Current Topics

Lipid Dynamics and Pathobiology in Membrane Lipid Rafts

Lipid Rafts: New Tools and a New Component

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Lipid rafts are liquid ordered membrane domains enriched with sphingolipids and cholesterol. After 20 years since the proposal of the original concept, the structure and function of lipid rafts are still obscure. Recently new tools to study lipid rafts have been developed. Lysenin is a sphingomyelin binding protein that specifically recognizes the lipid clusters. Poly(ethyleneglycol)-derivatized cholesterol ether (PEG-Chol) is a non-toxic cholesterol probe. These probes have revealed the heterogeneity of lipid rafts. The heterogeneity of lipid rafts is further supported by the discovery of a new lipid component, phosphatidylglucoside. Metabolic inhibitors are another useful tool. Sulfamisterin is a new addition to the serine palmitoyltransferase inhibitors. Recent findings have uncovered a previously unrecognized activity of a glycosphingolipid synthesis inhibitor, *D-threo*-1-phenyl-2-decanoylamino-3-morpholino-1-propanol (*D*-PDMP).

Key words lipid domain; lipid probe; sphingolipid; cholesterol; detergent insolubility; bis(monoacylglycerol)phosphate

1. INTRODUCTION

Lipid rafts are defined as liquid ordered (*lo*) lipid domains segregated from bulk liquid disordered (*ld*) membranes.^{1,2)} In the *lo* phase, the lateral mobility of the lipid molecules is similar to that in the *ld* phase, whereas the conformational order of the lipid hydrocarbon chains in the *lo* phase is similar to that in the solid ordered (*so*) phase. Cholesterol plays a crucial role in *lo* phase formation via interaction with the hydrocarbon chains of phospholipids or sphingolipids. Thus, in sphingomyelin-phosphatidylcholine with unsaturated fatty acid (such as palmitoylcholine phosphatidylcholine (POPC))-cholesterol ternary mixture, cholesterol facilitates phase separation of the sphingomyelin-rich *lo* phase and POPC-rich *ld* phase.

In biological membranes, lipid rafts have been operationally identified as the detergent insoluble membrane fraction.³⁻⁵⁾ Recent results have pointed out that the re-organization of membranes takes place during detergent treatment.⁶⁾ However, the discovery that *lo* lipids are resistant to detergent⁷⁾ suggests that the detergent method is a useful first screening of lipid raft components. To directly observe lipid rafts, specific probes are needed to mark the lipid raft components. Although several probes for raft proteins are available, lipid probes are limited. Since sphingolipids are major components of lipid rafts, inhibitors of sphingolipid metabolism are also useful tools to study lipid rafts. In this review, we will introduce new lipid raft probes and a new inhibitor of sphingolipid synthesis. We will also summarize the newly identified additional activity of a widely used glycosphingolipid synthesis inhibitor. In the last part of this review, we

will touch on the discovery of a new glycosphingolipid component of lipid rafts.

2. HOW TO DETECT LIPIDS IN LIPID RAFTS? —DEVELOPMENT OF NEW PROBES

Studies of the organization of lipids in lipid rafts have been hindered by the lack of appropriate lipid probes. For a long time, cholera toxin was the only useful probe to visualize raft lipids in living cells. The development of non-toxic sphingomyelin and cholesterol probes provided highly useful information on the distribution and dynamics of lipids in lipid rafts.

Lysenin Lysenin is an earthworm-derived 297 amino acid protein toxin that specifically binds sphingomyelin.⁸⁻¹¹⁾ Upon binding to sphingomyelin-containing membranes, lysenin assembles to oligomers. These oligomers form pores of 3 nm diameter in target membranes.¹²⁾ The binding of lysenin to sphingomyelin is dependent on the surface distribution of the lipid, *i.e.*, lysenin binds only when sphingomyelin is in the form of a cluster.¹³⁾ In model membranes, lysenin binds membranes composed of sphingomyelin and dioleoylphosphatidylcholine (diC18:1 PC), for which the gel to liquid crystalline phase transition temperature is -17°C . In these membranes, sphingomyelin and diC18:1 PC are phase separated and thus sphingomyelin forms clusters. In contrast, when diC18:1 PC is replaced with diC16:0 PC (phase transition temperature, 41°C), the binding of lysenin is not observed. In these membranes sphingomyelin and diC16:0 PC are well mixed and sphingomyelin does not form clusters. Isothermal calorimetry has revealed that the stoichiometry of

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lysenin: sphingomyelin is 1 : 5, indicating lysenin requires a cluster of sphingomyelin made up of at least a few lipid molecules in the membrane in order to bind the lipid.¹³⁾ The fact that most mammalian cells are sensitive to lysenin suggests that sphingomyelin forms clusters in most biomembranes. There are a few exceptions, such as the apical membranes of epithelial cells and the plasma membrane of melanoma cells. These membranes are highly enriched with glycosphingolipids for which the phase transition temperatures are higher than the physiological temperature. It is thought that sphingomyelin and glycosphingolipids are well mixed in these membranes.

A problem of using lysenin as a raft probe in living cells is its cytotoxicity. Using truncated mutants of lysenin, it is shown that the N-terminus of the protein is necessary for cytotoxicity whereas C-terminus is required to bind sphingomyelin. A non-toxic sphingomyelin probe was obtained by deleting the N-terminal 160 amino acids of the protein.¹⁴⁾ This protein binds sphingomyelin-containing membranes but does not oligomerize, suggesting that oligomerization is necessary for the cytotoxic activities of lysenin. The dissociation constant K_d of lysenin to sphingomyelin is 5.3×10^{-9} M, whereas that of non-toxic lysenin is 2.0×10^{-7} M. This is because of the high off-rate of non-toxic lysenin. This result indicates that the oligomerization stabilizes the binding of lysenin to sphingomyelin-containing membranes.

Cholera toxin specifically binds ganglioside GM1 (Gal β 1, 3GalNAc β 1,4(NeuAc α 2,3)Gal β 1,4GlcCer). The non-toxic B-subunit of cholera toxin has long been used as the only lipid probe for lipid rafts. In the recent study, living Jurkat T cells were doubly labeled with cholera toxin B-subunit and the non-toxic lysenin followed by fixation. The distribution of the toxins in the plasma membrane sheet was then examined by immunoelectron microscopy.¹⁴⁾ The experiment revealed that both sphingomyelin and GM1 form domains with a radius of 60–70 nm in Jurkat cells. However, there is no co-cluster of sphingomyelin and GM1 in the plasma membrane of Jurkat T cells. Jurkat cells are activated by crosslinking of the T-cell receptor or GM1. The crosslinking of sphingomyelin also activates the cells and induces calcium influx and ERK phosphorylation. However, unlike the activation *via* T cell receptor and GM1, the crosslinking of sphingomyelin is not accompanied by protein tyrosine phosphorylation. Sphingomyelinase hydrolyzes sphingomyelin to ceramide and phosphocholine. Cell surface sphingomyelinase treatment significantly decreases membrane sphingomyelin and thus disturbs sphingomyelin-rich membrane domains. The results of sphingomyelinase treatment together with lysenin experiment suggest the importance of sphingomyelin domains in G-protein coupled receptor signal transduction.¹⁴⁾ Thus, the sphingomyelin-rich membrane domain provides a functional signal cascade that is distinct from those provided by the T cell receptor or GM1. The combination of lysenin and cholera toxin revealed that lipid rafts are spatially and functionally heterogeneous. The existence of distinct raft domains has also been described in the promyelocytic cell line HL60.¹⁵⁾ The crosslinking of sphingomyelin induces apoptotic cell death, whereas GM1-crosslinking stimulates growth of the cell.

Poly(ethyleneglycol)-Derivatized Cholesterol Ether (PEG-Chol) Poly(ethyleneglycol)-derivatized cholesterol

ethers (PEG-Chols) are a unique group of non-ionic amphiphatic cholesterol derivatives. Because of their low toxicity, various PEG-Chols were initially used *in vivo* to disperse otherwise water-insoluble antibiotics. A high concentration of PEG(50)-Chol (50 is the average number of PEG repeats) selectively inhibits clathrin-independent, raft-dependent endocytosis.¹⁶⁾ Subsequent study with a fluorescein ester of PEG-Chol (fPEG-Chol) revealed that PEG-Chol has a high affinity for cholesterol-rich membrane domains (Fig. 1). Selective partition to cholesterol-rich membranes and the relatively low working concentration of fPEG-Chol makes it possible to use fPEG-Chol as a probe for cholesterol-rich membrane domains in living cells.¹⁷⁾ The antibiotic filipin is a well-known cholesterol marker for fixed cells, but due to its toxicity it cannot be used in living cells. fPEG-Chol can be applied as an aqueous dispersion. When added to cultured cells at low temperature, fPEG-Chol distributes exclusively in the outer leaflet of the plasma membrane. Although cholesterol is believed to be distributed to both outer and inner leaflet of the plasma membrane, the bulk PEG of PEG-Chol hinders transbilayer movement of PEG-Chol from the outer to the inner leaflet. Thus, fPEG-Chol monitors the dynamics of the outer leaflet cholesterol-rich membrane domains. Upon increasing the incubation temperature to 37 °C, fPEG-Chol is internalized together with GM1 and glycosylphosphatidylinositol (GPI)-anchored proteins.¹⁷⁾ When fixed and permeabilized human skin fibroblasts are labeled with fPEG-Chol, fluorescence is observed in the Golgi apparatus. In contrast, fibroblasts from a patient suffering from Niemann-Pick type C (NPC) disease display a strong labeling of late endosomes/lysosomes in addition to the Golgi apparatus. NPC is a congenital disease characterized by the intracellular accumulation of free cholesterol.¹⁸⁾ Interestingly, when fPEG-Chol is microinjected into the cytoplasm, both normal and NPC fibroblasts display only the Golgi labeling. These results indicate that in NPC, cholesterol is selectively accumulated in the lumen of the organelles. In contrast, in the Golgi apparatus, cholesterol is exposed to cytoplasmic (or cytoplasmic plus luminal) membranes.

Dihydroergosterol (DHE) is a naturally occurring fluorescent cholesterol derivative. Maxfield and co-workers studied the endocytic pathway of DHE. They showed the accumulation of DHE in recycling endosomes in cultured Chinese hamster ovary (CHO) cells.¹⁹⁾ In contrast to fPEG-Chol, DHE undergoes rapid transbilayer movement. fPEG-Chol and DHE internalized differently in CHO cells, suggesting that the cholesterol-rich membranes of the outer and inner leaflet behave differently.

3. METABOLIC INHIBITORS AS A TOOL TO STUDY SPHINGOLIPID FUNCTIONS

Specific inhibitors of sphingolipid biosynthesis are employed to disrupt lipid rafts. Inhibitors of the first step of sphingolipid synthesis, of ceramide synthesis and of glycosphingolipid synthesis have been reported. Using the serine palmitoyltransferase inhibitor, ISP-1, Kasahara *et al.* have suggested the association of GPI-anchored protein TAG-1 with src-family kinase Lyn in lipid rafts.²⁰⁾ Depleting glycosphingolipid by D-threo-1-phenyl-2-decanoylamino-3-morpholino-1-propanol (D-PDMP) also affects GPI-anchored

protein-mediated signal transduction.²¹⁾ The combination of ceramide synthase inhibitor fumonisin-B1, PDMP and sphingomyelinase treatments suggest that the formation of the scrapie prion protein correlate inversely with sphingomyelin levels.²²⁾ Here we introduce a new member of a serine palmitoyltransferase inhibitor, sulfamisterin. We also identify an additional activity of well-known glycosphingolipid synthesis inhibitor, D-PDMP. The results explain some interesting activities of D-PDMP to take place in a glycolipid-independent manner.

Sulfamisterin The early steps of the sphingolipid biosynthesis pathway are conserved from fungi to mammals. The first step is the condensation of serine and palmitoyl CoA. This reaction is catalyzed by serine palmitoyltransferase (SPT) [EC2.3.1.50].^{23,24)} This enzyme belongs to a family of pyridoxal 5'-phosphate-dependent enzymes and is reported to localize at the endoplasmic reticulum in mammalian cells. Several natural and synthetic inhibitors of SPT have been reported. ISP-1 (myriocin/thermozymocidin) from the fungus *Isalia sinclairii* is a widely used and highly selec-

tive SPT inhibitor.²⁵⁾ The structure of ISP-1 resembles that of sphingosine. ISP-1 inhibits sphingolipid synthesis in mammalian cells with an IC_{50} value in the nanomolar range. In contrast, a micromolar concentration of ISP-1 is required to inhibit sphingolipid synthesis in yeast, *Saccharomyces cerevisiae*. The relative resistance of yeast to ISP-1 is likely to be due to the inactivation of the inhibitor through *N*-acetylation by the recently identified Sli1 protein.²⁶⁾

Other SPT inhibitors are also reported. Sphingofungins isolated from two species of thermotolerant fungi, *Aspergillus fumigatus* and *Paecilomyces variotii*,^{27,28)} and lipoxamycin from *Streptomyces*, are antifungal compounds which inhibit SPT activity.²⁹⁾ Viridofungins from *Trichoderma viride* are potent inhibitors of mammalian SPT, but they also inhibit squalene synthase in mammalian and yeast cells.^{30,31)} L-Cycloserine³²⁾ and β -chloroalanine³³⁾ are synthetic inhibitors, which have sometimes been used to inhibit SPT in intact cells, but they are actually wide-range inhibitors of the pyridoxal 5'-phosphate-dependent enzymes.

Sulfamisterin is a newly identified SPT inhibitor derived from the fungus *Pycnidiaella* sp.³⁴⁾ The chemical structure of sulfamisterin resembles both that of sphingosine as well as ISP-1 (Fig. 2). Sulfamisterin inhibits SPT activity with an $IC_{50}=3$ nM *in vitro* in a cell lysate prepared from Chinese hamster ovary fibroblasts. Sulfamisterin markedly inhibits the biosynthesis of sphingolipids in intact Chinese hamster ovary cells and in yeast as monitored by radioactive precursors. Unlike the *in vitro* experiments, 10 μ M sulfamisterin is required for complete inhibition of sphingolipid synthesis *in vivo*. This concentration of sulfamisterin does not affect cell growth. Whereas ISP-1 is inactivated through the conversion to *N*-acetyl-ISP-1 by Sli1p in yeast, sulfamisterin is equally effective in wild type and Δ -sli1 mutant, suggesting that sul-

Fig. 1. PEG-Chol Labels the Golgi Apparatus in Human skin Fibroblasts

Cells were fixed, permeabilized and triply labeled with fPEG-Chol (green), anti-TGN46 (Golgi, red), and TOPRO-3(nucleus, blue). The lower right figure shows the merge. Bar, 20 μ m. Golgi labeling by PEG-Chol indicates the accumulation of cholesterol in the organelle in human skin fibroblast. Photograph courtesy of Kumiko Ishii.

Fig. 2. Structure of Sphingosine, ISP-1 and Sulfamisterin

Fig. 3. Accumulation of Multilamellar Bodies in Cells Treated with D-PDMP

Control fibroblast (left) and the cell treated with 10 μ M D-PDMP for 3 d. Bar, 0.5 μ m. Photograph courtesy of Motohide Murate.

Fig. 4. The Structure and a Molecular Model of Phosphatidylglucoside

Figure courtesy of Tomohiro Hayakawa.

famisterin is not a substrate for Sli1p. These results indicate sulfamisterin as an additional useful tool to study sphingolipid biosynthesis.³⁴⁾ Recently, total chemical synthesis of sulfamisterin and its derivatives has been reported.³⁵⁾ Biological assessment of all synthetic compounds revealed that natural sulfamisterin and its 3-epimer as well as their desulfonated derivatives possessing 2*S*-configuration strongly inhibit the serine palmitoyl transferase both *in vitro* and *in vivo*, whereas compounds with 2*R*-configuration were found to show much weaker inhibitory activity.

D-threo-1-Phenyl-2-decanoylamino-3-morpholino-1-propanol (D-PDMP) D-threo-1-Phenyl-2-decanoylamino-3-morpholino-1-propanol (D-PDMP) is a well-known inhibitor of UDP-glucose: ceramide glucosyltransferase (EC 2.4.1.80; GlcCer synthase (GCS/GlcT-1), the first step of glycosphingolipid synthesis.^{36,37)} This inhibitor has long been employed to study the roles of glycosphingolipids. In order to inhibit cellular GCS/GlcT-1 activities, the inhibitor should be transported to the ER and Golgi apparatus where glycosylation of ceramides occurs. Unexpectedly, however, a recent study revealed that D-PDMP is accumulated in late endosomes.³⁸⁾ A characteristic feature of this organelle is its multivesicular structure.^{39,40)} The internal membranes of late endosomes are highly enriched with a unique acidic phospholipid, lysobisphosphatidic acid (LBPA, also called as bis(monoacylglycerol)phosphate (BMP)).^{41–43)} The BMP-rich membrane domain has been shown to be involved in both membrane traffic from late endosomes and the degradation of sphingolipids within the organelle.^{44–46)} BMP is also involved in the formation of multivesicular membranes, suggesting that the characteristic membrane structure is indeed a prerequisite for the proper function of this membrane domain.⁴⁷⁾ Small-angle X-ray scattering indicates that the addition of D-PDMP at low pH, which resembles the pH of the lumen of late endosomes/lysosomes, changes the organization of BMP membranes from loosely packed lamellar structures to closely packed multilamellar ordering.³⁸⁾ Similarly, the accumulation of multilamellar endosomes is observed when cultured mammalian cells are treated with D-PDMP (Fig. 3). This structural change is also induced by L-PDMP, a stereoisomer of D-PDMP which does not inhibit glycosphingolipid synthesis.

However, *N*-butyl-deoxynojirimycin (NB-DNJ), another inhibitor of GCS, does not affect the structure of BMP.

Lysosomal acid lipase is dramatically activated in the presence of BMP. However, the addition of D-PDMP inhibits this activation. As a result, cells accumulate both free cholesterol and cholesterol esters in late endosomes. Again, the accumulation is observed upon the treating cells with L-PDMP but not with NB-DNJ. The inhibition of the degradation of low density lipoproteins (LDL) leads to the decrease of cell surface cholesterol. A similar effect of D-PDMP is obtained using a melanoma mutant which is defective in GCS,⁴⁸⁾ confirming that the effect of D-PDMP on cholesterol homeostasis is not dependent on the inhibition of GCS activity.

D-PDMP is known to sensitize multi-drug resistance (MDR) cells. A major cause of MDR is the activation of P-glycoprotein.^{49,50)} P-glycoprotein is a member of the ATP-binding-cassette (ABC) transporter superfamily and acts as an ATP-dependent efflux pump against a broad range of anti-cancer reagents. The lipid environment influences the ATPase activity of P-glycoprotein.^{51–55)} Modok *et al.*⁵³⁾ showed that P-glycoprotein retains function in liquid-ordered cholesterol and sphingolipid model membranes. Gayet *et al.*⁵⁵⁾ showed that cholesterol increases with the level of chemoresistance in human CEM acute lymphoblastic leukemia. They suggest that cholesterol is involved in the P-glycoprotein-induced MDR phenotype and controls both the ATPase and drug efflux activities of P-glycoprotein. The decrease of cell surface cholesterol by D- and L-PDMP reduces the P-glycoprotein activity and thus leads to the increase of the cellular content of anti-cancer reagent. These results indicate that D-PDMP alters both glycolipid and cholesterol homeostasis and the effect on cholesterol is independent on the inhibition of glycosphingolipid synthesis.

4. LIPID RAFTS ARE MORE HETEROGENEOUS THAN PREVIOUSLY ACKNOWLEDGED—A NEW COMPONENT OF LIPID RAFTS

Detergent insolubility has long been employed as a biochemical approach to isolate lipid rafts. Recent studies have

demonstrated that Triton X-100 may promote liquid ordered domain formation in model membranes.⁶⁾ It has also been shown that not all of the proteins that are in rafts are detergent insoluble.⁵⁶⁾ Thus, rafts and the detergent insoluble fraction are different. However, detergent insolubility does afford one of the few options to screen new *lo*-forming lipids, although there are caveats to using this approach. Nagatsuka *et al.* found a novel and unique type of glycerophospholipid, phosphatidylglucoside (PtdGlc), in the detergent insoluble fraction isolated from HL60 cells.¹⁵⁾ This glycolipid is unevenly distributed on the plasma membrane even under fluorescent microscopy. Crosslinking of this lipid generates the signal for granulocytic differentiation.

It is reported that the detergent insoluble fraction acts as an effective immunogen.^{57,58)} Based on a similar protocol for preparation of monoclonal antibodies, Yamazaki *et al.* generated mouse monoclonal antibody recognizing the PtdGlc-enriched domain by immunizing mice with detergent-insoluble fractions from HL60 cells.⁵⁹⁾ Using this novel antibody, they found that PtdGlc exists in rodent brains. Surprisingly, only a single molecular species of this lipid exists in the brain. The fatty acid composition of this lipid is stearic acid (C18:0) at the C1 position and there is an unusual arachidic acid (C20:0) at the C2 position of the glycerol backbone (Fig. 4).⁶⁰⁾ PtdGlc is considered to be a new astroglial marker and is strongly expressed in the fetal brain, in which neuronal cells are well known to proliferate and differentiate actively (Kinoshita *et al.*, The 28th annual meeting of the Japan Neuroscience Society, 2005). The discovery of the new raft lipid emphasizes the importance of carefully examining lipid structure in terms of both the hydrophilic head group and fatty acyl chains. Recent progress in lipidomics may uncover further as yet unidentified but biologically important lipid components of lipid rafts.

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REFERENCES

- London E., *Biochim. Biophys. Acta*, **1746**, 203—220 (2005).
- Simons K., Vaz W. L., *Annu. Rev. Biophys. Biomol. Struct.*, **33**, 269—295 (2004).
- Kobayashi T., Hirabayashi Y., *Glycoconj. J.*, **17**, 163—171 (2000).
- Edidin M., *Ann. Rev. Biophys. Biomol. Struct.*, **32**, 257—283 (2003).
- Mukherjee S., Maxfield F. R., *Annu. Rev. Cell Dev. Biol.*, **20**, 839—866 (2004).
- Heerklotz H., *Biophys. J.*, **83**, 2693—2701 (2002).
- London E., Brown D. A., *Biochim. Biophys. Acta*, **1508**, 182—195 (2000).
- Yamaji A., Sekizawa Y., Emoto K., Sakuraba H., Inoue K., Kobayashi H., Umeda M., *J. Biol. Chem.*, **273**, 5300—5306 (1998).
- Kiyokawa E., Makino A., Ishii K., Otsuka N., Yamaji-Hasegawa A., Kobayashi T., *Biochemistry*, **43**, 9766—9773 (2004).
- Ishitsuka R., Kobayashi T., *Anat. Sci. Int.*, **79**, 184—190 (2004).
- Ishitsuka R., Sato S. B., Kobayashi T., *J. Biochem. (Tokyo)*, **137**, 249—254 (2005).
- Yamaji-Hasegawa A., Makino A., Baba T., Senoh Y., Kimura-Suda H., Sato S. B., Terada N., Ohno S., Kiyokawa E., Umeda M., Kobayashi T., *J. Biol. Chem.*, **278**, 22762—22770 (2003).
- Ishitsuka R., Yamaji-Hasegawa A., Makino A., Hirabayashi Y., Kobayashi T., *Biophys. J.*, **86**, 296—307 (2004).
- Kiyokawa E., Baba T., Otsuka N., Makino A., Ohno S., Kobayashi T., *J. Biol. Chem.*, **280**, 24072—24084 (2005).
- Nagatsuka Y., Hara-Yokoyama M., Kasama T., Takekoshi M., Maeda F., Ihara S., Fujiwara S., Ohshima E., Ishii K., Kobayashi T., Shimizu K., Hirabayashi Y., *Proc. Natl. Acad. Sci. U.S.A.*, **100**, 7454—7459 (2003).
- Baba T., Rauch C., Xue M., Terada N., Fujii Y., Ueda H., Takayama I., Ohno S., Farge E., Sato S. B., *Traffic*, **2**, 501—512 (2001).
- Sato S. B., Ishii K., Makino A., Iwabuchi K., Yamaji-Hasegawa A., Senoh Y., Nagaoka I., Sakuraba H., Kobayashi T., *J. Biol. Chem.*, **279**, 23790—23796 (2004).
- Liscum L., *Traffic*, **1**, 218—225 (2000).
- Mukherjee S., Zha X., Tabas I., Maxfield F. R., *Biophys. J.*, **75**, 1915—1925 (1998).
- Kasahara K., Watanabe K., Kozutsumi Y., Oohira A., Yamamoto T., Sanai Y., *Neurochem. Res.*, **27**, 823—829 (2002).
- Nagafuku M., Kabayama K., Oka D., Kato A., Tani-ichi S., Shimada Y., Ohno-Iwashita Y., Yamasaki S., Saito T., Iwabuchi K., Hamaoka T., Inokuchi J., Kosugi A., *J. Biol. Chem.*, **278**, 51920—51927 (2003).
- Naslavsky N., Shmeeda H., Friedlander G., Yanai A., Futerman A. H., Barenholz Y., Taraboulos A., *J. Biol. Chem.*, **274**, 20763—20771 (1999).
- Merrill A. H., Jr., *J. Biol. Chem.*, **277**, 25843—25846 (2002).
- Hanada K., *Biochim. Biophys. Acta*, **1632**, 16—30 (2003).
- Miyake Y., Kozutsumi Y., Nakamura S., Fujita T., Kawasaki T., *Biochem. Biophys. Res. Commun.*, **211**, 396—403 (1995).
- Momoi M., Tanoue D., Sun Y., Takematsu H., Suzuki Y., Suzuki M., Suzuki A., Fujita T., Kozutsumi Y., *Biochem. J.*, **381**, 321—328 (2004).
- Horn W. S., Smith J. L., Bills G. F., Raghoobar S. L., Helms G. L., Kurtz M. B., Marrinan J. A., Frommer B. R., Thornton R. A., Mandala S. M., *J. Antibiot.*, **45**, 1692—1696 (1992).
- Zweierink M. M., Edison A. M., Wells G. B., Pinto W., Lester R. L., *J. Biol. Chem.*, **267**, 25032—25038 (1992).
- Mandala S. M., Frommer B. R., Thornton R. A., Kurtz M. B., Young N. M., Cabello M. A., Genilloud O., Liesch J. M., Smith J. L., Horn W. S., *J. Antibiot.*, **47**, 376—379 (1994).
- Mandala S. M., Thornton R. A., Frommer B. R., Dreikorn S., Kurtz M. B., *J. Antibiot.*, **50**, 339—343 (1997).
- Onishi J. C., Milligan J. A., Basilio A., Bergstrom J., Curotto J., Huang L., Meinz M., Nallin-Omstead M., Pelaez F., Rew D., Salvatore M., Thompson J., Vicente F., Kurtz M. B., *J. Antibiot.*, **50**, 334—338 (1997).
- Sundaram K. S., Lev M., *J. Neurochem.*, **42**, 577—581 (1984).
- Medlock K. A., Merrill A. H., Jr., *Biochemistry*, **27**, 7079—7084 (1988).
- Yamaji-Hasegawa A., Takahashi A., Tetsuka Y., Senoh Y., Kobayashi T., *Biochemistry*, **44**, 268—277 (2005).
- Sato H., Maeba T., Yanase R., Yamaji-Hasegawa A., Kobayashi T., Chida N., *J. Antibiot.*, **58**, 37—49 (2005).
- Radin N. S., Shayman J. A., Inokuchi J., *Adv. Lipid Res.*, **26**, 183—213 (1993).
- Sillence D. J., Puri V., Marks D. L., Butters T. D., Dwek R. A., Pagano R. E., Platt F. M., *J. Lipid Res.*, **43**, 1837—1845 (2002).
- Makino A., Ishii K., Murate M., Hayakawa T., Suzuki Y., Suzuki M., Ito K., Fujisawa T., Matsuo H., Ishitsuka R., Kobayashi T., *Biochemistry*, **45**, 4530—4541 (2006).
- Kobayashi T., Gu F., Gruenberg J., *Semin. Cell Dev. Biol.*, **9**, 517—526 (1998).
- Gruenberg J., *Nat. Rev. Mol. Cell Biol.*, **2**, 721—730 (2001).
- Kobayashi T., Stang E., Fang K. S., de Moerloose P., Parton R. G., Gruenberg J., *Nature (London)*, **392**, 193—197 (1998).
- Kobayashi T., Yamaji-Hasegawa A., Kiyokawa E., *Semin. Cell Dev. Biol.*, **12**, 173—182 (2001).
- Kobayashi T., Beuchat M. H., Chevallier J., Makino A., Mayran N., Escola J. M., Lebrand C., Cosson P., Gruenberg J., *J. Biol. Chem.*, **277**, 32157—32164 (2002).
- Kobayashi T., Beuchat M. H., Lindsay M., Frias S., Palmiter R. D.,

- Sakuraba H., Parton R. G., Gruenberg J., *Nat. Cell Biol.*, **1**, 113—118 (1999).
- 45) Kolter T., Sandhoff K., *Annu. Rev. Cell Dev. Biol.*, **21**, 81—103 (2005).
- 46) Le Blanc I., Luyet P. P., Pons V., Ferguson C., Emans N., Petiot A., Mayran N., Demaurex N., Faure J., Sadoul R., Parton R. G., Gruenberg J., *Nat. Cell Biol.*, **7**, 653—664 (2005).
- 47) Matsuo H., Chevallier J., Mayran N., Le Blanc I., Ferguson C., Faure J., Satori Blanc N., Matile S., Dubochet J., Sadoul R., Parton R. G., Vilois F., Gruenberg J., *Science*, **303**, 531—534 (2004).
- 48) Ichikawa S., Nakajo N., Sakiyama H., Hirabayashi Y., *Proc. Natl. Acad. Sci. U.S.A.*, **91**, 2703—2707 (1994).
- 49) Dean M., Hamon Y., Chimini G., *J. Lipid Res.*, **42**, 1007—1017 (2001).
- 50) Ambudkar S. V., Kimchi-Sarfaty C., Sauna Z. E., Gottesman M. M., *Oncogene*, **22**, 7468—7485 (2003).
- 51) Urbatsch I. L., Senior A. E., *Arc. Biochem. Biophys.*, **316**, 135—140 (1995).
- 52) Rothnie A., Theron D., Soceneantu L., Martin C., Traikia M., Berridge G., Higgins C. F., Devaux P. F., Callaghan R., *Eur. Biophys. J.*, **30**, 430—442 (2001).
- 53) Modok S., Heyward C., Callaghan R., *J. Lipid Res.*, **45**, 1910—1918 (2004).
- 54) Troost J., Albermann N., Emil Haefeli W., Weiss J., *Biochem. Biophys. Res. Commun.*, **316**, 705—711 (2004).
- 55) Gayet L., Dayan G., Barakat S., Labialle S., Michaud M., Cogne S., Mazane A., Coleman A. W., Rigal D., Baggetto L. G., *Biochemistry*, **44**, 4499—4509 (2005).
- 56) Harder T., Scheiffele P., Verkade P., Simons K., *J. Cell Biol.*, **141**, 929—942 (1998).
- 57) Katagiri Y. U., Ohmi K., Katagiri C., Sekino T., Nakajima H., Ebata T., Kiyokawa N., Fujimoto J., *Glycoconj. J.*, **18**, 347—353 (2001).
- 58) Katagiri Y. U., Ohmi K., Tang W., Takenouchi H., Taguchi T., Kiyokawa N., Fujimoto J., *Lab. Invest.*, **82**, 1735—1745 (2002).
- 59) Yamazaki Y., Nagatsuka Y., Oshima E., Suzuki Y., Hirabayashi Y., Hashikawa T., *J. Immunol. Methods*, **311**, 106—116 (2006).
- 60) Nagatsuka Y., Horibata Y., Yamazaki Y., Kinoshita M., Shinoda Y., Hashikawa T., Koshino H., Nakamura T., Hirabayashi Y., *Biochemistry*, in press (2006).