Acta Biochimica Polonica

Vol. 45 No. 2/1998

373–384

QUARTERLY

Review

Processing of sphingolipid activator proteins and the topology of lysosomal digestion*°

Konrad Sandhoff✉ and Thomas Kolter

Kekulé-Institut für Organische Chemie und Biochemie der Universität, Gerhard-Domagk-str. 1, D-53121 Bonn, Germany

Key words: endosomes, lysosomal storage diseases, saposins, sphingolipid activator proteins, sphingolipidoses

Plasma membrane derived glycosphingolipids (GSLs) destined for digestion are internalized through the endocytic pathway and delivered to the lysosomes. There, GSLs are degraded by the action of exohydrolases, which are supported, in the case of GSLs with short oligosaccharide chains, by sphingolipid activator proteins (SAPs). Four of the SAPs, SAP-A to -D (also called saposins) are synthesized from a single precursor protein (pSAP). Intracellular routing of pSAP and of the G_{M2} activator protein is only in part dependent on mannose-6-phosphate residues. Their endocytosis occurs in a carbohydrate-independent manner. The inherited deficiencies of individual activators, the G_{M2} activator, SAP-B, and SAP-C, as well as the deficiency of the precursor pSAP give rise to different, neuronal, white matter or visceral sphingolipid storage diseases. The analysis of cultured fibroblasts from corresponding patients suggests a new model for the topology of endocytosis and lysosomal digestion. It supports the hypothesis that endocytosis of plasma membrane-derived lipids occurs *via* small intraendosomal and intralysosomal vesicles and membrane structures, that are then digested within the lysosomes. In combined activator protein deficient cells nondegradable GSLs on the surface of intralysosomal vesicles protect them against lysosomal digestion. Mice with disrupted genes for activator proteins (SAP precursor −/−, G_{M2}A −/−) as well as disrupted genes for ganglioside G_{M2} degrading hexosaminidases (HEXA −/−, HEXB −/−) turned out to be useful models for known human diseases whereas double knock out mice (HEXA −/− and HEXB −/−) show a new phenotype of both mucopolysaccharidosis and gangliosidosis.

---

*Presented at the International Symposium on Structure, Metabolism, and Functions of Glycosphingolipids and Sphingolipids in Warsaw, Poland, September 4–6, 1997.

°The work performed in the laboratory of the authors was supported by the Deutsche Forschungsgemeinschaft (SFB 284).

✉To whom correspondence should be addressed: K. Sandhoff; phone int./228/73 53 46; telefax: int./228/737 778; e-mail: Sandhoff@uni-bonn.de

Abbreviations: G_{M2}A, G_{M2} activator protein; GSL, glycosphingolipid; HEX, hexosaminidase; SAP, sphingolipid activator protein; pSAP, SAP precursor.

STRUCTURE AND FUNCTION OF GLYCOSPHINGOLIPIDS

Glycosphingolipids (GSLs) are components of the plasma membrane of eukaryotic cells (Wiegandt, 1985). They contain a hydrophobic ceramide moiety that acts as a membrane anchor, and an extracellularly oriented glycan chain. Variations in the type, number and linkage of sugar residues give rise to a wide range of naturally occurring GSLs. These cell-type specific patterns at the cell surface change with cell growth, differentiation, viral transformation and oncogenesis (Hakomori, 1981). GSLs interact at the cell surface with toxins (Holmgren *et al.*, 1975), viruses (Markwell *et al.*, 1981), and bacteria (Karlsson, 1989) as well as with membrane bound receptors and enzymes (Nagai & Iwamori, 1995). They are also involved in cell type specific adhesion processes (Walz *et al.*, 1990; Phillips *et al.*, 1990), and, in addition, lipophilic products of GSL metabolism such as sphingosine and ceramide, play a role in signal transduction events (Spiegel *et al.*, 1996).

TOPOLOGY OF LYSOSOMAL DIGESTION

Catabolism of complex GSLs derived from the plasma membrane occurs in the lysosomes after endocytosis. In the conventional model, components of plasma membranes reach the lysosomal compartment by endocytic membrane flow *via* the early and late endocytic reticulum (Griffiths *et al.*, 1988). During this vesicular flow (see Fig. 1) molecules in the membrane are subjected to a sorting process which directs some of them to the lysoso-

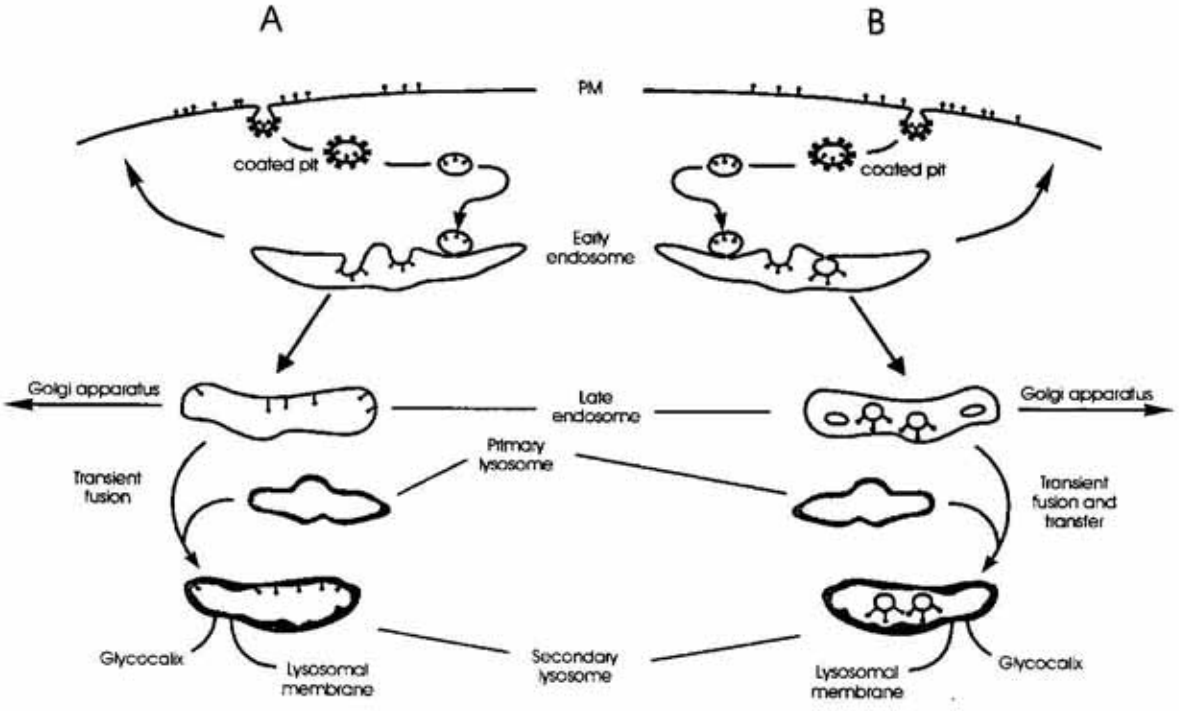

Figure 1. Two models for the topology of endocytosis and lysosomal digestion of glycosphingolipids (GSLs) derived from the plasma membrane (Sandhoff & Kolter, 1996).

Conventional model (A): degradation of GSLs derived from the plasma membrane occurs selectively within the lysosomal membrane. Alternative model for the topology of endocytosis and digestion of GSLs (B): during endocytosis glycolipids of the plasma membrane become incorporated into the membranes of intraendosomal vesicles (multivesicular bodies). The vesicles and derived membrane structures are transferred into the lysosomal compartment when late endosomes transiently fuse with primary lysosomes. PM, plasma membrane; ●, glycosphingolipid.

mal compartment, some others to the Golgi apparatus and yet others even back to the plasma membrane (Koval & Pagano, 1989). After a series of vesicle budding and fusion events through the endosomal compartment, the membrane fragments reach the lysosomal compartment. As a consequence of endocytic membrane flow, the plasma membrane fragments are incorporated into the lysosomal membrane. Lysosomal degradation of former components of the plasma membrane must then proceed selectively within the lysosomal membrane without destroying it. It is difficult to see how this could occur, especially as the inner leaflet of the lysosomal membrane is covered with a thick glycocalix composed of glycoproteins, the so called limps (lysosomal integral membrane proteins) and lamps (lysosomal associated membrane proteins) (Carlsson *et al.*, 1988).

In an alternative model for the topology of endocytosis (Fürst & Sandhoff, 1992), components of the plasma membrane pass through the endosomal compartment as intraendosomal vesicles that become intralysosomal vesicles on reaching the lysosomes (see Fig. 1). The vesicles are initially formed in the early endosome by selective invagination (budding-in) of endosomal membranes enriched in components of the plasma membrane. The surrounding endosome then passes along the endocytic pathway by the normal, successive events of membrane fission and fusion. The intraendosomal vesicles, however, are carried along as passengers and normally do not undergo fusion and fission. When the vesicle reaches the lysosome, glycoconjugates originating from the outer leaflet of the plasma membrane face the lysosol on the outer leaflet of intralysosomal vesicles and are thus in the correct orientation for degradation. In this model there is no glycocalix blocking the action of hydrolases. This hypothesis is supported by a series of observations (reviewed by Sandhoff & Kolter, 1996). Multivesicular storage bodies were found to accumulate in cells from a patient with a combined activator protein deficiency (Harzer *et al.*, 1989; Schnabel *et al.*, 1992). Analysis of cultured fibroblasts from this patient shows that the storage vesicles refer to late endosomal or lysosomal compartments. These compartments are still functionally active, except in their ability to degrade sphingolipids with short oligosaccharide head groups. After complementation of the medium of these cells with the missing SAP-precursor, not only the degradation block is abolished but also the morphological shape of the multivesicular bodies is reversed (Burkhardt *et al.*, 1997). This suggests a protective function of GSLs against the degradation of intralysosomal vesicles and membrane structures by lysosomal hydrolases. To date, membrane invagination and the subsequent budding-off of vesicles into the lumen of early endosomes has not been observed by microscopy. This membrane fission event is postulated to take place since this would explain both, the occurrence of intralysosomal vesicles and the selective degradation of glycosphingolipids derived from the plasma membrane, in contrast to those of the lysosomal membrane.

Within the lysosome, degradation of GSLs occurs by the step-wise action of specific acid exohydrolases (Sandhoff & Kolter, 1996) (see Fig. 2). If any of these enzymes is deficient, the corresponding lipid substrates accumulate and are stored in the lysosomal compartment. In these inherited deficiencies, lipid storage diseases, accumulation of lipids occurs mainly in those cell types and organs in which the lipids are predominantly synthesized. In Tay–Sachs disease, for example, β-hexosaminidase A is deficient. This causes accumulation of the ganglioside G_{M2} in neuronal cells, the main site for synthesis of gangliosides (sialic acid-containing GSLs) (Sandhoff *et al.*, 1989; Gravel *et al.*, 1995).

Detailed biochemical analysis of sphingolipidoses such as Metachromatic Leukodystrophy and Tay-Sachs disease demonstrated that the *in vivo* degradation of some glycolipids involves not only exohydrolases but also a sec-

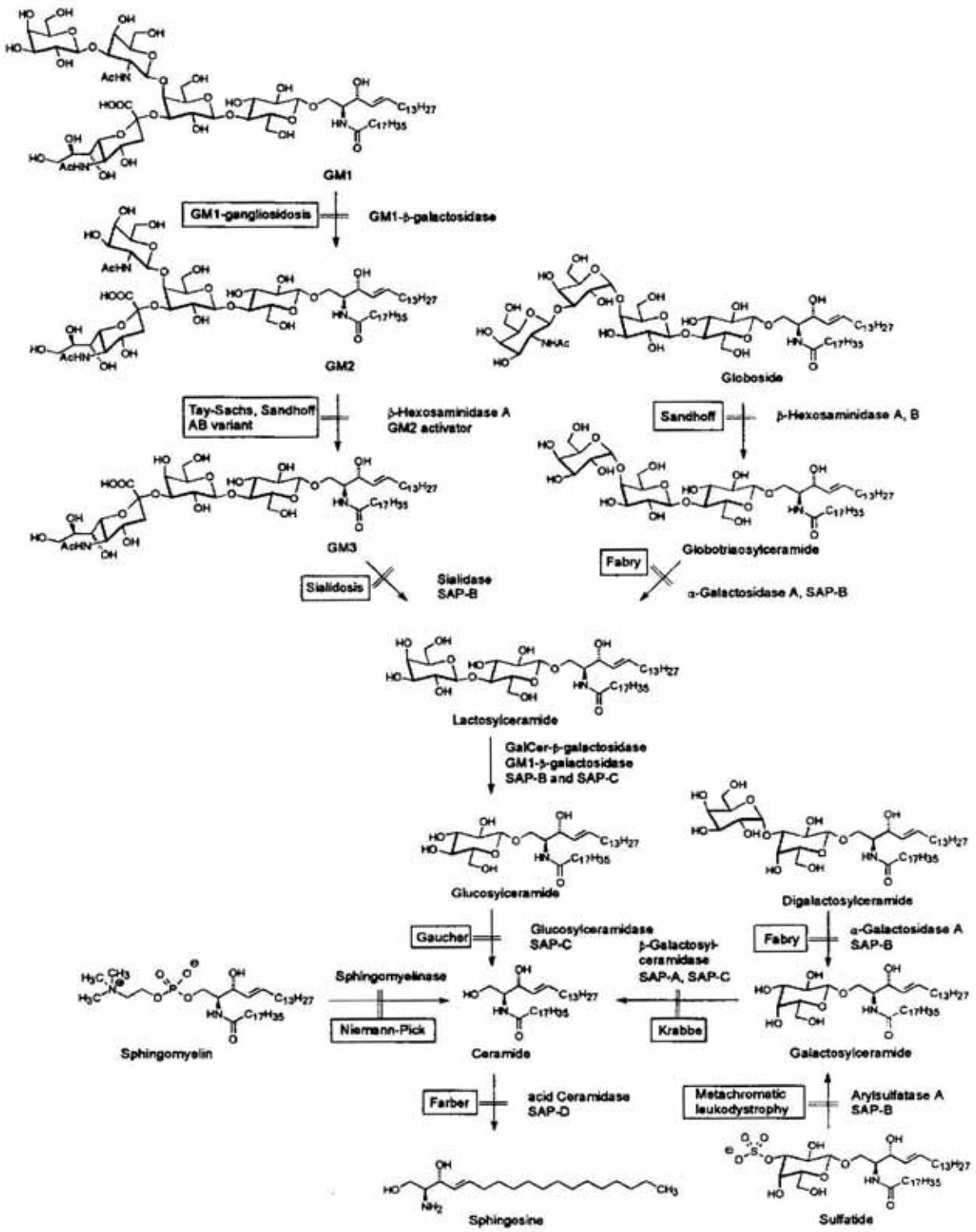

Figure 2. Lysosomal sphingolipid degradation (modified from Sandhoff & Kolter, 1996).

Sphingolipid activator proteins, exohydrolases and the eponyms of known storage diseases are shown. AB variant: AB variant of G_{M2} gangliosidosis (deficiency of G_{M2} activator protein); SAP, sphingolipid activator protein. Heterogeneity within the ceramide moiety due to varying degrees of saturation, hydroxylation and chain length is not shown.

ond type of effector molecule (Sandhoff *et al.*, 1995). In these cases lipid storage is observed in patients without any enzyme deficiency, but with a deficiency of a protein cofactor. The degradation of membrane bound GSLs with short oligosaccharide chains requires the cooperation of a water soluble exohydrolase and a sphingolipid activator protein. Several sphingolipid activator proteins are now known including the G_{M2} activator and saposins. A detailed study of these proteins has illuminated some of their main features and indicated the mechanisms by which they facilitate digestion of GSLs in the lysosome.

THE G_{M2} ACTIVATOR AND ITS ROLE IN LYSOSOMAL DIGESTION

The enzymatic degradation of ganglioside G_{M2}, the main storage material in Tay–Sachs disease, requires β-hexosaminidase A and a lysosomal ganglioside binding protein, the G_{M2} activator (Meier *et al.*, 1991). The GM2 activator binds ganglioside G_{M2} as well as related gangliosides forming water-soluble complexes (mostly in a 1:1 molar ratio). *In vitro* it functions as a ganglioside transfer protein transferring gangliosides from donor membranes to acceptor membranes (Conzelmann *et al.*, 1982). The G_{M2} activator recognizes G_{M2} within the membrane and, by binding to it, lifts the lipid out of the bilayer and presents it to the water soluble β-hexosaminidase A for degradation. Formation of the ternary complex presumably involves also a protein-protein interaction between the G_{M2} activator and β-hexosaminidase A (Kytzia & Sandhoff, 1985).

Point mutations within the structural gene of the G_{M2} activator have been identified in four patients with the AB-variant of G_{M2} gangliosidosis (Schröder *et al.*, 1991; 1993; Schepers *et al.*, 1996). The mutated proteins are proteolytically labile so that some of them are already degraded in the endoplasmic reticulum (ER) and/or in the Golgi compartment. The resulting loss of the G_{M2} activator causes a block within the G_{M2} degradation process in the lysosomes as demonstrated in metabolic studies on cultured fibroblasts of the patients. The block can be bypassed by feeding a native G_{M2} activator to the culture medium of the mutant fibroblasts (Klima *et al.*, 1993).

BIOSYNTHESIS, PROCESSING, AND INTRACELLULAR TRANSPORT OF THE G_{M2} ACTIVATOR

In human epidermal keratinocytes, the G_{M2} activator is synthesized as a 22 kDa precursor (see Fig. 3) bearing a single high mannose N-linked oligosaccharide chain on a peptide backbone of 18 kDa (Glombitza *et al.*, 1997). Processing of the N-glycans give rise to a high mannose type of 22 kDa, a complex type of 24 kDa and a multiantennary type of 25 to 27 kDa. About one third of the precursor population is secreted and consists in more than 90% of the 24 kDa form. The intracellular activator is processed to a mature protein of 20 kDa with a 17 kDa peptide bearing an multiantennary N-glycan without phosphate residues. Tunicamycin treatment revealed that nonglycosylated activator is delivered to the lysosomes with essentially the same kinetics as in nontreated cells (Glombitza *et al.* 1997). These data suggest a mannose-6-phosphate independent transport of the activator to the lysosomes. This observation is in agreement with the finding of Klima and coworkers that recombinant, nonglycosylated activator is efficiently endocytosed by fibroblasts from AB variant patients (Klima *et al.*, 1993). On the other hand, lysosomal enzymes are transported in a mannose-6-phosphate dependent manner (Braulke, 1996; Kornfeld & Mellman, 1989; von Figura & Hasilik, 1986).

OTHER ACTIVATOR PROTEINS

The first activator protein was identified in 1964 as a protein which is necessary for the hydrolytic degradation of glycosphingolipids carrying a sulfuric ester group (sulfatides) by lysosomal arylsulfatase A (Mehl & Jatzkewitz, 1964). This sulfatide activator, SAP-B (saposin B), is a small lysosomal glycoprotein consisting of 80 amino acids, with a N-linked carbohydrate chain and three disulfide bridges (Fürst *et al.*, 1990). Like the G_{M2} activator it binds GSLs but has a broader specificity. *In vitro* it behaves similarly to the G_{M2} activator in some aspects, i.e. it can recognize and bind several different GSLs on the surface of micelles by forming a stoichiometric complex and is then able to transfer the GSLs to the membranes of acceptor liposomes (Fürst & Sandhoff, 1992; Sandhoff *et al.*, 1995). SAP-B can also present GSLs to water-soluble enzymes as substrates (Li *et al.*, 1988). The inherited deficiency of the sulfatide activator leads to a lysosomal storage disease which resembles Metachromatic Leukodystrophy. However, unlike typical Metachromatic Leukodystrophy, not only sulfatide but also other glycolipids, e.g. globotriaosyl ceramide, accumulate owing to a blockage of degradation at several points in the catabolic pathway (Sandhoff *et al.*, 1995).

The sulfatide activator shares a sequence homology with three further activator proteins: the Gaucher factor (SAP-C), SAP-A and SAP-D. Molecular biological analysis has revealed that these four activator proteins are formed by proteolytic processing of a common precursor protein, the SAP-precursor (Fujibayashi & Wenger, 1986; Fürst *et al.*, 1988; O'Brien *et al.*, 1988; Nakano *et al.*, 1989). The four activator proteins, SAP-A -D show homology to each other and have similar properties, but differ in their function and their mechanism of action (Fürst & Sandhoff, 1992; Sandhoff *et al.*, 1995). The physiological function of these sphingolipid activator proteins is only partially clarified to date; most of our knowledge has emerged from studies on patients with atypical lipid storage diseases (Klein *et al.*, 1994). SAP-C deficiency causes an atypical form of Gaucher disease, where glucosyl ceramide accumulates (Christomanou *et al.*, 1986; Schnabel *et al.*, 1991). It can directly activate glucosylceramide β-glucosidase (Ho & O'Brien, 1971). In one patient with a complete deficiency of the whole SAP-precursor protein due to a homoallelic mutation within the start codon (AUG → UUG) (Schnabel *et al.*, 1992) there is a simultaneous storage of many sphingolipids, including ceramide, glucosyl ceramide, lactosyl ceramide, ganglioside G_{M3}, galactosyl ceramide, sulfatides, digalactosyl ceramide and globotriaosyl ceramide (Bradova *et al.*, 1993).

BIOSYNTHESIS, PROCESSING AND TARGETING OF pSAP

Sphingolipid activator proteins SAP A–D are derived from a 73 kDa glycoprotein, the SAP precursor (pSAP). Biosynthesis and processing of pSAP in cultured human skin fibroblasts (see Fig. 4) were analyzed by metabolic labeling and immunoprecipitation techniques with the aid of polyclonal anti-SAP-C antibodies (Vielhaber *et al.*, 1997). pSAP bears phosphate residues on noncomplex carbohydrate chains linked to the SAP-C and -D domains as well as sulfate residues on complex carbohydrate chains on the SAP-A, -C, and, possibly, on the SAP-D domain. pSAP is synthesized as a 65 kDa precursor with high mannose carbohydrate chains and converted to a 73 kDa form in the Golgi apparatus bearing complex carbohydrates. Forms of 30 to 50 kDa arise from the 65 kDa pSAP by proteolytic cleavage. The occurrence of other forms (Vielhaber *et al.*, 1997) is summarized in Fig. 4. Proteolytic processing starts at the stage of early endosomes. Normal fibroblasts internalize pSAP secreted from I-cell fibroblasts nearly as efficiently as pSAP secreted from normal fibroblasts. Moreover, deglycosylated

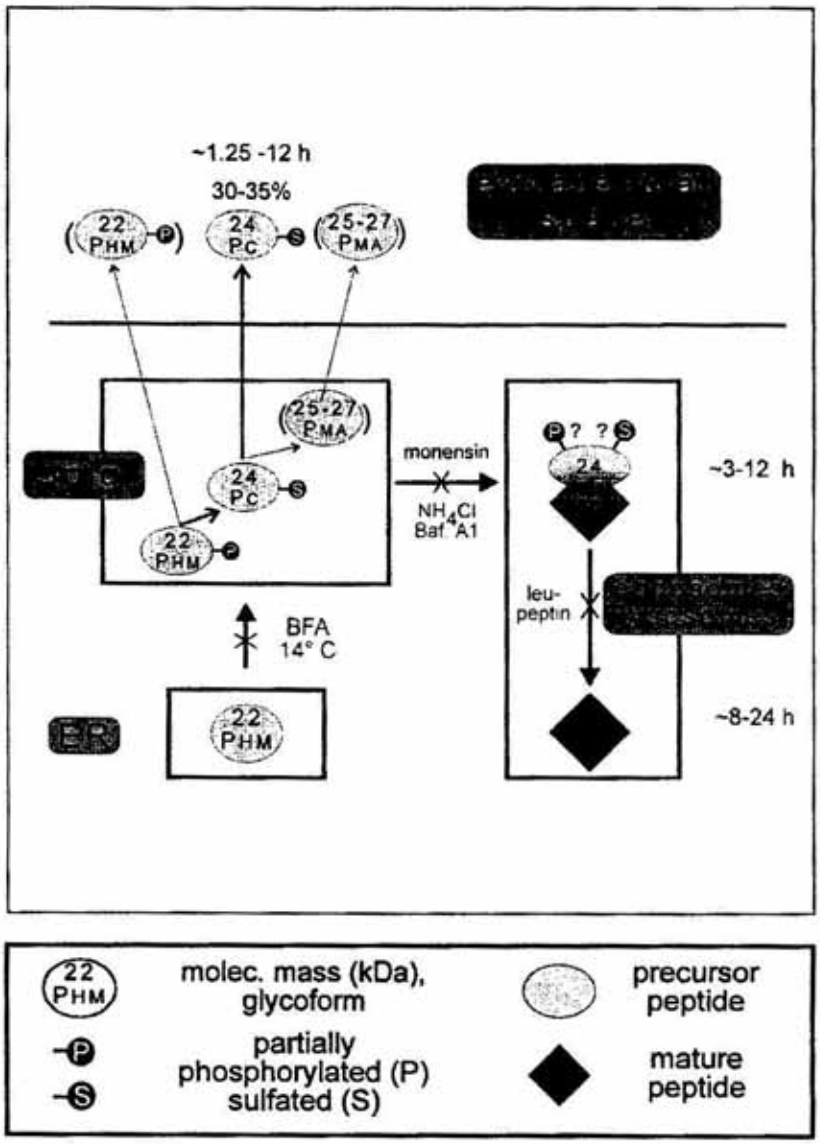

Figure 3. Schematic representation of G_{M2} activator protein processing in human epidermal keratinocytes (modified from Glombitza *et al.*, 1997).

The localization of individual glyco and protein forms of G_{M2} activator during transport and processing as determined by metabolic labeling and immunoprecipitation are shown. Thick arrows, major pathways; thin arrows, minor pathways. Approximate transit or conversion times are indicated for each step. Blocking agents are given for transitions that could be inhibited. BafA1, bafilomycin A1; PHM, precursor, high mannose; PC, precursor, complex; PMA, precursor with multiantennary N-glycan; M, mature; ER, endoplasmic reticulum.

pSAP is taken up by the mannose-6-phosphate receptor double knock out mouse fibroblasts more efficiently than the glycosylated protein. These data suggest that targeting of pSAP from the Golgi compartment to the lysosomes is only in part dependent on mannose-6-phosphate residues, while endocytosis occurs in a carbohydrate-independent manner (Vielhaber *et al.*, 1997).

ANIMAL MODELS OF G_{M2}-GANGLIOSIDOSES AND pSAP-DEFICIENCY

Animal models are useful means for the study of pathogenesis and in approaches towards therapy of sphingolipid storage diseases. The mouse model of Tay–Sachs disease (HEXA -/-) was generated by targeted dis-

ruption of the gene of the α-chains of β-hexosaminidase A in murine embryonic stem cells (Yamanaka *et al.*, 1994; Taniike *et al.*, 1995). Also the animal model of Sandhoff's disease (HEXB -/-) has been described, in which the β-chain of the hexosaminidases A and B are inactivated (Sango *et al.*, 1995). While the phenotypes of the two variants of G_{M2} gangliosidoses are only slightly different in humans, the animal models show drastic differences in severity and course of the disease. Mice with deficient β-hexosaminidase A (the Tay-Sachs mice) are completely asymptomatic. Although they show a mild accumulation of G_{M2} in the central nervous system, they do not express the neurological symptoms characteristic of the human case of Tay-Sachs disease. In contrast, mice with deficient β-hexosaminidase B (Sandhoff mice) develop severe neurological disorders. They heavily accumulate G_{M2} and G_{A2} and express neurological symptoms at about three months of age, with spasticity, muscle weakness, rigidity, tremor, and ataxia progressing to spastic quadriparesis and death within several weeks since the onset of the disease. The life span of the animals is strongly reduced. Lipid storage in HEXB -/- mice was far more extensive and widely distributed. The reason for this is the different specificity of sialidase in mouse

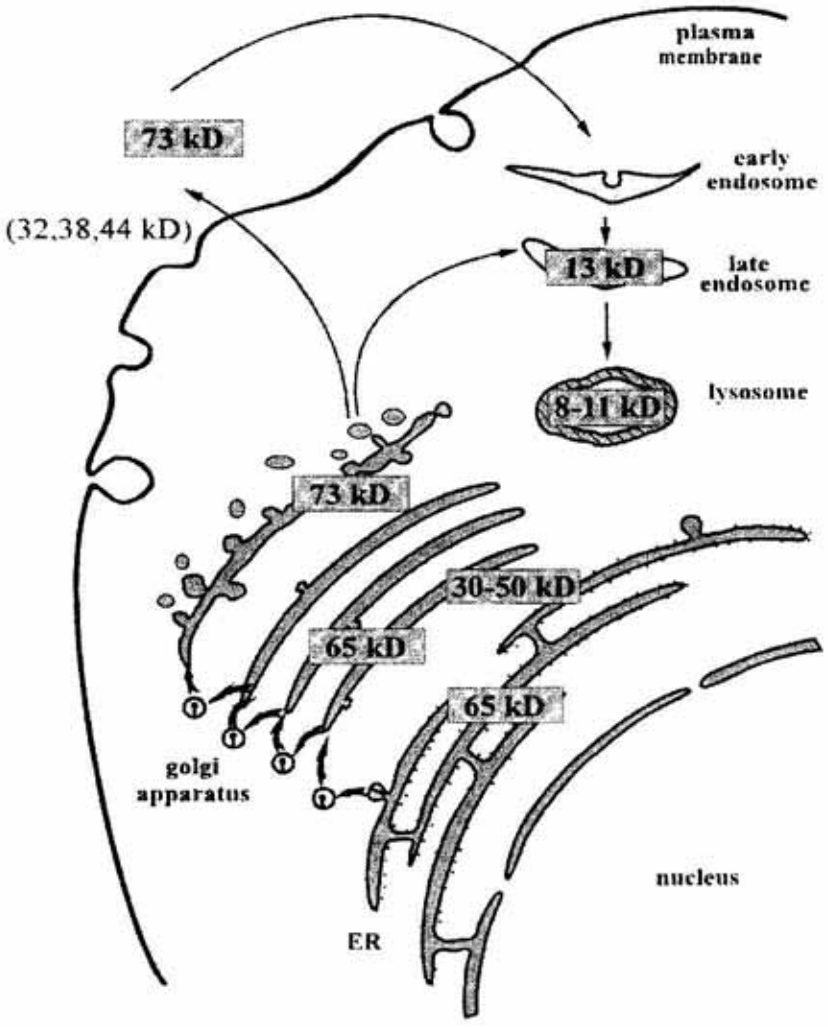

Figure 4. Schematic representation of pSAP processing and transport in cultured human fibroblasts.

The localization of individual protein forms of pSAP during transport and processing are shown. The formation of SAP-C in the endosomal/lysosomal compartment is indicated. ER, endoplasmic reticulum.

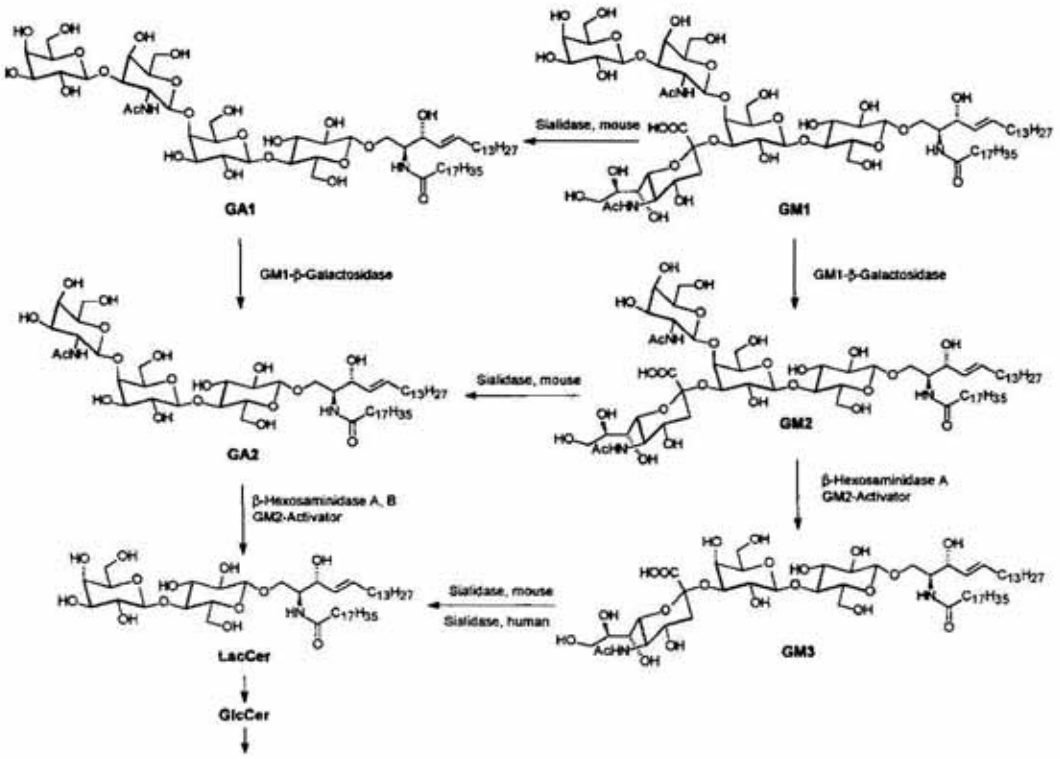

Figure 5. The ganglioside degradation pathway in mice and human (Sango *et al.*, 1995; Hahn *et al.*, 1997).

and in humans. Mouse sialidase accepts G_{M2} as a substrate and converts it to G_{A2}. In the human case, this metabolic pathway is of no significance. G_{A2} can be degraded by the still intact β-hexosaminidase B, so that in spite of a complete loss of β-hexosaminidase A the metabolic barrier is in part circumvented (see Fig. 5). Only the loss of both isoenzymes, hexosaminidases A and B, leads to a phenotype corresponding to the human Sandhoff disease. Although the mouse sialidase can still convert G_{M2} to G_{A2}, G_{A2} cannot be further degraded, since the responsible enzyme, β-hexosaminidase B, is also deficient. A double knock out mouse lacking *Hex* A, B and also *Hex* S ($\alpha\alpha$) shows a phenotype of both, mucopolysaccharidosis and gangliosidosis (Sango *et al.*, 1996). Mouse models for G_{M2}-activator deficiency express an intermediate phenotype between Tay-Sachs- and Sandhoff mice (Liu *et al.*, 1997). Besides storage of ganglioside G_{M2} and, to a lesser extent, G_{A2} in restricted regions of the brain, the animals develop defects in balance and coordination.

Also a mouse model for SAP-precursor deficiency has been described (Fujita *et al.*, 1996). The animals developed a clinical, pathological and biochemical phenotype closely resembling that of the human disease. Mice surviving birth and therefore exhibiting a later-onset phenotype developed rapidly progressive neurological signs at around 20 days of life and died at 35–38 days. At day 30, severe hypomyelination and storage materials were found throughout the nervous system and in abnormal cells in the liver and spleen. Most prominently lactosylceramide, and additionally ceramide, glucosylceramide, galactosylceramides, sulfatide and globotriaosylceramide were abnormally increased in the brain, liver, and kidney and their catabolism was abnormally slow in cultured fibroblasts.

In the future, animal models of gangliosidoses will serve as valuable tools for the development of novel therapeutic concepts for the treatment of these diseases. Recently, central nervous lipid storage was reported to be retarded in the animal model of Tay-Sachs dis-

ease by a substrate deprivation strategy (Platt *et al.*, 1997).

REFERENCES

Bradova, V., Smid, F., Ulrich-Bott, B., Roggendorf, W., Paton, B.C. & Harzer, K. (1993) Prosaposin deficiency: Further characterization of the sphingolipid activator protein-deficient sibs. Multiple glycolipid elevations (including lactosylceramidosis), partial enzyme deficiencies and ultrastructure of the skin in this generalized sphingolipid storage disease. *Hum. Genet.* **92**, 143–152.

Braulke, T. (1996) Origin of lysosomal proteins; in *Subcellular Biochemistry* (Lloyd, I.B. & Mason, R.W., eds.) vol. 27, *Biology of the Lysosome*, pp. 15–49, Plenum Press, New York.

Burkhardt, J.K., Hüttler, S., Klein, A., Möbius, W., Habermann, A., Griffiths, G. & Sandhoff, K. (1997) Accumulation of sphingolipids in SAP-precursor (prosaposin) deficient fibroblasts occurs as intralysosomal membrane structures and can be completely reversed by treatment with human SAP-precursor. *Eur. J. Cell Biol.* **73**, 10–18.

Carlsson, S.R., Roth, J., Piller, F. & Fukuda, M. (1988) Isolation and characterization of human lysosomal membrane glycoproteins, h-lamp-1 and h-lamp-2. *J. Biol. Chem.* **263**, 18911–18919.

Christomanou, H., Aignesberger, A. & Linke, R.P. (1986) Immunochemical characterization of two activator proteins stimulating enzymic sphingomyelin degradation in vitro–Absence of one of them in a human Gaucher disease variant. *Biol. Chem. Hoppe-Seyler* **367**, 879–890.

Conzelmann, E., Burg, J., Stephan, G. & Sandhoff, K. (1982) Complexing of glycolipids and their transfer between membranes by the activator protein for degradation of lysosomal ganglioside G_{M2}. *Eur. J. Biochem.* **123**, 455–464.

von Figura, K., Hasilik, A. (1986) Lysosomal enzymes and their receptors. *Annu Rev. Biochem.* **55**, 167–193.

Fujibayashi, S. & Wenger, D.A. (1986). Biosynthesis of the sulfatide/GM1 activator protein (SAP-1) in control and mutant cultured skin fibroblasts. *Biochim. Biophys. Acta* **875**, 554–562.

Fujita, N., Suzuki, K., Vanier, M.T., Popko, B., Maeda, N., Klein, A., Henseler, M., Sandhoff, K., Nakayasu, H. & Suzuki, K. (1996) Targeted disruption of the mouse sphingolipid activator protein gene: A complex phenotype, including severe leukodystrophy and wide-spread storage of multiple sphingolipids. *Hum. Mol. Genet.* **5**, 711–725.

Fürst, W. & Sandhoff, K. (1992) Activator proteins and topology of lysosomal sphingolipid catabolism. *Biochim. Biophys. Acta* **1126**, 1–16.

Fürst, W., Machleidt, W. & Sandhoff, K. (1988) The precursor of sulfatide activator protein is processed to three different proteins. *Biol. Chem. Hoppe-Seyler* **369**, 317–328.

Fürst, W., Schubert, J., Machleidt, W., Meyer, E.H. & Sandhoff, K. (1990) The complete amino-acid sequences of human ganglioside GM2 activator protein and cerebroside sulfate activator protein. *Eur. J. Biochem.* **192**, 709–714.

Glombitza, G.J., Becker, E., Kaiser, H.W. & Sandhoff, K. (1997) Biosynthesis, processing, and intracellular transport of GM2 activator protein in human epidermal keratinocytes. The lysosomal targeting of the GM2 activator is independent of a mannose-6-phosphate signal. *J. Biol. Chem.* **272**, 5199–5207.

Gravel, R.A., Clarke, J.T.R., Kaback, M.M., Mahuran, D., Sandhoff, K. & Suzuki, K. (1995) The GM2 ganglisidoses; in *The Metabolic and Molecular Basis of Inherited Disease* (Scriver, C., Beaudet, A.L., Sly, W.S. & Valle, D., eds.) pp. 2839–2879, McGraw-Hill.

Griffiths, G.W., Hoflack, B., Simons, K., Mellman, I.S. & Kornfeld, S. (1988) The mannose-6-phosphate receptor and the biogenesis of lysosomes. *Cell* **52**, 329–341.

Hahn, C.N., del Pilar, M., Schröder, M., Vanier, M.T., Hara, Y., Suzuki, K., Suzuki, K. & d'Azzo, A. (1997) Generalized CNS disease and massive G(M1)-ganglioside accumulation in

mice defective in lysosomal acid beta-galactosidase. *Hum. Mol. Genet.* **6**, 205–211.

Hakomori, S. (1981) Glycosphingolipids in cellular interactions, differentiation and oncogenesis. *Annu. Rev. Biochem.* **50**, 733–764.

Harzer, K., Paton, B.C. & Poulos, A. (1989) Sphingolipid activator protein (SAP) deficiency in a 16-week old atypical Gaucher disease patient and his fetal sibling; biochemical signs of combined sphingolipidoses. *Eur. J. Pediatr.* **149**, 31–39.

Ho, M.W. & O'Brien, J.S. (1971) Gaucher's Disease: Deficiency of 'acid' beta-glucosidase and reconstitution of enzyme activity *in vitro*. *Proc. Natl. Acad. Sci. U.S.A.* **68**, 2810–2813.

Holmgren, J., Lönnroth, I., Mansson, J.-E. & Svennerholm, L. (1975) Interaction of cholera toxin and membrane GM1 ganglioside of small intestine. *Proc. Natl. Acad. Sci. U.S.A.* **72**, 2520–2524.

Karlsson, K.-A. (1989) Animal glycosphingolipids as membrane attachment sites for bacteria. *Annu. Rev. Biochem.* **58**, 309–350.

Klein, A., Henseler, M., Klein, C., Suzuki, K., Harzer, K. & Sandhoff, K. (1994) Sphingolipid activator Protein D (*sap*-D) stimulates the lysosomal degradation of ceramide *in vivo*. *Biochem. Biophys. Res. Commun.* **200**, 1440–1448.

Klima, H., Klein, A., van Echten, G., Schwarzmann, G., Suzuki, K. & Sandhoff, K. (1993) Over-expression of a functionally active human G_{M2}-activator protein in *Escherichia coli*. *Biochem. J.* **292**, 571–576.

Kornfeld, S. & Mellman, I. (1989) The biogenesis of lysosomes. *Annu. Rev. Cell. Biol.* **5**, 483–525.

Koval, M. & Pagano, R.E. (1989) Lipid recycling between the plasma membrane and intracellular compartments: Transport and metabolism of fluorescent sphingomyelin analogues in cultured fibroblasts. *J. Cell Biol.* **108**, 2169–2181.

Kytzia, H.-J. & Sandhoff, K. (1985) Evidence for two different active sites on human hexosaminidase–interaction of G_{M2} activator protein with hexosaminidase A. *J. Biol. Chem.* **260**, 7568–7572.

Li, S.-C., Sonnino, S., Tettamanti, G. & Li, Y.-T. (1988) Characterization of a nonspecific activator protein for the enzymatic hydrolysis of glycolipids. *J. Biol. Chem.* **263**, 6588–6591.

Liu, Y., Hoffmann, A., Grinberg, A., Westphal, H., McDonald, M.P., Miller, K.M., Crawley, J.N., Sandhoff, K., Suzuki, K. & Proia, R.L. (1997) Mouse model of G_{M2} activator deficiency manifests cerebellar ganglioside storage and motor impairment. *Proc. Natl. Acad. Sci. U.S.A.* **94**, 8138–8143.

Markwell, M.A.K., Svennerholm, L. & Paulson, J.C. (1981) Specific gangliosides function as host cell receptors for Sendai virus. *Proc. Natl. Acad. Sci. U.S.A.* **78**, 5406–5410.

Mehl, E. & Jatzkewitz, H. (1964) Eine Cerebrosidsulfatase aus Schweineniere. *Hoppe-Seyler's Z. Physiol. Chem.* **339**, 260–276.

Meier, E.M., Schwarzmann, G., Fürst, W. & Sandhoff, K. (1991) The human G_{M2} activator protein: A substrate specific cofactor of β-hexosaminidase A. *J. Biol. Chem.* **266**, 1879–1887.

Nagai, Y. & Iwamori, M. (1995) Cellular biology of gangliosides; in *Biology of the Sialic Acids* (Rosenberg, A., ed.) pp. 197–241, Plenum Press, New York.

Nakano, T., Sandhoff, K., Stümper, J., Christomanou, H. & Suzuki, K. (1989) Structure of full-length cDNA coding for sulfatide activator, a co-beta-glucosidase and two other homologous proteins: Two alternate forms of the sulfatide activator. *J. Biochem.* **105**, 152–154.

O'Brien, J.S., Kretz, K.A., Dewji, N., Wenger, D.A., Esch, F. & Fluharty, A.L. (1988) Coding of two sphingolipid activator proteins (SAP-1 and SAP-2) by same genetic locus. *Science* **241**, 1098–1101.

Phillips, M.L., Nudelman, E., Gaeta, F.C.A., Perez, M., Singhal, A.K., Hakomori, S. & Paulson, J.C. (1990) ELAM 1 mediates cell adhesion by recognition of a carbohydrate ligand, sialyl-Le^x. *Science* **250**, 1130–1132.

Platt, F.M., Neises, G.R., Reinkensmeier, G., Townsend, M.J., Perry, V.H., Proia, R.L., Winchester, B., Dwek, R.A. & Butters, T.D. (1997) Prevention of lysosomal storage in Tay-Sachs

mice treated with *N*-butyldeoxynojirimycin. *Science* **276**, 428–431.

Sandhoff, K. & Kolter, T. (1996) Topology of glycosphingolipid degradation. *Trends Cell Biol.* **6**, 98–103.

Sandhoff, K., Conzelmann, E., Neufeld, E., Kaback, M.M. & Suzuki, K. (1989) The GM2 gangliosidoses; in *The Metabolic Basis of Inherited Disease* (Scriver, C., Beaudet, A.L., Sly, W. S., Valle, D., eds.) pp. 1807–1839, McGraw-Hill.

Sandhoff, K., Harzer, K. & Fürst, W. (1995) Sphingolipid Activator Proteins; in *The Metabolic and Molecular Basis of Inherited Disease* (Scriver, C., Beaudet, A.L., Sly, W.S. & Valle, D., eds.) pp. 2427–2441, McGraw-Hill.

Sango, K., Yamanaka, S., Hoffmann, A., Okuda, Y., Grinberg, A., Westphal, H., McDonald, M.P., Crawley, J.N., Sandhoff, K., Suzuki, K. & Proia, R.L. (1995) Mouse models of Tay-Sachs and Sandhoff diseases differ in neurologic phenotype and ganglioside metabolism. *Nature Genetics* **11**, 170–176.

Sango, K., McDonald, M.P., Crawley, J.N., Mack, M.L., Tifft, C.J., Skop, E., Starr, C.M., Hoffmann, A., Sandhoff, K., Suzuki, K. & Proia, R.L. (1996) Mice lacking both subunits of lysosomal beta-hexosaminidase display gangliosidosis and mucopolysaccharidosis. *Nature Genetics* **14**, 348–352.

Schepers, U., Glombitza, G.J., Lemm, T., Hoffmann, A., Chabàs, A., Ozand, P. & Sandhoff, K. (1996) Molecular analysis of a G_{M2}-activator deficiency in two patients with G_{M2}-gangliosidosis AB variant. *Am. J. Hum. Genet.* **59**, 1048–1056.

Schnabel, D., Schröder, M. & Sandhoff, K. (1991) Mutation in the sphingolipid activator protein 2 in a patient with a variant of Gaucher disease. *FEBS Lett.* **284**, 57–59.

Schnabel, D., Schröder, M., Fürst, W., Klein, A., Hurwitz, R., Zenk, T., Weber, J., Harzer, K., Paton, B.C., Poulos, A., Suzuki, K. & Sandhoff, K. (1992) Simultaneous deficiency of sphingolipid activator proteins 1 and 2 is caused by a mutation in the initiation codon of their common gene. *J. Biol. Chem.* **267**, 3312–3315.

Schröder, M., Schnabel, D., Suzuki, K. & Sandhoff, K. (1991) A mutation in the gene of a glycolipid-binding protein (GM2 activator) that causes G_{M2}-gangliosidosis variant AB. *FEBS Lett.* **290**, 1–3.

Schröder, M., Schnabel, D., Hurwitz, R., Young, E., Suzuki, K. & Sandhoff, K. (1993) Molecular genetics of G_{M2} gangliosidosis AB variant: A novel mutation and expression in BHK cells. *Hum. Genet.* **92**, 437–440.

Spiegel, S., Foster, D. & Kolesnick, R. (1996) Signal transduction through lipid second messengers. *Curr. Opin. Cell Biol.* **8**, 159–167.

Taniike, M., Yamanaka, S., Proia, R.L., Langaman, C., Bonc-Turentine, T. & Suzuki, K. (1995) Neuropathology of mice with targeted disruption of Hexa gene, a model of Tay-Sachs disease. *Acta Neuropathol.* **89**, 296–304.

Vielhaber, G., Hurwitz, R. & Sandhoff, K. (1997) Biosynthesis, processing, and targeting of sphingolipid activator protein (SAP) precursor in cultured human fibroblasts. Mannose 6-phosphate receptor-independent endocytosis of SAP precursor. *J. Biol. Chem.* **272**, 32438–32446.

Walz, G., Aruffo, A., Kolanus, W., Bevilacqua, M. & Seed, B. (1990) Recognition by ELAM-1 of the sialyl-Le^x determinant on myeloid and tumor cells. *Science* **250**, 1132–1135.

Wiegandt, H. (1985) Gangliosides; in *New Comprehensive Biochemistry* (Neuberger, V.A. & van Deenen, L.L.M., eds.) vol. 10, pp. 199–260, Elsevier, Amsterdam.

Yamanaka, S., Johnson, M.D., Grinberg, A., Westphal, H., Crawley, J.N., Taniike, M., Suzuki, K. & Proia, R.L. (1994) Targeted disruption of the *Hexa* gene results in mice with biochemical and pathologic features of Tay–Sachs disease. *Proc. Natl. Acad. Sci. U.S.A.* **91**, 9975–9979.