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Lipid membrane domains in cell surface and vacuolar systems

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Detergent insoluble sphingolipid-cholesterol enriched 'raft'-like membrane microdomains have been implicated in a variety of biological processes including sorting, trafficking, and signaling. Mutant cells and knockout animals of sphingolipid biosynthesis are clearly useful to understand the biological roles of lipid components in raft-like domains. It is suggested that raft-like domains distribute in internal vacuolar membranes as well as plasma membranes. In addition to sphingolipid-cholesterol-rich membrane domains, recent studies suggest the existence of another lipid-membrane domain in the endocytic pathway. This domain is enriched with a unique phospholipid, lysobisphosphatidic acid (LBPA) and localized in the internal membrane of multivesicular endosome. LBPA-rich membrane domains are involved in lipid and protein sorting within the endosomal system. Possible interaction between sphingolipids and LBPA in sphingolipid-storage disease is discussed.

Keywords: **sphingolipid, cholesterol, raft, endocytosis, lysobisphosphatidic acid, sphingolipidosis**

Introduction

Sphingolipids have emerged as an essential lipid for development and cell homeostasis. Ceramide, for example, was identified as an intracellular signaling molecule mediating variety of cellular processes such as apoptotic cell death, survival, and differentiation (for review see ref. [1–6]. Although there is still a debate on action mechanisms of ceramide, identification of sphingosine-1-phosphate, a ceramide degradation product, as a ligand of EDG (Endothelial Differentiation Gene) gives a strong indication that sphingolipids play significant roles in signal transduction for cell survival and differentiation [7,8]. Another recent progress in sphingolipid research is the discovery of the existence of sphingolipid microdomains. Membrane sphingolipids make the microdomains by clustering of cholesterol and glycosyl-phosphatidyl inositol (GPI)-anchored proteins. The formation of the domains is solely dependent upon physical interactions between lipids and proteins [9–13]

Sphingolipids are proposed to be critical components of microdomains on cell membranes that serve as rafts for the attachment and sorting of proteins to the surface membrane. Of particular interest, the complex includes intracellular proteins attaching on inner leaflet of plasma membrane such as palmitoyl acylated src-family kinases. The domains are, thus, proposed to be platforms where intracellular signaling is generated. Within the past few years, studies have provided a new concept or idea that lipid domains are involved in several pathological conditions such as sphingolipidosis and multi-drug resistance.

Formation of sphingolipid-cholesterol-enriched domains partially explains the complex distribution of lipids within cell membranes. However, enormous molecular diversity of lipids as structure constituents of cell membranes suggests the existence of lipid membrane domains other than sphingolipid-rich domains. In fact, recent studies suggest that lysobisphosphatidic acid (LBPA)-rich membrane domains in the internal membranes of late endosomes play an important role in lipid and protein sorting from the organelle. In this review, we will discuss mainly the role of sphingolipids on the formation and function of raft-like microdomains. We will especially focus on the genetic results using mutant cell lines defective in sphingolipid synthesis. We will also summarize internalization of sphingolipids along different endosome membrane domains.

Sphingolipid domains in plasma membranes and the Golgi apparatus

Requirement of sphingolipids for raft-like lipid domains

Glycosphingolipid (GSL)-enriched domains have been isolated from plasma membranes by non-ionic detergents such as Triton X-100 at 4°C. This method has been used in the

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biochemical characterization of rafts or caveolae. GSL-enriched domains have also been called the detergent-insoluble GSL-enriched domains (DIGs), GSL-enriched membranes or microdomains (GEMs), detergent-resistant membranes (DRMs), Triton-insoluble membranes (TIMs), or GSL (or sphingolipid-cholesterol) rafts. Here, we often call 'raft-like lipid or sphingolipid microdomains. Such domains have been isolated from a wide variety of cells including fibroblasts, epithelial cells, neuroblastoma and neuronal cells.

There are accumulating evidences suggesting that sphingolipids are essential components of raft-like lipid domains. In fact, depletion of sphingolipids in ts-mutant cells of CHO (SPB-1) results in the disappearance of a GPI-anchored protein in the detergent insoluble fraction [14]. However, since there are two major complex sphingolipids, glycosphingolipids (GSLs) and sphingomyelin in mammals (Figure 1), it was not clear which class of sphingolipids is involved in the formation of rafts. Previously, it was suggested that GSLs tend to form self-aggregation through hydrogen bonds between oligosaccharide groups and between the hydroxy groups of sphingosine base and hydroxy of fatty acyl groups [15,16]. On the other hand, Brown and her group proposed the importance of acyl chains interactions in the rafts, and that sphingolipids, irrespective of head groups, exist in the liquid-ordered phase (Io) in membranes. Studies with artificial model membranes support that sphingolipid- and cholesterol-rich membranes can form detergent-resistant membranes [17]. In this case, sugar-dependent hydrogen bondings are not essential for the lipid domain formation.

Metabolic inhibitors for sphingolipid biosynthesis are useful tools to understand implication of GSLs in raft-like lipid domain formation. Shu et al. introduced a specific inhibitor of glucosylceramide synthase, D-threo-1-phenyl-2-palmitoyl-3-pyrrolidino-propanol, in order to test whether or not glycosphingolipids are required for the sorting of proteins to rafts [18]. When GSLs in NIH 3T3 cells were depleted with the treatment of the inhibitor, the caveolae structure remained intact, suggesting that protein sorting to caveolae in fibroblasts occurs independently of GSL synthesis. However, the use of inhibitors cannot always give a conclusive interpretation on the results obtained.

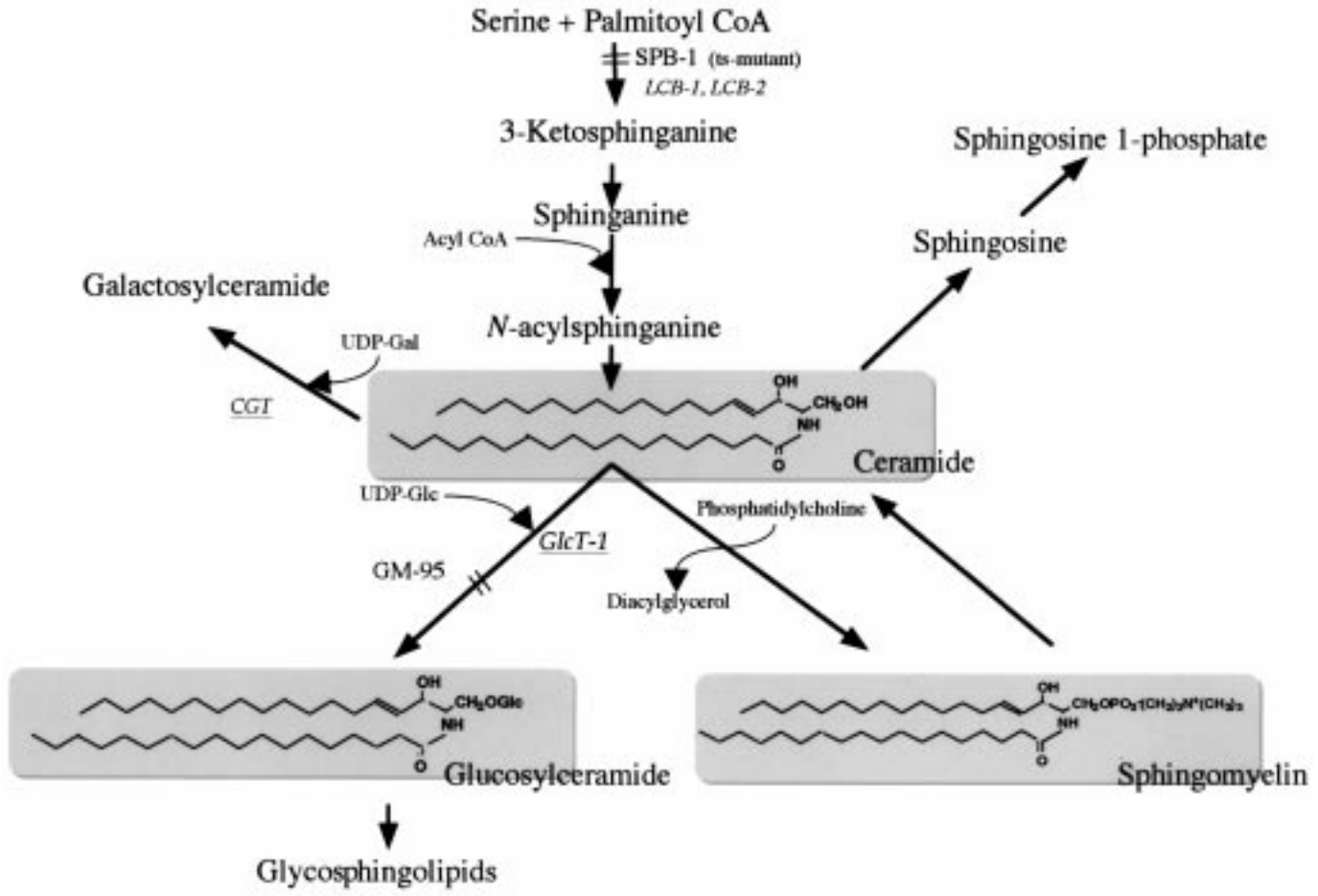


Figure 1. Synthetic pathway of mammalian sphingolipids. In mammals, there are two major classes of sphingolipids, sphingomyelin and glycosphingolipid (GSL). Both have ceramide as hydrophobic backbone structure in common. Ceramide is also converted to sphingosine by ceramidase.

Very recently, Ostermeyer et al. introduced GM-95 melanoma mutant cells lacking all GSLs due to deficiency of ceramide glucosyltransferase (GlcT-1) [19] to test whether the head group of GSLs is directly involved in the raft formation [20]. Biochemical analysis of the detergent resistant membranes showed that there were no significant changes in protein profiles between GM-95 and its parental cell line, MEB-4. A GPI-anchored protein was also transported to the cell surface membrane at similar rates between GM-95 and MEB-4 cells. Based on these results, they concluded that GSLs are not a crucial component for the raft's formation. Since sphingomyelin instead of GSLs was enriched in the detergent resistant membrane of GM-95, the presence of either GSLs or sphingomyelin in the outer leaflet of membrane may be sufficient to form and maintain the raft-like lipid domain [21, see also 2–4]. Functional studies of sphingomyelin in the rafts remain to be addressed.

The sites of raft-like lipid domain formation

Sphingolipids are highly enriched in plasma membranes. For understanding the raft-like lipid domain distribution, it is important to know where and how each component is synthesized and transported to targeted membranes. All lipid domain components including GPI-anchored proteins, cholesterol and ceramide are synthesized in the ER. Riezman et al. suggested a possible presence of the rafts in the ER membrane as a pre- or immatured form [22]. Ceramide is then transported and modified further with glycosyltransferases in the Golgi membrane. Little is known about the transport system of ceramide from the Golgi to ER membranes. Recently, Fukasawa et al. isolated a CHO mutant (LY-A) defective in sphingomyelin biosynthesis and found that the mutant was defective in ER-Golgi apparatus trafficking of ceramide for sphingomyelin synthesis [23]. This transport pathway was ATP-dependent and, interestingly, specific for sphingomyelin synthesis but not GSLs, although both synthesis occur at the Golgi apparatus. ER-to-Golgi trafficking of GPI-anchored or membrane-spanning proteins in the mutant cells appeared to be normal, indicating again the specific trafficking for ceramide to sphingomyelin.

Importantly, glucosylceramide is synthesized at the cytosolic surface of the Golgi membranes [24–30]. The glucosylceramide formed is flipped into the lumenal leaflet of the Golgi membrane. There, the lipid is further glycosylated to form higher GSLs. GSLs are then transported to cell surface via vesicular traffic. Direct transport of glucosylceramide from the site of synthesis to the cytoplasmic leaflet of the plasma membrane is also suggested [30,31]. It is an interesting issue whether the direct trafficking of glucosylceramide is involved in the formation of the raft-like lipid domains of the inner leaflet.

Posttranscriptional modifications with unsaturated fatty acyl chains are also essential process for association of Ras isoforms at the inner face of the plasma membrane (for review see, ref. [32]. However, little is known about the biochemistry and enzymology of the modification enzymes such as palmitoyltransferases.

Structure of sphingolipid domains in plasma membrane–Sphingolipid raft is not homogeneous

Recently, two groups independently demonstrated that microdomains containing GPI-anchored proteins are present in living cells [33,34]. One domain contains at least 15 molecules of this type of protein [33]. This finding indicated that the size of ordered domains is much smaller than that found in DRMs. DRMs isolated from cells by detergent has 1 μm in diameter [35].

Most recent studies on distribution of GPI-anchored proteins on cell surface membranes indicated the diverse structure of the domains [36]. Iwabuchi et al. reported that two distinct fractions, one enriched in GM3 ganglioside and the other containing caveolin, could be separated from low density detergent-insoluble membrane fraction prepared by sucrose density gradient centrifugation of postnuclear fraction of mouse melanoma B16 cells [37]. The GM3-enriched subfraction isolated by anti-GM3, contained sphingomyelin, cholesterol, c-Src, and Rho A but not caveolin. In contrast, the caveolin-containing subfraction, separated by anti-caveolin, contained glucosylceramide, Ha-Ras, a very small quantity of sphingomyelin, and a large quantity of cholesterol. It is interesting to note that the latter caveolin-rich fraction contained glucosylceramide together with Ha-Ras. These biochemical results indicate the possible presence of two functionally distinct domains in the surface membrane of B16 melanoma. Each domain may be trafficked to the membrane through independent intracellular processes, although we cannot exclude the possibility of contamination of other intracellular membranes or an artifact caused by the use of detergent.

Roles of GSLs in raft-like lipid domain–Lessons from GM-95 mutant cells

Mouse melanoma mutant GM-95 gives valuable information about roles of GSLs in the raft-like lipid domains. Sphingolipids are enriched in the detergent-insoluble substrate attachment matrix of both GM-95 and its transfectant with GlcT-1 (Figure 2). Hidari et al. found that when both cells were treated with sphingomyelinase, only GM-95 cells were detached from cell culture dishes covered with ECM proteins. GSLs re-expression in GM-95 cells by introduction of GlcT-1 make the cells resistant to sphingomyelinase-mediated detachment (Figure 3). These results indicate clearly that sphingolipids, irrespective of their head groups, play roles in cell-substratum adhesion. In the case of GM-95 cells, sphingomyelin synthesis compensated for the depletion of GSLs [21].

As discussed above, the melanoma mutant cell line, GM95 shows that GSLs are not essential for the lipid domain formation and also cell proliferation *in vitro.* These results did

Figure 2. Localization of sphingolipids in substrate attachment matrix (SAM). Staining with anti-GM3 (M2590 antibody) shows that GM3 ganglioside is localized in the clustered form in SAM of B16 melanoma cells. In case of the GSL-negative GM-95 cells, sphingomyelin is concentrated in SAM (see details in ref. [21]).

not mean that GSLs have no biological functions *in vivo*. Indeed, a very recent GlcT-1 knockout study by Yamashita et al. demonstrated that the GSL synthesis is essential for *in vivo* development and growth [38]. We got the same results with GlcT-1 knockout mouse (Ichikawa S, Hirabayashi, Y and Miyoshi I, unpublished observation). However, it is not clear yet why disruption of GlcT-1 in mouse leads to embryonic lethality mainly because no biochemical data is available about sphingomyelin synthesis during mouse development. If sphingomyelin synthesis is not switched on at the early developmental stages, then the elimination of GlcT-1 gene might cause disruption of the raft-like lipid domains and eventually of their signaling or cell-substratum adhesion system as seen in GM-95 cells when treated with sphingomyelinase [21]. Molecular characterization and roles of the raft-like lipid domain during *in vivo* differentiation and development has not as-yet been examined.

Glucosylceramide and multidrug resistance

Cabot and his research group demonstrated specific accumulation of glucosylceramide in cancer cells due to up-regulation of GlcT-1 in association with multidrug resistance (MDR) [39-41]. Caveolin-1 (adaptor protein of caveolae) and caveolin-2 were also over-expressed in multidrug resistant HT-29 and adriamycin-resistant MCF-7 cells, respectively [42]. Interestingly, P-glycoprotein (P-gp) was shown to co-localize with caveolins in detergent insoluble membrane domains. It is not clear, however, at present whether glucosylceramide is required for the structural organization of the caveolae and the caveolae glycolipid contributes to acquisition of multidrug resistance.

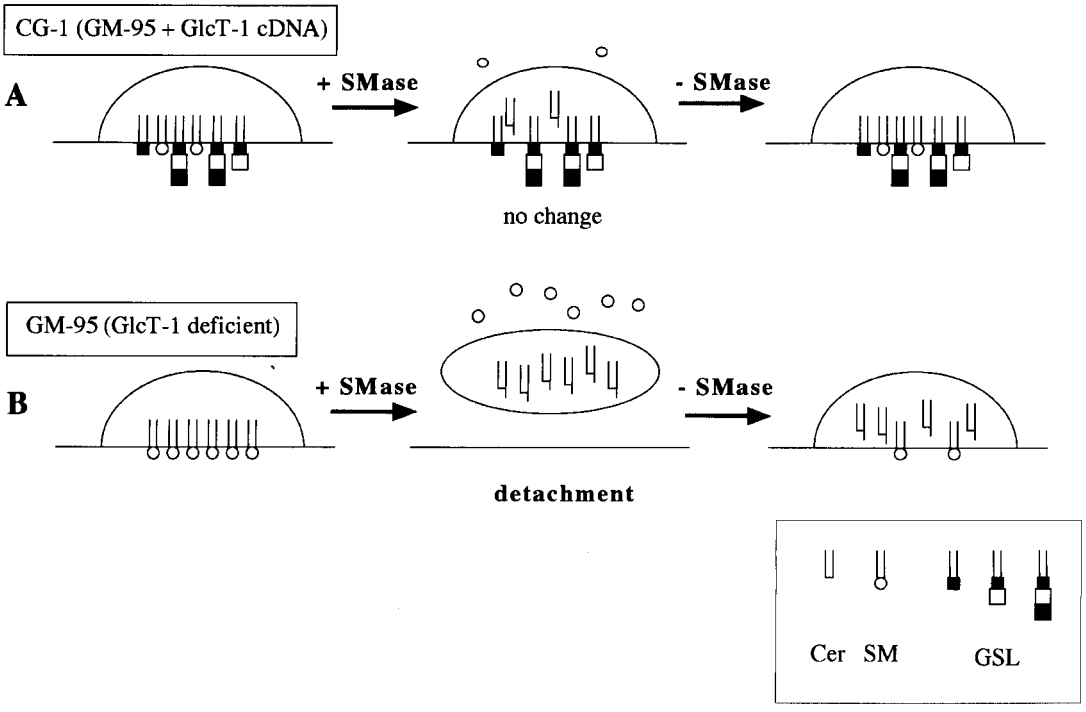


Figure 3. Treatment of melanoma mutant GM-95 with sphingomyelinase. Since sphingomyelin is a sole sphingolipid in the mutant, sphingomyelinase treatment could remove all sphingolipids leaving ceramide from the surface membrane as illustrated in panel B. Interestingly, ceramide generated is quickly glycosylated by GlcT-1 to form GSLs (panel A), but hardly converted to sphingomyelin (panel B).

Intracellular lipid microdomains

The endocytic pathway and late endosome membrane domains

After internalization, both plasma membrane lipids and proteins are first transported to peripheral early endosomes (Figure 4). Previous biochemical characterization showed that early endosomes contain cholesterol and GSLs and that their overall lipid composition is similar to that of the plasma membrane [43], although the difference in fatty acid composition is reported [44].

Recent results support the presence of raft components in early endosomes. A GPI-anchored protein delivered to early endosomes recycles back to the plasma membrane more slowly than bulk membrane. However, after depletion of cholesterol, the proteins recycle as fast as bulk membrane [45]. These results suggest that the association of GPI-anchored proteins with rafts in the endocytic pathway may slow their recycling.

Annexin II is an abundant protein which is present in the cytosol and on the cytoplasmic face of plasma membrane and early endosomes [46,47]. Several lines of evidence indicate that annexin II is involved in both the regulated exocytic pathway [48] and the endocytic pathway [49,50]. Annexin II distributes in a non-random manner on early endosome membranes, and is concentrated in morphologically distinct regions [51]. Harder et al. showed that annexin II is membrane-associated via a novel mechanism depending on membrane cholesterol [51]. Distribution of cholesterol within membranes is not well characterized. However, it is currently accepted that sphingolipid and cholesterol-rich domains assemble in the exoplasmic side of the plasma membranes. Since annexin II associates cytoplasmic side of the membrane, these results suggest that cholesterol-rich domains exist in the cytoplasmic leaflet of early endosomes.

From early endosomes, most of the internalized molecules are rapidly recycled back to the plasma membranes. However, selected ligands, such as down-regulated EGF receptor, are efficiently transported to late endosomes and lysosomes for degradation. A striking feature of endosomes along the degradation pathway is the accumulation of internal membranes within their lumen. These often appear like vesicles and tubules or onion-like arrangements, hence they are named multivesicular or multilamellar [10,52-58]. Although internal membranes can be observed at all stages of the pathway, their accumulation is far more pronounced in late endosomes. Very little is known about biogenesis and function of these membranes. Components of late endosomes are not equally distributed within the multivesicular system of the organelle. Extensively glycosylated membrane protein, lgp120/lamp1 is restricted to the limiting membrane [59–61]. whereas tetraspanins are enriched in the internal membranes of late endosomes [62–65]. Tetraspanins are proteins from the transmembrane 4-superfamily, TM4SF. They form membrane complex with integrin receptors and are implicated in integrin-mediated cell migration [66]. Interestingly, these tetraspanins are also enriched in exosomes, vesicles released by cells of the haemopoietic lineage and presumably derived from internal membranes of multivesicular endosomes [62,67–69].

In contrast to early endosomes, late endosomes do not contain significant amounts of cholesterol, phosphatidylserine and sphingomyelin but contain high amounts of neutral lipids such as triglycerides and cholesterol ester. Late endosomes are also enriched with a unique, non-degradable, highly hydrophobic, acidic phospholipid, lysobisphosphatidic acid (LBPA),

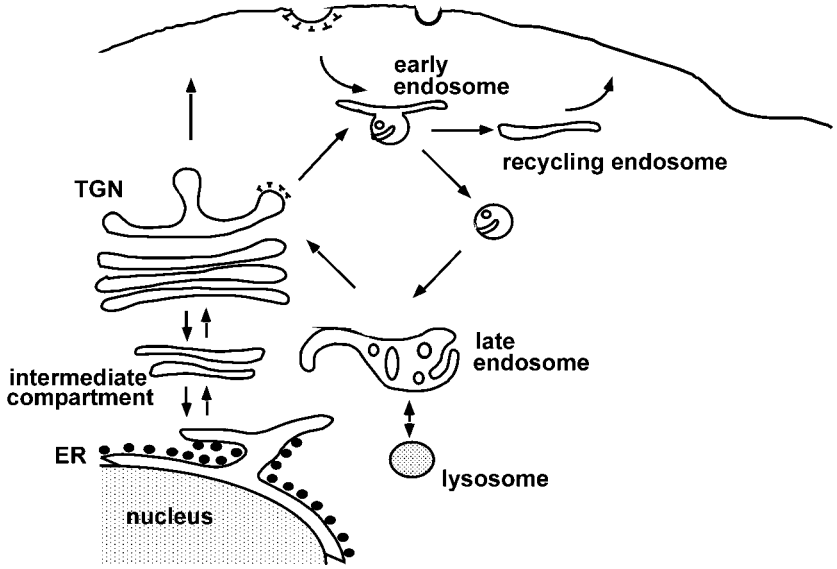
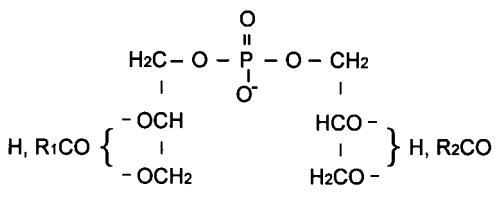


Figure 4. Lipid membrane domains in vacuolar system. Cholesterol and sphingolipid-enriched microdomains are distributed in the plasma membranes, early endosomes, and possibly in recycling endosomes and trans-Golgi network. Lysobisphosphtidic acid-rich domains are restricted in internal membranes of late endosomes. In Niemann-Pick type C cells, cholesterol is accumulated in late endosomes. LBPA-rich domains function as distribution device for LDL-derived cholesterol.

also called bis(monoacylglycero)phosphate (Figure 5). In baby hamster kidney (BHK) cells, LBPA accounts for ~1.5% of total phospholipids whereas this lipid is ⩾ 15% of late endosome phospholipids [61,70]. Although LBPA is a structural isomer of phosphatidylglycerol, its backbone is uncommon *sn*-1-glycerophospho-*sn*-1′-glycerol stereoconfiguration [71–73]. Since phospholipases recognize the *sn*-3 stereoconfiguration, LBPA is resistant to most phospholipases. Each glycerol of LBPA contains one fatty acid. Contradictory results were obtained on the position of fatty acids [74–76]. Enrichment of polyunsaturated fatty acids in LBPA suggests the binding of fatty acid acids to the beta-position of glycerol moiety [76]. Immunogold labeling of cryosections using a monoclonal antibody against LBPA showed that the lipid is restricted to the internal membranes of late endosomes [61,77–79]. These results suggest that lipid composition of internal membranes is different from that of limited membranes in late endosomes. Our results also suggest that the internal membranes form specialized microdomains within endosomes.

Lysosomes presumably correspond to the last station in the degradation pathway. In contrast, late endosomes exhibit protein sorting functions. Our recent results suggest that LBPA-rich internal membranes play an important role in the sorting function of late endosomes. Multifunctional receptor for mannose-6-phosphate-bearing ligands and insulin-like growth factor 2 (IGF2/MPR) delivers newly synthesized lysosomal enzymes, which carry the mannose-6-phosphate signal, from the trans-Golgi network (TGN) to late endosomes and recycle back to the TGN for re-utilization [80]. Antibodies against LBPA, when internalized from the medium, alter both the organization of internal membranes and the trafficking of IGF2/MPR through late endosomes [61]. In contrast, antibodies against lgp120/lamp 1, which is abundant on the limiting membrane of the same compartment, are without effect. These results suggest that LBPA-rich internal membrane domains contribute to IGF2/MPR sorting/trafficking.

Human autosomal recessive Niemann-Pick type C disease (NPC) is accompanied by intracellular accumulation of unesterified cholesterol [81]. The precise function of the protein encoded by the NPC gene is not known, although it is presumably involved in the regulation of cholesterol transport



Lysobisphosphatidic acid (LBPA)

Figure 5. Structure of lysobisphosphatidic acid (LBPA). LBPA has unique sn-1-glycerophospho-sn-1′-glycerol backbone. Each glycerol has one fatty acid. The positions of fatty acids are not yet determined.

[82–88]. In skin fibroblasts from NPC patients, accumulated cholesterol is present within vesicles containing both the small GTPase Rab7, a late endosome marker [89], and LBPA, suggesting that NPC is not only a lysosomal, but also an endosomal, storage disorder [77]. Cholesterol accumulation in late endosomes is also caused by intraluminal accumulation of cations, which is predicted to affect the internal membranes, as well as by highly specific perturbations of internal membranes with a mouse monoclonal or with human antibodies recognizing LBPA [77]. These results suggest that LBPA-rich internal membranes function as distribution device for LDL-derived cholesterol. Cholesterol accumulation is accompanied by inhibition of IGF2/MPR-mediated sorting and trafficking. In endothelial cells, cholesterol accumulation in late endosomes and the resulting sorting/trafficking defect also alter the transport of P-selectin and tetraspanin CD63/lamp3 from late endosomes to Weibel-Palade bodies, specific secretory granules of these cells [65].

The anti-phospholipid syndrome is characterized by various clinical manifestations accompanied by the presence of humoral anti-phospholipid antibodies [90–93]. The lipid target of anti-phospholipid antibodies is not yet established. We observed that anti-phospholipid antibodies bind LBPA [61,79]. These human antibodies, when endocytosed, also perturb the IGF2/MPR cycle, suggesting that the syndrome is associated, at least in part, to an endosomal defect. These data indicate that LBPA-rich membrane domains are involved in lipid and protein sorting within the endosomal system. It is attractive to propose that endosome internal and limiting membranes interact in a highly dynamic manner, perhaps via intra-luminal fusion/fission events. Recently it was shown that the phosphatidylinositol (PI) 3-kinase inhibitor wortmannin reduced the number of internal membranes in multivesicular bodies. This observation suggests the involvement of PI 3-kinase in multivesicular body morphogenesis [94].

Internalization of plasma membrane sphingolipids

Internalization of plasma membrane sphingolipids has been followed indirectly using glycolipid-specific toxin [95] and directly using fluorescent analogs of sphingolipids. These fluorescent sphingolipids contain a fluorophore of molecular weight 200–500, whereas molecular weight of sphingomyelin is 700–800. Since this bulky fluorescent group may alter physicochemical and biological properties of sphingolipids, sometimes control experiments should be performed before drawing any conclusions [96,97]. Fluorescent analogs of sphingomyelin and glucosylceramide, when inserted into the outer leaflet of the plasma membranes, are internalized into early endosomes and then the bulk is rapidly recycled back to the cell surface [98–101]. Sphingolipid analogs labeled with 4, 4-difluoro-5,7-dimethyl-4-bora-3a, 4a-diaza-s-indacene-3-pentanoic acid (BODIPY FL C5) exhibit a concentration-dependent shift in their fluorescence emission maximum from green to red wavelengths that can be used to estimate the

relative concentration of an analog in the intracellular membranes of living cells by quantitative fluorescence microscopy [102,103]. When these fluorescent sphingolipids were added to the plasma membranes, membranes exhibited green fluorescence. However, after internalization, within the same cell some endosomes exhibited green fluorescence, whereas others emitted red-orange fluorescence. The apparent concentration of the lipid analog in the red-orange endosomes was several-fold higher than its initial concentration at the plasma membrane, suggesting selective internalization of the lipid into a subset of early endosomes.

In polarized HepG2 cells, exogenously added fluorescent sphingolipid analogs are internalized and accumulated in a subapical endosomal compartment (SAC). From there, sphingomyelin analog is transported to the basolateral membrane whereas fluorescent glucosylceramide is targeted to the apical (bile canalicular) membranes [104]. It is believed that lipid sorting occurs within SAC [105]. Using different inhibitors recently it is suggested that separate sphingolipid domains exist at the luminal side of the SAC membranes in HepG2 cells [106].

Exogenous short chain ceramide modulates endocytic pathway. Incubation of Chinese hamster ovary cells with C6-ceramide slowed horseradish peroxidase and LDL transport from early endosomes to lysosomes [107]. C6-ceramide also causes enlarged late endosomes and lysosomes in mouse fibroblasts [108]. The mechanism of ceramide-induced enlargement is not clear. Ceramide metabolites sphingosine, sphingosine 1-phosphate, C6-Cer sphingomyelin, C2-Cer cerebroside, and C2-Cer GM3 gave no effect.

Transport of sphingolipids to the later compartments of endocytosis is also reported. Using immuno-electron microscopy, Mobius et al. showed that biotin-labeled derivative of the ganglioside GM1 was preferentially transported to late endosomes and lysosomes [109].

Sphingolipidosis and intracellular lipid membrane domains

Sphingolipidosis is a genetic disease in which a mutation of one of the lipid hydrolases or activator proteins blocks sphingolipid degradation, leading to lysosomal accumulation of endogenous sphingolipids. A fluorescent analog of the GSL lactosylceramide (BODIPY-LacCer) is internalized from the plasma membrane to the Golgi apparatus in normal human skin fibroblasts, but is targeted predominantly to endosomes and lysosomes in fibroblasts from patients with various sphingolipid-storage diseases [110]. Altered trafficking of BODIPY-LacCer was accompanied by the accumulation of cholesterol in late endosomes/lysosomes and altered distribution of NPC1 [111]. When patient fibroblasts were grown in lipoprotein-free medium, the labeling of the endosomes and lysosomes by fluorescent LacCer is eliminated and the labeling of the Golgi complex was concomitantly enhanced, suggesting that altered cholesterol levels or distribution may be responsible for the perturbation in fluorescent sphingolipid internalization in the cells derived from sphingolipid storage diseases. It is unexpected but very interesting that cholesterol is accumulated in various sphingolipid-storage disease cell types examined. Since LBPA-rich internal membrane domain of late endosomes plays an important role in cholesterol exit from endosomes, it is attractive to speculate specific interaction between sphingolipids and LBPA. Sphingolipids differ from glycerolipids in that they possess both hydrogen bond-accepting and -donating groups. Together with acyl group, these groups facilitate extensive formation of hydrogen-bonded networks [13]. In contrast, glycerolipids offer only hydrogen bond-accepting capacity. Although LBPA is a glycerolipid, unlike predominant glycerolipids in membranes, it possesses free hydroxyl groups which offer hydrogen bond-donating capacity. This may facilitate interaction between LBPA and sphingolipids in the internal membranes of late endosomes.

Korkotian et al. reported that accumulation of intracellular glucosylceramide after the treatment of hippocampal neurons with a chemical inhibitor of glucocerebrosidase caused the increase of tubular endoplasmic reticulum (ER) and calcium release from the ER [112]. This observation is important since the increase of calcium release is supposedly involved in neuronal cell death of Gaucher brain. The mechanism of the ER changes is not known.

Perspectives

Most of the genes involved in sphingolipid biosynthesis have been cloned and subsequently their knockout animals have revealed that sphingolipids are essential component for multicellular organization and maintenance. In contrast, the transport system of sphingolipids and their precursors, including a flippase for glucosylceramide, is poorly understood at molecular genetical level. For example, the possible existence of specific transporter of ceramide, which is required for sphingomyelin synthesis in the Golgi apparatus, has been proposed by Hanada et al. [23]. Molecular characterization of this and other factors involved in sphingolipids and sphingolipid-precursor traffic may facilitate our understanding of assembly and regulation of raft-like sphingolipid domains. The discovery of LBPA-rich membrane domains in the internal membranes of late endosomes indicates the existence of physically and biologically different lipid membrane domains within the cell. Future studies will unravel other, as yet unknown, lipid membrane domains which possess specific structures and functions, as well as the dynamic interaction between different lipid membrane domains.

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