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Biosynthesis and degradation of mammalian glycosphingolipids

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Glycolipids are a large and heterogeneous family of sphingolipids that form complex patterns on eukaryotic cell surfaces. This molecular diversity is generated by only a few enzymes and is a paradigm of naturally occurring combinatorial synthesis. We report on the biosynthetic principles leading to this large molecular diversity and focus on sialic acid-containing glycolipids of the ganglio-series. These glycolipids are particularly concentrated in the plasma membrane of neuronal cells. Their *de novo* synthesis starts with the formation of the membrane anchor, ceramide, at the endoplasmic reticulum (ER) and is continued by glycosyltransferases of the Golgi complex. Recent findings from genetically engineered mice are discussed. The constitutive degradation of glycosphingolipids (GSLs) occurs in the acidic compartments, the endosomes and the lysosomes. Here, water-soluble glycosidases sequentially cleave off the terminal carbohydrate residues from glycolipids. For glycolipid substrates with short oligosaccharide chains, the additional presence of membrane-active sphingolipid activator proteins (SAPs) is required. A considerable part of our current knowledge about glycolipid degradation is derived from a class of human diseases, the sphingolipidoses, which are caused by inherited defects within this pathway. A new post-translational modification is the attachment of glycolipids to proteins of the human skin.

Keywords: biosynthesis; glycolipids; lysosomes; sphingolipid activator proteins; sphingolipid

1. INTRODUCTION

Glycolipids are minor components of biological membranes and are composed of a carbohydrate moiety linked to a hydrophobic aglycon (Kopitz 1997). They can be divided into glycoglycerolipids that are abundant in bacteria and plants, and GSLs, the major glycolipids in animals. Most animal glycolipids contain a hydrophobic ceramide building block and a hydrophilic, extracellular oligosaccharide chain (Kolter & Sandhoff 1999; figure 1). Ceramide itself consists of a long chain amino alcohol, D-*erythro*-sphingosine, which is *N*-acylated with a fatty acid. It is also a component of a plasma membrane phospholipid, sphingomyelin. Variations in type, number, linkage and further modification of sugar- and sialic acid residues within the oligosaccharide chain—but also within the lipid moiety—give rise to a combinatorial variety (Kolter *et al.* 2002) of naturally occurring GSLs. They can be classified into series, which are characteristic for a group of evolutionarily related organisms (Kolter & Sandhoff 1999). Apart from the species dependence, GSLs form cell-type specific patterns on the cell surface. In particular sialic acid containing GSLs of the ganglio-series, the gangliosides, are abundant on neuronal cells and contribute to the function of the nervous system (Ledeen & Wu 1992). Cell surface glycolipid patterns change with cell growth, differentiation, viral transformation, ontogenesis and oncogenesis (Hakomori 1981). Cell-type-specific glycolipid expression and stable lipid patterns indicate a tight regulation of their biosynthesis, degradation and intracellular transport (Van Meer & Lisman 2002). In addition to their function *per se* and as components of microdomains (Van Meer & Lisman 2002), animal glycolipids serve as metabolic precursors for sphingolipids that contribute to the water permeability barrier of the skin (Wertz & Van den Bergh 1998) and are involved in the transduction of extracellular signals into the interior of cells (Huwiler *et al.* 2000). In humans, inherited defects of GSL- and sphingolipid catabolism give rise to lysosomal storage diseases, the sphingolipidoses. In higher organisms, the function of animal glycolipids has been analysed in cells from human patients with defects in sphingolipid catabolism, or in genetically engineered mutant mice with defects in distinct biosynthetic steps (Kolter *et al.* 2002). For example, human patients of a rare case of Gaucher disease (collodian babies) with a complete deficiency of lysosomal glucocerebrosidase die shortly after birth and exhibit an ichthyosiform dermatitis phenotype (Liu *et al.* 1988). In experimental approaches, sphingolipid biosynthesis can also be interrupted with the aid of chemical inhibitors (Kolter & Sandhoff 1999) or by the generation of mutant cells (Futerman 1994), which allows investigation of the function of glycolipids and their metabolic intermediates. Also, data obtained in yeast might improve understanding of glycolipid function in animals (Dickson 1998). The conservation of the overall structure of animal glycolipids during evolution, the absence of inherited diseases affecting their biosynthesis, and the severe phenotypes of mice with biosynthetic defects in this pathway (see below) indicate their functional importance for the living organism.

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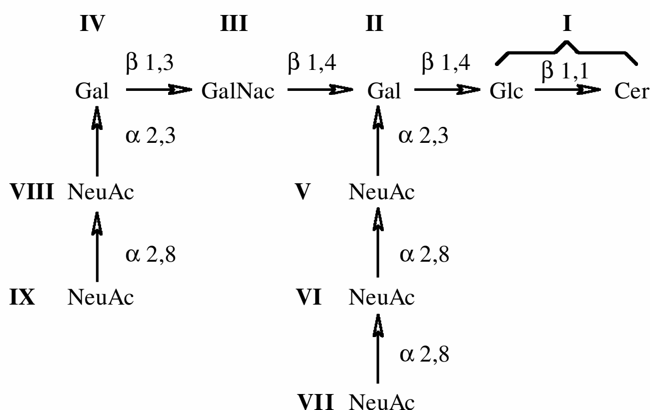

Figure 1. Structure of gangliosides (van Echten & Sandhoff 1993). Terminology for gangliosides (IUPAC 1998) e.g. GP1c (NeuAcα2 → 8NeuAcα2 → 3Galβ1 → 3GalNAcβ1 → 4(NeuAcα2 → 8NeuAcα2 → 8NeuAcα2 → 3)Galβ1 → 4Glcβ1 → 1Cer): **II** - inner galactose, **IV** - outer galactose. The structures of GSL derived from LacCer are part of the GP1c structure, containing the following sugar residues: GlcCer - glucosylceramide, **I**, LacCer - lactosylceramide, **I**, **II**, GM3 - **I**, **II**, **V**, GD3 - **I**, **II**, **V**, **VI**, GT3 - **I**, **II**, **V–VII**, GA2 - **I–III**, GM2 - **I–III**, **V**, GD2 - **I–III**, **V**, **VI**, GT2 - **I–III**, **V–VII**, GA1 - **I–IV**, GM 1a- **I–V**, GD1b - **I–VI**, GT1c - **I–VII**, GM1b - **I–IV**, **VIII**, GD1a - **I–V**, **VIII**, GT1b - **I–VI**, **VIII**, GQ1c - **I–VIII**, GD1c - **I–IV**, **VIII**, **IX**, GT1a - **I–V**, **VIII**, **IX**, GQ1b - **I–VI**, **VIII**, **IX**, GP1c - **I–IX.**

2. GLYCOSPHINGOLIPID BIOSYNTHESIS IN ANIMALS

Glycolipid biosynthesis in animals requires the intracellular formation of the membrane anchor (Merrill 2002) and the subsequent addition of single carbohydrate residues (Kolter *et al.* 2002). Both events are coupled to intracellular movement of metabolic intermediates and final products to the plasma membrane (Van Meer & Lisman 2002). The combinatorial variety of naturally occurring glycolipids can be largely attributed to the combination of glycosyltransferase activities found in different species and cell types. Apart from the skin, where much less is known about glycolipid metabolism, the brain is the organ where functional aspects of glycolipids are most evident (Ledeen & Wu 1992). Therefore, we focus on the metabolism of GSLs of the ganglio-series, which are especially enriched in the mammalian nervous system (Ledeen & Wu 1992).

(a) *Ceramide and sphingomyelin biosynthesis*

De novo biosynthesis of GSLs (Merrill 2002) starts with the formation of the membrane anchor, ceramide, at the membranes of the ER. The condensation of the amino acid L-serine with a fatty acyl coenzyme A, usually palmitoyl coenzyme A, to 3-ketosphinganine is catalysed by the enzyme serine palmitoyl transferase (Braun & Snell 1968; Mandon *et al.* 1991; Nagiec *et al.* 1994; Zhao *et al.* 1994; Weiss & Stoffel 1997).

In the following NADPH-dependent reaction, 3-ketosphinganine is reduced to D-*erythro*-sphinganine by 3-ketosphinganine reductase (Stoffel *et al.* 1968). Sphinganine is subsequently acylated to dihydroceramide by a N-acyltransferase (Rother *et al.* 1992; Merrill & Wang 1986; Shimeno *et al.* 1998). The major part of the dihydroceramide pool is desaturated to ceramide in the dihydroceramide desaturase reaction (Geeraert *et al.* 1997; Michel *et al.* 1997; Mikami *et al.* 1998). The parent compound of the sphingolipids, sphingosine, is not an intermediate of sphingolipid biosynthesis but is formed during sphingolipid degradation. The enzymes involved in ceramide biosynthesis are localized on the cytosolic leaflet of the ER membrane (Mandon *et al.* 1992; Michel & van Echten-Deckert 1997). A sphinganine derivative with an additional hydroxyl group in 4-position (*R*-configuration), phytosphingosine, is the structural constituent of many plant, yeast and mammalian epidermis sphingolipids. Details on phytosphingosine biosynthesis in animals are far from clear; a gene responsible for phytosphingosine formation in yeast has been characterized (Haak *et al.* 1997). Ceramide is the common precursor of GSLs and sphingomyelin. Sphingomyelin biosynthesis occurs on the luminal part of Golgi membranes (Futerman *et al.* 1990), but also other sites of sphingomyelin formation have been discussed (Miro Obradors *et al.* 1997). It is formed by the transfer of phosphorylcholine from a phospholipid, phosphatidylcholine, on the 1-hydroxyl group of ceramide. Diacylglycerol is liberated in this reaction. Intracellular formation of glycolipids also occurrs by salvage pathways within recycling processes (Gillard *et al.* 1998). Sphinganine N-acyltransferase is also able to acylate sphingosine that is liberated in this pathway (Morell & Radin 1970).

(b) *Lactosylceramide*

Most animal glycolipid series are biosynthetically derived from lactosylceramide. In glucosylceramide, a glucose residue is β-glycosidically linked to the 1-position of ceramide. The glucosylceramide synthase uses uridine-diphospho-glucose as the glycosyl donor and has been isolated from rat liver (Paul *et al.* 1996). The human enzyme has been cloned (Ichikawa *et al.* 1996) and is located on the cytosolic leaflet of the Golgi apparatus (Coste *et al.* 1986; Jeckel *et al.* 1992). From here, GlcCer can reach the plasma membrane by direct transport (Warnock *et al.* 1994) or can be modified by further glycosylation in the Golgi apparatus. The enzymes that transfer glucose and galactose on ceramide catalyse related reactions, but have different topologies of their active sites and share no sequence homology (Ichikawa & Hirabayashi 1998). During human keratinocyte development, glucosylceramide-synthase is upregulated at the transcriptional level (R. Watanabe *et al.* 1998). LacCer, the common precursor for the GSL series found in vertebrates, is formed by the addition of a galactose moiety from UDP-Gal to GlcCer catalysed by galactosyltransferase I. The enzyme has been purified and cloned from rat brain (Nomura *et al.* 1998). LacCer formation and also the reactions leading to higher glycosylated lipids occur on the luminal leaflet of Golgi membranes (Lannert *et al.* 1996). Therefore, GlcCer has to translocate through the Golgi membrane for the synthesis of higher glycolipids. The orientation of the glycan chains is topologically equivalent to the situation on the plasma membrane, where complex glycolipids exclusively face the extracellular space.

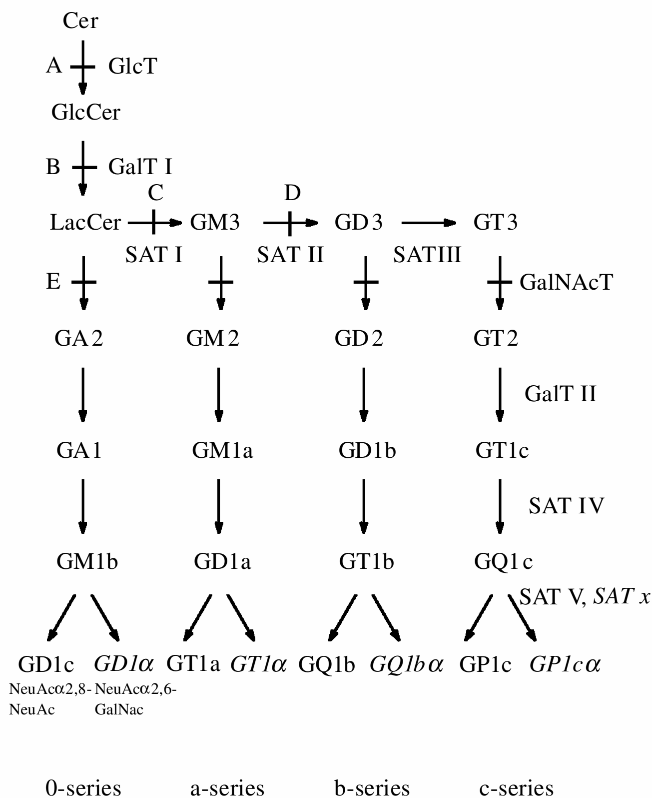

Figure 2. Scheme of ganglioside biosynthesis. Enzyme defects in knockout mice are indicated. A, ceramide glucosyltransferase −/−; B, galactosyltransferase I −/− (not available yet); C, sialyltransferase I −/−; D, sialyltransferase II −/−; E, GalNAc-transferase −/−; 'GM3-only'- mouse = D + E; 'LacCer-only'-mouse = C + E. GD1α, GT1α, GQ1bα and GP1cα with a sialic acid residue α2,6-glycosidically linked to N-acetylgalactosamine are formed by a yet uncharacterized sialyltransferase, while GD1c, GT1a, GQ1b and GP1c are formed by SAT V. 'T', transferase (modified from Kolter *et al.* 2002). Nomenclature according to IUPAC (1998).

(c) *Biosynthesis of complex gangliosides*

A GSL series that is especially abundant on neuronal cells is the ganglio series. The biosynthesis of sialic acid-containing GSLs of this series, the gangliosides, is catalysed by glycosyltransferases in the lumen of the Golgi apparatus (Kaufman *et al.* 1968; Yip & Dain 1969; Roseman 1970; Lloyd & Furukawa 1998; Maccioni *et al.* 1999; Kolter *et al.* 2002). With the exception of ganglioside GM4, a major component of the myelin (Ledeen *et al.* 1973), gangliosides are structurally and biosynthetically derived from lactosylceramide (figure 2). Lactosylceramide and the hematosides GM3, GD3 and GT3, serve as precursors for complex gangliosides of the 0-, a-, b- and c-series. In adult human tissues, gangliosides from the 0- and c-series are found only in trace amounts. The transferases that catalyse the first steps in ganglioside biosynthesis show high specificity towards their glycolipid substrates, i.e. for the formation of LacCer, GM3 and GD3. The relative amounts of these glycolipids in the steady state seem to determine the amount of 0-series glycolipids, which are derived only from LacCer, a-series gangliosides which are only derived from ganglioside GM3, and b-series gangliosides which are only derived from ganglioside GD3.

It was an unexpected discovery that the stepwise glycosylation of these precursors is performed by only a few glycosyl transferases of limited specificity. Like on an assembly line, they transfer carboydrate- and sialic acid residues to glycosyl acceptors that differ only in the number of sialic acid residues bound to the inner galactose. Sialyltransferases I and II are much more specific for their glycolipid substrates than sialyltransferases IV and V, or than galactosyltransferase-II and GalNAc transferase. It was assumed that different transferases catalyse the formation of homologous gangliosides of different series occurring in figure 2. The identity of these transferases was demonstrated by enzyme kinetics (Pohlentz *et al.* 1988; Iber *et al.* 1992) and resulted in the actual biosynthetic scheme which demonstrates the major anabolic routes. Rat liver Golgi was used as enzyme source, which excludes the contribution of glycosyl transferases present in other membranes (Stern & Tiemeyer 2001), which have no contact to metabolic intermediates under physiological conditions. After determination of the kinetic constants for each individual reaction, the reaction rates were calculated in a two-substrate system for two cases (figure 3): one active site catalyses two reactions, e.g. the formation of ganglioside GM2 from GM3 and of ganglioside GD2 from GD3 (case one) or that two independent active sites—for each reaction a specific one—are present in the membrane preparation (case two). The calculated reaction rates were expressed in dependence of partial substrate concentrations and compared with the experimentally observed reaction rates (figure 3). The conclusion was that, for example, GM2 and GD2 are formed by the same enzyme and that GM3 and GM1 are formed by different enzymes. The results have been confirmed later on by the analysis of genetically engineered animals (see below).

The complex gangliosides with sialic acid moieties α2,6-glycosidically linked to N-acetylgalactosamine residues have been added later to the biosynthetic scheme (Hidari *et al.* 1994; Irie *et al.* 1994). Based on experiments in cultured cells, it has been concluded that the cDNAs for GD3 and GT3 synthase are identical. Therefore, both gangliosides, GD3 and GT3, should be biosynthesized by the same enzyme (Nakayama *et al.* 1996). There is, however, no accumulation of ganglioside GT3 in the GalNAc-transferase knockout mouse (see below), so that it has to be assumed that an elusive sialyltransferase different from SAT II is required for the formation of GT3 *in vivo*. This would also explain the occurrence of gangliosides of the c-pathway in early mammalian development and in the adult brain of vertebrates like birds and fish.

(d) *Regulation*

It is not clear how the relative amounts of the various GSLs are controlled. The kinetic parameters of the transferases, their topological organization within the Golgi apparatus, or spatial neighbourhood to other transferases will influence the resulting ganglioside pattern. Furthermore, it cannot be excluded that the flow rates through the Golgi apparatus and the *trans* Golgi network differ for the different glycolipid metabolites. The glycolipid patterns might also be modified by transcriptional regulation of transferases and translocators, and other means.

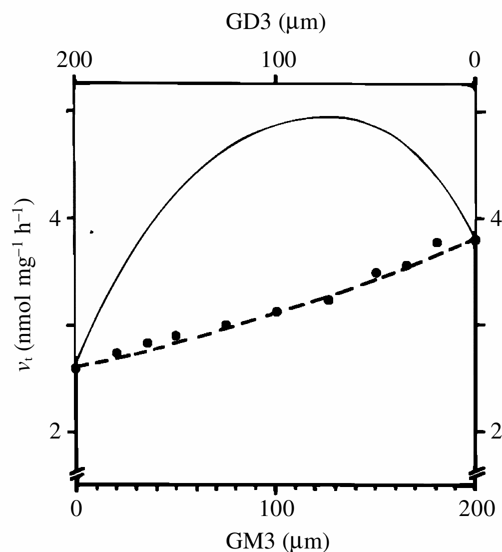

Figure 3. Evidence from enzyme kinetics that gangliosides GM2 and GD2 are synthesized by the same enzyme in rat liver Golgi. Gangliosides GM3 and GD3 were used as acceptors for GalNAc-transferase in various partial concentrations, keeping total substrate at 200 μM. Total reaction velocity v_t as experimentally determined (circles), as calculated for two different active sites (solid line), or as calculated for one active site (dashed line). Reproduced, with permission, from Pohlentz *et al.* (1988).

Two enzymes accept LacCer as a substrate: sialyltransferase I which forms ganglioside GM3 and GalNAc-transferase that forms glycolipid GA2. Based on *in vitro* experiments, it has been postulated that the biosynthesis of 0-series glycolipids occurs in this way, but these lipids are usually not observed *in vivo*. They have only been recently demonstrated in sialyltransferase I knockout mice, as predicted in the biosynthetic scheme (see below). The selective formation of ganglioside GM3 can be explained by different possibilities. Owing to the different enzyme-kinetic constants, much more GM3 is formed than GA2 (Lutz *et al.* 1994). Representative Michaelis–Menten constants for LacCer are 72 μM for SAT I and 437 μM for GalNAc transferase, as determined from rat liver Golgi in a micellar assay (Iber 1988). An initial attempt has been made to calculate glycolipid pattern on the bases of the kinetic constants of the transferases, which were estimated from the steady-state concentrations of the glycolipid substrates in intact cells (Bieberich & Yu 1999). However, it is possible that ganglioside GM3 might be synthesized in an earlier compartment than glycolipid GA2, as suggested by experiments with inhibitors of vesicular membrane flow. The addition of brefeldin A, which causes a fusion of the ER mainly with parts of the *cis* and medial Golgi, leads to a reduced biosynthetic labelling of the gangliosides GM1a, GD1a, GD1b, GT1b and GQ1b as well as to a lesser extent of sphingomyelin (van Echten *et al.* 1990). A direct location of GalNAc transferase by inhibition of GM2 synthesis was not possible because brefeldin A causes in part conversion of membrane-bound GalNAc transferase into a soluble form which has minimal capability to produce GM2 in whole cells (Young *et al.* 1999). Monensin, a cationic ionophore, inhibits membrane flow between proximal and distal Golgi cisternae and leads to an increased biosynthetic galactose labelling of GlcCer, LacCer, GM3, GD3 and GM2, while the labelling of complex gangliosides is reduced. Although glycosyltransferase activity has been detected in many Golgi subcompartments (Iber *et al.* 1992), current data suggest that the precursor molecules GM3 and GD3 are formed in early Golgi compartments, and the complex gangliosides predominantly in the *trans* Golgi network (Lannert *et al.* 1998; Giraudo *et al.* 1999; Allende *et al.* 2000). These findings have been confirmed to some extent by biochemical and immunohistochemical studies that, for example, localized SAT I to the medial and *trans*-Golgi cisternae (Lannert *et al.* 1998; Stern *et al.* 2000). An additional possibility is that galactosyltransferase I and sialyltransferase I form a functional complex and that lactosylceramide is transferred directly after its formation to the next enzyme. Saul Roseman predicted that enzymes of ganglioside biosynthesis might form such functional complexes (Roseman 1970). This has been confirmed for GalNAc-transferase and galactosyltransferase II by coimmunoprecipitation (Giraudo *et al.* 2001). This enzyme complex may accept ganglioside GM3 and finally release ganglioside GM1. This might explain why the brain contains larger amounts of gangliosides GM1 and GD1a, but little ganglioside GM2. These type II glycosyltransferases interact via their N-terminal domains. Because GalNAc-transferase forms disulphide-bonded homodimers (Li *et al.* 2000), the glycosyltransferases might occur as multicomponent complexes. During ontogenesis and cell transformation a correlation between GSL expression and the activity of glycosyltransferases that lead to their synthesis has been observed. Therefore, control of glycosyltransferases, possibly at the transcription level, appears to be a significant regulation point. Because most glycosyltransferases have been cloned in the recent past (Kapitonov & Yu 1999), it is expected that information for an understanding of the control at the transcription level will be available in the near future. In addition to regulation at a genomic level, some observations also suggest epigenetic regulation mechanisms. Feedback control of several glycosyltransferases either by their respective reaction product or by an end product of the respective GSL series has been observed at least *in vitro* (Yusuf *et al.* 1987). The phosphorylation status of glycosyl transferases and the pH value of their environment (Iber *et al.* 1990) can also affect their activity.

(e) ***Genetically engineered mice***

A significant advance towards understanding the function of the complex ganglioside pattern found on eukaryotic cells is the development of mice with defects in distinct biosynthetic steps (Furukawa *et al.* 2001; Proia 2003). Blocks in the early steps of this pathway have been indicated 'A–E' in figure 2.

In 1994, a mouse melanoma cell line deficient in glycolipids was described. The cells were viable and showed only minor changes in cellular morphology and growth rate. From these observations it was concluded that glycolipids including gangliosides might not be essential for survival (Ichikawa *et al.* 1994). Later, the development of animals deficient in glycolipids led to the conclusion that they are indeed required for the development of a multicellular organism: mice with targeted disruption of the

ceramide glucosyltransferase gene (A in figure 2) are not viable and die around day 7.5 of embryonic development, presumably by increased apoptosis in the ectoderm. There was no cellular differentiation beyond the primitive germ layers. Embryonic stem cells, with both alleles of the ceramide glucosyltransferase gene disrupted were also severely compromised in their ability to form mature differentiated tissues in teratomas grown in mice. This indicates that formation of glucosylceramide, and subsequently higher glycolipids, might be essential events in the process of differentiation and development (Yamashita *et al.* 1999). However, the possibility that accumulation of the upstream substrate, ceramide, might account for some of the characteristics of the knockout has not been ruled out.

The phenotype of the knockout animals was not evident from cell culture experiments using ceramide glucosyltransferase deficient embryonic stem cells. The mutant cells displayed similar growth curves as normal control cells and showed the ability to differentiate *in vitro*, which indicates that *in vitro* models may be of limited use to elucidate the function of metabolites that are directly or indirectly involved in cell–cell interactions in the complex morphogenesis of multicellular aggregates (Kolter *et al.* 2000).

Negatively charged glycolipids of the cell surface are not necessarily derived from glucosylceramide, but also from galactosylceramide. Knockout mice, in which the gene of ceramide galactosyltransferase was inactivated and which consequently cannot form any galactosylceramide, sulphatide or ganglioside GM4, are still able to form myelin of essentially normal structure. In place of the absent GalCer they incorporate a GlcCer with hydroxylated fatty acid moieties into their myelin. Stability and function of these myelin sheets, however, was considerably impaired and the animals suffered from generalized tremor, ataxia, conduction deficits and further symptoms (Bosio *et al.* 1996; Coetzee *et al.* 1996). Spermatogenesis was impaired in these mice, owing to a defect in the formation of monogalactosylalkylglycerol and, subsequently, seminolipid (Fujimoto *et al.* 2000). Also, mice deficient in the sulphotransferase, which is required for the formation of sulphatide, sulpholactoslyceramide and seminolipid, have been generated (Honke *et al.* 2002). The animals showed similar neurological abnormalities to the animals with defective galactosylceramide formation.

Two different strains of mice have been generated which are not able to synthesize the major gangliosides especially found in the brain of the animals (Proia 2003). In accordance with the scheme of ganglioside biosynthesis, mice with deficiency of GalNAc-transferase (E in figure 2) are not able to form the major gangliosides GM2, GD2, GM1a, GD1b, GD1a, GT1b, GT1a and GQ1b and mice with deficient sialyltransferase II (D in figure 2) lack gangliosides GD3, GD2, GD1b, GT1b and GQ1b. Surprisingly, both strains showed only subtle impairment of brain function. GalNAc-transferase-deficient homozygous mutant mice (Takamiya *et al.* 1996) developed normally without significant histological defects in the central nervous system. Their lifespans were nearly normal. Further examination revealed axonal degeneration and myelination defects in the nervous system (Sheikh *et al.* 1999). Consistent with these findings, the mutant mice displayed progressive defects in motor function (Chiavegatto *et al.* 2000). Unexpectedly, defects were also found in non-neuronal tissues. The mutant male mice were sterile and showed morphological and functional defects in the testis (Takamiya *et al.* 1998). Within their immune system, the response of spleen T cells to interleukin 2 was impaired (Zhao *et al.* 1999). The molecular bases for these observations are currently unknown.

Disruption of the gene encoding sialyltransferase II, which is required for the formation of ganglioside GD3 and subsequently the b-series gangliosides, led to homozygous animals with a normal lifespan and without detectable developmental defects (Kawai *et al.* 2001; Proia 2003). The dramatic changes in the expression of GD3 and other b-series gangliosides during neuronal development and morphogenesis have led to the belief that these gangliosides might be required for neuronal differentiation. However, it has been shown before that embryonic stem cells with a disrupted sialyltransferase II gene underwent neuronal differentiation even in the absence of b-series gangliosides (Kawai *et al.* 1998). This is also in agreement with the phenotype of the knockout animals.

To further restrict ganglioside expression, sialyltransferase II mutant mice were crossbred with mice carrying a disrupted gene encoding β1,4-GalNAc-transferase. The double mutant mice (D and E in figure 2) expressed only ganglioside GM3 as their major ganglioside. In contrast to sialyltransferase II mutant mice, the double mutants were extremely susceptible to induction of lethal seizures by sound stimulus and displayed a sudden death phenotype. This indicates a vital role of physiological ganglioside pattern for neuronal function and enables further functional studies (Proia 2003).

A recent mouse model generated is a sialyltransferase-I-deficient strain (C in figure 2). The mice are not able to form ganglioside GM3 and, subsequently, none of the gangliosides found in the nervous systems of humans and mice, at least of the ganglio-series (T. Yamashita, A. Hashiramoto, N. Werth, R. Sandhoff, K. Sandhoff and R. L. Proia, unpublished data). As could have been predicted from the scheme of ganglioside biosynthesis, they form gangliosides of the 0-series (GM1b, GD1c and GD1α) in amounts that correspond to the total ganglioside content of normal animals. The knockout mice display altered glucose homeostasis with an accelerated insulin receptor signalling pathway. The results indicate that ganglioside GM3 is an important regulator of insulin-mediated signalling and they support recent evidence that GM3 over-expression could contribute to type-II diabetes (Tagami *et al.* 2002; Proia 2003).

The most recently developed animal model is the double knockout mouse with deficient GalNAc transferase and sialyltransferase I (C and E in figure 2) (T. Yamashita, N. Werth, R. Sandhoff, K. Sandhoff and R. L. Proia, unpublished data). These severely diseased mice are not able to form any glycolipid of the ganglio-series. Lactosylceramide concentrations are up to 100-fold elevated in the brains of the animals. In addition, lactosylceramide sulphate with sphingoid- and fatty acid-composition similar to gangliosides has been detected in these mice. The usually found ganglio-series gangliosides are completely absent, whereas traces of other sialic acid-containing glycolipids that are also present in a normal brain were still found (Proia 2003).

3. GLYCOLIPID CATABOLISM

(a) *Lysosomal glycolipid degradation*

Constitutive glycolipid degradation occurs in the acidic compartments of the cells, the endosomes and the lysosomes. Within the digestion of cellular membranes, cellular glycolipids are also cleaved into their building blocks. For this purpose, parts of the plasma membrane are endocytosed and traffic through the endosomal compartment to the lysosomes. Lysosomal glycosidases sequentially cleave off the sugar residues from the non-reducing end of their glycolipid substrates. The resulting monosaccharides, sialic acids, fatty acids and sphingoid bases can leave the lysosome, are used within salvage processes or are further degraded.

(b) *Topology*

Plasma membrane components—lipids and proteins—reach the lysosome by endocytotic vesicular flow. Parts of the membrane enriched in GSL bud into coated pits that are internalized, uncoated and subsequently fuse with early endosomes. Degradation of sphingolipids derived from the cell surface that become part of the limiting membrane of endosomes and lysosomes is prevented by the thick glycocalyx, which protects the membrane from the attack by the lipid-degrading enzymes present in the lysosol (Peters & von Figura 1994). This glycocalyx is composed of the carbohydrate part of lysosomal integral membrane proteins and lysosomal associated membrane proteins (Carlsson *et al.* 1988) which are highly N-glycosylated with lactosamine units. The enzyme/activator system required for degradation cannot be expected to reach their substrates through this glycocalyx. This explains why glycolipids of the perimeter membrane are much more resistant towards degradation than plasma membrane-derived GSL (Henning & Stoffel 1973).

In 1992, we assumed that the major part of GSL catabolism occurs on small intra-endosomal and intra-lysosomal vesicles (Fürst & Sandhoff 1992). This assumption is supported by a series of experimental observations (Sandhoff & Kolter 1996). Parts of the endosomal membrane enriched in components derived from the plasma membrane, invaginate and bud into the endosomal lumen. Recently, this has also been recognized by the cell biology community (Van der Goot & Gruenberg 2002). GSLs originating from the outer leaflet of the plasma membrane should reach the lumen of the endosomes on the surface of intra-endosomal vesicles or other lipid aggregates and not as parts of the perimeter membrane. One of the observations leading to the proposal of the model was that multivesicular structures accumulate in cells of sphingolipidosis patients especially in cells with SAP-precursor (prosaposin) deficiency. They refer to the late endosomal–lysosomal compartment (Burkhardt *et al.* 1997). Additional evidence for the proposed route came from experiments in human fibroblasts: biotin-labelled ganglioside GM1 derived from the plasma membrane is mainly targeted to intra-lysosomal structures and much less to the lysosomal perimeter membrane (Möbius *et al.* 1999). If degradation occurs on the surface of these intra-lysosomal membrane structures, their respective size, lipid composition and lateral pressure should influence the degradation process. Analysis of these molecular details supports the initial hypothesis of GSL degradation at intra-lysosomal membrane structures.

(c) *Enzymology*

According to the model discussed above, the degrading lysosomal enzymes cleave substrates that are part of intralysosomal membrane structures. In the absence of detergents that solubilize the lipids, GSL with carbohydrate chains of one to four residues (Wilkening *et al.* 2000) are not sufficiently accessible to the hydrolytic water-soluble enzymes in the absence of a membrane-perturbing activator protein.

In ganglioside degradation, ganglioside GM1 is degraded to ganglioside GM2 by a β-galactosidase in the presence of either the GM2-AP or SAP-B (Wilkening *et al.* 2000). The resulting ganglioside GM2 is cleaved to ganglioside GM3 and N-acetyl-galactosamine mainly by the β-HEX isoenzymes, especially HEX A. The heterodimer HEX A (αβ) has two active sites that differ in their substrate specificity (Kytzia & Sandhoff 1985). The other subunit combinations in the HEXs are HEX B (ββ-homodimer) and HEX S (αα-homodimer). The crystal structure of HEX B has been determined very recently (T. Maier, N. Straeter, C. Schuette, R. Klingenstein, K. Sandhoff and W. Saenger, unpublished data). The catalytic centre of the β-subunit cleaves predominantly neutral sugars chains with terminal N-acetyl-β-D-galactosaminyl and N-acetyl-β-D-glucosaminyl residues. Towards uncharged substrates, the active site on the α-subunit is less active than the β-unit, but cleaves sugars from negatively charged substrates (Hepbildikler *et al.* 2002). In addition to the β-HEXs A or S, degradation of ganglioside GM2 requires the GM2-AP (see figure 4). This activator is essential for the *in vivo* degradation of this ganglioside (Sandhoff *et al.* 2001). Historically this was the first example for the absolute requirement of an activator for the *in vivo* degradation of a glycolipid. Its absence leads to the so-called AB-variant of the GM2-gangliosidoses (Conzelmann & Sandhoff 1978, 1979). In the absence of additional factors like detergents or activator proteins, the enzyme is only able to degrade water soluble substrates or such membrane-bound substrates with sugar chains that reach sufficiently far from the lipid core into the aqueous space (Meier *et al.* 1991).

A sialidase cleaves ganglioside GM3 into lactosylceramide and sialic acid—a reaction stimulated by SAP-B (Fingerhut *et al.* 1992), before the galactose is split off by either galactosylceramide-β-galactosidase or GM1-β-galactosidase to yield glucosylceramide in the presence of either SAP-B or -C (Zschoche *et al.* 1994). The stepwise cleavage of the hydrophilic headgroups from these glycolipids finally leads to ceramide which is cleaved by acid ceramidase in the presence of SAP-D (Linke *et al.* 2001*b*) into sphingosine and a fatty acid. Together with the other cleavage products, these two metabolites are able to leave the lysosome.

For the non-glycosylated sphingolipids like ceramide and sphingomyelin, non-lysosomal degradation steps are known which apparently do not need the assistance of an activator protein. Sphingomyelin is cleaved to ceramide and phosphorylcholine. Ceramide later on is degraded into sphingosine and a fatty acid by ceramidases of different subcellular localization. In the cytosol, sphingosine can

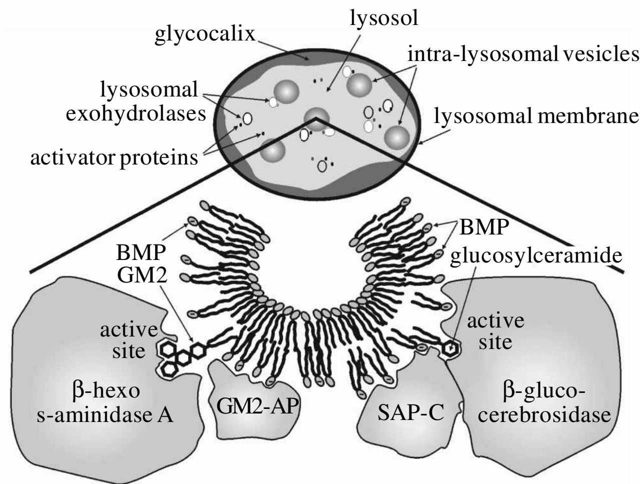

Figure 4. Model for the lysosomal degradation of membrane-bound GSLs by water-souble lysosomal exohydrolases and membrane-active activator proteins. Given are two examples: GM2-degradation by β-HEX A and the GM2-AP; glucosylceramide-degradation by β-glucocerebrosidase and SAP-C (or saposin C). The model emphasizes the topology of degradation, the properties of the intra-lysosomal membrane structure, and the interactions between membrane surface, activator protein, hydrolytic enzyme and substrate. (Modified from Macheleidt *et al.* (2003).)

be phosphorylated to sphingosine-1-phosphate or can be re-acylated to ceramide. These highly regulated degradation processes occur at several subcellular places to produce various signal molecules (Huwiler *et al.* 2000; Hannun & Obeid 2002; Spiegel & Milstien 2002).

(d) *GM2-activator protein*

The GM2-AP was identified in a patient with a massive storage of ganglioside GM2 and glycolipid GA2 in the brain despite the presence of HEXs A and B (Sandhoff 1977). In the presence of a surfactant, the isolated enzymes from the patient were able to cleave the storage material *in vitro*. The search for the essential '*in vivo* detergent' defective in the tissues of the patient led to the identification of the GM2-AP (Conzelmann & Sandhoff 1978). This activator has been purified and structurally and functionally characterized (Conzelmann & Sandhoff 1979; Fürst & Sandhoff 1992). It is a lysosomal glycoprotein of 17.6 kDa in the deglycosylated form, bears a N-glycosidically bound oligosaccharide chain (Fürst *et al.* 1990), and contains four disulphide bridges (Schütte *et al.* 1998). Recently, the crystal structure of the human GM2-AP recombinantly expressed in *Escherichia coli* has been solved (Wright *et al.* 2000). The structure of the non-glycosylated protein contains a cavity for the ceramide moiety and an adjacent possible recognition site for the oligosaccharide chain. It also has a flexible hydrophobic loop which may facilitate two functions discussed below, the interaction with the membrane and the extraction of the GM2 ganglioside. The cDNA and the gene on chromosome 5 are known (Klima *et al.* 1991; Swallow *et al.* 1993; Schepers *et al.* 2000). A non-functional pseudogene was mapped to chromosome 3 (Xie *et al.* 1992) and an alternatively spliced variant was found (Wu *et al.* 1996). Until now, five different mutations in the structure gene of the GM2-activator have been identified. Point mutations (Schröder *et al.* 1991, 1993) lead to the exchange of one amino acid and to the complete deficiency of the protein. The mutated activator proteins are of limited stability and the mutations cause premature degradation (Schepers *et al.* 1996).

The GM2-AP can be regarded as a weak detergent with high selectivity and lifts ganglioside GM2 or GM1 (Wilkening *et al.* 2000) out of the membrane so that they become accessible for the active site of the degrading enzyme. GM2-AP can insert into and disturb intra-lysosomal vesicle membranes. HEX A recognizes the complex of ganglioside GM2 and GM2-AP, hydrolyses ganglioside GM2 and releases ganglioside GM3. Synthetic analogues of ganglioside GM2 with shortened fatty acid chains and therefore of higher water solubility are degraded by HEX A already in the absence of the GM2-AP or detergents (Meier *et al.* 1991). GM2-AP recognizes the hydrophobic ceramide moiety (Conzelmann *et al.* 1982), the sialic acid-, and the N-acetyl-galactosamine-moiety of ganglioside GM2 (Smiljanic-Georgijev *et al.* 1997) and might also cleave an intramolecular hydrogen bond between the terminal residues to facilitate the degradation of the ganglioside (Wu *et al.* 1994). This latter mechanism, however, does not explain that the degradation of short chain analogues of ganglioside GM2 and of ganglioside GD1aGalNAc by HEX A already occurs in the absence of GM2-AP. The cleavage of two glycolipids which apparently do not contain this intramolecular hydrogen bond is also stimulated by GM2-AP: GM2-AP stimulates the HEX A and HEX S catalysed degradation of sulphoglycolipid SM2 (Hepbildikler *et al.* 2002), and glycolipid GA2 accumulates in human patients (Sandhoff *et al.* 1971) and in knockout mice deficient in the GM2-AP (Liu *et al.* 1997).

Apart from its function in hydrolysis of ganglioside GM2, further physiological roles have been attributed to GM2-AP (Mahuran 1998). It is suggested to act as a factor that stimulates phospholipase D-activity through enhancing the association between the enzyme and enzyme activators (Nakamura 1998; Sarkar *et al.* 2001). Another potential role of the GM2-AP is the regulation of proton pumps in intercalated cells of the kidney (Mundel *et al.* 1999). Because a cytosolic localization of the GM2-AP has not been convincingly demonstrated so far, functional roles within the cytosol, as mentioned above, remain ambiguous.

Apart from activator proteins, other factors also influence the digestion of glycolipids on intra-lysosomal vesicles. As determined in tissues from SAP-deficient patients, the diameter of these vesicles is in the range of 50–100 nm (Bradova *et al.* 1993) and can be mimicked in *in vitro* experiments by vesicles of a comparable size. The convex curvature favours spreading of the GSL headgroups and makes them more easily accessible for exohydrolases in the presence of activator proteins. The geometry of the membrane vesicles depends on their lipid composition (Kobayashi *et al.* 1998), the chain length and the degree of saturation of the amphiphilic lipids. Owing to steric and electrostatic repulsion, lipids with large and negatively charged hydrophilic headgroups favour a strong curvature and a smaller size of the vesicles. This should

result in a relatively low lateral pressure of such membrane structures.

For recognition and binding of gangliosides, the GM2-AP has to insert into the lipid bilayer. Physico-chemical measurements with lipid monolayers show that the GM2-AP protein inserts only if the lateral pressure of the monolayer is below a critical value of 15–25 mN m^{-1}, depending on the lipid composition (Giehl *et al.* 1999). Therefore it can be assumed that the GM2-AP is not able to penetrate into membranes like the perimeter membrane of the lysosome. The lateral pressure of biological membranes is in the range between 30 and 35 mN m^{-1} (Marsh 1996). Albeit there are no data on the lateral pressure of intralysosomal vesicles available, it can be expected to be below the critical value of 25 mN m^{-1}.

In addition to size, curvature and lateral pressure the lipid composition of the inner membranes of late endosomes and lysosomes appear to be unique. They contain BMP as analysed by immunogold electron microscopy (Kobayashi *et al.* 1998) and subcellular fractionation (Becker 1999). BMP is an anionic phospholipid with a lysolipid structure and is synthesized in the acidic compartment of the cell (Amidon *et al.* 1996). It has been described as a marker lipid of lysosomes (Brotherus & Renkonen 1977). Owing to its unusual sn1,sn1′-configuration, it is more resistant to the action of phospholipases than normal phospholipids (Matsuzawa & Hostetler 1979). BMP is presumably formed during the degradation of phosphatidylglycerol and cardiolipin which are derived from mitochondria that enter the lysosomal compartment by autophagy. During this degradation process, BMP should be formed on intra-lysosomal vesicles and distinguishes these membranes from the perimeter membrane. Apart from BMP, smaller amounts of phosphatidylinositol (Kobayashi *et al.* 1998) and dolicholphosphate (Chojnacki & Dallner 1988) have also been found as anionic lipids within the lysosomal compartment.

BMP and other anionic lysosomal phospholipids dramatically stimulate the interfacial hydrolysis of membrane-bound glycolipids by their water-soluble exohydrolases in the presence of GM2-AP; ganglioside GM2 by HEX A (Werth *et al.* 2001), ganglioside GM1 by β-galactosidase (Wilkening *et al.* 2000), and the sulphated gangliotriaosylceramide SM2 by HEX A and HEX S (Hepbildikler *et al.* 2002). BMP did not enhance binding of the GM2-AP to the membrane but increased its ability to solubilize lipids. This ability should lead to a destabilization of the membranes and facilitate the attack of water-soluble exohydrolases.

In vitro, glucosylceramide degradation is drastically enhanced by the presence of negatively charged lipids such as phosphatidylserine, phosphatidylglycerol and phosphatidic acid (Berent & Radin 1981; Sarmientos *et al.* 1986; Salvioli *et al.* 2000).

(e) *Sphingolipid activator proteins derived from prosaposin*

Apart from the GM2-AP, four additional enzymatically inactive proteins are required for the degradation of GSLs with short oligosaccharide chains. These proteins are called SAPs (Mehl & Jatzkewitz 1964) or saposins (Morimoto *et al.* 1988). Having different specificity, they facilitate the degradation of membrane-bound sphingolipids by water-soluble exohydrolases.

All four saposins, or SAP-A to -D, are derived from a single protein, SAP-precursor or prosaposin, by proteolytic processing (Fürst *et al.* 1988; O'Brien *et al.* 1988; Nakano *et al.* 1989).

The SAP-precursor is synthesized in the ER and transported through the Golgi stacks, where it is glycosylated to a 70 kDa protein. It is secreted to the extracellular space and can be endocytosed by the low density lipoprotein receptor-related protein (Hiesberger *et al.* 1998). The SAP-precursor itself is reported to have neurotrophic and neuroprotective properties of its own (Hiraiwa *et al.* 1997) which are, however, still disputed (Lapchak *et al.* 2000; Schuette *et al.* 2001). It is found in several body fluids, including human milk and semen (Hineno *et al.* 1991; Kondoh *et al.* 1991).

In the acidic compartment of the cell, the individual domains of the precursor protein are proteolytically processed to the individual SAPs A–D (Vielhaber *et al.* 1997). The four SAPs show homology to each other and have similar properties, but differ in their specificity and their mode of action. Their sequences (all about 80 amino acids) contain six highly conserved cysteins and a conserved *N*-glycosylation site (Kishimoto *et al.* 1992). The disulphide bridges are essential for the activity and might account for the unusual stability of the proteins (Vaccaro *et al.* 1995*b*). A structural motif known as saposin-like domain has been identified in several proteins (Munford *et al.* 1995). It is characterized by three intradomain disulphide linkages and a subset of conserved amino-acid residues with hydrophobic side chains. 'Saposin-like proteins' carry out diverse functions on a common backbone structure. The three-dimensional structure of one SAP-like domain has been solved by nuclear magnetic resonance spectroscopy (Liepinsh *et al.* 1997). In the so-called 'swaposins', carboxy- and aminoterminal halves of the domains have been swapped (Ponting & Russell 1995). There is evidence that the precursor of pulmonary surfactant protein B contains three SAP-like domains (Zaltash & Johansson 1998). All proteins of this group share a lipid binding and a membrane perturbing property.

The first SAP was discovered in 1964 by Mehl and Jatzkewitz and called sulphatide activator protein (SAP-B or saposin B), which is required for the degradation of sulphatide (galactosylceramide-3-sulphate) by arylsulphatase A. SAP-B is a small lysosomal glycoprotein with one N-glycosidically linked oligosaccharide chain and three disulphide bridges (Fischer & Jatzkewitz 1975; Fürst *et al.* 1990). These cross linkages might account for the unusual stability against pH (1.5–12), heat (up to 95 °C) and proteases (Gärtner *et al.* 1983). Until now, five point mutations on the SAP-B domain of the SAP-precursor gene have been identified (Wrobe *et al.* 2000).

Like the GM2-AP, SAP-B behaves like a physiological detergent and stimulates sulphatide degradation by solubilizing the membrane-bound substrate. *In vitro*, it was found to be a transport protein for sulphatides and other GSLs (Vogel *et al.* 1991). The inherited defect of SAP-B leads to the accumulation of sulphatides, digalactosylceramide and globotriaosylceramide (Sandhoff *et al.* 2001). The phenotype of the patients resembles a variant form of

metachromatic leukodystrophy with late infantile or juvenile onset (Kretz *et al.* 1990).

SAP-C or saposin C is required for the lysosomal degradation of glucosylceramide by glucosylceramide-β-glucosidase. SAP-C is a 20 kDa protein, obviously in homodimeric structure, and was first isolated from spleen of Gaucher patients (Ho & O'Brien 1971). In contrast to SAP-B, it binds not only to lipids and membranes but also interacts with glucosylceramide-β-glucosidase and stimulates the enzyme directly (Berent & Radin 1981; Fabbro & Grabowski 1991). The β-glucosidase is a water soluble lysosomal enzyme that can associate to membranes. *In vitro* it requires detergents or negatively charged phospholipids for its full enzymatic activity (Wilkening *et al.* 1998). The activator enhances the activity against the natural lipid substrate as well as against synthetic water-soluble substrates *in vitro* (Sarmientos *et al.* 1986; Vaccaro *et al.* 1997). SAP-C apparently does not bind glucosylceramide but the degrading enzyme. Kinetic data suggest an allosteric activation of the enzyme (Morimoto *et al.* 1990). Under acidic pH, the affinity of SAP-C to membranes is strongly increased (Vaccaro *et al.* 1995*a*). At these pH values, SAP-C (and SAP-D) also destabilizes phospholipid membranes of large unilamellar vesicles *in vitro* (Wilkening *et al.* 1998). This could facilitate the association of glucosylceramide-β-glucocerebrosidase with membranes that favour the degradation of glucosylceramide (Vaccaro *et al.* 1999). *In vitro*, SAP-C also stimulates the degradation of galactosylceramide by galactosylceramide-β-galactosidase (Wenger *et al.* 1982), sphingomyelin by acid sphingomyelinase (Tayama *et al.* 1993; Linke *et al.* 2001*a*), and of ceramide by acid ceramidase (Linke *et al.* 2001*b*). The deficiency of SAP-C leads to an abnormal juvenile form of Gaucher disease and an accumulation of glucosylceramide (Christomanou *et al.* 1986; Schnabel *et al.* 1991). Feeding of purified SAP-C to patients' fibroblasts reduces the level of glucosylceramide storage, whereas SAP-A, -B and -D were not effective (Klein *et al.* 1994).

Until now, no human disease is known which is based on the isolated defect of SAP-A (Morimoto *et al.* 1989) or SAP-D (Fürst *et al.* 1988). *In vitro*, SAP-A can bind to gangliosides GM1 and GM2 (Hiraiwa *et al.* 1992) and stimulate to some extent the enzyme-catalysed hydrolysis of glucosyl- and galactosylceramide (Morimoto *et al.* 1990). *In vivo*, it is required for the degradation of galactosylceramide: mice carrying a mutation in the SAP-A domain of the SAP-precursor accumulate galactosylceramide and suffer from a late-onset form of Krabbe disease (Matsuda *et al.* 2001).

SAP-D contains 78 amino acids and one N-linked oligosaccharide chain. It stimulates lysosomal ceramide degradation by acid ceramidase in cultured cells (Klein *et al.* 1994) as well as *in vitro* (Linke *et al.* 2001*b*). An activating effect is also described for the acid sphingomyelinase, but this seems not to be necessary for the *in vivo* degradation of sphingomyelin (Morimoto *et al.* 1988; Linke *et al.* 2001*a*). At the appropriate pH, SAP-D binds to vesicles containing negatively charged lipids and solubilizes membranes (Ciaffoni *et al.* 2001).

A lipidosis patient with a pleiotrophic sphingolipid accumulation, but without an enzyme defect, showed a complete lack of the whole SAP-precursor-protein. The patient died within 16 weeks (Harzer *et al.* 1989). The morphological analysis revealed a storage of vesicles and other membranes within the endosomal and lysosomal compartments (Bradova *et al.* 1993; Burkhardt *et al.* 1997). The molecular analysis revealed a homoallelic mutation in the start codon of the SAP-precursor protein from ATG to TTG (Schnabel *et al.* 1992). This led to the complete loss of SAP-A–D. Neutral glycolipids were stored in the liver, in the kidney and in cultured skin fibroblasts (Bradova *et al.* 1993). Sulphatide was stored in the kidney and free ceramide accumulated in the liver and kidney. The levels of gangliosides GM2 and GM3 were elevated in the liver, but not in the brain, whereas sphingomyelin and phospholipids were not affected.

The *in vivo* role of the different SAPs A–D for sphingolipid degradation was investigated in cultured fibroblasts from this patient in metabolic labelling studies (Klein *et al.* 1994). Accumulation of lactosylceramide could be normalized by feeding of SAP-B (stimulating GM1-β-galactosidase catalysed reaction) as well as SAP-C (stimulating galactosylceramide-β-galactosidase catalysed reaction); this was likewise confirmed *in vitro* (Zschoche *et al.* 1994). SAP-B also reduces the accumulation of ganglioside GM3 and glucosylceramide. The exogenous addition of SAP-D stimulates ceramide degradation in cultured cells and *in vitro* (Linke *et al.* 2001*b*). Recently, a second case with complete deficiency of the SAP-precursor and the mature SAPs has been reported. Here, a deletion within the SAP-B domain leads to a frame shift and a premature stop codon (Hulkova *et al.* 2001).

(f) *Sphingolipids in epidermal barrier formation*

Another area in which sphingolipids play an important role is the formation of the water permeability barrier of the skin. The barrier is essential for mammalian terrestrial life, because it restricts excess transepidermal water loss and therefore protects the organism from desiccation. It is localized in the outermost stratum corneum layer of the epidermis where corneocytes are embedded in a matrix of extracellular lipid membranes mainly consisting of long chain ceramides, free fatty acids and cholesterol. Some of these ceramides possess a ω-hydroxyl group on their long chain *N*-acyl moiety and are covalently attached to proteins on the surface of corneocytes, probably forming the hydrophobic scaffold (lipid-bound envelope) on which the extracellular lipid membranes are initially deposited (Wertz & Downing 1987).

So far, the formation of the epidermal barrier to water loss is not completely understood. It is known that glucosylceramides together with other polar lipids are secreted into the extracellular space of the stratum corneum by lamellar bodies and are subsequently processed by a set of co-localized lysosomal lipid hydrolases into a more-hydrophobic mixture, enriched in ceramides (Wertz & Van den Bergh 1998). Although most human patients with a partial defect in β-glucocerebrosidase do not display any visible skin phenotype, there are some reports of so-called 'collodion babies', infants whose skin feels sticky and who die shortly after birth. In several cases this phenotype has been shown to be associated with a severe infantile form of Gaucher disease, characterized by a complete deficiency of β-glucocerebrosidase (Liu *et al.* 1988).

These findings are confirmed by a mouse model for this disease which has been created by introducing point mutations into the murine gene encoding β-glucocerebrosidase. Owing to an almost complete loss of β-glucocerebrosidase activity, these mice show a severely abnormal skin and die 24 h after birth. When analysing the epidermal lipid composition of these mice, it was found that the amount of glucosylceramides was increased whereas the amount of ceramides was reduced (Doering *et al.* 1999*a*). These observations demonstrate that the processing of glucosylceramide to ceramide is essential for barrier competence but that only a nearly complete loss of enzymatic activity leads to epidermal pathology in humans.

Lipid analysis of the β-glucocerebrosidase-deficient mice and of mice with a complete deficiency of the SAP precursor protein which are therefore also deficient in SAP-C, a cofactor of β-glucocerebrosidase, revealed the existence of a new glycoconjugate: it was demonstrated by our group that ω-hydroxylated glucosylceramides are covalently attached to corneocytes (Doering *et al.* 1999*a*,*b*). In the SAP precursor protein- and β-glucocerebrosidase-deficient epidermis this novel glycoconjugate accounted for 30% and 70% of the total protein-bound lipid, respectively. Concomitantly, levels of related protein-bound ceramides and fatty acids were decreased in the epidermis of these mice, all of which show an abnormal epidermal permeability barrier.

These data support the concept that free ω-hydroxylated glucosylceramides are transferred to carboxylated side chains on surface proteins of the corneocytes and are subsequently processed by the sequential action of β-glucocerebrosidase and acid ceramidase which need SAPs (C and D) to enhance their activity against these rather hydrophobic substrates.

The results obtained demonstrate how the study of metabolic diseases can lead to insights into related metabolic pathways, in this case the formation of the epidermal barrier. Further investigations in this area may give rise to a better understanding of skin malfunctions as they are observed in diseases like psoriasis or atopic dermatitis.

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GLOSSARY

BMP: bis-(monoacylglycero)-phosphate
ER: endoplasmic reticulum
GlcCer: glucosylceramide
GM2-AP: GM2-activator protein
GSL: glycosphingolipid
HEX: hexosaminidase
SAP: sphingolipid activator protein