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Functional roles of glycosphingolipids in signal transduction via lipid rafts

Kohji Kasahara* and Yutaka Sanai

The Tokyo Metropolitan Institute of Medical Science, Tokyo Metropolitan Organization for Medical Research, Bunkyo-ku, Tokyo 113-8613, Japan. E-mail: kasahara@rinshoken.or.jp; sanai@rinshoken.or.jp

The formation of glycosphingolipid (GSL)-cholesterol microdomains in cell membranes has been proposed to function as platforms for the attachment of lipid-modified proteins, such as glycosylphosphatidylinositol (GPI)-anchored proteins and src-family tyrosine kinases. The microdomains are postulated to be involved in GPI-anchored protein signaling via src-family kinase. Here, the functional roles of GSLs in signal transduction mediated by the microdomains are discussed. Antibodies against GSLs co-precipitate GPI-anchored proteins, src-family kinases and several components of the microdomains. Antibody-mediated crosslinking of GSLs, as well as that of GPI-anchored proteins, induces a rapid activation of src-family kinases and a transient increase in the tyrosine phosphorylation of several substrates. Enzymatic degradation of GSLs reduces the activation of src-family kinase and tyrosine phosphorylation by antibody-mediated crosslinking of GPI-anchored protein. Furthermore, GSLs can also modulate signal transduction of immunoreceptors and growth factor receptors in the microdomains. Thus, GSLs have important roles in signal transduction mediated by the microdomains.

Keywords: **lipid rafts, caveolae, microdomains, glycosphingolipid, ganglioside, signal transduction**

Introduction

Glycosphingolipids (GSLs) are components of the outer leaflet of the plasma membrane of all vertebrate cells. The expression of GSLs is developmentally regulated, and exogenously administered GSLs induce cell differentiation, suggesting that they may play a fundamental role during development [1–4]. GSLs have also been shown to be modulators of signal transduction [5,6], leading to the hypothesis that GSLs may regulate cell proliferation and differentiation through modified signal transduction. However, the physiological roles of GSLs remain to be clarified.

Recent studies suggest that GSLs exist in clusters and form microdomains containing cholesterol at the surface of the plasma membrane. The GSL- and cholesterol-rich microdomains are variously referred to as lipid rafts, detergent-resistant membranes, detergent-insoluble glycosphingolipid-enriched domains, and caveolae membranes [7–12]. Lipid rafts have been implicated in signal transduction, because signaling molecules such as the src-family kinases and trimeric G proteins, are associated with them [13,14]. These studies provide new insights into the functions of GSLs.

A number of studies have examined the influence of cholesterol on the physical properties and signaling in lipid rafts [12,15]. Cholesterol depletion leads to perturbation of raft-mediated signaling. On the other hand, few studies have examined the influence of GSLs on the signaling mediated by lipid rafts [16–18]. In this review, we discuss the functional roles of GSLs in signal transduction mediated by lipid rafts.

*Author for correspondence: Department of Biochemical Cell Research, The Tokyo Metropolitan Institute of Medical Science, Tokyo Metropolitan Organization for Medical Research, 3-18-22 Honkomagome, Bunkyo-ku, Tokyo 113-8613, Japan. Tel: 81-3-3823-2101 (ext 5235); Fax: 81-3-3828-6663

Lipid rafts

Recent studies suggest that plasma membrane lipids are not homogeneously distributed and that the membranes may contain microdomains or compartments [19]. Low-density, detergent-insoluble membrane fractions can be isolated from cells by sucrose density gradient centrifugation. These membrane fractions are rich in GSLs, sphingomyelin, cholesterol, glycosylphosphatidylinositol (GPI)-anchored proteins, and a variety of signaling molecules (Table 1). These

Table 1. Association of signaling molecules with lipid rafts.

Molecules	*Methods*	*Cell/Tissue*	*Ref*
A Receptor			
Trk	(i)	rat brain	[100]
	(ii) anti-GM1	PC12 cells	[53]
EGF receptor	(i)	Rat-1 fibroblasts	[39]
PDGF receptor	(i)	human fibroblasts	[40]
insulin receptor	(i)	CHO transfectants	[101]
p75(NTR)	(i)	pheochromocytoma PC12	[102]
TNF receptor 1	(i)	Histiocytic lymphoma U937	[103]
acetylcholine receptor	(i)	cardiac myocytes	[104]
adenosine receptor	(i)	rat ventricular cardiomyocytes	[26]
angiotensin II receptor	(i)	vascular smooth muscle cells	[105]
bradykinin receptor	(i)	DDT_1MF-2 smooth muscle cells	[106]
β-adrenergic receptor	(i)	COS7 transfectant	[107]
calcium-sensing receptor	(i)	parathyroid cells	[108]
endothelin receptor	(i)	primary astrocytes	[109]
T-cell receptor	(i)	mouse thymocytes	[35]
FcεRI	(i)	basophilic leukemia RBL-2H3	[110]
B GPI-anchored protein			
Thy-1	(i)	basophilic leukemia RBL-2H3	[111]
CD59	(i)	human thymoma HPB-ALL	[112]
	(ii) anti-GM3	human thymoma HPB-ALL	[113]
TAG-1	(i)	primary DRG neuron	[99]
	(ii) anti-GD3	primary cerebellar cultures	[45]
F3	(i)	mouse cerebellum	[114]
C Ion channel & pump			
IP_3 receptor	(i)	lung epithelium	[115]
Ca^{2+}ATPase	(i)	lung epithelium	[115]
H^{+}ATPase	(i)	kidney epithelial MA104 cells	[116]
D Non-receptor tyrosine kinase			
c-src	(i)	mouse lung	[117]
	(ii) anti-GM3	mouse melanoma B16	[118]
	(ii) anti-DSGG	renal carcinoma TOS-1	[119]
Lyn	(i)	mouse lung	[117]
	(ii) anti-GD3	primary cerebellar cells	[64]
	(ii) anti-αGalGD1b	basophilic leukemia RBL-2H3	[43]
Lck	(i)	mouse lung	[117]
Fyn	(i)	mouse lung	[117]
Yes	(ii) anti-Gb3	renal tubular ACHN cells	[120]
Cbl	(i)	Jurkat cells	[36]
FAK	(ii) anti-GM3	mouse melanoma B16	[118]
Csk	(i)	Neuro2A neuroblastoma	[73]
E GTP-binding protein			
trimeric Gα	(i)	mouse lung	[117]
Ras	(i)	Rat-1 fibroblasts	[39]
Rho	(ii) anti-GM3	mouse melanoma B16	[118]
	(ii) anti-DSGG	renal carcinoma TOS-1	[119]
Rac1	(i)	Rat-1 fibroblasts	[121]
Rap1	(i)	mouse lung	[117]
F Adaptor			
Shc	(i)	human fibroblasts	[40]
Grb2	(i)	Rat-1 fibroblasts	[39]
Sos	(i)	Rat-1 fibroblasts	[39]
Vav	(i)	basophilic leukemia RBL-2H3	[30]

Table 1. *(continued)*

Molecules	*Methods*	*Cell/Tissue*	*Ref*
Syp	(i)	human fibroblasts	[40]
PSD-95	(i)	COS transfectants	[122]
GRIP	(i)	HEK cells	[123]
G Effector			
adenylate cyclase	(i)	primary human fibroblasts	[124]
Raf-1	(i)	Rat-1 fibroblasts	[39]
MAP kinase	(i)	Rat-1 fibroblasts	[39]
PI 3 kinase	(i)	human fibroblasts	[40]
phospholipase Cγ	(i)	Jurkat cells	[36]
phospholipase D	(i)	HaCaT human keratinocytes	[125]
H Ca^{2+} or calmodulin-binding protein			
calmodulin	(i)	pulmonary artery endothelial cells	[126]
protein kinase C	(i)	mouse lung	[117]
eNOS	(i)	pulmonary artery endothelial cells	[126]
GAP43	(i)	rat brain	[127]
NAP22	(i)	rat brain	[127]
I Scaffolding protein			
caveolin	(i)	mouse lung	[117]
	(ii) anti-GD3	CHO transfectants	[64]
LAT	(i)	Jurkat cells	[128]
J Lipid			
PI (4)P	(i)	epidermoid carcinoma A431	[129]
$PI(4,5)P_2$	(i)	epidermoid carcinoma A431	[129]
ceramide	(i)	human fibroblasts	[130]

Methods: (i) Localization in raft fraction on density gradient
(ii) Co-immunoprecipitation with anti-GSL antibody

observations indicate the possible presence of lipid rafts in cells and their involvement in signal transduction. The presence of lipid rafts *in vivo* is supported by several lines of evidence, including the results of a single-particle tracking study [20], a fluorescence resonance energy transfer study [21], and a chemical crosslinking study [22]. The lipid rafts may range in size from 20~70 nm in diameter. Clustered GSLs, as observed electron microscopically, are presumed to represent lipid rafts [23–25]. Caveolae, which are flask-shaped invaginations of the plasma membrane that contain the marker protein caveolin, can also be isolated using similar methods and appear to contain many of the same components as rafts. Caveolae are thought to be built around rafts with caveolin [9,10].

The basic forces driving the formation of rafts are thought to be lipid interactions, which are weak and transient [8]. GSLs are relatively rich in saturated fatty acyl chains, which allows tight packing and confers the characteristic of a high melting temperature. On the other hand, phospholipids are relatively rich in *cis*-unsaturated fatty acyl chains (kinked structure), which prevents tight packing and confers the characteristic of a low melting temperature. Lipid rafts may exist as phase-separated domains in the membrane. The lipid composition renders the rafts and their constituent proteins resistant to solubilization with non-ionic detergents. Hydrogen bonding among the carbohydrate moieties in GSLs is also thought to contribute to the formation of lipid rafts [11].

There are four modes of association of signaling molecules with lipid rafts; (i) constitutive presence in lipid rafts, (ii) agonist-induced translocation of membrane protein to lipid rafts, (iii) agonist-induced translocation of cytosolic protein to lipid rafts, (iv) agonist-induced translocation out of lipid rafts [26]. It is also known that some signaling molecules such as CD45 and trimeric G protein $\beta\gamma$ subunits are excluded from lipid rafts [27,28]. GPI-anchored proteins generally have saturated acyl chains, which are likely to be inserted preferentially into lipid rafts. Src-family kinases, trimeric G protein α subunits and endothelial nitric oxide synthase (eNOS) are modified by saturated-chain lipids: palmitoylation and myristoylation, which are likely to be inserted preferentially into lipid rafts. Caveolin and LAT are transmembrane scaffolding proteins in lipid rafts, which undergo palmitoylation on three and two residues, respectively. Palmitoylation of Ras, β-adrenergic receptors, endothelin receptors, PSD-95 and

GAP43, or myristoylation of NAP-22, may help their association with lipid rafts [29]. Agonist-induced translocation of several adaptors or effectors, such as Grb 2, Vav, phospholipase C (PLC) and PI 3 kinase, to lipid rafts is probably due to their association with the raft protein, LAT, which has multiple tyrosine phosphorylation sites, via src homology 2 (SH2) modular interactions [30].

Lipid rafts are thought to function as platforms for the dynamic association of signaling molecules. Concentration of the receptors for interaction with ligands and effectors on both sides of the membrane can allow efficient and rapid coupling of activated receptors to the effector system and prevent inappropriate crosstalk between pathways [7].

Signal transduction in lipid rafts

GPI-anchored protein signaling

Several GPI-anchored proteins have been implicated in transmembrane signaling via the src-family tyrosine kinases [31]. In the immune and nervous system, anti-GPI-anchored protein antibodies co-immunoprecipitate src-family kinases. Antibody-mediated crosslinking of GPI-anchored proteins induces activation of src-family kinases and transient tyrosine phosphorylation of several substrates, concomitantly with cell activation. GPI-anchored proteins are restricted to the outer leaflet of the lipid bilayer, and are integrated into the membrane by the GPI anchor. The lipid anchors are not in direct contact with the cytoplasm. Src-family kinases anchor onto the inner leaflet of the lipid bilayer via N-terminal lipid modification. How do GPI-anchored proteins transduce signals via src-family kinases? This is thought to be mediated via lipid rafts [32,33]. In support of this concept, the crosslinking of GPI-anchored proteins and GSLs leads to the formation of membrane patches which is enriched in the src-family kinases and tyrosine-phosphorylation of proteins in lymphoid cells [34].

Immunoreceptor signaling

Lipid rafts are involved in signaling by immunoreceptors, such as the T-cell receptors and IgE receptors (FcεRI) [35–37]. Efficient T-cell activation requires one signal from a T-cell receptor and a second signal from the costimulatory molecule. The costimulation leads to the recruitment of lipid rafts to the site of cell-cell contact between the T cell and antigen-presenting cell. The concentration of src-family kinases, LAT and downstream molecules, and exclusion of tyrosine phosphatase CD45 allow strong and stable tyrosine phosphorylation of several substrates [38]. These observations suggest that cell-cell contact can modulate raft-mediated signaling. Aggregation of IgE-FcεRI by antigen induces coalescence of FcεRI with lipid rafts, activation of the src-family kinases and histamine release from mast cells.

Growth factor receptor signaling

Epidermal growth factor (EGF) receptor, platelet-derived growth factor (PDGF) receptor and Ras are present in lipid rafts. Application of EGF and PDGF induces (i) tyrosine phosphorylation of their receptors and adaptors, (ii) interaction with ras of Raf-1, as mitogen-activated protein (MAP) kinase kinase kinase, and (iii) activation of MAP kinase in the lipid rafts. These observations suggest that lipid rafts may be the initiation site of the MAP kinase cascade [39,40].

Caveolin-regulated signaling

Caveolin can directly interact with a variety of signaling molecules such as trimeric Giα proteins, eNOS, src-family kinases, EGF receptors and protein kinase C (PKC)α, via the so-called caveolin scaffolding domain. The interaction holds the signaling molecules in an inactive conformation [10]. Caveolae may inactivate signaling events initiated at the cell surface. Caveolin is a substrate for v-src and c-src [41,42].

GSL functions in signal transduction via lipid rafts

GSLs have been shown to be modulators of signal transduction in a variety of systems (Table 2). However, the underlying molecular mechanism remains to be explored. GSLs, the core lipids of rafts, may modulate the signal transduction in lipid rafts. Most of the signaling molecules modulated by GSLs are actually present in lipid rafts (Table 1).

GPI-anchored protein signaling

Antibody-mediated crosslinking of GSLs is known to activate src-family kinases. GSL signaling and GPI-anchored protein signaling share many properties [18,32,33]. (i) Anti-GSL antibody, as well as anti-GPI-anchored protein antibody, co-precipitate protein kinase activity. Proteins of 80 and 60-53; src-family kinases and 40-kDa are phosphorylated at tyrosine residues as revealed by immune complex kinase assays. (ii) Antibody-mediated crosslinking of GSLs as well as GPI-anchored proteins induce activation of src-family kinases; (iii) both induce transient tyrosine phosphorylation of several substrates, including PLCγ and an 80-kDa protein, (iv) both induce Ca^{2+} flux, and (v) both induce cell activation. Treatment with anti-GSL antibody can mimic GPI-anchored protein signaling, suggesting that GPI-anchored protein and GSLs transduce signals using the same signaling pathway. The 80-kDa phosphoprotein recognized in the immune complex kinase assays is identical to the 80-kDa protein phosphorylated by antibody crosslinking [43]. Tyrosine phosphorylation of the 80-kDa protein is detected in lipid rafts, which is attenuated in the presence of a selective inhibitor for src-family kinases and in src-family kinase-deficient mice [44]. These observations suggest that the 80-kDa protein may be a membrane protein in lipid rafts and a putative substrate for src-family kinases. Thus, src-family kinases, 80 and 40-kDa proteins are sets of

Table 2. Signal transduction mediated by glycosphingolipids.

GSL	*Agonist*	*Responding cell*	*Type of response*	*Ref*
GalCer	anti-GalCer	oligodendrocytes	Ca^{2+} flux	[93]
LacCer	exogenousLacCer	aortic smooth muscle cells	activation of Ras and MAP kinase phosphorylation of Raf, proliferation	[131]
GM3	anti-GM3	mouse melanoma B16	tyrosine phosphorylation of FAK activation of Ras, Rho, growth inhibition	[118]
		Neuro2A neuroblastoma	cAMP accumulation, differentiation	[132]
		T51B liver epithelial cells	Ca^{2+} flux	[133]
	exogenous GM3	Neuro2A neuroblastoma	activation of c-src, MAP kinase, decrease of Csk level in rafts fraction neuritogenesis	[73]
		human blood lymphocytes	phosphorylation of CD4, activation of PLA_2 activation of PKCδ,θ	[134]
		human platelets	Ca^{2+} flux	[135]
		epidermoid carcinoma A431	inhibition of autophosphorylation of EGF receptor proliferation	[51]
GD3	anti-GD3	human peripheral T cells	tyrosine phosphorylation of PLCγ1 PI turnover, Ca^{2+} flux, ras activation	[136]
		primary cerebellar cells	activation of src-family kinase Lyn, MAP kinase tyrosine phosphorylation	[64]
9-O-actyl GD3	anti-9-O-actylGD3	human mononuclear cells	tyrosine phosphorylation of Syk, PI turnover	[137]
GM1	anti-GM1	Neuro2A neuroblastoma	PI turnover, Ca^{2+} flux	[132]
	cholera toxin B	jurkat T cell line	tyrosine phosphorylation of PLCγ1, Ca^{2+} flux	[138]
			tyrosine phosphorylation of ZAP-70, LAT Ca^{2+} flux, activation of MAP kinase	[139]
		rat thymocytes	Ca^{2+} flux, proliferation	[140]
		N18 neuroblastoma	Ca^{2+} flux, differentiation	[141]
		taenia coli myocytes	inhibition of β-adrenergic receptor-mediated maxi-K^+ channel activity	[142]
	exogenous GM1	jurkat T cell line	activation of src-family kinase Lck tyrosine phosphorylation of PLCγ1	[72]
		Neuro2A neuroblastoma	Ca^{2+} flux, differentiation	[84]
		PC12 cells	increase in autophosphorylation of Trk	[53]
			enhancement of depolarization-mediated Ca^{2+} flux and phosphorylation	[54]
		U-1242 MG glioma	activation of MAP kinase, S6 kinase DNA synthesis	[143]
		Sf9 cells	inhibition of β-adrenergic receptor-induced cAMP formation	[144]
GD1a	exogenous GD1a	Swiss 3T3 cells	inhibition of autophosphorylation of PDGF receptor proliferation	[52]
GT1b	exogenous GT1b	bovine mammary gland	enhancement of annexin I phosphorylation	[145]
GQ1b	exogenous GQ1b	mouse primary keratinocytes	PI turnover, Ca^{2+} flux, activation of PKC differentiation	[146]
		GOTO human neuroblastoma	protein phosphorylation, neuritogenesis	[147]
αGalGD1b	anti-αGalGD1b	basophilic leukemia RBL-2H3	activation of src-family kinase Lyn tyrosine phosphorylation of PLCγ1 PI turnover, Ca^{2+} flux	[47]
Gb3	Shiga toxin	renal tubular ACHN cells	activation of Yes	[120]
	anti-Gb3 verotoxin B	Burkitt's lymphoma cell line	Ca^{2+} flux, cAMP accumulation, activation of cAMP dependent kinase	[148]
SPG	exogenous SPG	1M9 lymphoid cell line	inhibition of autophosphorylation of insulin receptor and proliferation	[149]

signaling components in lipid rafts for both GPI-anchored proteins and GSLs.

Enzymatic removal of the carbohydrate moiety from cell-surface GSLs impairs activation of the src-family kinase Lyn and tyrosine phosphorylation of the 80-kDa protein by antibody-mediated crosslinking of TAG-1, a GPI-anchored neuronal cell adhesion molecule. This observation suggests that GSLs are involved in GPI-anchored protein signaling [45].

Immunoreceptor signaling

Aggregation of IgE-FcεRI by an antigen induces activation of the src-family kinase Lyn, tyrosine phosphorylation of PLCγ and focal adhesion kinase (FAK), Ca^{2+} flux, phosphatidylinositol (PI) turnover and histamine release from RBL-2H3 cells. Pretreatment with anti-ganglioside αGalGD1b antibody inhibits the binding of IgE to FcεRI and FcεRI-mediated histamine release [46]. Treatment with anti-ganglioside αGalGD1b antibody induces Lyn activation and tyrosine phosphorylation of PLCγ, but not tyrosine phosphorylation of FAK and histamine release from RBL-2H3 cells [47]. Furthermore, one of the RBL-2H3 variants, that was initially selected for deficiency in FcεRI-mediated histamine release, was found to lack expression of the ganglioside αGalGD1b. Antigen stimulation did not induce Ca^{2+} flux, PI turnover and histamine release in the variant, even though it expresses FcεRI [48,49]. Exogenous gangliosides enhance FcεRI-mediated histamine release [50]. These observations suggest the gangliosides have an important role in FcεRI signaling.

Growth factor receptor signaling

Exogenous ganglioside GM3 inhibits EGF-induced autophosphorylation of the EGF receptor and proliferation of A431 epidermoid carcinoma cells [51]. Exogenous ganglioside GD1a inhibits PDGF-induced autophosphorylation of the PDGF receptor and proliferation of Swiss 3T3 cells, even though it had no effect on PDGF binding to the receptor [52]. Exogenous ganglioside GM1 increases NGF-induced autophosphorylation of Trk [53] and enhancement of depolarization-mediated Ca^{2+} flux in PC12 cells [54].

Endogenous GSLs can also affect growth-factor-receptor signaling in lipid rafts. In GM3-deficient cells, *de novo* synthesized GM3 also inhibits autophosphorylation of the EGF receptor [55]. Changes in the amount of endogenous ganglioside GM1 associated with Trk can modulate receptor kinase activity [56]. In the brain cell membrane in cases of GM1 and GM2 gangliosidosis, PLC activity is impaired and adenylate cyclase activity is enhanced [57].

Taken together, these observations suggest that GSLs have a significant role in signal transduction mediated by lipid rafts.

Modulation mechanism of signal transduction by GSLs in lipid rafts

Contribution of GSLs to the physical properties of lipid rafts

Lipid rafts are not detected in the presence of octylglucoside. This is attributed to the resemblance of the detergent to GSLs [15]. Inhibition of sphingolipid biosynthesis leads to increased solubility of GPI-anchored proteins in non-ionic detergents [58]. Single-particle tracking of a GPI-anchored protein and GSLs in a native membrane showed transient confinement in patches, which are thought to represent lipid rafts *in vivo*. The size of the confining domain for the GPI-anchored protein is reduced by treatment with inhibitors of GSL biosynthesis [59]. Furthermore, exogenous gangliosides abolished clustering of the GPI-anchored protein in lipid rafts and increased their detergent solubility [60]. These observations suggest that GSLs can modulate the physical properties of lipid rafts. Such modulation of the physical properties of lipid rafts may affect signal transduction.

Tumor cell mutants, deficient in GSL also have lipid rafts, suggesting that GSLs are not required for the formation of lipid rafts *in vitro* [61,62]. Up-regulated expression of sphigomyelin in the mutant probably compensates for the lack of GSLs. This observation does not conflict with the concept of GSL functions in lipid rafts *in vivo*, because GSL-deficient mice are embryonic-lethal [63]. GSLs are essential for embryonic development.

Possible modulation mechanism of raft-mediated signaling by GSLs

An activation model of src-family kinase Lyn by crosslinking of GSLs [64] is shown in Figure 1. It is assumed that GSL crosslinking leads to coalescence of lipid rafts. This may induce clustering of Lyn and transphosphorylation of tyrosine residue in the kinase domain (activating site Tyr 397; homologous to c-src Tyr 416) [65]. Exclusion of protein tyrosine phosphatase CD45 from lipid rafts prevents dephosphorylation of the phosphotyrosine at the activation site and allows Lyn activation and phosphorylation of the 80-kDa protein. Consistent with this model, active Lyn was selectively enriched within lipid rafts [66]. It was proposed that an adaptor molecule must exist that spans the membrane to link exoplasmic GSLs with cytoplasmic src-family kinases. Crosslinking of GPI-anchored proteins seems to activate src-family kinases via the same mechanism [32,33].

It should be noted that anti-GSL antibody treatment represents artificial stimulation. Several natural ligands for GSLs have been reported, such as selectin [67], sialoadhesin [68], myelin-associated glycoprotein [69], gliolectin [70] and galectin [71], suggesting that cell-cell contact via the carbohydrate moieties of GSLs may induce signal transduction in lipid rafts [17]. However, the physiological significance of these molecules remains to be elucidated.

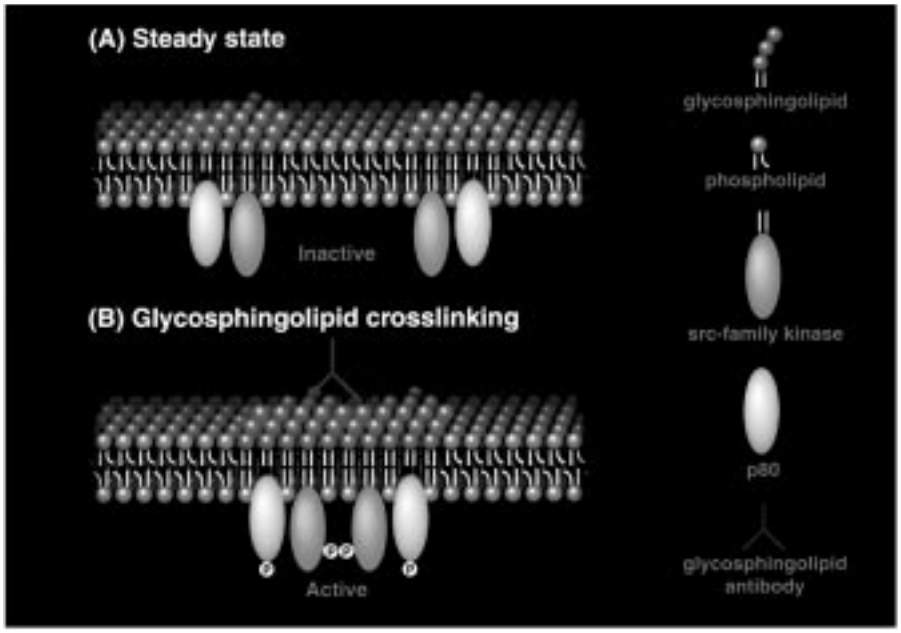

Figure 1. Activation model of Src-family tyrosine kinase by glycosphingolipid crosslinking.

Exogenously administered GSLs also induce the activation of src-family kinases, transient tyrosine phosphorylation of several substrates, and Ca^{2+} flux [72,73]. This activation is probably due to a decrease of Csk levels, a negative regulator of src-family kinases in lipid rafts [73]. These findings suggest that exogenous GSLs can affect the distribution of raft proteins and signal transduction in lipid rafts.

Change of lateral distribution of GSLs by antibody-mediated crosslinking induces intracellular signaling. Interestingly, intracellular signaling also induces changes of the lateral distribution of GSLs on the cell surface. For example, activation of PKC decreases ganglioside susceptibility to sialidase on the cell surface [74] and affects the dynamics of lipid rafts [75] and causes morphological changes of caveolae [76]. These observations suggest the presence of reciprocal regulation of lipid rafts on either side of the membrane.

Cell-free experiments using purified enzymes show that one of the modulation mechanisms of signal transduction by GSLs is direct interaction in plasma membrane. Ganglioside GM1 enhances Trk kinase activity [53]. Ganglioside GM3 increase Ca^{2+} ATPase activity [77]. CD38 NADase is also inhibited by gangliosides [78]. Taken together, these observations suggest that GSL could regulate signal transduction by affecting the functions of lipid rafts *in vivo*.

Possible role of GSLs in lipid rafts during development

In the nervous system, where gangliosides are especially enriched, the 'amounts' and 'species' of gangliosides undergo profound changes during development. Biochemical studies indicate (i) no or very low ganglioside expression in the neural tube, (ii) increased expression of ganglioside GD3 in glial and neural progenitor cells in parallel with the spatiotemporal increase in proliferation activity, (iii) decrease of GD3 and expression of highly sialylated gangliosides, which are complex gangliosides, in neurons which are migrating or extending neurites, (iv) severalfold increase of total neuronal ganglioside synthesis during growth spurts and synaptogenesis, (v) establishment of final pattern with GM1, GD1a, GD1b and GT1b as the major ganglioside components of the adult nervous system [79]. The spatial and temporal expression of gangliosides has also been demonstrated by immunohistochemical staining using specific monoclonal antibody [80]. Gangliosides are synthesized by sequential catalytic reaction of multiple glycosyltransferases. The ganglioside profiles during development are due to spatio- and stage-specific expression of these glycosyltransferases [81].

The accumulation of gangliosides within neurons in ganglioside storage diseases, GM2 gangliosidosis, results in extensive neurite growth [82]. Exogenously administered gangliosides have been shown to accelerate regeneration of the central nervous system *in vivo* after lesioning [83]. The addition of exogenous gangliosides to cultures of primary neurons and neuroblastoma cells *in vitro* has been shown to stimulate cellular differentiation with concomitant neurite sprouting and extension [84–86]. Glucosylceramide (GlcCer) synthesis, the first glycosylation step of GSL synthesis, is required for axonal growth of hippocampal neurons [87]. Transfection of the ganglioside GD3 synthase cDNA induced continuous activation of Trk and MAP kinase in PC12 cells [88] and cholinergic differentiation of Neuro 2A neuroblastoma cells with neurite sprouting [89]. Complex-ganglioside-deficient mice exhibit axonal degeneration [90]. These data

suggest that gangliosides are involved in neural cell differentiation and brain development.

The association of GPI-anchored proteins with lipid rafts is developmentally regulated during oligodendrocyte maturation [91,92]. In oligodendrocyte progenitor cells which do not synthesize galactocerebroside (GalCer), a major GSL in myelin, the GPI-anchored neuronal cell adhesion molecule, F3, is not present in lipid rafts and is not associated with the src-family kinase Fyn. In contrast, F3 from maturing oligodendrocytes and from myelin is present in lipid rafts and is associated with Fyn. Treatment with anti-GalCer antibody induces Ca^{2+} flux [93] in oligodendrocytes and inhibits differentiation of oligodendrocyte progenitor cells [94]. Interestingly, functional breakdown of the lipid bilayer of the myelin membrane is observed in GalCer-deficient mice [95,96]. These observations suggest that 'amounts' of GSLs can be involved in the regulation of raft-mediated signaling and oligodendrocyte development.

Compositional and functional heterogeneity is known to exist among lipid rafts. In melanoma, two membrane subfractions were separated from the low-density, detergent-insoluble fractions by sucrose density gradient centrifugation. There was a clear difference in their GSL compositions. The GM3-rich fraction, separated using the anti-GM3 antibody, contained sphingomyelin, cholesterol and c-src, but not caveolin. In contrast, the caveolin-containing fraction, separated using the anti-caveolin antibody, contained GlcCer, a large quantity of cholesterol and Ras [97]. In hippocampal neurons, association of Thy-1 with lipid rafts seems to require a developmental increase in the expression of sphingomyelin, but not GlcCer [98]. In dorsal root ganglionic neurons, also two subfractions were separated by immunoisolation. The GPI-anchored prion protein, located primarily in the cell body, was relatively soluble in detergent. Thy-1, abundantly expressed in neurites, was highly resistant to detergent solubilization. Thus, functionally different GPI-anchored proteins are organized in rafts of different lipid compositions [99]. These observations suggest that different 'species' of GSLs might be involved in the regulation of raft-mediated signaling and neuronal development. Lending support to this idea, GSL-modulation of signal transduction is dependent on the species of GSL (Table 2).

In conclusion, the GSL changes during development may regulate cellular events by affecting signal transduction in lipid rafts.

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