ1→4Glcβ1→1Cer	Bovine brain[ac], porcine brain[ad], mini pig brain[ae], skate brain[af], mouse digestive tract and testis[ag], boar testis[ah]
	Fuc-Gal-GM1	Fucα1→2Galα1→3Galβ1→3GalNAcβ1→4 (Neu5Acα2→3)Galβ1→4Glcβ1→1Cer	Rat brain dentate gyrus[ai,aj]
	Fuc-GD1b	Fucα1→2Galβ1→3GalNAcβ1→4(Neu5Acα2→8Neu5Acα2→3)Galβ1→4Glcβ1→1Cer	Pig brain[ak], Mini pig brain[ae]
	Fuc-Gal-GD1b	Fucα1→2Galα1→4Galβ1→3GalNAcβ1→4 (Neu5Acα2→8Neu5Acα2→3)Galβ1→4Glcβ1→1Cer	Neurological disease[al]
Galactosylated	Gal-GD1b	Galα1→4Galβ1→3GalNAcβ1→4(Neu5Acα2→8Neu5Acα2→3)Galβ1→4Glcβ1→1Cer	Rat basophilic leukemia cells[am]
	Gal-GalNAc-GM1b	Galβ1→3GalNAcβ1→4(Neu5Acα2→3)Galβ1→3GalNAcβ1→4Galβ1→4Glcβ1→1Cer	T-lineage cells[an]
	GalNAc-GM1	GalNAcβ1→4Galβ1→3GalNAcβ1→4(Neu5Acα2→3)Galβ1→4Glcβ1→1Cer	Squamous cell cancer
	GalNAc-GM1b	GalNAcβ1→4(Neu5Acα2→3)Galβ1→3GalNAcβ1→4Galβ1→4Glcβ1→1Cer	T-lineage cells[an], mouse thymus, spleen and lymph node[ao]
	GalNAc-GD1b	GalNAcβ1→4Galβ1→3GalNAcβ1→4(Neu5Acα2→8Neu5Acα2→3)Galβ1→4Glcβ1→1Cer	Motor neurons[ap]
	Gb3Cer	Galβ1→4Galβ1→4Glcβ1→1Cer	Human Erythrocytes, Kidney, Spleen, serum, and other extraneural tissues[aq]
	Gb4Cer	GalNAcβ1→3-Galβ1→4Galβ1→4Glcβ1→1Cer	Human Erythrocytes, Kidney, Spleen, serum, and other extraneural tissues

Sialic acid containing glycosphingolipids are underlined in yellow. Cer, ceramide; Gal., galactose, GalNAc, N-acetylgalactosamine; GG, ganglioside; Glc, glucose; Neu5Ac, N-acetylneuraminic acid. All human gangliosides and nonhuman brain gangliosides contain exclusively or predominantly Neu5Ac, whereas nonhuman extraneural mammalian gangliosides often contain Neu5Gc. a. [62]; b. [275]; c. [26]; d. [276]; e. [277]; f. [278]; g. [6]; h. [279]; j. [280]; k. [152]; l. [281]; m. [282]; n. [283]; o. [284]; p. [283]; q. [284]; r. [285]; s. [265]; t. [126]; u. [286]; v. [287]; w, [288]; x. [289]; y. [290]; z. [291]; aa. [292]; ab. [293]; ac. [294]; ad. [274]; ae. [295]; af. [296]; ag. [17]; ah. [297]; ai. [298]: aj. [299]; ak. [300]; al. [301]; am. [302], an. [303]; ao. [304]; ap. [305]; aq. [278].

membrane-bound enzymes should interact within the plane of the membrane by diffusion following a two-dimensional form of the Michaelis–Menten equation [63]. On the other hand, the electrostatic interaction of protonated and positively charged catabolic hydrolases and the sphingolipid activator proteins (SAPs) with the negatively charged surface of lipid substrate carrying ILVs should increase the concentration of the reaction partners and thereby accelerate the catabolic reaction rate, too.

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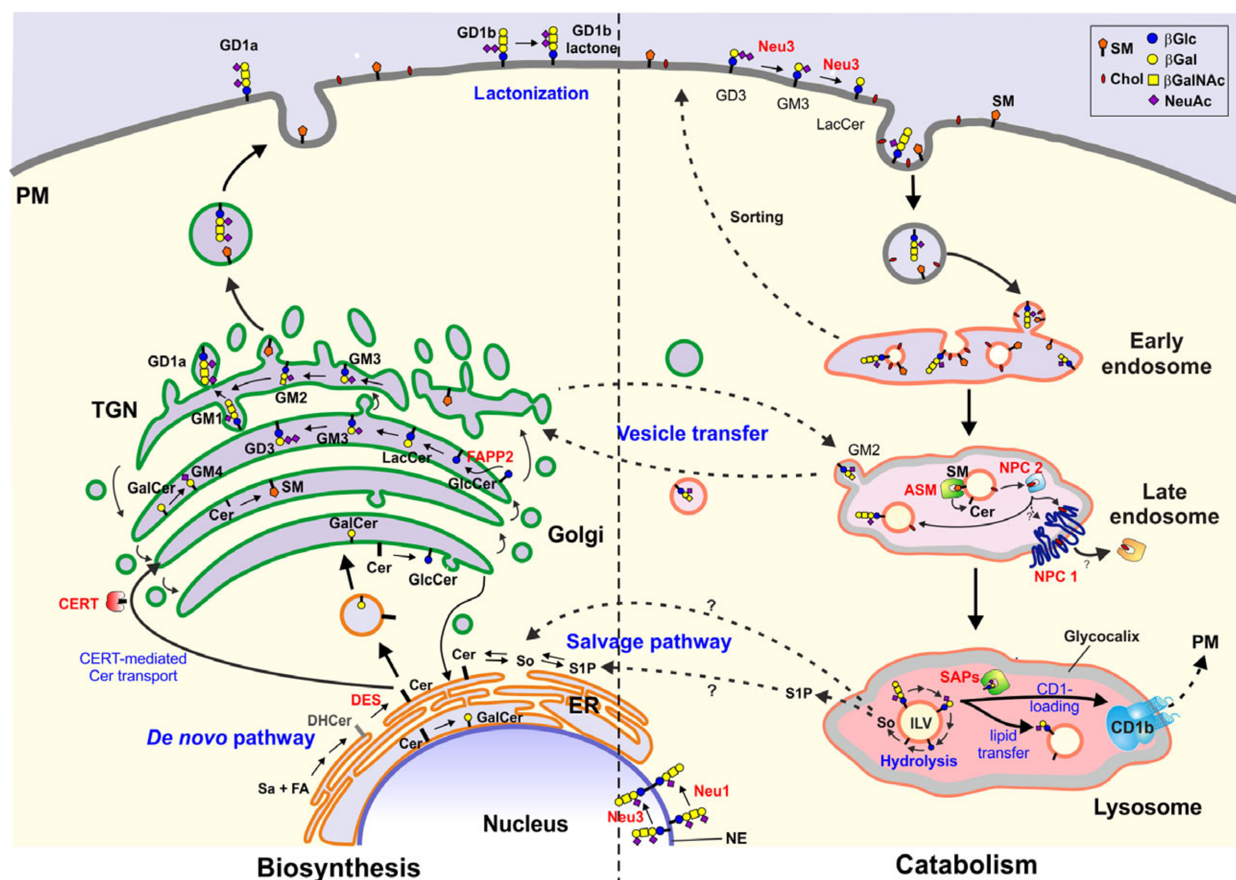

Fig. 3. Subcellular compartmentalization and trafficking in Ganglioside Metabolism. Ganglioside biosynthesis and secretion starts at the endoplasmic reticulum (ER) and finalizes in the lumen of the Golgi system before gangliosides are carried by vesicular transport to the plasma membrane (anabolism, left side). Besides vesicular transport, some sphingolipids such as Cer and GlcCer are also transported between the ER and the Golgi system by lipid transfer proteins like CERT and FAPP2, respectively [110]. Upon endocytosis and incorporation into intraendolysosomal luminal vesicles (ILVs) GG are degraded into monosaccharides, free fatty acids, and sphingoid bases (catabolism, right side), which are recycled for sphingolipid synthesis by the salvage pathway (modified after [62]). ASM, acid sphingomyelinase; Cer, ceramide; CERT, ceramide transfer protein; DES, dihydroceramide desaturase; DHCer, dihydroceramide; FA, fatty acid; GlcCer, glucosylceramide; ER, endoplasmatic reticulum; FAPP2 (PLEKHA8), four-phosphate adaptor protein 2 (pleckstrin homology domain containing A8); LacCer, lactosylceramide; NE, nuclear envelope; NEU, neuraminidase; NPC, Niemann–Pick disease type C protein; PM, plasma membrane; Sa, sphinganine; SAP, sphingolipid activator protein; SM, sphingomyelin; So, sphingosine; S1P, sphingosine-1-phosphate; TGN, trans Golgi network.

Emerging topology of glycolipid biosynthesis

Enzyme reactions for the formation of sphingoid bases and the hydrophobic dihydroceramides and ceramides [64] occur at the cytosolic leaflet of the ER membranes, from where (dihydro)ceramides are transferred to the cytosolic leaflet of Golgi membranes serving as substrates of the glucosylceramide synthase. The generated glucosylceramides are transferred from the cytosolic leaflet to the luminal leaflet of the Golgi membranes, where they are converted by one of two lactosylceramide synthases to lactosylceramide (LacCer) [65–67]. LacCer itself is substrate for six transferases guiding GSL synthesis into different GSL series. Two of them, the GM3 synthase as well as the GA2-synthase will guide anabolism into ganglio-series GSLs/GGs. These integral membrane-spanning GTs require catalytic activity at the luminal leaflet of Golgi and TGN membranes. The luminal oriented GSLs/GGs reach the plasma membrane by vesicular transport and consequently are mainly located on the extracellular leaflet [68,69].

Generation of ganglioside GM4

Ganglioside GM4 is the only ganglioside derived from galactosylceramide (GalCer, Fig. 2). GalCer is

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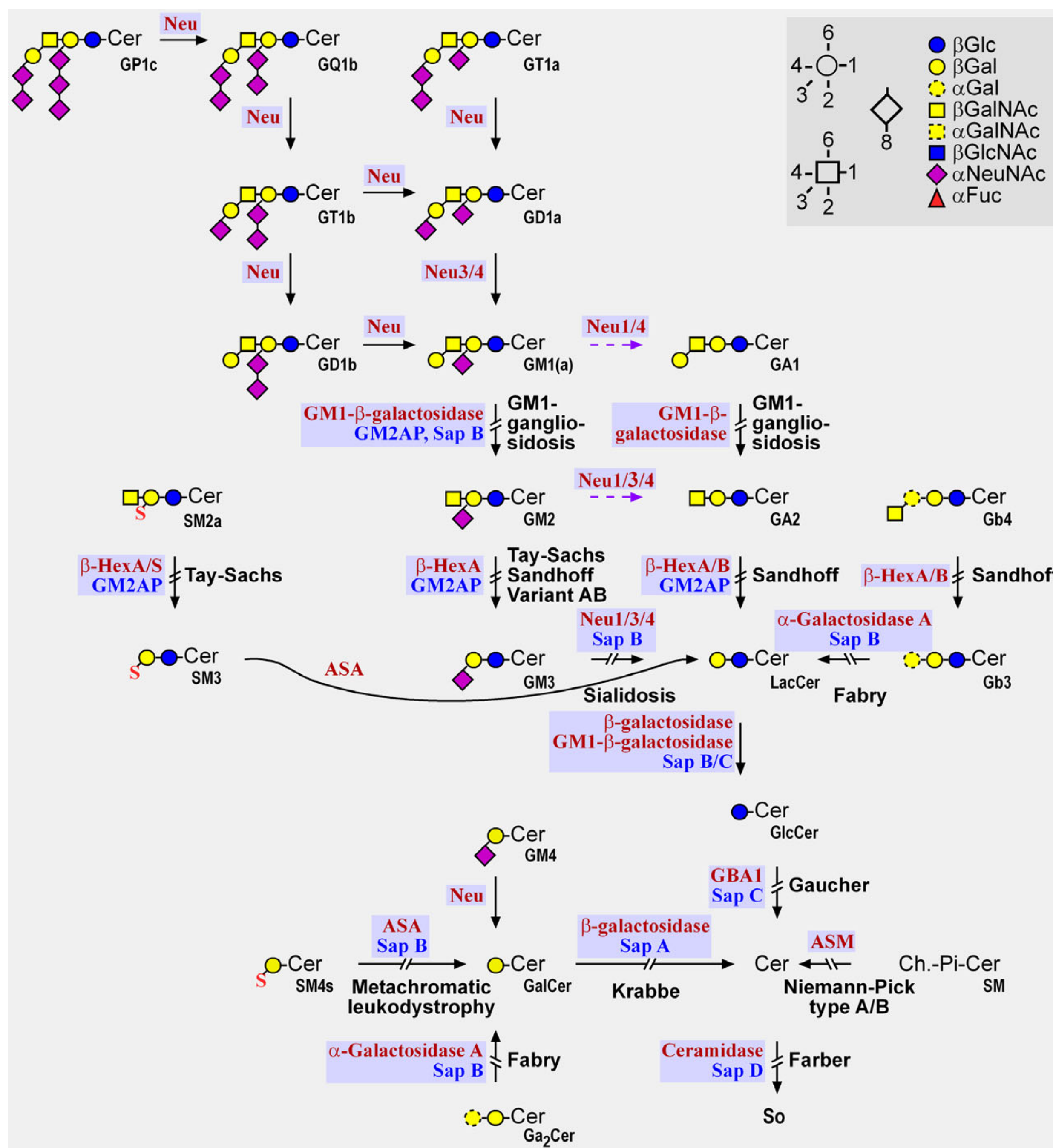

Fig. 4. Lysosomal ganglioside catabolism. Gangliosides are degraded in a stepwise manner by lysosomal glycosidases/hydrolases (red) in the presence of activator proteins (blue). Malfunction of these proteins causes metabolic diseases, as subsequent hydrolases do not act on presubstrates. Metabolic diseases are indicated in the scheme. Broken purple arrows indicate the bypass pathway of the ganglioside GM1 degradation in mice, mainly caused by neuraminidase 3 (Neu3). Sialidoses caused by different inherited sialidase (Neu) deficiencies are not marked in the figure due to their overlapping oligosialo-ganglioside storage pattern caused by promiscuous and overlapping substrate specificities of mammalian sialidases. ASA, arylsulfatase A; ASM, acid sphingomyelinase; Ch.-Pi-Cer (SM), sphingomyelin; GBA1, lysosomal β-glucocerebrosidase; Hex A/B/S, β-hexosaminidase A/B/S; Neu, neuraminidase; Sap, saposin.

synthesized by the beta-galactosylceramide synthase (UGT8a), which is located in the ER with its active site facing the luminal side [70] (Fig. 3). After vesicular transport to the Golgi, a sialic acid from CMP-sialic acid is transferred in α2,3 linkage to the galactosyl moiety of GalCer by GM3-synthase (GM3S, St3gal5)

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forming the most simple ganglioside GM4 [71], but eventually only when St3gal5 is not in complex with lactosylceramide synthase [72]. GM4 is a minor component of the brain, and, otherwise, detected in low concentrations in erythrocytes, kidney, and intestine [73,74]. Little is known about its biological function, but it may interact with the myelin basic protein [62,75].

Generation of cell-type-specific ganglioside patterns

There are several factors emerging to contribute to the generation of cell-type-specific pattern on the surface of mammalian cells.

Most of the enzymes involved in biosynthetic pathways of GGs and GSLs have a rather poor specificity for their lipid and glycolipid substrates, for example, the dihydroceramide and ceramide synthases accepting sphingoid bases and fatty acids of different chain length, saturation, and hydroxylation grade as well as the GTs – GalNAc-transferase, for example, converts LacCer as well as the GGs GM3, GD3, and GT3 into higher GGs of the ganglio-series (Fig. 2). Thereby the combinatorial activity of only few promiscuous GTs, their levels are mostly controlled by transcriptional regulation, allows the formation of cell-type-specific GSL pattern on cellular surfaces [76]. The combinatorial action of GTs is mirrored by the interplay of ceramide synthases with the cell-type-specific machinery for sphingoid bases and fatty acids to define their ceramide anchor pattern [77].

Cell-specific GG and GSL patterns and metabolism have even been observed for different neurons, including granule neuron, pyramidial neurons, and Purkinje cells [26,78–82]. Cell-type- and cell-specific expression of GG and GSL are obviously obtained by a combination of several factors. Mayor players are (a) the cell-type-specific expression of often promiscuous biosynthetic enzymes (SPT subunits, ceramide synthases, and GTs) and (b) the availability of local acyl-CoAs donors and glycolipid acceptors at the organellar membranes of the secretory pathway. Of course, further well-known factors like pH value, ionic strength, ion composition, and the availability of soluble enzyme substrates like activated sugar nucleotides in the lumen of the Golgi and TGN compartment are also of great importance.

Emerging factors like membrane fluidity, lipid, and protein composition of organellar membranes, and nutritional state will presumably gain more importance for the regulation of biosynthetic and remodeling steps in GG and GSL metabolism. For example, the anionic lipid phosphatidylglycerol (PG) stimulates GG biosynthesis in detergent-free *in vitro* assays [83,84] and an increase in membrane fluidity as triggered by the addition of general anesthetics (e.g., halothan, Xenon gas) or a series of fatty acids with declining acyl chain lengths stimulates desialylation of oligosialo-GGs by membrane-bound sialidases [63]. Finally, high fat or so called western diet may cause increased production of C16-sphingolipids [85] including, for example, ganglioside GM3, which in turn can downregulate insulin receptor activity thereby supporting metabolic syndrome and type 2 diabetes mellitus [44].

Enzyme catalysis at membrane surfaces

Biosynthetic steps are catalyzed by integral membrane-bound enzymes, which can interact with their lipid substrates by diffusion within the plane of the membrane. This has to be expected for enzymes of the sphinganine (Sa) and ceramide formation in the cytosolic leaflet of the ER and their membrane-bound lipid substrates, whereas the soluble serine substrate can interact with the membrane-bound SPT through the aqueous space (Fig. 2) [14,86–89]. GTs are also membrane-spanning proteins, which should interact with their lipid substrates by lateral diffusion within the plane of the membrane, and directly with the water soluble activated sugar nucleotides in the lumen of the Golgi and TGN.

The kinetics of lipid substrate turnover by membrane-bound GTs at the lipid-water interphase, however, has been only poorly studied, mostly in the presence of detergents, which profoundly obscure the physiological interaction between membrane-bound substrate and enzyme. A model case has been investigated, however, for the interaction of plasma membrane-bound sialidase (presumably Neu3) and the radiolabeled GG GD1a in isolated synaptosomal membranes [90–94]. As expected, the GG hydrolysis does not follow the well-known Michaelis–Menten kinetics, but its two-dimensional version. A reproducible Michaelis–Menten constant was obtained only when the substrate concentration was presented as amount of GD1a per membrane-surface available in the incubation assay and not as amount of GD1a per incubation volume, as usually done for solutes [63,95].

The analysis also revealed a stimulation of the GG hydrolysis by sialidase in isolated neuronal membranes with increasing membrane fluidity [63,95,96] modulated by the addition of free FAs with different chain lengths or general anesthetics like halothane or Xenon gas [95,96]. The concept of two-dimensional kinetics,

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however, does not apply to the lysosomal GG catabolism catalyzed by mostly water-soluble mammalian exohydrolases. Their interaction with membrane-bound GG and other lipid substrates is facilitated at the surface of the lipid phase by a mixture of small promiscuous lipid-binding proteins, the SAPs.

Ganglioside biosynthesis and salvage pathways

Ceramide synthesis

De novo synthesis of sphingolipids (SLs) starts with the condensation of an activated fatty acid (mainly palmitoyl-CoA) with L-serine at the cytosolic leaflet of the ER membrane. The rate limiting step is catalyzed by the serine-palmitoyl-CoA-transferase (SPT) [97], which is regulated in a feedback loop by inhibitory ORM proteins in yeast [98,99], and ORMDL proteins in mammals [100,101]. Interestingly, the chain length of the product 3-keto-sphinganine also known as 3-keto-dihydrosphingosine (KDS) is influenced by the presence of either of the two small subunits SPTssa and SPTssb [102,103]. KDS is reduced with the help of NADPH by the 3-keto-dihydrosphingosine reductase (KDSR) to Sa, which is a substrate of ER-bound ceramide synthases (CerSs). They convert sphingoid bases, for example, Sa and sphingosine (So), from *de novo* and salvage pathways at the ER to the respective dihydroceramides and ceramides. In mammals, there are six genes leading to different CerS isoforms, which are cell type specifically expressed. In combination with the available acyl-CoA profile the expressed CerS pattern leads to characteristic ceramide anchor profiles of SLs and GGs in different cell types and organs [18,104–109]. The activities of CerS are regulated at the transcriptional and the protein level [110,111]. The ceramide anchors of neuronal GGs contain mainly stearic acid due to high expression levels of CerS1 [112–114]. Enzyme activities of CerS2-6 are regulated manifold [115], for example, by phosphorylation. They contain a HOX-domain [108], which may have a regulatory role as a nuclear DNA-binding protein as has been shown for the single CerS of drosophila [116,117]. Transcripts of several genes involved in lipid metabolism and cell division in mouse liver are also regulated by CerS2 activity, suggesting a role of very long acyl chain ceramides in the nucleus for the transcriptional regulation of target genes ([118,119].

Dihydroceramide (DHCer) formed by the acylation of Sa is subsequently converted (e.g., in neurons) by the dihydroceramide desaturase 1 with the cosubstrates NAD(P)H and oxygen to ceramide by generating a trans-double bond in 4-position of the sphingoid base [120,121]. DHCer can also be converted to phytoceramides especially in intestinal tissue [122–124].

Ganglioside synthesis

Synthesis of complex GSLs and GGs occurs by the stepwise addition of monosaccharides to ceramides at the membranes of the Golgi and TGN [14,125] (Fig. 2). Ceramides reach the Golgi membranes either by vesicular transport or by the interorganellar transfer protein CERT, preferentially transporting ceramides with C14 to C30 acyl chains [110].

GSL formation is initiated by the addition of glucose to ceramide by UDP-glucose ceramide glucosyltransferase, the glucosylceramide synthase at the cytosolic leaflet of early Golgi membranes [126–128]. Generated glucosylceramide can be translocated to the luminal face of Golgi membranes [111], where it is converted to LacCer by one of two lactosylceramide synthases [66,67].

LacCer is the precursor of different GSL series, that is, ganglio-, asialoganglio-, sulfoganglio-, globo-, isoglobo-, and (iso)lacto series (Fig. 2, Table 1). These series are formed by cell-type-specific glycosyltransferases, integral membrane proteins at the Golgi and TGN membranes, which are organized in distinct multienzyme complexes [129,130].

In neurons, the biosynthesis of a- and b-series gangliosides starts with the transfer of sialic acid from CMP-sialic acid to the galactosyl residue of LacCer in 2,3 linkage to generate the simple ganglioside GM3 [131] (Fig. 2). Further sialylation of GM3 generates GD3 and finally GT3 [132,133], the precursors of b- and c-series gangliosides, respectively.

As suggested by Saul Roseman, biosynthesis of GG is often mediated by multiglycosyltransferase complexes [84,134–138] located in Golgi and TGN membranes (Fig. 3), which stabilize enzymes and improve glycolipid synthesis [139,140]. GG glycosyltransferases are type II membrane glycoproteins [141], some of them are S-acylated at conserved cysteine residues, which may be involved in the formation of homodimers through disulfide bonds [142].

Enzymatic steps for the synthesis of GGs had already been identified in the 1960s and 70s [143,144]. It was expected that the formation of a- and b-series gangliosides is catalyzed by different GTs. Kinetic competition experiments between the lipid acceptors GM3 (of the a-series) and GD3 (of the b-series), however, proved that both lipid substrates competed for the same active site of a quite unspecific GalNAc-transferase [145]. All GTs involved in the biosynthesis

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of complex gangliosides turned out to be rather promiscuous for their lipid substrates [146–148], resulting in the assembly line given in Fig. 2. Further modifications of the formed gangliosides (see Fig. 2) by different forms of sialylation, 0-acetylation, and lactonization have been reviewed [62].

GG biosynthesis is cell-type specific and regulated at the transcriptional and protein level [18,46,76,84,93,95,97,99–101,106,107,149,150] and by a sequential organization and coordination of glycosyltransferases with the traffic of biosynthetic intermediates through the membranes of the secretory pathway [151]. Blocks in GG biosynthesis as generated in GM3-only KO mice and other mouse models indicate no feedback inhibition due to accumulating intermediates [150]. With respect to defects in GG biosynthesis, mouse models and inherited human defects not necessarily correlate: Inherited defects of ganglioside GM2 biosynthesis cause severe infantile epilepsy and spastic paraplegia [152], whereas mice lacking major gangliosides due to a GM2/GD2 deficiency develop manifestations of parkinsonism [153]. Whereas human defects in GM3 synthesis [154,155] cause infantile-onset symptomatic epilepsy syndrome or refractory epilepsy and mitochondrial dysfunction, GM3 synthase-deficient mice appeared rather normal but are deaf [156,157].

Remodeling and recycling of cell surface gangliosides

Complex GSLs and GGs reach the cellular surface by vesicular transport. They are enriched in the extracellular leaflet of PMs, GGs especially at synaptic surfaces, where they undergo lateral associations and transinteractions [33].

GG interactions may be regulated by PM located sialidases, like the PM-bound Neu3 [158] and sialyltransferase activity, both of which are present in synaptosomal membranes [90–94].

Polysialogangliosides expressed on cellular surfaces can serve as ligands for lectins and substrates for enzymes [159]. Their composition can be adjusted to changing membrane functions by desialylation mostly catalyzed by Neu3 [160–162] and by recycling. Radiotracer techniques and ESR spectroscopy studies on cultured GM2 gangliosidosis fibroblasts demonstrated that the exogenously added radiolabeled GG GM2 can be incorporated into the PM and directly glycosylated to form radiolabeled GGs GM1 and GD1a despite a complete cellular block in GM2 degradation, thus excluding transfer through the endolysosomal compartment. A direct glycosylation of labeled GGs GM2 and GM1 was also observed in normal control cells, presumably after trafficking from endosomes to the TGN compartment [163,164] (Fig. 3), which may involve the retromer complex [165].

Intracellular trafficking especially of GG GM1 having unsaturated or shorter ceramide anchors [48,49] has been observed as an important aspect of cellular uptake of cholera toxin and metabolism. Recycling of fluorescent labeled glucosylceramide has also been observed from the PM and back [166] as well as the glycosylation of nondegradable glucosylceramide analogs involving trafficking from proximal to distal Golgi cisternae to form complex gangliosides [167]. Gangliosides have also been identified in the nucleus, their metabolism and functions, however, remain so far mostly unknown. GGs GM1 and GD1a are located in both membranes of the nuclear envelope together with two neuraminidases [168].

Emerging concepts on the constitutive degradation of gangliosides and glycosphingolipids at ILVs

Location and topology of sphingomyelin, glycosphingolipids, and ganglioside catabolism

The constitutive degradation of membrane lipids, sphingolipids (SLs), phospholipids, and other membrane components, most likely takes place at the surface of ILVs. They can be reached by SLs and GGs of the PM by endocytosis (Fig. 3). This emerging concept is suggested by biochemical and immunoelectrohistochemical studies on the endocytosis of biotinylated and radiolabeled ganglioside GM1 [169] and is required to understand drug-induced phospholipidosis. Most steps of membrane lipid catabolism are catalyzed by rather promiscuous hydrolases (lipases, phospholipases, glycosidases), in case of SLs often with the essential help of lipid-binding proteins, the SAPs.

Endocytosis of gangliosides

Desialylation of complex polysialylgangliosides is catalyzed by membrane-bound sialidases, Neu1, Neu4, and Neu3, to generate ganglioside GM1 [170,171]. This redundancy may explain no complex poysialylgangliosides to accumulate in the brain of 2-month-old mice deficient either in Neu1, Neu3, Neu4 or Neu3, and Neu4 [162]. The desialylation is an initial step in the ganglioside degradation in mammalian tissues [172] and can take place at the plasma membrane or later in the endosomes and lysosomes. The lysosomal sialidase Neu1 is a member of a multienzyme complex together

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with protective protein/cathepsin A (PPCA, a stabilizing protein for sialidase Neu1), and the GM1 cleaving β-galactosidase [62]. Inherited defects of Neu1 leading to sialidosis cause accumulation of sialylated metabolites [173], including an amyloidogenic processing of an oversialylated amyloid precursor protein in lysosomes and an extracellular release of Aβ-peptides [174], whereas genetic defects in PPCA trigger a storage of GM1 besides oligosaccharides and other glycolipids [175,176].

Lysosomes degrade a variety of macromolecules and complex lipids and release their components into the cytosol as nutrients for use in energy metabolism and biosynthetic salvage pathways [177]. Macromolecules and complex lipids can reach the lysosomes and the ILVs by endocytotic pathways [177], and probably also by phagocytosis and autophagy [178]. Metabolic studies and immunoelectron microscopic observations indicate that PM-bound radio- and biotin-labeled ganglioside GM1 reaches ILVs for final degradation [169] (Fig. 3). Released components like So, fatty acids, and monosaccharides can leave the lysosomes to reach the ER and the cytosol. Released So can be incorporated again into newly synthesized SLs and GGs [164,179–181], but may also be phosphorylated to generate S1P for final degradation of the sphingoid base by S1P-Lyase [182,183].

Emerging concept on the generation of ILVs during endocytosis

As proposed [184], ILVs are generated by an inward budding of the endosomal membrane and budding off of ILVs into the luminal space, which is catalyzed by ESCRT proteins [185] (Fig. 3). Components of the endosomal membrane sorted into the ILVs can be catabolized by the digestive juice in lysosomes. ILV degradation will destabilize and destroy the bilayer structure of ILV membranes and generate transiently micellar and other amphiphilic aggregates, which either may dissolve or fuse with new incoming ILVs.

Catabolism of GSLs, SLs, and GGs at ILVs can be blocked by genetic defects of lysosomal proteins [177], causing lysosomal lipid and membrane storage diseases. An inherited defect of prosaposin in patients, the precursor of four lipid-binding proteins, the saposins A, B, C, and D, triggers an excessive accumulation of GSLs, SLs and intralysosomal ILVs with an average diameter of 90 nm within the lumen of late endosomes and lysosomes. The blocked lipid catabolism can be restored and the pathological lipid and ILV accumulation can be completely reversed by feeding nanomolar concentrations of the missing prosaposin to patients' cultured fibroblasts [62,186–190]. ILVs are obviously the main location for degradation of SLs including GGs and membranes.

Maturation of ILVs and lipid sorting

Whereas ILVs can be attacked by the lysosomal juice, the lysosomal perimeter membrane is protected by a thick glycocalix, which covers its luminal surface and prevents hydrolases and SAPs to reach and digest the perimeter membrane. The coat is generated by integral glycoproteins, heavily N-glycosylated with almost digestion-resistant polylactosamine units [191]. Defects in the biosynthesis of the lysosomal perimeter membrane glycoproteins, however, increase their turnover [191] and may attenuate their barrier function. Most of the degradation products released within the lysosomal compartment need membrane carriers to leave the lysosol and to reach the cytosol [62,191], where they can be used for biosynthesis of new macromolecules or as fuel for the energy metabolism.

However, attack of the lysosomal juice is substantially facilitated by a lipid sorting process leading to maturation of ILVs already at the level of late endosomes [177,192]: membrane stabilizing lipids of the plasma membrane, sphingomyelin (SM) and cholesterol, are removed. Acid sphingomyelinase (ASM) cleaves several phospholipids, but mainly SM, thereby releasing ceramide [193,194] and a minor amount of diacylglycerol. Reconstitution studies *in vitro* suggest that the decrease in inhibiting SM levels and the concomitant increase in stimulating ceramide levels are prerequisites for effective secretion of the nondegradable cholesterol from the ILVs by the transfer and sterol-binding glycoprotein NPC2 [195]. The anionic lysopholipid bis(monoacylglycero) phosphate (BMP) facilitates membrane binding of NPC2, which is counteracted by SM [194–196]. Besides BMP, also other anionic phospholipids like phosphatidic acid (PA), PG, and phosphatidylinositol (PI) stimulate cholesterol transfer by NPC2. BMP itself is a lysosome-specific lipid as it is a transient intermediate of PG degradation within the ILVs. As it is rather slowly catabolized significant steady state levels of BMP build up within lysosomes [62,192,195].

Lowering SM and cholesterol levels and generating anionic BMP during maturation of ILVs appear to be substantial to reach physiological rates of GSL and SL degradation in the endolysosomal pathway [197].

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BMP formation contributes significantly to the quintessential negative surface charge on ILVs

Reaching ILVs in the lysosol, membrane lipids are usually readily degraded in active lysosomes. The catabolism of PG, however, generates an intermediate, the anionic bis-lysolipid BMP, which is only slowly catabolized and therefore increases to up to 40–60 mol% of ILV-phospholipids [62,192,198,199]. Like other phospholipids (PA, PG, and PI) BMP is negatively charged, even at pH values as low as 4, [194,200]. Reconstitution studies *in vitro* reveal it to generate a negative surface potential on ILVs [194]. This potential should force binding and concentration of lysosomal enzymes and SAPs on the surface of ILVs by electrostatic interaction as these proteins are protonated in the acidic lysosol with pH values around 4–5 [194,201]. Importantly, surface-bound lysosomal enzymes like ASM [202], acid ceramidase [203], HexA, HexB, and other glycoproteins are partially protected against premature proteolytic digestion in the lysosome. Compensation of the negative surface charge of ILVs favors release of these enzymes and activator proteins and subsequently their proteolytic digestion. This is observed when feeding cationic amphiphilic drugs (such as desipramine) to cultured fibroblasts, which causes a drop in lysosomal degradation activity and triggers a phospholipid storage disease, that is an induced phospholipidosis [62,201,202,204]. The catabolism of membrane lipids by the concerted action of lipases and glycosidases supported by the mixture of five known lipid binding and membrane disturbing SAPs, will destroy the lipid bilayer and topology of ILVs. Micelles and smaller lipid aggregates may well be generated as intermediates of ILV degradation.

Removal of inhibitory lipids from ILVs

The plasma membrane stabilizing lipids, SM and cholesterol, inhibit GSL and GG catabolism and are removed from ILVs during endocytosis.

Defective NPC2-mediated cholesterol efflux and a reduced ASM activity in the lysosomes of Niemann–Pick disease type C2 may well increase cholesterol and sphingomyelin levels [205,206], which inhibit the BMP-enhanced hydrolysis of GM2 and glucosylceramide strongly [207,208]. Catabolism of inhibitory sphingomyelin by ASM, however, generates stimulatory ceramide, which enhances cholesterol transfer by NPC2 tremendously [194]. Sphingomyelin degradation is obviously an initial step required for physiological secretion of cholesterol from the late endosomal compartment, which in turn is a prerequisite for lysosomal GSL and GG digestion [62]. The secondary storage of LacCer and ganglioside GM3 in NPC patients [206] might also be explained by cholesterol inhibiting the function of SAPs, Sap A [209], Sap B [210] and GM2 activator protein [207] to mobilize lipids, since the inhibition of GSL biosynthesis by Miglustat improves the clinical course of the disease [211,212].

Catabolism of gangliosides at ILVs

Catabolism of gangliosides, SLs and SM proceeds at the surface of ILVs (Figs 3 and 4) [177]. In contrast to the GG biosynthesis at the membranes of the secretory pathways, no membrane-spanning enzymes are directly involved in lysosomal phospholipid and GG catabolism. The lipids of the ILVs are catabolized by soluble lysosomal glycoproteins, lysosomal hydrolases and lipid-binding sphingolipid activator proteins (SAPs).

Stepwise degradation of complex gangliosides starts in mammalian tissues with the removal of terminal sialic acid units from the oligosaccharide chain by neuraminidase(s) to generate GM1 (Fig. 4). GM1 degradation continues with the removal of terminal galactose by GM1-β-galactosidase, supported by the GM2 activator protein or saposin B to yield GM2 [62,213]. Thereafter the terminal N-acetylgalactosamine residue is split off by β-hexosaminidase A with the help of the GM2 activator protein, to form GM3 (Fig. 5), which can be degraded to LacCer by an α-sialidase together with Sap B. In mouse, the first two steps can be bypassed by the removal of sialic acid from GM1 and GM2, producing the corresponding asialo-derivates GA1 and GA2, respectively. The latter are degraded to form LacCer by a β-galactosidase and β-hexosaminidase A and B, respectively, with the assistance of the GM2 activator protein [62,214]. Consequently, HexA deficiency in mice causes a very mild gangliosidosis and does not correlate with the infantile form of Tay–Sachs disease [214,215], whereas additional deficiency of Neu3 on top of the loss of HexA function blocks this bypass quite efficiently resulting in strong GM2 gangliosidosis [216]. The Sap B or Sap C-assisted action of β-galactosidase degrades LacCer to GlcCer, whose glucosyl residue is removed by Sap C-assisted β-glucosidase. Finally, ceramide is cleaved by acid ceramidase aided mainly by Sap D to sphingoid base (mainly sphingosine) and free fatty acid [62]. The rate of hydrolytic steps of GM1 catabolism is supported by several factors, such as low pH values (e.g., only a narrow range of pH 3.8–4.5 allows hydrolysis of membrane-bound GM2 by HexA and GM2AP [217]), surface tension, and electrostatic binding of protonated and positively charged enzymes and SAPs

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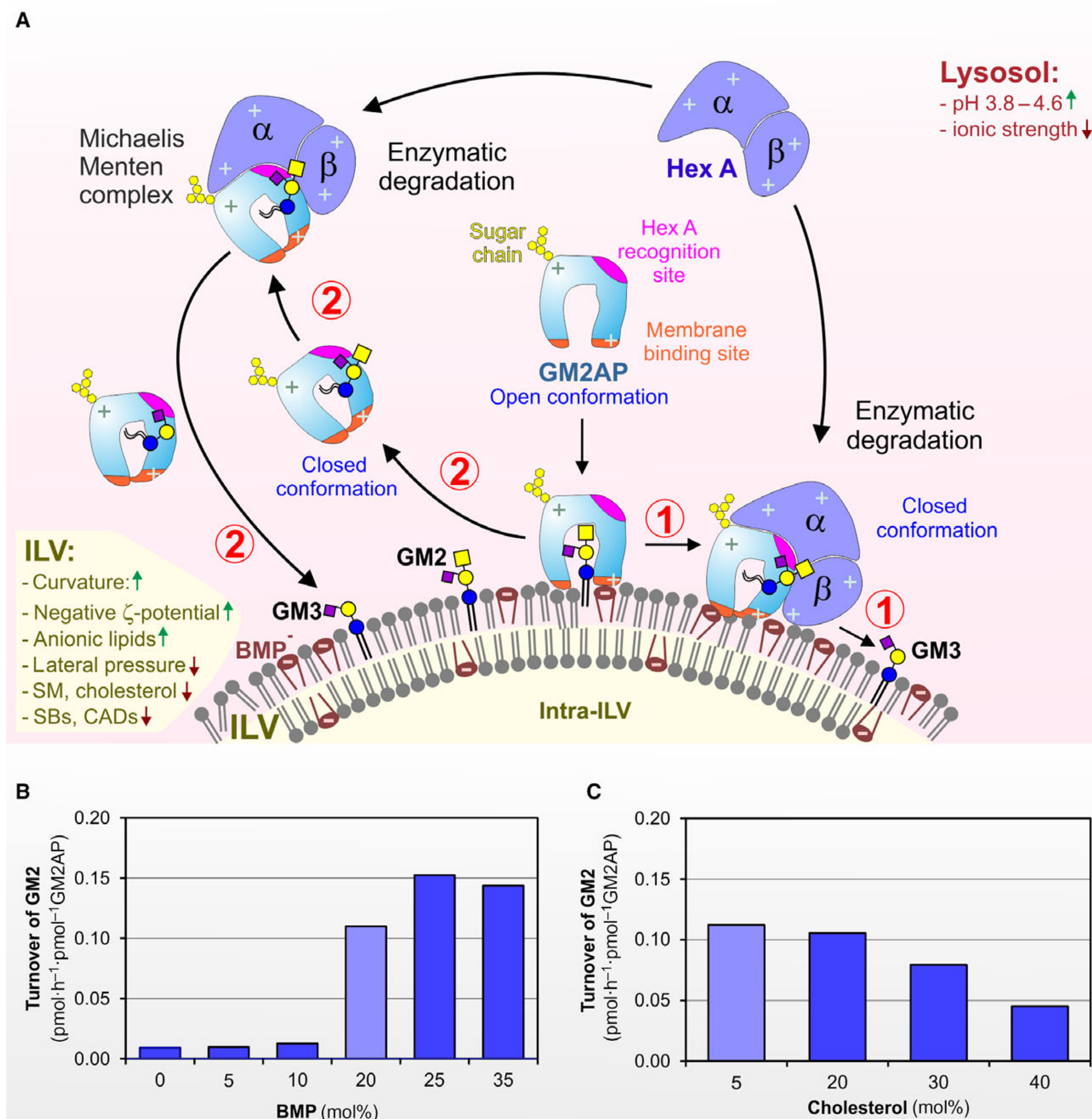

Fig. 5. Model of GM2 degradation by human β-hexosaminidase A (Hex A), and the cofactor GM2AP. (A) GM2AP contains a hydrophobic cavity lined by surface loops and a single short helix. The cavity is suitable for the ceramide anchor of GM2 and other lipids. In its open conformation GM2AP binds to the membrane using the hydrophobic loops (orange) and penetrates to a certain extent into the hydrophobic region of the bilayer. The lipid recognition site of the activator then interacts with the substrate, and its ceramide portion can move inside the hydrophobic cavity. At this point, the lipid-loaded activator may change to its closed conformation, thus the complex becomes more water soluble and can stay (1) or leave the membrane (2), exposing GM2 to the water-soluble Hex A for degradation (modified after [62]). Besides the cofactor GM2AP, lysosomal degradation is stimulated (green arrow pointing upwards) by the acidic lysosomal pH leading to a net positive charge of hydrolases and activator proteins as well as increasing curvature, negative ζ-potential, and anionic lipids of ILVs, whereas increasing lysosomal ionic strength, too high lateral pressure, and the presence of sphingomyelin (SM), cholesterol, free sphingoid bases (SBs), and cationic amphiphilic drugs (CADs) inhibit (red arrow pointing down) lysosomal lipid (GM2) catabolism. The lysosomal-specific anionic lipid BMP serves the net negative charge, reduced lateral pressure, and increased negative ζ-potential of ILVs. (B, C) *In vitro* turnover rates of GM2 hydrolysis supporting the stimulatory influence of the anionic lipid BMP (B) and the inhibitory effect of cholesterol (Chol) (C). Liposomes contained 5 mol% cholesterol, 0–35 mol% BMP or phosphatidylcholine as host lipid (B) and 5–40 mol% cholesterol, 20 mol% BMP and phosphatidylcholine as host lipid (C) [207]. The lighter blue columns represent identical liposomal conditions in (B) and (C).

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to negatively charged surfaces of ILVs. The needed combination of all these factors restricts ganglioside catabolism almost exclusively to the ILVs of the lysosol and protects cells from spontaneous self-degradation.

The salvage pathway, which utilizes lysosomal degradation products for biosynthesis, for example, sphingosine for the formation of the growth factor sphingosine-1-phosphate, plays an important role in many cells and may explain that ablation of acid ceramidase blocks cell cycle progression and fertilization of oocytes [218,219].

A schematic overview of these and other degradation steps and corresponding inherited diseases, which are described elsewhere in more details is given in Fig. 4 [220,221].

SAPs are essential cofactors for lysosomal ganglioside catabolism

Soluble lysosomal hydrolases hardly attack lipophilic membrane components of ILVs directly due to a solubility barrier between the aqueous and the lipid phase, also known as the lipid phase problem: Hydrophobic lipids or lipid moieties of SLs like their ceramide residues are insoluble in aqueous phases, whereas hydrophilic molecules like water-soluble proteins, carbohydrates or oliogosaccharide chains of GSLs and GGs are insoluble in lipid phases.

Therefore the catabolism of GSLs and GGs by water-soluble enzymes needs the help of membrane perturbing lipid-binding proteins, the SAPs (saposins A, B, C, and D, and GM2AP), small glycoproteins with amphipatic properties, some with lipid transfer and others with fusogenic functions at low pH values [177]. Their inherited defects cause rare, but fatal, often degenerative brain diseases [150,221,222] with a clinical picture quite similar to that of the respective enzyme deficiencies [195,222,223].

The GM2 activator protein (GM2AP) and the dimeric saposin B (Sap B) are promiscuous lipid-binding and intervesicular lipid transfer proteins [14]. GM2AP is an essential cofactor for the catabolism of GM2 by hexosaminidase A (HexA; Fig. 5). Its inherited deficiency causes a fatal GM2 gangliosidosis (variant AB of GM2-gangliosidosis) with a clinical picture almost indistinguishable from Tay–Sachs disease. GM2AP can extract GGs from vesicular membranes at low pH values, forming a stoichiometric and soluble GG-protein complex, which is recognized by HexA as substrate, forming a Michaelis–Menten complex (Fig 5). Furthermore, it facilitates the degradation of GA2 [224] and of its sulfated derivative, SM2a, which appeared in the liver of a Tay–Sachs patient [225]. Film balance experiments show that amphiphilic GM2AP inserts into lipid monolayers only when the lateral surface pressure is below a critical value of about 25 $mN \cdot m^{-1}$ [226], a value significantly below that of most biological membranes, which ranges between 30–35 $mN \cdot m^{-1}$. Due to their small diameter of around 90 nm and high curvature, ILVs are more susceptible to an enzymatic attack, as demonstrated for the cleavage of membrane-bound ceramide by acid ceramidase in cooperation with saposin D [227]. These membrane factors, lateral pressure and membrane curvature obviously ensure that GM2AP interacts preferentially with ILVs. Besides well-known factors like pH value and ionic strength, the GM2AP facilitated hydrolysis of membrane-bound GM2 by HexA is strongly stimulated by anionic lipids in the GM2-carrying membranes like BMP, PA, PG, PI, fatty acids, and also by ceramide, lyso-phosphatidylcholine, and diacylglycerol, whereas membrane stabilizing lipids like SM and cholesterol, cationic lipids like sphinganine and sphingosine and CADs like desipramine, chlorpromazine, imipramine, and chloroquine, as well as mucopolysaccharides as primary storage compounds in mucopolysaccharidoses like chondroitin sulfate, dermatansulfate, and hyaluronan, effectively inhibit GM2 hydrolysis [207] (Anheuser *et al.* in preparation). These factors also affect the ability of GM2AP to solubilize and mobilize membrane lipids, but hardly affect the hydrolysis of water-soluble synthetic substrates like 4-methyl-umbelliferyl-β-D-N-acetylglucosaminide-6-sulfate (MUGS; Anheuser *et al.* in preparation), which are often used for the diagnosis of HexA deficiency in patients with inherited GM2 gangliosidosis.

The homodimeric saposin B is also a promiscuous lipid-binding and transfer protein, which is an essential cofactor for the lysosomal hydrolysis of sulfatide SM4s by arylsulfatase A. It binds sulfatides in soluble stoichiometric complexes, which are recognized by arylsulfatase A as substrates [228,229]. On the other hand, saposin C [230–232] and D [233] are lipid-binding proteins, which can fuse lipid vesicles at low pH values, by that eventually bringing together newly formed ILVs with already processed, hydrolase loaded and BMP containing ILVs. The four saposins A, B, C, and D are generated in the lysosomal compartment by proteolytic cleavage of their precursor protein, prosaposin [234]. They share a similar folding pattern containing four helices stabilized by three conserved disulfide bridges. They are also involved in the presentation of lipid antigens onto the Cluster of Differentiation 1 (CD1) receptor protein (Fig. 3) [235].

All hydrolases and SAPs are N-glycosylated and glycosylation regulates their function, as has been shown for Sap B. The unglycosylated mutant Sap B causes a fatal sphingolipid storage disease just as in patients with classical metachromatic leukodystrophy, despite the presence of the unglycosylated protein in the lysosomal compartment and its ability to stimulate the enzymatic hydrolysis of sulfatides *in vitro* even better than the glycosylated wild type Sap B. However, unglycosylated Sap B lost its ability to extract membrane lipids at acidic pH, which is essential for SL degradation at ILVs *in vivo* [210]. An inherited deficiency of prosaposin, the precursor of four SAPs, the saposins A, B, C, and D, triggers a perinatal fatal disease with accumulation of ILVs, ceramide and several GSLs (Fig. 4), and a loss of the water permeability barrier of the skin, where SAPs are required in the extracellular processing of barrier lipids, which embed corneocytes of the epidermis [62,236–238].

Further information on the function of SAPs and corresponding diseases is given in [62].

Membrane lipid modifiers regulate glycosphingolipid and ganglioside catabolism

In contrast to membrane-spanning GTs catalyzing the biosynthesis of GGs at the ER-, Golgi-, and TGN-membranes, water-soluble hydrolases are the main players in the catabolism of GGs. Due to a lipid phase problem they can interact only poorly with their GSL and GG substrates being membrane components of the ILVs and having short oligosaccharide chains of up to four monosaccharides. They need the support of homodimeric SAPs as lipid binding, membrane disturbing and lipid extracting glycoproteins to allow physiologically relevant catabolic rates.

Some SAPs, (Sap B and GM2AP) can bind lipids at low pH values, lift and extract them to form stochiometric soluble complexes like with sulfatides and ganglioside GM2, respectively, to present them as Michaelis–Menten substrate to their respective hydrolases for degradation [239,240]. Other SAPs, Sap A, C, and D, disturb membrane structures, trigger vesicle fusion and are needed for the catabolism of GalCer, GlcCer, and ceramides, respectively [177,207,208,232,241]. However, GSLs and GGs with long hydrophilic oligosaccharide chains having more than five monosaccharides extending far enough from the membrane surface can be attacked directly by a hydrolase as observed for GG IV^4-GalNAc-GD1a by HexA [242].

Despite the presence of activator proteins, membrane-bound complex GSLs like GM1 (Fig. 6) and GM2 (Fig. 5) are hardly hydrolyzed at all in the absence of anionic lipids, such as bis(monoacylglycero) phosphate (BMP), phosphatidic acid, phosphatidylglycerol, PI, or PS, by their respective hydrolases, and even less so in the absence of both, SAPs and anionic lipids [62,207,243]. The addition of anionic lipids to substrate carrying liposomal membranes can stimulate the catabolic rate immensely *in vitro*. Quantitative data on the synergetic stimulation of ganglioside GM1 hydrolysis by β-galactosidase in the presence of SAPs (GM2AP and Sap B) and anionic lipids are given in Fig. 6.

A model of GM2 degradation by human β-hexosaminidase A, stimulated by GM2AP is presented in Fig. 5A. Reconstitution experiments using liposomes to mimic ILVs of the late endosomal/lysosomal compartment demonstrate the essential role of anionic membrane lipids, like BMP, PI, and PG, in the vesicular GM2-carrying membranes to achieve

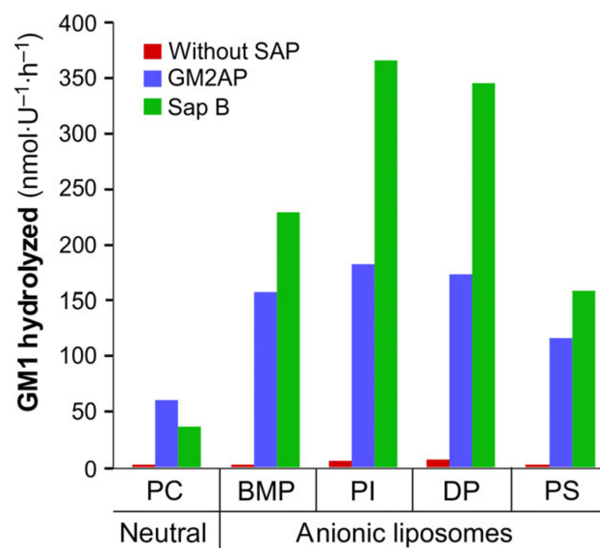

Fig. 6. The presence of anionic lipids in ILVs enhances the degradation of GM1 by β-galactosidase. In the absence of anionic lipids and SAPs, β-galactosidase hardly hydrolyzes any detectable amounts of ganglioside GM1 in phosphatidylcholine (PC) containing neutral liposomes (red bar, far left side). Addition of a SAP, either Sap B (green bars) or GM2AP (blue bars) together with the incorporation of anionic lipids [BMP, phosphatidylinositol (PI), dolicholphosphate (DP), phosphatidylserine (PS)] into GM1-carrying liposomes stimulate GM1 hydrolysis up to 100-fold. As indicated, lysosomal anionic lipids (10 mol%) were incorporated in lipid unilamellar vesicles, composed of 10 mol% ganglioside GM1, 20 mol% cholesterol, and 60 mol% PC. Assays were carried out in the absence of an activator protein and in the presence of 5 mM of GM2AP or 5 mM of Sap B, both isolated from human spleen. Mean error was determined to be less than 10% [62,213].

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physiologically relevant catabolic rates of GM2 degradation [62,207,243] (Fig. 5B). *In vivo*, however, the entire lipid composition of ILVs within endosomes and lysosomes is changing constantly due to ongoing lipid digestion and sorting out of cholesterol along the endocytic pathway. Therefore, *in vivo* rates of GG catabolism should also change permanently and will be hard to calculate.

The degradation of sphingolipids with short glycan chains is also regulated by the surrounding lipids of the substrate carrying vesicular membrane. Glucosylceramide is the primary storage lipid in the lysosomes of Gaucher patients and a secondary one in Niemann–Pick disease types A, B, and C. Glucosylceramide can already be cleaved by β-glucosidase in the absence of SAPs when anionic lipids are present [62,200,208]. Anionic lipids stimulate its hydrolysis up to 1000-fold (Fig. 7), whereas cationic lipids inhibit its degradation, especially sphingoid bases and the minor cytotoxic storage compound glucosylsphingosine [244], a biomarker for Gaucher disease [245]. Since sphingoid bases are the final product of lysosomal SL degradation, they need to be exported from lysosomes efficiently. Ceramides, fatty acids, monoacylglycerol, and diacylglycerol also stimulate glucosylceramide hydrolysis in the presence of anionic lipids, whereas sphingomyelin, sphingosine, and sphinganine play strong inhibitory roles, thereby explaining the secondary storage of glucosylceramide in Niemann–Pick diseases [62,208].

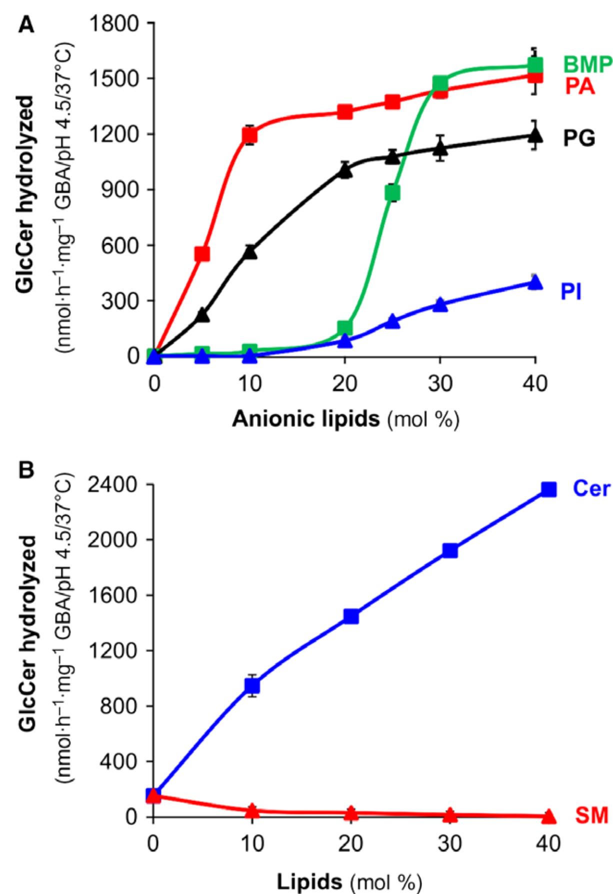

Fig. 7. The presence of anionic lipids in ILVs enhances and the presence of sphingomyelin inhibits the degradation of glucosylceramide by lysosomal β-glucosylceramidase in the absence of saposin C. Anionic lipids stimulate membrane bound GlcCer hydrolysis by GBA1 in a concentration-dependent manner (A). SM strongly inhibits membrane bound GlcCer hydrolysis by GBA1 in the presence of 20 mol% of BMP, while its degradation product ceramide plays a strong stimulatory role (B). Liposomes in (A) contain 5 mol% cholesterol, 0 to 40 mol% anionic lipids and phosphatidylcholine as host lipid and in (B) 5 mol% cholesterol, 20 mol% anionic BMP, phosphatidylcholine as host lipid, and ceramide or sphingomyelin as indicated [62,208].

Gangliosides and glycosphingolipids as secondary storage compounds in Niemann–Pick diseases

GGs are the primary and dominant storage compounds in GM1 and GM2 gangliosidoses [222]. Small GGs like GM2 and GM3 and small GSL like GlcCer and LacCer also accumulate as secondary storage compounds in different forms of Niemann–Pick disease [205]. Their lysosomal catabolism apparently is inhibited by primary storage compounds. For instance in Niemann Pick type A and B diseases (NPA and NPB, respectively), caused by deficient acid sphingomyelinase, the accumulating sphingomyelin (SM) strongly inhibits the sterol transfer protein NPC2 and thereby the secretion of cholesterol from the lysosomal compartment [194,195]. The combined storage lipids in Niemann–Pick diseases, SM and cholesterol, inhibit subsequently the catabolism of GM2 [207], GlcCer [208], and the function of several SAPs, GM2AP [207], Sap A [209] and B [210], and thereby the catabolism of further GSLs.

Ganglioside metabolism and disease

Dysfunctional genes in SPT, involved in sphinganine biosynthesis can lead to neurodegeneration and sensory and autonomic neuropathy type 1 [246]. These mutations, either in SPTLC1 or SPTLC2 enable a higher incorporation rate for other amino acids than L-serine, mainly L-alanine and cause increased levels of 1-deoxy-SLs, mainly 1-deoxy-dihydroceramides, which cannot be converted to sphingomyelin or GSLs/GGs and appear to cause mitochondrial dysfunction [247]. The big subunits of SPT are associated with a small subunit, either SPTssa or SPTssb, which

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confer distinct acyl-CoA substrate specificities. In Stellar mice a mutation in SPTssb results in increased production of C20-sphingoid bases and corresponding GGs in the brain causing neurodegeneration [103]. A human correlate is so far unknown. Downstream of SPT, KDSR reduces KDS to sphinganine. Mutations of this gene have recently been reported to cause a spectrum of keratinization disorders [248,249], although the overall epidermal ceramide production appeared not to be dramatically affected. The latter finding might also explain the absence of systemic failures. In contrast to KDSR, ceramide synthases comprise a family of six genes in mammals, CERS1-6. In humans mutations of CERS3, which is expressed mainly in stratified epithelia and male germ cells, were described to cause autosomal recessive ichthyosis [250,251] and CerS3-deficient mice die after birth due to breakdown of the lipid based skin barrier [109,252]. In mice, germ-cell-specific deletion of CerS3 affects SLs, including complex GGs and GSLs of the 0- and a-series resulting in a differentiation arrest of spermatids and male infertility [19]. Mutations in CERS1, abundantly expressed in neurons cause progressive myoclonus epilepsy and are associated with decreased production of ceramides containing stearic acid *in vitro*. How this mutation affects neuronal GG pattern in humans, however, remains to be elucidated [253]. Corresponding mouse models revealed reduced levels of neuronal ceramides [254], sphingomyelins and GGs with stearic acid, leaving about half the neuronal GG levels [255]. Deficiencies of CerS2 [118,256–258], of CerS4 [259], of CerS5 [260], and of CerS6 [261,262] as well as that of glucosylceramide synthase [19,31,38,47,263–265] have been analyzed so far only in genetically engineered mice and are reviewed briefly elsewhere [62].

Inherited defects in GG biosynthesis cause a severe infantile epilepsy syndrome (GM3-S deficiency) [155,266,267] and progressive hereditary spastic paraplegia (GM2-S deficiency) [152]. A wide spectrum of defects has been described in mutant mice [62] and it will be a matter of time until corresponding inherited human diseases will be elucidated.

Ganglioside degradation can be blocked at almost every catabolic step, either by an inherited deficiency of a hydrolase or the respective activator protein. The rare and fatal gangliosidoses are dominated by neuronal ganglioside, GSL and lysoGSL storage, triggering neurodegeneration [221,222,268]. GG and GSL storage diseases have been briefly reviewed in [62] and in Rosenberg's Molecular and Genetic Bases of Neurological and Psychiatric Disease, 5th edition (Ed. R. N. Rosenberg, J. M. Pascual) AP 2015. Therapeutic approaches for lysosomal storage diseases have been summarized recently [269].

Acknowledgement

The work of the authors was supported by grants of the DFG (German Research Association).

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