Dynamics & Biogenesis of Membranes

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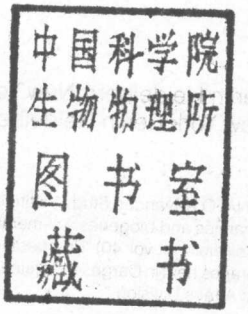

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PREFACE

The NATO Advanced Study Institute on "Dynamics and Biogenesis of Membranes" intended to provide an overview on both the structural and dynamic aspects of individual membrane constituents as well as on the processes and mechanisms involved in the assembly of these compounds into well organised biological membranes. Emphasis was laid in particular on the mutual interactions between the proteins, lipids and their glycosylated derivatives. The progressive increase in our knowledge of membrane biogenesis is due in particular to the application of new techniques and approaches in biophysics, genetics, biochemistry and molecular biology. In order to illustrate this and to achieve an extensive overview and a broad perspective of this complex field, lecturers originating from various disciplines contributed to the ASI. The cross fertilization of these disciplines and the combination and integration of the new developments is reflected in the present proceedings.

Much progress has been made in recent years in the elucidation of the structure, biosynthesis and functional properties of fatty acylated proteins and of complex, glycosylated lipids such as the glycosphingolipids and the glycosylated phosphatidylinositols. Furthermore, the biosynthetic routes of glycosylated proteins, in particular the topological aspects, have been studied in great detail. A substantial portion of this information originated from studies of mutants and detailed overviews on these and other important aspects of glycolipid and glycoprotein biogenesis are presented.

Mutual interactions between lipids and between lipids and proteins are the major stabilizing forces responsible for the structure of biological membranes. The various physico-chemical aspects of these interactions, including the possible occurrence of non-bilayer configurations of membrane lipids, are presented and the implications of the data for both membrane protein function as well as for protein insertion and protein translocation are discussed in detail.

Another aspect of membrane biogenesis which has drawn much attention in the past decade concerns the routes and mechanisms of intracellular traffic of membrane(fractions) and individual membrane constituents. Continuous movement of membranous material is essential for membrane repair, membrane multiplication during cell division, endo- and exo-cytosis and various other intracellular events. In particular the mechanisms by which proteins move through the cell and its membranes have been studied in great depth. A fair number of reports and overviews, dealing with protein signalling, targeting, export, translocation and insertion in a variety of biological systems, gives an impression of the current state of affairs in this fast evolving area of research.

Utrecht, October 1989

Jos A.F. Op den Kamp

CONTENTS

GLYCOLIPIDS - INTRACELLULAR MOVEMENT AND STORAGE DISEASES

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Glycosphingolipids (GSL) are components of plasmamembranes of animal cells. They are anchored in the cellular membrane by their hydrophobic ceramide (N-acylsphingosine) moiety, while the hydrophilic mono- or oligosaccharide part faces the extracellular space. Together with glycoproteins and glycosaminoglycanes glycosphingolipids form the glycocalix of cell surfaces. The GSL-patterns are characteristic for individual cell types, stages of differentiation and oncogenic transformation (Hakomori 1980; van Echten and Sandhoff, 1989). Though some of the sialic acid containing GSL have been identified as binding sites for toxins and viruses (Yamakawa and Nagai, 1978; Markwell et al., 1981), their physiological functions remain obscure. The structure of about 100 GSL could be elucidated thus far. Sialic acid-containing GSL, called gangliosides, are typical lipids of neuronal surfaces and are predominant in the grey matter (Lowden and Wolfe, 1964; Derry and Wolfe, 1967). Sulfatide and galactosylceramide as main components of myelin prevail on oligodendrocytes while glycolipids of the globo-series predominate on fibroblasts. Any disorder in metabolism of GSL would mainly affect those tissues in which the correspondent GSL is concentrated.

In order to get information about localization, intracellular movement and metabolism of GSL in cultured cells, different derivatives such as spin-, radio-, fluorescent- and biotin-labelled GSL-species, especially of gangliosides, were synthesized (Schwarzmann and Sandhoff, 1987). Studies with exogenous spin-labelled gangliosides in cell culture showed that they are

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slowly incorporated into the plasma membrane, where they obtain a position which is similar to that of endogenous gangliosides (Schwarzmann et al., 1983, 1984).

Feeding and metabolic experiments with different derivatives of gangliosides show that they participate in intracellular membrane flow after insertion into the plasma membrane. They may enter the lysosomal compartment to get degraded by the action of hydrolases, or, to a smaller extent, reach the Golgi, where they can be used for the biosynthesis of more complex GSL (Sonderfeld et al., 1985; Sandhoff et al., 1987). Experiments with double labelled gangliosides indicate that those GSL reaching the Golgi-complex are transported directly to this compartment, without passing through lysosomes (Klein et al., 1988). Direct glycosylation in the Golgi was observed with gangliosides G_{M2}, G_{M1} and G_{D1a} as well as with the synthetic amides of G_{M2} and G_{M1}. Electron microscopy studies with biotinylated ganglioside G_{M1} in fibroblasts support this view (Schwarzmann et al., 1986). In contrast, when Farber disease cells were fed with ganglioside G_{M3} or normal cells with G_{M3}-amide respectively, no synthesis of more complex gangliosides could be observed. This indicates that these GSL do not enter the biosynthetic pathway even when reaching the Golgi complex (Klein et al., 1987). However, due to the low activity of N-acetylgalactosaminyl-transferase in Golgi vesicles from rat liver (Pohlentz et al., 1988; Iber et al., 1989) it cannot be ruled out that reglycosylation of these GSL takes place very slowly, below the detection limits of the procedures used. The current understanding of intracellular routing of GSL is summarized in figure 1.

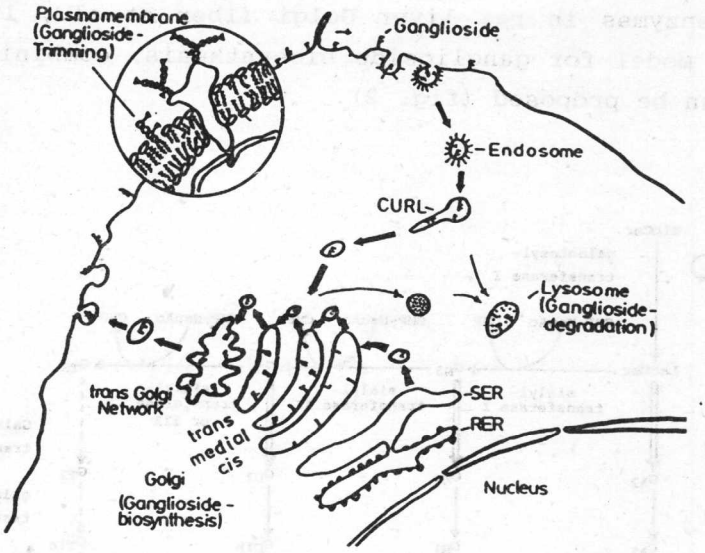

Fig. 1. Model of intracellular transport of exogenous gangliosides in cultured cells (Sandhoff et al., 1988).

Biosynthesis of GSL starts with ceramide formation in the endoplasmatic reticulum. Wether the synthesis of either glucosylceramide or galactosylceramide also takes place in the endoplasmatic reticulum (Suzuki et al., 1984) or at the cytosolic side of the Golgi apparatus (Coste et al. 1985, 1986) is a question which yet has to be clarified. All the other glycosyl- and sialyltransferases involved in GSL-biosynthesis are located in Golgi-cisternae. These membrane-bound enzymes act by sequential addition of galactose, N-acetylgalactosamine and sialic acid to the growing oligosaccharide chain, using the corresponding sugar nucleotides. Inhibitors of sugar nucleotide transport across the Golgi membrane such as tunicamycin, block ganglioside biosynthesis (Yusuf et al., 1983). Competition experiments using lactosylceramide, ganglioside G_{M3} and ganglioside G_{D3} as substrates suggested that N-acetylgalactosamine transfer in rat liver Golgi, leading to gangliosides G_{A2}, G_{M2} and G_{D2}, respectively, is catalyzed by a single enzyme. The same could be shown for the reaction leading to gangliosides G_{M1b}, G_{D1a} and G_{T1b} (Pohlentz et al., 1988). Very recent studies proved G_{A1}-, G_{M1}- and G_{D1b}-synthases to be

identical enzymes in rat liver Golgi (Iber et al., 1989), so that a new model for ganglioside biosynthesis, combining those results, can be proposed (fig. 2).

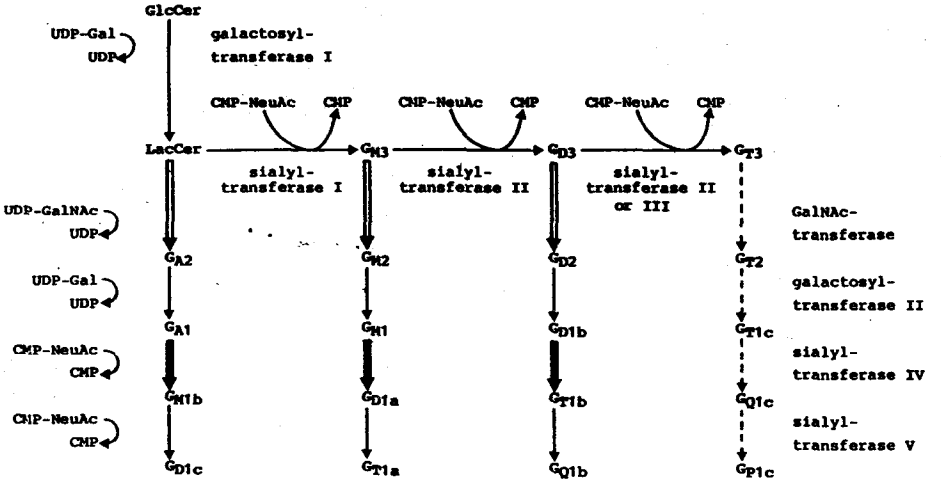

Fig. 2. Proposed model for ganglioside biosynthesis (Pohlentz et al., 1988; Iber et al., 1989). In this model successive transfer of N-acetylgalactosamine, galactose, and sialic acid to lactosylceramide, G_{M3} and G_{D3} (and possibly G_{T3}) leading to the asialo, a and b (and possibly c series) gangliosides is catalyzed by the same set of glycosyltransferases. ⟶, reactions not yet demonstrated in rat liver Golgi. ⟹, reactions catalyzed by one and the same N-acetylgalactosaminyl-transferase. ➡, reactions catalyzed by one and the same sialyltransferase.

Biosynthesis of glycosphingolipids is accompanied by an intracellular vesicle-bound membrane flow. The growing molecule is transported from the endoplasmatic reticulum through the Golgi cisternae to the plasma membrane, following the principle that individual biosynthetic steps correlate with compartmentation. Drugs affecting intracellular membrane flow or modulators of the cytoskeleton have strong inhibitory effects on ganglioside biosynthesis in murine cerebellar cells (van Echten and Sandhoff, 1989). However, the regulation of ganglioside biosyn-

thesis is poorly understood. There is evidence, that ganglioside biosynthesis is under transcriptional control of the respective glycosyltransferases (Hashimoto et al., 1989 a, b; Nakakuma et al., 1984). On the other hand, in vitro studies allow for an additional feedback control (Yusuf et. al., 1987).

GSL degradation takes place in the lysosome and is catalyzed by exohydrolases, which remove the sugar residues in a stepwise manner from the non-reducing end of the oligosaccharide chain (Sandhoff and Conzelmann, 1984; Sandhoff et al., 1987) (fig. 3).

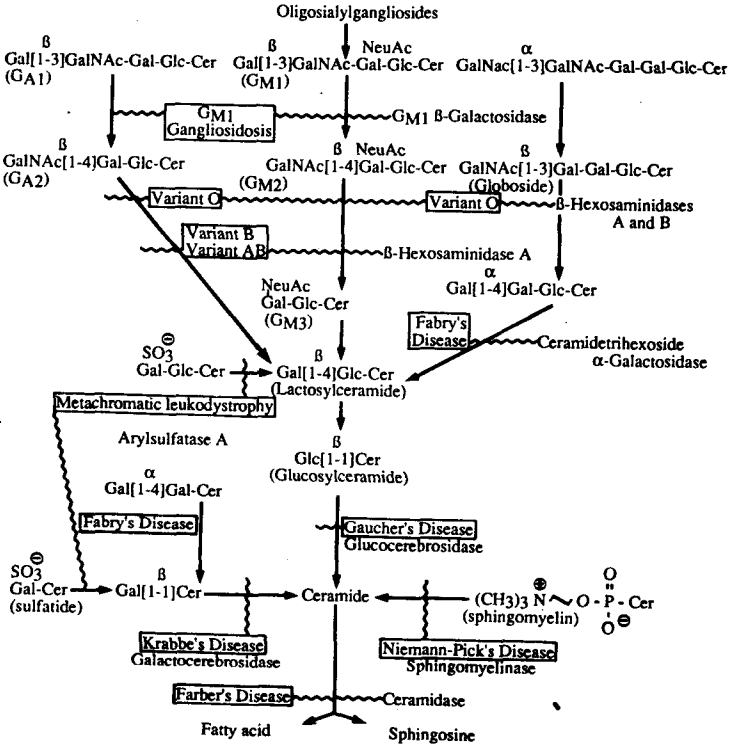

Fig. 3. Degradation scheme of sphingolipids denoting metabolic blocks of known diseases (Sandhoff and Christomanou, 1979).

Any defect of one of these enzymes results in the accumulation of the lipid compounds, which, due to their poor water solubility, precipitate within the lysosome, leading to progressive storage, swelling and finally dysfunction and death of the cells. The clinical symptoms of the resulting diseases depend on the kind of affected tissue or cell type. Though the reason for sphingolipidoses is simply an inherited defect of one catabolic step, there is a great heterogeneity of the respective diseases at the biochemical as well as at the clinical level (for reviews, see, e.g. Conzelmann and Sandhoff, 1984, Sandhoff et al. 1989).

In vitro analysis revealed that many of the lysosomal hydrolases are stimulated by the action of sphingolipid activator proteins (Conzelmann and Sandhoff, 1987 a, b). The function of some activator proteins is to extract the lipid from the membrane, forming a water-soluble complex, which enables the lysosomal enzyme to act upon its substrate. The G_{M2} activator protein is one of the best characterized lysosomal sphingolipid binding proteins; it is a monomeric glycoprotein consisting of one subunit containing 162 amino acids and one N-linked oligosaccharide chain (Fürst et al., 1989). This activator protein forms a stoichiometric complex with ganglioside G_{M2} and its analogues, utilizing a hydrophobic binding site for the ceramide residue and a hydrophilic one for the N-acetylgalactosamine and sialic acid residues of ganglioside G_{M2}. The whole complex is the substrate for the enzyme hexosaminidase A (Hex A) (Sandhoff and Conzelmann, 1984).

Hexosaminidase A is composed of two subunits, α and β, each possessing one catalytic site (Kytzia and Sandhoff, 1985). Only the α subunit is able to degrade activator-bound ganglioside G_{M2}, explaining why patients with variant B_1 of G_{M2}-gangliosidosis accumulate ganglioside G_{M2} in spite of showing Hex A activity against is synthetic substrate 4-methylumbelliferyl-β-D-N-acetylglucosaminide. The Hex A of those patients carries a point mutation in the α active site, the β-chain active site

being unaffected (Kytzia et al., 1983, 1985; Tanaka et al., 1988). The model shown in Fig. 4 explains the biochemical heterogeneinity of G_{M2}-gangliosidosis. Mutations in any of the three polypeptides (α-subunit, β-subunit or activator protein) would lead to a defect in G_{M2}-ganglioside catabolism, causing accumulation of this lipid in neuronal tissues of the affected patient.

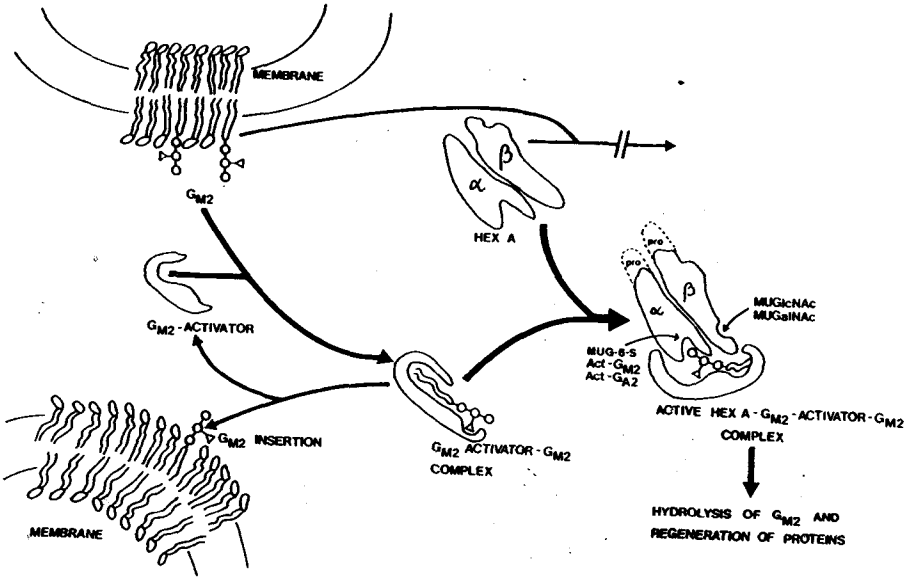

Fig. 4 Model for the lysosomal catabolism of ganglioside G_{M2} (Conzelmann and Sandhoff, 1979; Sandhoff and Conzelmann, 1984; Conzelmann et al., 1982). Hexosaminidase A cannot attack membrane-bound ganglioside G_{M2}. Instead, the ganglioside is extracted from the membrane by the activator protein and the water-soluble activator/lipid complex is the substrate for the enzymatic reaction. Of the two catalytic sites on hexosaminidase A, only the one on the α-subunit cleaves ganglioside G_{M2}. The hexosaminidase precursor ("prohex A") is also fully active on the activator/G_{M2} complex (Hasilik et al., 1982). After the reaction the product, ganglioside G_{M3}, is reinserted into the membrane and the activator protein is available for another round of catalysis.

Other activator proteins and cofactors in GSL-catabolism have been described. (Conzelmann and Sandhoff 1987 b; d'Azzo et al.,

1982). In contrast to the G_{M2} activator protein, the other proteins examined thus far are less specific and their mechanism of function is less clear. For example, the sulfatide-G_{M1} activator protein is able to bind a variety of glyco- and phospholipids (Conzelmann and Sandhoff, 1987 b). It has been proposed that it acts as a kind of physiological detergent, solubilizing the lipids that would otherwise not be accessible to water-soluble hydrolases (Fischer and Jatzkewitz, 1978). The sulfatide-G_{M1} activator protein is formed by proteolytic cleavage of a large precursor protein (Fürst et al., 1988). Recently it has been shown that this precursor protein is processed to four homologous proteins, the sulfatide activator protein, an activator protein for glucosylceramidase (A_1 activator) (Kleinschmidt et al., 1987), and two proteins with unknown function, respectively (Fürst et al., 1988; O'Brien et al., 1988; Nakano et al., 1989).

Allelic mutations affecting proteins involved in sphingolipid degradation may have consequences on synthesis of mRNA, formation of pre-pro-proteins, their intracellular targeting and processing, catalytic activities and substrate specifities of the mature enzymes, their stability against lysosomal proteases, temperature and / or pH-changes (Scriver et al., 1989). The biochemical heterogeneity is increased by the possibility of alternate splicing of hn-mRNA transcripts (Quintern et al., 1989), which was also observed in the case of α- and β-chain mutations of hexosaminidase A (Ohno and Suzuki, 1988; Navon and Proia, 1989; Sandhoff et al., 1989).

However, the molecular analysis of lysosomal storage diseases at the protein or genomic levels so far could not explain the frequently observed heterogeneity of clinical syndromes, such as the occurrence of infantile, juvenile and adult forms.

In G_{M2}-gangliosidosis as in metachromatic leukodystrophy, a disorder in sulfatide catabolism, these clinical forms show small but consistent differences in the residual activities of

the affected enzymes, hexosaminidase A and arylsulfatase A, respectively (Conzelmann et al., 1983; Lee-Vaupel and Conzelmann, 1987). In order to understand the significance of variations in the lower range of residual enzyme acitivities for the development of different clinical forms of a disease, a hypothesis was proposed, based on model calculations performed on the steady-state substrate concentration and the degradation rate of the lipid substrate as a function of the residual enzyme activity (Conzelmann and Sandhoff, 1983/84) (Fig. 5).

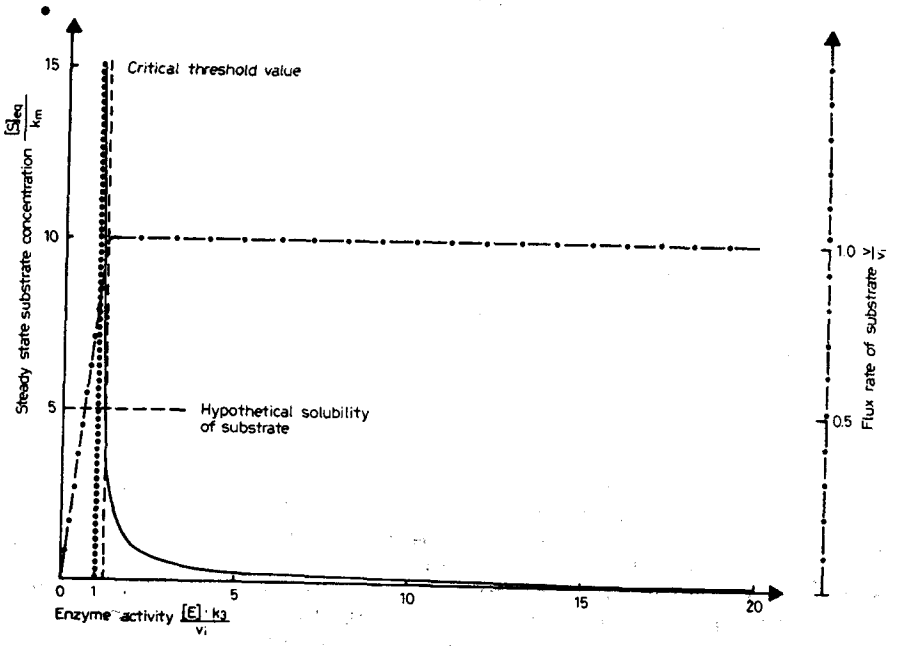

Fig. 5. Steady state substrate concentration as a function of enzyme concentration and activity (Conzelmann and Sandhoff, 1983/84). The model underlying this theoretical calculation assumes influx of the substrate into a compartment at a constant rate (v_i) and its subsequent utilization by the enzyme. ——— = $[S]_{eq}$, steady state concentration; ····· = theoretical threshold of enzyme avtivity; --- = critical threshold value, taking limited solubility of substrate into account; ·-·-· = turnover rate of substrate (flux rate)

Measurements on the degradation rate of lipid substrates and residual enzyme activities in cultured skin fibroblasts from patients with different clinical forms of metachromatic leukodystrophy or G_{M2}-gangliosidosis, variant B, respectively, could confirm this hypothesis (Conzelmann et al., 1988; Leinekugel P., Michel S., Conzelmann, E., Sandhoff K. manuscript in preparation). Supporting the proposed model, the results show that variation of the enzyme activity lead within a wide range only to changes in the steady state substrate concentration but not to accumulation of substrate. However, if the enzyme activity falls below a critical threshold, the decrease of activity can no longer be compensated for by a higher saturation of the enzyme, thus lysosomal storage of the substrate occurs. Below this critical threshold, small differences in residual enzyme activities have significant effects on substrate accumulation. This may explain that patients with small differences in the activities of the affected enzyme show a wide variation in age of onset, progression and severity of the disease.

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