Ceramide Selectively Displaces Cholesterol from Ordered Lipid Domains (Rafts)

IMPLICATIONS FOR LIPID RAFT STRUCTURE AND FUNCTION*

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Ceramide is a membrane lipid involved in a number of crucial biological processes. Recent evidence suggests that ceramide is likely to reside and function within lipid rafts; ordered sphingolipid and cholesterol-rich lipid domains believed to exist within many eukaryotic cell membranes. Using lipid vesicles containing co-existing raft domains and disordered fluid domains, we find that natural and saturated synthetic ceramides displace sterols from rafts. Other raft lipids remain raft-associ-ated in the presence of ceramide, showing displacement is relatively specific for sterols. Like cholesterol-containing rafts, ceramide-rich “rafts” remain in a highly ordered state. Comparison of the sterol-displacing abil-ities of natural ceramides with those of saturated diglycerides and an unsaturated ceramide demonstrates that tight lipid packing is critical for sterol displacement by ceramide. Based on these results, and the fact that cholesterol and ceramides both have small polar headgroups, we propose that ceramides and cholesterol compete for association with rafts because of a limited capacity of raft lipids with large headgroups to accommodate small headgroup lipids in a manner that pre-vents unfavorable contact between the hydrocarbon groups of the small headgroup lipids and the surrounding aqueous environment. Minimizing the exposure of cholesterol and ceramide to water may be a strong driving force for the association of other molecules with rafts. Furthermore, displacement of sterol from rafts by ceramide is very likely to have marked effects upon raft structure and function, altering liquid ordered properties as well as molecular composition. In this regard, certain previously observed physiological processes may be a result of displacement. In particular, a direct connection to the previously observed sphingomyel-i-nase-induced displacement of cholesterol from plasma membranes in cells is proposed.

Ceramide is a membrane lipid involved in many biological processes. Sphingomyelinase action can result in a rapid increase in ceramide levels due to the action of sphingomyelinase upon the plasma membrane have important effects upon bacterial pathogenesis, cholesterol homeostasis, and apoptosis (3–9). Ceramide-triggered apoptosis is of particular interest because it seems to play an important role in the sensitivity of tumor cells to chemotherapy and radiotherapy (7, 10, 11) and in the development of atherosclerosis (12). Ceramide location within lipid rafts is an important factor in ceramide action (3, 5, 6, 13). Lipid rafts are liquid ordered state membrane do-mains rich in cholesterol and saturated polar lipids (usually sphingolipids). They can co-exist with disordered fluid state membrane domains rich in unsaturated lipids (14, 15). Ceram-ide both stabilizes and associates strongly with lipid rafts (16, 17). It can also induce the formation of unusually large raft domains (“platforms”) in plasma membranes (3).

In this study, we found that natural ceramides or ceramides with long saturated acyl chains, and analogous diglycerides, efficiently displace cholesterol from rafts in model membranes. Other raft-associating molecules were not displaced from rafts by ceramide or diglyceride. A model for the origin of this displacement phenomenon is proposed. This model may provide insights into how various molecules, including proteins, participate in raft formation. Displacement of sterols from rafts is likely to have a variety of important consequences for raft function. For example, displacement of cholesterol from rafts can explain the displacement of cholesterol from plasma membranes upon ceramide generation, and may have implications for the association of cholesterol-bound proteins with rafts.

EXPERIMENTAL PROCEDURES

Materials and Sample Preparation—Unlabeled lipids (including sphingosine-based ceramides) were purchased from Avanti Polar Lipids (Alabaster, AL). Dehydroergosterol (DHE) and 1,6-diphenyl-1,3,5-hexatriene (DPH) were purchased from Sigma-Aldrich. 1-palmitoyl-2-(3-diphenylhexatrienyl)propanoyl-phosphatidylcholine (DPH-PC) was purchased from Molecular Probes (Eugene, OR). 22-(Diphenylhexatrien- yl)docosyltrimethylammonium, (long chain TMADPH, LcTMADPH) was a kind gift from G. Duportail and D. Heissler (Université Louis Pasteur, Strasbourg). Lipids and probes were stored dissolved in etha-nol at −20 °C. Concentrations were determined by weight. Radi-o-labeled dioleoylphosphatidylcholine (DOPC) and dipalmitoylphospha-tidylcholine (DPPC) were purchased from Amersham Biosciences (Piscataway, NJ). Radiolabeled palmitoyl (C16:0) ceramide was pur-chased from American Radiolabeled Chemicals (St. Louis, MO). Lipid (including radiolabeled lipid) purity was confirmed by TLC. Acetyl-K,W,L,AL,L,W,K,–amide (liquid-phase) was purchased from Invitrogen (Carlsbad, CA) and purified as described previously (18).

Multilamellar vesicles (MLV) were prepared at a concentration of

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† The abbreviations used are: DHE, dehydroergosterol; PBS, phosphate-buffered saline; SM, sphingomyelin; cer, ceramide; DRM, de-tergent-resistant membrane; DPH, 1,6-diphenyl-1,3,5-hexatriene; DOPC, dioleoylphosphatidylcholine; DPPC, dipalmitoylphosphatidylcholine; LeTMADPH, 22-(diphenylhexatrienyl)docosyltrimethylammonium; MLV, multilamellar vesicles; 12 SLPC, 1-palmitoyl-2-(12-doxyl)-stearoyl-phosphatidylcholine; SUV, small unilamellar vesicles.
500 μl lipid in PBS (10 mM sodium phosphate, 150 mM NaCl, pH 7) as described previously (19). Dried lipid mixtures were dispersed in buffer at 70 °C using a multilobe vortexer for 15 min and cooled to 25 °C. Small unilamellar vesicles (SUV) at a concentration of 50 μM lipid in PBS were prepared by ethanol dilution as described previously (20). Lipids mixed in ethanol were diluted slightly more than 50-fold in PBS buffer heated to 70 °C, briefly vortexed, incubated at 70 °C for about 5 min, re-vortexed and cooled to 25 °C.

Fluorescence Measurements—Fluorescence was measured on a SPEX Fluorolog 3. Excitation and emission wavelength sets used (in nm) were: (358, 427) for DPH and LcTMADPH, (358, 436) for DPH-PC, (324, 376) for DHE, and (280, 340) for LP peptide. Fluorescence intensity in background samples lacking fluorophore was subtracted when significant. Correlated fluorescent concentrations were corrected by the ratio % pelleting/% OD. Fluorescence periods for background fluorescence were 0.1 mol% for LcTMADPH and DPH-PC and 1.5 mol% for LP peptide.

Insolubility Assay of Raft Content—The detergent-resistant membrane (DRM) were isolated by insolubility at 23 °C as described previously (19). The DRM fraction from a 1-mL MLV sample was collected after 2 h of incubation at 1000 rpm for 15 min in an Eppendorf 5415C tabletop centrifuge. The fluorescence of 970–990 μl of supernatant and of the pellet suspended in 975 μl of PBS were compared. For calculating % pelleting corrections were made for differences in fluorophore intensity (quantum yield) in lipids and Triton X-100. Radioactive tracers were used at 0.02–0.1 Ci per sample. Average values and the range for duplicate experiments were calculated. (In most cases, for these and the fluorescence studies described below, additional experiments that are not reported gave similar results.)

Fluorescence Quenching Assays—Quenching of fluorophores incorporated into SUV was measured at 23 °C as described previously (17, 20). Fluorescence (F) of samples containing 12SLPC was normalized (F0) to that in samples in which DOPC replaced 12SLPC. Control experiments confirmed that quenching of DHE was not affected by the length of time that samples were incubated after preparation, or by whether MLV or SUV samples were prepared. Average values and the range for duplicate experiments were calculated. (In most cases, for these and the fluorescence studies described below, additional experiments that are not reported gave similar results.)

Polarization Measurements—Anisotropy measurements were made at 22 °C using a SPEX Glan-Thompson automated polarizer accessory. SUV samples were prepared as described above except that 0.3 mol% DPH or LcTMADPH was used. Background intensities were negligible. Average values and the range for duplicate experiments were calculated.

Measurement of DHE to LcTMADPH Energy Transfer—SUN samples were prepared with either LcTMADPH or with LcTMADPH plus DHE. Samples containing DHE were composed of 12:61:15 (mol:mol) DPPC:cer:DOPC:cholesterol (17, 19). Samples containing 0.3 mol% LcTMADPH were heated at about 3 °C per min, and fluorescence periodically measured during heating. Average values for duplicate experiments were calculated.

Detection of Displacement of Sterol from Rafts by Ceramide using Fluorescence Quenching—DRM isolated by use of Triton X-100 can overestimate or under estimate raft levels, and % OD is a crude measure of total raft levels (25, 26). For these reasons, studies were also carried out using fluorescence quenching. Fluorescent probes were incorporated into vesicles containing both raft-forming lipids and 1-palmitoyl-2-12-doxylsticearoyl PC (12SLPC), a fluorescence quenching lipid that, like DOPC, is relatively excluded from rafts (20). 12SLPC is a short-range quencher that has to be in near contact with a fluorescent molecule in order to quench its fluorescence (30, 31). When rafts and non-raft regions co-exist in 12SLPC-containing vesicles the intensity of fluorescence from a membrane-bound probe depends on whether it associates with rafts or not. In mixtures of DPPC (or sphingomyelin) with cholesterol and 12SLPC, rafts have a low % of 12SLPC while the remainder of the bilayer, which is in a disordered fluid state, is enriched in

Ceramide and Implications for Raft Structure
Fig. 1. Ceramide-induced displacement of molecules from rafts assayed by insolubility in Triton X-100 at 23 °C. Samples lacking ceramide were composed of 1:1.0.35 (mol:mol) DPPC: DOPC:sterol dispersed in PBS. In samples containing cer, DPPC was replaced by equivalent mole amounts of cer. Mol% cer shown is always relative to the total lipid concentration. Sterol was cholesterol except in samples containing DHE in which a 1:1 mol:mol cholesterol:DHE mixture was used. A and B, normalized content of fluorescent probes (A) or radioactive lipids (B) in detergent-insoluble fraction in absence (open bars) or presence (shaded bars) of 18 mol% C 18:0 cer. Abbreviations: DP, DOPC, DO, DOPC, Ch, cholesterol. C and D, effect of ceramide concentration upon displacement of DHE (C) or [3H]cholesterol (D) from the detergent-insoluble fraction. Symbols: squares, % OD remaining after detergent addition; triangles, % insoluble radioactivity or fluorescence; circles, % pellet/% OD. The % pellet/% OD equals the ratio of the % of the radioactive tracer or fluorescent probe in the Triton X-100 insoluble pellet fraction to the % of unsolubilized lipid as judged by OD at 400 nm. The average and range of duplicate samples are shown in this and most of the following figures. In most cases, additional experiments that are not shown gave similar results.

Partial substitution of DPPC with ceramide had little effect on quenching of LcTMA-DPH and peptide fluorescence, showing the locations of these molecules was not greatly altered by ceramide. (The small changes observed in their quenching at high ceramide concentrations may reflect a small change in raft association or ceramide-induced displacement of some of the residual raft-associated 12SLPC from rafts.) In contrast, as ceramide concentrations increased, confirming DHE displacement from rafts. DPH-PC fluorescence showed a much smaller increase in quenching as ceramide concentration was increased, indicating a more modest extent of displacement. Overall, the fluorescence quenching experiments are in agreement with those based upon detergent insolubility.

Utilizing quenching, ceramide-induced displacement of sterol from rafts was also observed when brain sphingomyelin (SM) and a brain ceramide mixture were substituted for DPPC and C18:0 ceramide, respectively (Fig. 2B). This shows that displacement is similar for analogous chemically defined and natural lipid mixtures. Comparison of the ability of different sphingolipids to displace sterol (Fig. 2C) showed that partial substitution of SM for DPPC had no effect on quenching of DHE fluorescence, whereas partial substitution of a cerebroside mixture for DPPC increased quenching, but to a much lesser degree than substitution of DPPC by C18:0 ceramide. The behavior of cerebroside-containing mixtures suggests that sterols associate with cerebroside-rich rafts to much higher degree than to ceramide-rich rafts but to a lesser degree than those formed by SM or DPPC, consistent with previous studies (17).

Comparison of Diglyceride and Ceramide Behavior—The effect of ceramide and diglyceride on raft stability and sterol displacement were compared. Both of these lipids contain two hydrocarbon chains and relatively small polar headgroups, but they differ in the details of their polar headgroup structure. In one experiment, their effects on raft stability were compared when added to mixtures of DPPC, 12SLPC, cholesterol, and LcTMA-DPH. As noted above, when rafts are present they bind LcTMA-DPH so that its fluorescence is only weakly quenched by 12SLPC (17, 20). However, at temperatures above that at which rafts are stable all lipids mix in a relatively homogeneous fashion, and thus quenching increases because LcTMA-DPH molecules come into increased contact with 12SLPC molecules. The temperature dependence of quenching can be used to monitor the thermal stability of rafts. The midpoint of a quenching versus temperature curve (raft “melting” temper-
Ceramide-rich Domains Are Highly Ordered—Fluorescence anisotropy measurements were made on samples containing LcTMADPH and DPH incorporated into various lipid bilayers to probe the effect of ceramide upon lipid order (Fig. 4A). As expected, for both probes anisotropy values were high in vesicles composed solely of lipids forming ordered states (DPPC or DOPC/cholesterol) and low in vesicles composed of lipids existing in the disordered liquid state (DOPC or DOPC/cholesterol). In vesicles composed of DPPC, DOPC, and cholesterol mixtures under conditions in which both ordered and disordered domains co-exist (see above) Fig. 4A shows anisotropy of LcTMADPH fluorescence remained as high as that in fully ordered bilayers regardless of ceramide concentration. Since LcTMADPH partitions strongly into ordered phases this indicates that the more ordered domains remain highly ordered in the presence of ceramide. DPH, which partitions into both ordered and disordered phases, gave fluorescence with intermediate anisotropy values in vesicles containing both ordered and disordered states. There was a decrease in DPH anisotropy in the presence of ceramide. This decrease can be explained by partial displacement of DPH from ceramide-rich ordered domains. Partial displacement of DPH by ceramide was confirmed by quenching experiments (data not shown).

Effect of Sterol Concentration upon Displacement of Sterol from Rafts by Ceramide and upon Displacement of Ceramide from Rafts by Sterol—Because sterol levels in the plasma membrane are high, the effect of cholesterol concentration upon displacement by ceramide was assessed (Fig. 4B). Even at a concentration as high as 40 mol% cholesterol, the presence of 18 mol% ceramide was able to displace the great majority of cholesterol from rafts. We were also interested to see if sterol could displace ceramide from rafts. Fig. 4C shows that cholesterol can displace some C16:0 ceramide from ordered domains both when ceramide is present in trace amounts (open bars) or when it forms a large (18 mol%) fraction of the lipid bilayer (closed bars). This indicates sterol and ceramide are competing for association with rafts. However, displacement of ceramide by cholesterol was much less effective than displacement of cholesterol by ceramide. For example, upon introduction of 25 mol% cholesterol into the model membrane vesicles, ceramide concentrations in the ordered domains only decreased by one-third when samples contained 18 mol% C18:0 ceramide. This should be contrasted to the roughly 4-fold decrease of cholesterol concentration within ordered domains induced by the introduction of 18 mol% ceramide into bilayers containing 25 mol% cholesterol (Fig. 4C). The observation that ceramide displaces cholesterol more efficiently than cholesterol displaces ceramide confirms that ceramide has a much stronger affinity for ordered domains than cholesterol.

Energy Transfer Analysis of Domain Organization—To confirm that sterol displacement was not a sample preparation artifact, we used energy transfer to examine whether sterol and raft-forming lipids were present in the same vesicles. In homogeneous control bilayers containing DOPC, sterol, and LcTMADPH, energy transfer from DHE to LcTMADPH signif-

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2) Megha and E. London, unpublished observations.
significantly enhanced LcTMADPH fluorescence. The increase in LcTMADPH emission in the presence of DHE was somewhat temperature dependent, decreasing from a 42% increase at 20 °C to a 26% increase at 45 °C. At low temperatures the increase in LcTMADPH fluorescence in bilayers containing DPPC and ceramide was much less than that in the control samples (Fig. 4D). This was expected because, as shown by the studies above, LcTMADPH should be located in the DPPC/ceramide-rich rafts while the displaced DHE should be located in disordered fluid domains. However, upon melting of the rafts at high temperature, energy transfer approached levels observed in control samples lacking raft-forming lipids. This should not have been observed if there was one set of vesicles containing raft-forming lipids and LcTMADPH and a separate population containing DHE. Instead, this indicates that the raft-forming lipids and DHE were in a single set of vesicles.

Additional experiments showed that the raft melting temperature detected by energy transfer was close to that detected by 12SLPC quenching of LcTMADPH fluorescence (Fig. 4D), indicating that the lipid composition of the bulk sample and the vesicles containing DHE was similar. If the DHE had been in a different subset of vesicles than the raft-forming lipids, energy transfer should have shown a very different thermal dependence than that exhibited by quenching of LcTMADPH.

**DISCUSSION**

**Origin of the Displacement of Sterol from Ordered Domains by Ceramide**—What is the origin of sterol displacement from rafts by ceramide? Both cholesterol and natural ceramides have hydrocarbon structures allowing tight packing with phospholipids and/or sphingolipids. Tight packing involving sterols may be also energetically driven by the fact that the small polar headgroup (the OH) of cholesterol is insufficient to shield the sterol rings from water, and tight packing with other lipids allows the sterol rings to hide under an “umbrella” formed by lipids with large polar headgroups (33). Because ceramides also have a small polar headgroup we propose a similar phenomenon applies to them, and that the limited ability of normal polar lipids to simultaneously shield hydrocarbon groups on both ceramides and cholesterol from water results in a competition between ceramide and sterol for inclusion in ordered domains. An analogous model has been proposed to explain the ability of ceramide in disordered fluid domains to increase the binding of cholesterol to a cytolysin protein (46). The observation that DPH-PC, which has a large polar headgroup (but also a rather bulky fluorophore-labeled acyl chain, was partially displaced from rafts by ceramide suggests the possibility that especially tight packing of hydrocarbon chains in ceramide-containing ordered domains relative to those containing saturated lipid and cholesterol can also contribute to displacement by squeezing out molecules that cannot pack as well as ceramide. On the other hand, hydrogen bonding appears to be less important for displacement. Diglycerides have a very different set of hydrogen bonding groups than do ceramide, and yet a saturated diglyceride displaced sterol more effectively than a ceramide with a similar hydrocarbon chain structure.

An alternative explanation of displacement is that ceramide forms cholesterol-poor ordered domains that are separate from the ordered domains formed by DPPC (or SM). When ceramide is substituted for DPPC there would be a lower amount of the DPPC-rich ordered domains into which sterol could incorpo-
rate. The observations that increasing ceramide concentration induces only a gradual increase in raft melting temperature, and that melting remains a single step process, argue against multiple types of ordered domains. A separate ceramide-rich phase would likely give rise to a separate melting event at a distinct temperature. Furthermore, inclusion of ceramide increased the fraction of DPPC associated with ordered domains while displacing sterol (Fig. 1B). This would not be expected if ceramide and DPPC formed separate ordered domains, but would if ceramide and DPPC interact in a single type of mixed ordered domain. Finally, the partial displacement of ceramide from ordered domains by cholesterol is much easier to rationalize if ceramide and cholesterol are competing for inclusion in a single type of ordered domain.

Physiologically Relevant Ceramide Levels Displace Cholesterol from Plasma Membranes in Cells and Are Equivalent to Values Sufficient to Displace Cholesterol from Rafts—Our data show that displacement of cholesterol from rafts is significant (roughly 50%) when cer comprises 9 mol% of the total bilayer lipid and that displacement can be nearly complete when cer levels reach 18 mol%. These values are comparable to those reached in the plasma membrane under physiological conditions. In cases of bacterial infections, viral infection and tumor necrosis factor action it has been found that 40% of all-cellular SM is digested by ceramide-producing cellular SMase in as little as a few minutes (1, 34, 35). It has also been shown that for each molecule of SM digested roughly one molecule of ceramide appears (2, 34). Thus, we can approximate ceramide levels in the plasma membrane. If we estimate that the plasma membrane lipid is typically 15% SM, and that almost all SM is in the outer leaflet of the membrane, then about 30% of the outer leaflet of the plasma membrane is SM. In that case 40% digestion by SMase means that 12% of the plasma membrane outer leaflet lipid is converted to ceramide. This number is only a lower estimate because it ignores the existence of SM pools in internal membranes [lysosomes, endosomes, Golgi]. If one-half of cellular SM is in internal membranes, and thus insensitive to SMase, then there would be 80% conversion of plasma membrane SM to ceramide by SMase. This would double the value for ceramide levels produced in the outer leaflet to 24 mol%.

Fig. 4. Assay of vesicle properties in the presence of cer. A, effect of inclusion of cer in rafts upon fluorescence anisotropy of 1 mol% DPH (black bars) or 1 mol% LcTMADPH (striped bars) at 23 °C. Ceramide-containing samples were composed of 1:1:0.35 [DPPC+cer]:DOPC:cholesterol dispersed in PBS. For calibration purposes, anisotropy values in vesicles in the liquid disordered (DOPC ± 15 mol % cholesterol), and gel (DOPC) states at 23 °C are shown. Also shown: DPPC + 15 mol % cholesterol. Abbreviations: DP, DPPC; DO, DOPC; Ch, cholesterol. B, effect of cholesterol concentration on its displacement by C18:0 cer measured by insolubility in Triton X-100 at 23 °C. Samples composed of 1:1 [DPPC+cer]:DOPC with various mol fractions of cholesterol. Shaded bars, 18 mol% cer; open bars, 0% cer. C, displacement of [3H]C16:0 ceramide from rafts as a function of cholesterol concentration as measured by insolubility in Triton X-100. Shaded bars, 18 mol% C18:0 cer; open bars, 0% C18:0 cer. D, comparison of the effect of temperature upon energy transfer from DHE to LcTMADPH and upon DHE quenching. Squares, the ratio of the fractional increase in LeTMADPH fluorescence due to energy transfer from DHE in vesicles composed of 12:12:61:7.5:7.5 (mol/mol) DPPC:cer:DOPC:DHE:cholesterol relative to the fractional increase in energy transfer in vesicles composed of 85:7.5:7.5 DOPC:DHE:cholesterol. Triangles, ratio of LeTMADPH fluorescence (F) in vesicles composed of 12:12:30.5:30.5:15 (mol/mol) DPPC:cer:DOPC:12SLPC:cholesterol to fluorescence (F0) of vesicles composed of 12:12:6:15 (mol/mol) DPPC:cer:DOPC:cholesterol. Samples also contained 1 mol % LcTMADPH. Vesicles were dispersed in PBS.
Several other studies have shown that SMase digestion also leads to displacement of cholesterol from the plasma membrane (1, 2, 4, 9, 36). There appears to be a direct relationship between these events. The rate of cholesterol displacement from the plasma membrane follows the rate of SM digestion without a significant time lag, and plasma membrane cholesterol levels recover at the same rate that SM is resynthesized from ceramide (2, 36). Even the step in which internalized cholesterol is esterified follows ceramide generation within minutes (1, 2, 9).

Detailed data about the relationship between ceramide generation and plasma membrane cholesterol levels was very recently presented by Al-Makdissy et al. (36). These investigators directly assayed both SM and cholesterol levels in isolated plasma membrane. Digestion of 25% of plasma membrane SM led to a loss of 50% of plasma membrane cholesterol, similar to what we would predict based on the data in this report. In addition, the authors measured DPH anisotropy to measure membrane fluidity. They found DPH anisotropy dropped when ceramide was produced. In other words, DPH found itself in a more disordered lipid environment upon ceramide generation. The connection between DPH anisotropy and ceramide levels is direct, as anisotropy values returned to higher values in parallel with the reconversion of ceramide to SM (36). Our anisotropy data shows a similar relationship between DPH anisotropy and ceramide levels. As we noted, quenching data shows that this drop in anisotropy is due to DPH displacement from the ordered ceramide-rich rafts and thus transfer to the disordered lipid regions of the bilayer.

The excellent fit between ceramide levels and both cholesterol displacement and DPH anisotropy data in cells and model membranes strengthens our conclusion that the same physical processes are involved. Furthermore, one group has reported an experiment showing a decrease in cholesterol in detergent-resistant membrane fractions from rat astrocytes treated with SMase (37). Thus, we propose that displacement of cholesterol from plasma membranes is a direct consequence of ceramide-induced displacement of cholesterol from rafts such that the cholesterol released from rafts enters a pool that is relatively rapidly internalized. This displacement provides a more precise molecular mechanism for the previously proposed idea that internalization is linked to the loss of favorable SM-cholesterol interactions when plasma membrane SM levels decrease (4). It is also in agreement with the previous proposal that in cholesterol homeostasis “excess” plasma membrane cholesterol is rapidly internalized (38).

Our model is also supported by a very recent study showing the digestion of plasma membrane SM by sphingomyelinase D (which converts SM to ceramide phosphate, a molecule with a significantly more polar headgroup than ceramide) does not induce cholesterol movement out of the plasma membranes (39). This shows that cholesterol internalization is linked to ceramide production, and not SM degradation.

Other Biological Implications of Sterol Displacement—Conditions in which rafts become ceramide-rich and displace sterol may result in additional alterations of raft physical properties and composition. The relatively rapid lateral diffusion characteristic of the sterol-rich liquid ordered state (40) may be dramatically altered in ceramide-rich cholesterol-poor rafts. Additionally, protein association with rafts may be affected, especially for proteins linked to cholesterol, such as hedgehog (41), or proteins that interact with cholesterol, as some bacterial cytolysins and NAP-22 (42, 43).

It should be noted our model predicts that cholesterol and ceramides should be able to mix under conditions in which hydration can be avoided. An example may be the lipid layers of the stratum corneum of skin, which are primarily composed of a cholesterol/ceramide mixture (44). The tendency of both cholesterol and ceramide to avoid excessive hydration presumably contributes functionally to the action of the stratum corneum as a barrier to water.

The tendency of cholesterol and ceramide to avoid exposure to water also might be a driving force for the association of other types of molecules with rafts. It could contribute, along with saturated acyl chain packing interactions, to the raft-association of molecules that can cover the bilayer surface, such as carbohydrate anchors of GPI-anchored proteins, or glycosphingolipids with large headgroups. It could also contribute to the ability of protein domains that can cover hydrophobic sites on the bilayer surface to bind to rafts (or to any membranes with high cholesterol or ceramide levels).

In this regard, it should be noted that such interactions should be enhanced at high cholesterol levels. It has been proposed that the ability of phospholipids to form a complex with cholesterol (45) or to maintain a spatial distribution that prevents contact of cholesterol hydrocarbon with water (33) would become overwhelmed at high cholesterol concentrations.

For all these reasons, it will be important in future studies to investigate the behavior of ceramide-rich rafts in cells. We plan to undertake studies both of their lipid composition, protein composition, and physical behavior by examining the behavior of detergent-resistant membranes in normal and SMase-treated cells.

REFERENCES
1. Chatterjee, S. (1984) J. Biol. Chem. 259, 879–882
2. Ridgway, N. D., Lagace, T. A., Cook, H. W., and Byers, D. M. (1998) J. Biol. Chem. 273, 31621–31628
3. Grassme, H., Jendrossek, V., Riehle, A., von Kurthy, G., Berger, J., Schwarz, H., Weller, M., Kolesnick, R., and Gullbins, E. (2003) Nat. Medicine 9, 322–330
4. Ridgway, N. D. (2000) Biochim. Biophys. Acta 1484, 129–141
5. Gullbins, E. (2000) Pharmacol. Res. 47, 393–399
6. Grassme, H., Jende, A., Riehle, A., Schwarz, H., Berger, J., Sandhoff, K., Kolesnic, R., and Gullbins, E. (2001) J. Biol. Chem. 276, 20589–20596
7. Ruvolo, P. F. (2003) Pharmacol. Res. 47, 383–392
8. Lange, Y., and Steck, T. L. (1997) J. Biol. Chem. 272, 13103–13108
9. Slote, J. P., Hedstrom, G., Rannstrom, S., and Ekman, S. (1989) Biochim. Biophys. Acta 995, 80–96
10. Crighton, B., and Hannun, Y. A. (2001) Drug Resist. Updat. 4, 368–377
11. Michael, J. M., Lavin, M. F., and Watters, D. J. (1997) Cancer Res. 57, 3600–3605
12. Leidig, A., Sevenik, E., Reisenerhuber, G., Deigner, H.-G., and Hermetter, A. (2003) J. Biol. Chem. 278, 32921–32928
13. Cresmetti, A. E., Goni, F. M., and Kolesnic, R. (2002) FEBS Lett. 531, 47–53
14. Brown, D. A., and London, E. (1990) J. Membr. Biol. 143, 133–114
15. Rietveld, A., and Simons, K. (1998) Biochim. Biophys. Acta 1376, 467–479
16. Wang, T.-Y., and Silvis, J. R. (2003) Biochim. Biophys. Acta 1588, 182–195
17. Xu, X., Rittman, R., Duportail, G., Heisser, D., Vilchez, C., and London, E. (2001) J. Biol. Chem. 276, 33540–33546
18. Fastenberg, M. E., Shugomori, H., Xu, X., Brown, D. A., and London, E. (2003) Biochemistry 42, 12576–12580
19. Xu, X., and London, E. (2000) Biochemistry 39, 843–849
20. Ahmed, S. N., Brown, D. A., and London, E. (1997) Biochemistry 36, 10944–10951
21. Niu, S. L., and Litzman, B. J. (2002) Biochim. Biophys. Acta 1538, 3403–3415
22. Leventis, R., and Silvis, J. R. (2001) Biochim. Biophys. Acta 81, 2257–2267
23. Brown, D. A., and Rose, J. K. (1992) Cell 68, 533–544
24. Schröder, B., London, E., and Brown, D. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 12130–12134
25. Heerklotz, H., Szadkowska, H., Anderson, T., and Seelig, J. (2003) J. Mol. Biol. 329, 783–799
26. London, E., and Brown, D. A. (2000) Biochim. Biophys. Acta 1508, 182–195
27. Wang, T.-Y., Leventis, R., and Silvis, J. R. (2001) Biochemistry 40, 13031–13040
28. Hale, J. E., and Schroeder, F. (1982) Eur. J. Biochem. 122, 649–661
29. Beck, A., Heisser, D., and Duportail, G. (1993) Chem. Phys. Lipids 66, 135–142
30. Christofoletty, A., and London, E. (1987) Biochemistry 26, 39–45
31. Abrams, F. S., and London, E. (1992) Biochemistry 31, 5312–5322
32. London, E. (2002) Curr. Opin. Struct. Biol. 12, 480–486
33. Huang, J., and Feigenson, G. W. (1999) Biochemistry 38, 2142–2157
34. Grassme, H., Ghulbins, E., Brenner, B., Flemm, K., Sandhoff, K., Harker, K., Lang, F., and Meyer, T. F. (1997) Cell 91, 605–615
35. Jan, J. T., Chatterjee, S., and Griffin, D. E. (2000) J. Viral. 74, 6425–6432
36. Al-Makdissy, N., Yousisi, M., Pierre, S., Zagler, O., and Donner, M. (2003) Cell. Signal. 15, 1019–1030
37. Ito, J.-I., Nagayasu, Y., and Yokoyama, S. (2000) J. Lipid Res. 41, 894–904

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Ceramide and Implications for Raft Structure

38. Lange, Y., Ye, J., Rigney, M., and Steck, T. L. (1999) J. Lipid Res. 40, 2264–2270
39. Subbaiah, P. V., Billington, S. J., Jost, B. H., Senger, J. G., and Lange, Y. (2003) J. Lipid Res. 44, 1574–1580
40. Almeida, P. F., Vaz, W. L., and Thompson, T. E. (1992) Biochemistry 31, 6739–6747
41. Porter, J. A., Young, K. E., and Beachy, P. A. (1996) Science 274, 255–259
42. Khan, T. K., Yang, B., Thompson, N. L., Maekawa, S., Epand, R. M., and Jacobson, K. (2003) Biochemistry 42, 4780–4786
43. Tweten, R. K., Parker, M. W., and Johnson, A. E. (2001) Curr. Top. Microbiol. Immunol. 257, 15–33
44. ten Grotenhuis, E., Demel, R. A., Ponec, M., Boer, D. R., van Miltenburg, J. C., and Bouwstra, J. A. (1996) Biophys. J. 71, 1389–1399
45. Radhakrishnan, A., and McConnell, H. M. (2000) Biochemistry 39, 8119–8124
46. Zitzer, A., Bittman, R., Verbicky, C. A., Erukulla, R. K., Bhakdi, S., Weis, S., Valeva, A., and Palmer, M. (2001) J. Biol. Chem. 276, 14628–14633
