Chapter 14
Role of Gangliosides and Plasma Membrane-Associated Sialidase in the Process of Cell Membrane Organization

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Glycosphingolipids are amphiphilic membrane lipids characterized by the presence of a long-chain (C18 or C20) amino alcohol, which has the trivial name "sphingosine." Glycosphingolipids are components of all eukaryotic cell membranes, and gangliosides (glycosphingolipids containing sialic acid residues in their oligosaccharide chains) are particularly abundant in the plasma membranes of neurons. As sphingolipids are concentrated at the subcellular level in the plasma membrane, where they reside asymmetrically in the extracellular leaflet, they are relatively abundant in this district. Keeping in mind that sphingolipids are not homogeneously distributed throughout the membrane plane but rather are concentrated in restricted membrane areas [1] due to their spontaneous segregation with respect to glycerophospholipids, it can be predicted that their local concentration in specific "lipid membrane domains" would be very high.

14.1 Regulation of Plasma Membrane Glycosphingolipid Composition by Biosynthesis and Degradation

The regulation of plasma membrane glycosphingolipid composition is of crucial importance for cell biology and is mainly dependent on the biosynthetic and catabolic processes occurring within the cells. Both biosynthesis and degradation take place in intracellular districts, thus the turnover of plasma membrane sphingolipids is intimately connected with the bidirectional flow of molecules from and to the plasma membrane that mainly occurs via vesicular traffic, even if nonvesicular

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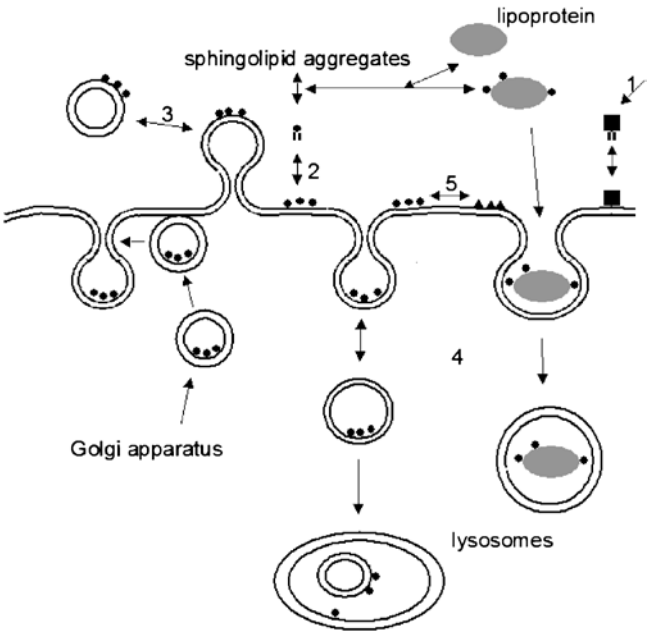

Fig. 14.1 Different metabolic pathways possibly involved in changing plasma membrane glycosphingolipids. The following pathways can be altered by modulating the enzyme activities/expressions or process rates. (1) Plasma membrane uptake of extracellular glycolipids shed by different cells; (2) shedding of glycolipid monomers (some directly reenter the membrane, while others interact with the extracellular proteins or lipoproteins and are subsequently taken up by the cells and catabolized into lysosomes); (3) release of glycolipid-containing vesicles from the plasma membrane; (4) membrane endocytosis followed by sorting to lysosomes and lysosomal catabolism; (5) biosynthetic modifications by plasma membrane-associated glycosyltransferases and glycosidases

transport via sphingolipid binding proteins plays an important role in specific steps (Fig. 14.1) [2–4].

The de novo biosynthetic pathway of sphingolipids starts at the cytosolic face of the endoplasmic reticulum, where enzyme activities responsible for the reaction sequence leading to the formation of ceramide are localized. The neosynthesized ceramide reaches the Golgi apparatus by a not-yet-known mechanism, where it is used as the common precursor of glycosphingolipids. Different membrane-bound glycosyltransferases are responsible for the sequential addition of sugar residues to the ceramide, leading to the growth of the oligosaccharide chain. Glucosylceramide is the first glycosylated product, formed by a ceramide glucosyltransferase activity localized at the cytosolic side of the early Golgi membrane. Glucosylceramide can either directly reach the plasma membrane [5], presumably transported in a nonvesicular way, or be translocated to the luminal side of the Golgi, where it is further glycosylated by other glycosyltransferases located in this cellular district to generate more complex glycosphingolipids. Neosynthesized glycosphingolipids move through the Golgi apparatus to the plasma membrane following the mainstream exocytotic vesicular traffic.

The enzymology and the intracellular topology of the de novo biosynthesis of glycosphingolipid have been unveiled in their details, whereas very little is known about its regulation, which has been regarded for a long time as the main mechanism responsible for the formation of a specific glycosphingolipid pattern. It is generally assumed that glycosphingolipid synthesis is mainly regulated at the transcriptional level through the control of the expression levels of all the enzymes involved in the synthesis (glycosyltransferases) or the trafficking among the different intracellular districts (transporter proteins). Indeed, changes in the expression of glycosyltransferases have been observed in several phenomena characterized by changes in cellular glycosphingolipid patterns, such as those occurring during neuronal development, oncogenic transformation, or acquisition of drug resistance in tumor cells. However, the possibility that differential intracellular flows of different glycosphingolipids could influence the resulting glycosphingolipid patterns (independently from the expression levels of relevant glycosyltransferases) should not be neglected [6]. In other words, the regulation of intracellular sphingolipid traffic might be as important as the control of synthetic enzyme expression in determining the final glycosphingolipid composition of the plasma membrane.

Another important point about the regulation of plasma membrane glycosphingolipid composition is the degradation that takes place in the lysosomes, where glycosphingolipids are transported by the endocytic vesicular flow through the early and late endosomal compartment to be catabolized. During this retrograde transport from plasma membrane to lysosomes, some glycosphingolipids, originally residents in the plasma membrane, can be diverted to different intracellular sites (presumably the Golgi apparatus), where they undergo direct glycosylation with the formation of more complex products and thus are able to return to the plasma membrane. It has been suggested that this process might be quantitatively relevant for certain cell types, including neurons [7], thus being another potential mechanism for regulating plasma membrane ganglioside composition at the level of intracellular traffic. Analogously, intermediate or final degradation products can escape the lysosomes and be recycled along the biosynthetic pathway. The salvage pathways for gangliosides in neurons should not be neglected from the quantitative point of view [8], but very little is known about the mechanisms of escape from the lysosome, the transfer of these intermediates to the Golgi or other cellular districts, or the regulation of these processes. The presence of soluble ganglioside–protein complexes in the cytosol, as reported by some authors [9–12], might reflect the intracellular traffic linked to the recycling of these intermediates.

14.2 Biological Functions of Glycosphingolipids and the Importance of Their Local Concentration

Glycosphingolipids are essential for the survival, proliferation, and differentiation of eukaryotic cells within complex multicellular systems (i.e., tissues). This becomes particularly evident when cellular or animal models, lacking the activity of some of the enzymes involved in glycosphingolipid metabolism, are used.

Important observations about the vital importance of glycosphingolipids in the "social life" of cells, i.e., in cells that are dealing with a multifaceted extracellular reality, have been made in comparing cellular models lacking ceramide glucosyltransferase activity with the corresponding animal model. In the GM-95 mutant melanoma cell line [13], ceramide glucosyltransferase activity is absent. This enzyme catalyzes the synthesis of glucosylceramide, a common glycosylation step in the biosynthetic pathway of all glucosylceramide-based complex glycosphingolipids. In the same way, embryonic stem cells derived from ceramide glucosyltransferase knockout mice [14] become glycolipid- deficient cells. Like the GM-95 cells, they are able to survive, grow, and undergo in vitro differentiation as well as their counterparts expressing the enzyme activity. However, ceramide glucosyltransferase knockout mice are embryonically lethal and showed no cellular differentiation beyond the primitive germ layers [15].

As already mentioned, glycosphingolipids are not randomly distributed along the membrane surface, but they are rather highly segregated with cholesterol in lipid domains with specialized signaling functions [1], typically referred to as "lipid rafts." In these lipid domains, glycosphingolipids modulate the functional features of several membrane proteins through direct specific lipid–protein interactions or through the maintenance of a dynamic membrane organization. Thus, these complex membrane lipids participate in the modulation of several processes, such as cell proliferation, survival, adhesion, and cell differentiation.

A high local concentration of glycosphingolipids in the plasma membrane has important implications with regard to their ability to engage both *trans* and *cis* functional interactions with other cellular components. In the first case, the recognition of lipid-bound oligosaccharides by soluble ligands (such as antibodies or toxins) or by complementary carbohydrates and carbohydrate-binding proteins (such as selectins, siglecs, and other lectins) belonging to the interfacing membrane of adjacent cells is strongly affected by their degree of dispersion (or segregation) [16]. In the same way, sphingolipid-enriched membrane domains could favor *cis* interactions, i.e., direct lateral interactions with plasma membrane proteins or short-range alterations of the lipid microenvironment of plasma membrane proteins [16].

During the development of the nervous system and along differentiation in cultured neurons, the glycosphingolipid patterns undergo deep qualitative and quantitative modifications [17–25], and this reflects the crucial role played by gangliosides in controlling various aspects of neural cell function [26, 27], as suggested by several experimental observations.

The study of glycosphingolipid biological functions has been pursued for a long time by several experimental approaches; one of the widely used experimental models is the exogenous administration of gangliosides dissolved in culture medium to intact cells or membrane preparations. The binding, uptake, and metabolic fate of exogenous gangliosides under different experimental conditions have been well characterized [4, 28], and it has been shown that, after removing the amount of administered ganglioside loosely bound to the membrane, a portion of the stably associated ganglioside was inserted into the membrane. As a consequence,

the subsequent cellular events can be ascribed to the resulting modifications of membrane composition and organization [29–32]. The addition of exogenous gangliosides resulted in the modulation of the biological activity of several proteins as tyrosine kinase receptors, protein kinases and phosphatases, ion channels, and pumps. Moreover, this exogenous addition is able to exert neuritogenic, neurotrophic, and neuroprotective effects on cultured neurons and neurotumor cell lines [20, 26, 30].

As supported by many study results, the differentiation and function of neurons in culture is strongly dependent on sphingolipid biosynthesis. In neuroblastoma cell lines, for example, the ability to extend neurites in response to various stimuli was correlated with the cellular gangliotetraose content [33], and treating neuroblastoma cells with *Clostridium perfringens* sialidase increased surface expression of GM1 and potentiated PGE1-induced neurite formation [33, 34].

Several pharmacological approaches could influence neuronal function and normal differentiation, causing either inhibition or upregulation of specific enzymes involved in glycosphingolipid metabolism. The inhibition of glycosphingolipid biosynthesis by synthetic inhibitors of glucosylceramide synthase (D-threo-1-phenyl-2-decanoylamino-3-morpholino-1-propanol [D-PDMP] and analogs) [35] or by inhibitors of sphinganine N-acyltransferase (the enzyme that catalyzes the synthesis of dihydroceramide, the biosynthetic precursor of ceramide and of all complex sphingolipids) [36], such as *Fusarium moniliforme* mycotoxins (fumonisins), caused a reduction in axonal elongation and branching in cultured hippocampal and neocortical neurons [37–39], and nerve growth factor (NGF)-induced neurite outgrowth in human neuroblastoma and PC12 cells [40, 41]. Conversely, upregulation of glycosphingolipid biosynthesis by L-PDMP stimulated neurite outgrowth in cultured cortical neurons [39, 42]. In the same cellular model, D- and L-PDMP exerted opposite effects on the formation of functional synapses and synaptic activity [42]. Induced expression of GD3 synthase was able to switch neuroblastoma cells to a differentiated phenotype [43]. NGF- and forskolin-induced differentiation in PC12 was accompanied by the upregulation of several glycosyltransferase activities (GalGb3-, GM3-, GD1a-, and GM2 synthases) [44], and basic fibroblast growth factor (bFGF)-stimulated axonal growth in cultured hippocampal neurons resulted in the activation of ceramide glucosyltransferase [45].

As mentioned above, the role of glycosphingolipids in the maintenance of neuronal structure and function can be explained, at least in part, by their ability to laterally interact with specific proteins (including growth factor receptors and neuronal adhesion molecules) at the level of the plasma membrane and to modulate their activity (*cis* interactions). Possible functionally significant interactions between gangliosides and plasma membrane proteins have been intensively studied in the past [27, 46, 47], and they usually resulted in being highly specific.

Well-studied examples are represented by the interactions of epidermal growth factor receptor (EGFr) or insulin receptor with tyrosine kinases. In the first case, the phosphorylation on tyrosine residues and the dimerization of EGFr are inhibited by GM3 but uninfluenced by GM1 [48]; the insulin receptor is inhibited by GM3 but not by GD1a [49]. In the nervous system, it has been reported that GM1 is able

to potentiate the neuritogenic effect of NGF in PC12 cells, i.e., it is able to induce neuronal differentiation in the presence of an NGF concentration that is ineffective by itself [50–52].

Within sphingolipid- and cholesterol-enriched membrane domains, glycosphingolipids and signaling proteins colocalize, and many papers have indicated that this could be sufficient for the realization of functional links, even in the absence of direct, strong, and specific glycosphingolipid–protein interactions. Thus, it is clear how the overall lipid raft dynamics, as determined by the peculiar (and possibly regulated) lipid composition of these domains, might be rather responsible for the functional modulation of raft-associated signaling proteins [1, 53–57]. Within lipid membrane domains isolated from cultured neural cells (neurons, oligodendrocytes, astrocytes, and neurotumor cell lines), brain tissues, myelin, and synaptic plasma membranes, it has been shown that sphingolipids (glycosphingolipids, sphingomyelin, and ceramide) and cholesterol segregate together with many classes of proteins involved in mechanisms of signal transduction that are relevant for neural cell biology (including receptor tyrosine kinases, G protein-coupled receptors, nonreceptor tyrosine kinases of the Src family, adapter and regulatory molecules of tyrosine kinase signaling, heterotrimeric and small guanosine triphosphate (GTP)-binding proteins, protein kinase C isoenzymes, cell adhesion molecules, ion channels, proteins involved in neurotransmitter release, and postsynaptic density complex proteins) [55, 58–66]. This specific protein enrichment of lipid membrane domains is in accordance with the functional role played by these domains in several aspects of nervous system development and functional specialization. Many pieces of evidence demonstrated that sphingolipid- and cholesterol-enriched membrane domains have been involved in neurotrophic factor signaling [55, 64–66], cell adhesion and migration [55, 67, 68], axon guidance, synaptic transmission [55, 69], neuron–glia interactions [56, 70], and myelin genesis [71]. In some cases, it has been shown that signal initiation and propagation in neural cells involve receptors and effectors that permanently reside in lipid membrane domains [55, 64–66, 72, 73]. Alternatively, the activation of membrane receptors is followed by the translocation of the receptors themselves or effector signaling proteins to or from the domain to other cellular districts [55, 64, 66, 68]. In both cases, these events imply changes in the reciprocal interactions among lipid membrane domain components. Sphingolipids play an active role in the regulation of these interactions, as has been reported in several papers. In rat cerebellar granule cells, an increase in the surface occupied by the sphingolipid- and cholesterol-enriched membrane domains during the different stages of development in culture has been observed [25]. In particular, during axonal sprouting and neurite extension, the sphingolipid–glycerophospholipid molar ratio more than doubled, and the maximum ganglioside density was reached in fully differentiated neurons. On the contrary, a high content of ceramide was found in the domains of aging neurons. By different experimental approaches, some interactions between gangliosides and proteins have been identified within the lipid membrane domains. Ganglioside GM3 has been found to be closely associated with c-Src and Csk in neuroblastoma Neuro2a cells [74], and in rat brain and cerebellar granule cells, GD3 was associated with the Src-family kinase Lyn and

the neural cell adhesion molecule TAG-1 [60, 75]. In these cells, a complex lipid environment seems to be essential for the interaction on the domain of c-Src, Lyn, Fyn, TAG-1, and prion protein [25, 59, 63, 76].

In differentiated rat cerebellar neurons, the membrane environment of PrP^C has been studied by immunoprecipitation experiments [76]. In the separated PrP^C-rich membrane domains, about 50% of the sphingolipids, cholesterol, and phosphatidylcholine present in the detergent-resistant sphingolipid-enriched membrane fraction have been found. The enrichments of all main sphingolipids in the PrP^C-rich membrane domains, including sphingomyelin, neutral glycosphingolipids, and gangliosides, were very similar to those in the detergent-resistant sphingolipid-enriched membrane fraction. Moreover, a complex pattern of proteins was associated with the PrP^C-enriched membrane domains, in a way depending on the existence of lipid-mediated interactions. Thus, the prion protein plasma membrane environment in differentiated neurons resulted in being a complex entity, with its integrity requiring a network of lipid-mediated noncovalent interactions rather than (or as well as) specific direct molecular interactions.

Further supporting the notion that glycosphingolipids are essential in lipid domain-dependent cellular events was the multifaceted evidence that experimental manipulations able to change the concentration or pattern of glycosphingolipids in the plasma membrane profoundly affect – together with the organization of lipid membrane domain – the association of protein components with the domain itself and lipid domain-dependent signal transduction. Administration of exogenous GM1 and GM3 induced dissociation of Csk (the physiological inhibitor of Src kinases) from the lipid domain in neuroblastoma cells, followed by c-Src activation and neuritogenesis [74].

Treatment with fumonisin B1 or with ceramide glucosyltransferase inhibitors was able to deplete a detergent-insoluble lipid membrane domain from the glycosphingolipids and GPI-anchored proteins (e.g., Thy-1 in hippocampal neurons) [77–82] and to impair lipid domain-mediated biological functions [83–90]. Selective depletion of cell-surface sphingolipids, achieved by treating living cells with bacterial sphingomyelinases [84, 91] or with endoglycoceramidase (which are able to remove the oligosaccharide chain from cell-surface glycosphingolipids) [72] reduced the amount of sphingomyelin in detergent-insoluble membrane fractions in neuroblastoma cells [84] and inhibited TAG-1 signaling in cerebellar neurons, respectively [72].

14.3 Local Plasma Membrane Events and Their Role in the Modulation of Glycosphingolipid Composition

The reduced molecular heterogeneity of the sphingolipid simple breakdown products – ceramide, sphingosine, and sphingosine-1-phosphate – greatly clarified the biological roles of this class of lipids, particularly when compared with the more complex glycosphingolipids. Moreover, the regulation of cellular sphingoid

levels in response to physiological stimuli is relatively simple for this kind of molecule and relies on the activity of a limited number of metabolic enzymes.

For a long time, the production of bioactive ceramide was regarded as being caused exclusively by sphingomyelin hydrolysis by sphingomyelinases [92]. It soon became clear that "signaling" sphingomyelinases are residents in the plasma membrane (as is the case for the Mg^{2+}-dependent neutral SMase) or translocated to it from intracellular sites upon stimulus (as happens for the acid SMase, usually described as the lysosomal enzyme involved in the catabolic degradation of sphingomyelin) and are active on plasma membrane sphingomyelin pool(s) [93, 94]. More recently, a sphingomyelin synthase enzyme activity (SMS2), encoded by a different gene than that of the Golgi enzyme, was also shown to be present at the plasma membrane [95]. Thus, ceramide and sphingomyelin levels within the plasma membrane are regulated by two different enzyme activities acting – in opposite directions – directly on the plasma membrane in response to changes in cellular physiology, without needing to sort any of the substrates to intracellular sites of metabolism. The sphingomyelin–ceramide interconversion on the plasma membrane leads to changes in membrane curvature, as schematically reported in Fig. 14.2. Sphingomyelin, as a component of the external leaflet of the membrane, participates with its large and hydrophilic head group to confer a positive curvature

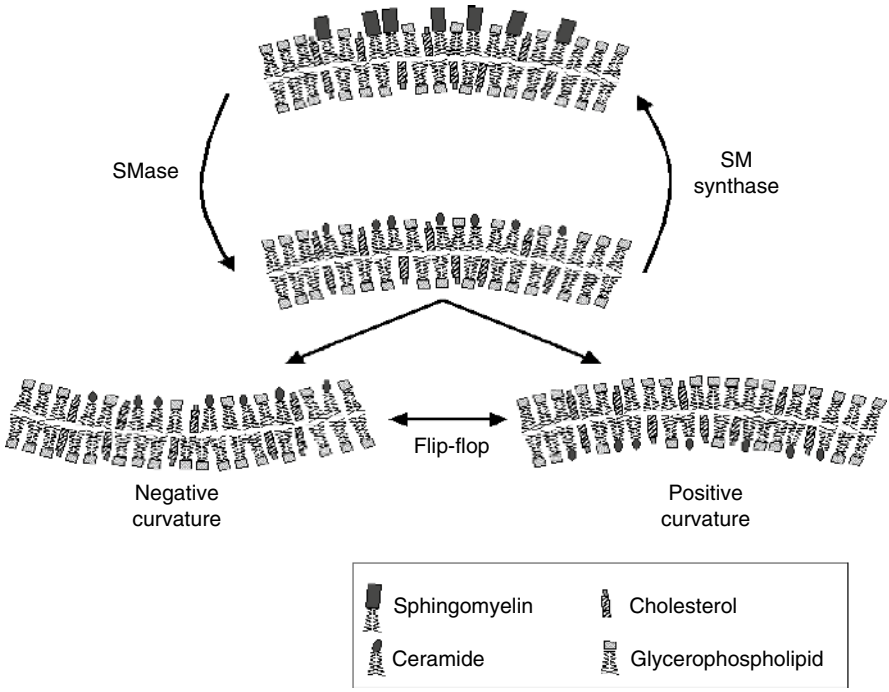

Fig. 14.2 Changes of membrane geometry and organization following plasma membrane-associated sphingomyelin–ceramide interconversion

to the cell surface. Ceramide, due to its much stronger hydrophobic character, is likely associated with a less positive, or negative, membrane curvature. Accordingly, ceramide formed enzymatically at the plasma membrane very rapidly should determine the formation of membrane areas with a negative curvature (or move to preexisting areas with these features). A further possibility is that a flip-flop process is thermodynamically favored, allowing ceramide to move from a positive to a negative membrane curvature. The information on sphingomyelin synthase is very scant, and no information is available on the membrane topology of this enzyme. In any case, due to geometrical considerations, sphingomyelin formed from ceramide belonging to a membrane area with a negative curvature will return to being a component of the extracellular leaflet of the membrane with positive curvature.

Similar observations have been made for other enzymes responsible for regulating bioactive sphingoid levels. Plasma membrane-associated ceramidases and sphingosine kinases have been described as putatively responsible for the generation of sphingosine and/or sphingosine-1-phosphate at the cell surface [96–98]. Thus, in the case of simple sphingoids, the role of the plasma membrane as the site for those metabolic events responsible for locally regulating sphingoid levels in response to specific biological events is well established, even if not fully unveiled.

In the case of glycosphingolipids, the number of enzymes responsible for their metabolism that have been shown to be associated with the plasma membrane is growing very rapidly, as is the information on their features, allowing for a precise characterization of some of them. A long time ago, it was shown that synaptosomal membranes, a subset of neuronal membranes highly enriched in gangliosides, carry both sialidase [99–102] and sialyltransferase [103] activity.

However, the existence of a plasma membrane-associated sialidase distinct from the lysosomal enzyme was suggested by enzymatic and immunological studies [104–109], as well as by metabolic studies of intact cells; cultured rat cerebellar granule and human neuroblastoma cells possessed the capability to desialylate exogenously added GM3, GD1a, and GD1b under experimental conditions, preventing ganglioside internalization and lysosomal function [110, 111]. In human neuroblastoma SK-N-MC cells, the desialylation of GM3 and polysialogangliosides, but not of GM1, was strongly inhibited by a cell-impermeable sialidase inhibitor [112]. The membrane-bound sialidase was purified from human brain gray matter [113] and from bovine brain [114] and further characterized [115]. In 1999, the existence of a specific membrane-linked sialidase, distinct from other known sialidases, was unambiguously proven by Miyagi's group, who cloned the complementary DNA (cDNA) sequence for human [116], bovine [117], and mouse [118] plasma membrane-associated sialidase, subsequently termed Neu3 [119]. Following studies elucidated the role of this enzyme in modifying the cell-surface ganglioside composition, causing a shift from polysialylated species to GM1, a decrease of GM3, and a parallel increase in lactosylceramide, with deep consequences on very important cellular events such as neuronal differentiation and apoptosis in colon cancer. In mouse and human neuroblastoma cells, Neu3 expression increased during pharmacologically induced neuronal differentiation [120],

and Neu3 gene transfection accompanied by a corresponding increase in the enzyme activity enhanced the extension or branching of neurites induced by 5-bromodeoxyuridine [118] or by dibutyryl cAMP treatment, and was sufficient by itself to induce neurite outgrowth [120]. Conversely, inhibition of plasma membrane sialidase activity resulted in the loss of neuronal differentiation markers [111, 121]. In cultured hippocampal neurons, the activity of the plasma membrane-associated ganglioside sialidase locally regulated GM1 surface levels and was essential for axonal growth and regeneration after axotomy [122]. In these cells, Neu3 activity was asymmetrically concentrated at the end of one single neurite and determined the neurite's axonal fate by a local increase in TrkA activity [123]. In colon and renal cancer, this sialidase seemed to be responsible for maintaining high cellular levels of lactosylceramide that would exert a Bcl-2-dependent antiapoptotic effect, contributing to the survival of cancer cells and consequent tumor progression [124, 125]. The nonrandom distribution of Neu3 at the cellular surface was confirmed by the observation that this ganglioside sialidase associated with Triton X-100 insoluble glycosphingolipid-enriched membranes [126] and closely associated with caveolin-1 in Neu3-transfected COS-1 cells [127]. The colocalization of Neu3 and its putative substrates at the cell surface is probably not surprising; on the contrary, it raises the possibility that the biological effects of this enzyme are due to the local reorganization of glycosphingolipid-based signaling units (Fig. 14.3).

Remarkably, the ability of Neu3 to modulate the cell-surface glycolipid composition was not restricted to *cis* interactions. In fact, mouse Neu3 overexpressed in COS-7 cells was able to hydrolyze ganglioside substrate belonging to the surface of neighboring cells [128]. Subsequently, it has been shown that Neu3 was able to modulate the production of bioactive ceramide at the cell surface when overexpressed in cultured skin fibroblasts, providing the first direct evidence on a link between glycosphingolipid metabolism and ceramide-mediated signaling [129]. Neu3-assisted cell-surface ceramide generation from ganglioside GM3 indirectly demonstrates the presence of the other two active glycosyl hydrolases, β-glucosidase and β-galactosidase, in the same plasma membrane district.

The presence of active β-hexosaminidase A in the external leaflet of plasma membrane has been also demonstrated in cultured fibroblasts [130]. Different from the sialidase, the immunological and biochemical characterization of the membrane-associated β-hexosaminidase suggested that this enzyme has the same structure as the lysosomal enzyme. Since it has been shown that the regulated fusion of lysosomes with the plasma membrane might be a general mechanism of repair for the plasma membrane [131], these observations open the possibility that other lysosomal glycolipid-metabolizing enzymes could reach the cell surface and play an active role in remodeling its glycolipid composition. However, the existence of specific membrane-associated isoenzymes for glycosyl hydrolases, other than sialidases, cannot be excluded. A lot of further experimental work will be needed to fully understand the real significance of these events, but it is indubitable that cell-surface hydrolysis of complex glycosphingolipids does occur.

Some information is also available about the in situ sialylation of gangliosides at the cell surface. The original report on the existence of a synaptosomal membrane

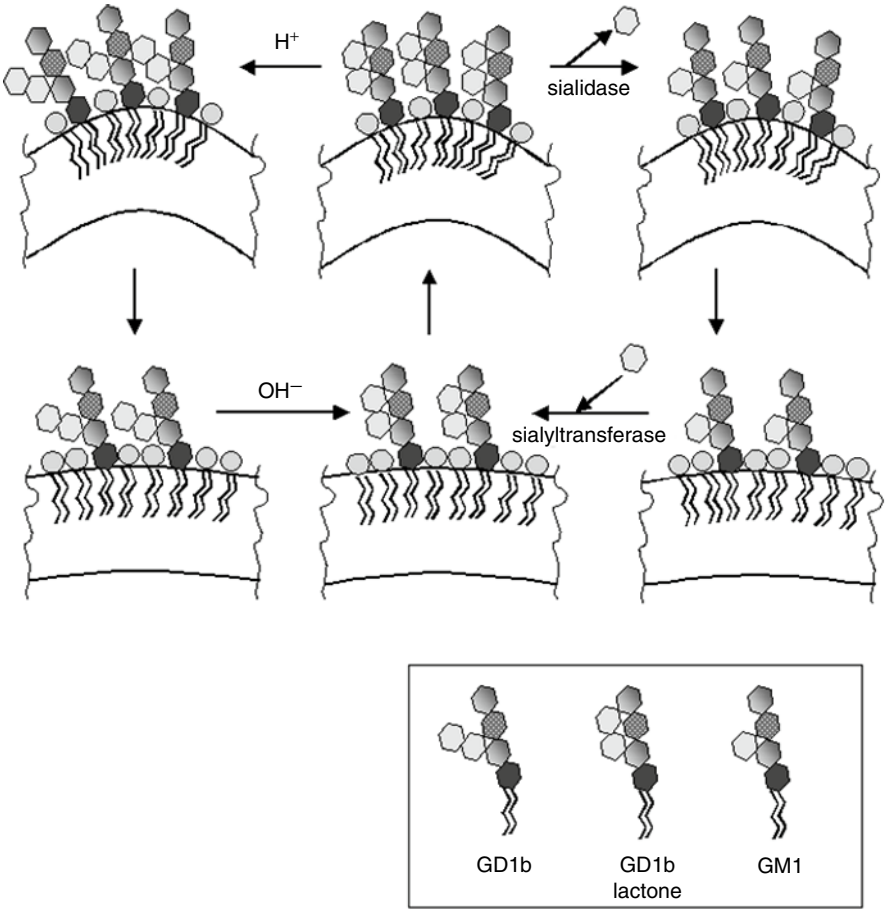

Fig. 14.3 Changes of membrane geometry and organization following plasma membrane-associated GD1b–GM1, or GD1b–GD1b lactone interconversion

sialyltransferase in calf brain [103] has been confirmed by metabolic studies in chicken embryos [132] and rat brain [133, 134]. More recently, it has been shown that dexamethasone treatment markedly increased GM3 synthesis due to enhanced gene expression and increased enzyme activity of GM3 synthase. Radiolabeling metabolic studies indicated that this event was localized at the plasma membrane [135], thus confirming that glycolipid sialylation might occur outside the Golgi compartment, contributing to the local modulation of cell-surface glycolipid patterns.

Glycosylation and deglycosylation pathways are not the only chance to modify the plasma membrane glycosphingolipid composition. In this sense, a very intriguing (even if very poorly understood) mechanism is the possible lactonization of gangliosides containing a disialosyl residue, such as GD1b. Ganglioside lactones are present as minor components in vertebrate brains [136, 137]. GD1b monolactone

formation in the presence of catalytic proton concentrations has been studied in vitro [138], and it has been shown that the lactonization process profoundly influenced the conformational, aggregational [139], and biological properties of GD1b [140]. GD1b is able to directly interact with several cellular proteins [59] and to modulate several plasma membrane-associated protein kinase activities [140]. But when gangliosides were lactonized, these properties were strongly reduced or lost [140, 141]. This suggests that lactonization/delactonization might be a localized event that is able to trigger specific ganglioside-mediated cellular events. Unfortunately, no information is available about the possible mechanism responsible for this conversion in vivo.

Metabolic remodeling is not the only local event that could contribute to the surface composition and organization of cell glycosphingolipids. It is known that glycosphingolipids and sphingolipids can be released from the cell surface to the extracellular milieu in the form of monomers or aggregates, including shedding vesicles [142–145]. Glycolipid-containing shedding vesicles seemed to originate from caveolin- and glycolipid-enriched membrane areas, thus their release could be used by the cell to modify the lipid membrane domain composition and organization. On the contrary, it has been suggested that shed gangliosides could be taken up by neighboring cells, modifying their lipid composition [146]. However, the metabolic fate of shed glycolipids after reuptake seems oriented toward degradation, thus the contribution of this event to the determination of cell lipid composition remains unclear [144].

14.4 Summary

A long time ago, it was shown that synaptosomal membranes, a subset of neuronal membranes highly enriched in glycosphingolipids, particularly gangliosides, carry both a sialidase and a sialyltransferase activity on sialoglycolipids. The existence of a plasma membrane-associated ganglioside sialidase distinct from the lysosomal enzyme has been suggested by enzymatic, immunological, and metabolic studies, and then unambiguously proven by cloning the cDNA sequence for the human, bovine, and mouse enzyme, subsequently termed Neu3. In neuroblastoma cells, Neu3 expression increased during pharmacologically induced neuronal differentiation, and Neu3 gene transfection, accompanied by a corresponding increase in the enzyme activity, enhanced the extension or branching of neurites induced by 5-bromodeoxyuridine and was sufficient by itself to induce neurite outgrowth. Conversely, inhibition of plasma membrane sialidase activity resulted in the loss of neuronal differentiation markers. In cultured hippocampal neurons, the activity of the plasma membrane-associated ganglioside sialidase locally regulated GM1 surface levels and was essential for axonal growth and regeneration after axotomy. In colon and renal cancer, this sialidase seemed to be responsible for maintaining high cellular levels of lactosylceramide, which would exert a Bcl-2-dependent antiapoptotic effect, contributing to the survival of cancer cells and

consequent tumor progression. Remarkably, the ability of Neu3 to modulate the cell-surface glycolipid composition was not restricted to *cis* interactions. In fact, mouse Neu3 overexpressed in COS-7 cells was able to hydrolyze ganglioside substrate belonging to the surface of neighboring cells. More recently, it has been shown that Neu3 was able to modulate the production of bioactive ceramide at the cell surface when overexpressed in cultured skin fibroblasts, providing the first direct evidence of a link between glycosphingolipid metabolism and ceramide-mediated signaling. Neu3-assisted cell-surface ceramide generation from gangliosides indirectly implies the presence of other glycosyl hydrolases (β-glucosidase and β-galactosidase) in the same plasma membrane district. In addition to this, the presence of active β-hexosaminidase A in the external leaflet of plasma membrane has been demonstrated in cultured fibroblasts.

Much less information is available about the possible in situ sialylation of gangliosides at the cell surface. Nevertheless, the original report on the existence of a synaptosomal membrane sialyltransferase has been confirmed by several metabolic studies.

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