thematic review

Membrane dynamics and cell polarity: the role of sphingolipids

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Abstract **In recent years, glycosphingolipids (GSLs) have attracted widespread attention due to the appreciation that this class of lipids has a major impact on biological life. Inhibition of the synthesis of glucosylceramide, which serves as a precursor for the generation of complex glycosphinglipids, is embryonic lethal. GSLs play a major role in growth and development. Metabolites of sphingolipids, such as ceramide, sphinganine, and sphingosine, may function as second messengers or regulators of signal transduction that affect events ranging from apoptosis to the (co)regulation of the cell cycle. In addition, GSLs can provide a molecular platform for clustering of signal transducers. The ability of sphingolipids, with or without cholesterol, to form microdomains or rafts is critical in sorting and membrane transport that underlies the biogenesis of polarized membrane domains.** Here, a brief summary is presented of some recent developments in this field, with a particular emphasis on raft assembly and membrane transport in the establishment of membrane polarity.—Hoekstra, D., O. Maier, J. M. van der Wouden, T. A. Slimane, and S. C. D. van IJzendoorn. **Membrane dynamics and cell polarity: the role of sphingolipids.** ***J. Lipid Res.*** **2003.** 44: **869–877.**

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Glycosphingolipids (GSLs) are a class of lipids that are present in relatively minor abundance when compared with the abundance of phospholipids and cholesterol in eukaryotic cell membranes. GSLs contain a ceramide moiety as their core (**Fig. 1**). Addition of a phosphocholine headgroup generates sphingomyelin (SM). The addition of sugars to the ceramide moiety generates GSLs. If the headgroup contains the negatively charged sugar sialic acid, the resulting lipids are referred to as gangliosides. If the headgroup lacks sialic acid, they are called neutral GSLs (Fig. 1).

Although commonly contributing less than 5% to the total cellular lipid pool, GSLs are highly enriched in the outer leaflet of the apical plasma membrane domain of polarized epithelial cells. Here they may provide mechanical stability and protect certain membranes, such as the apical bile canalicular membrane of liver hepatocytes, from being solubilized by detergent-like bile acids. In addition, GSLs and their metabolites perform numerous other functions important in cellular functioning, tissue development, and physiology in general. This is emphasized by the fact that failure of GSL biosynthesis is lethal to embryonic development (1).

GSLs display a dynamic behavior. They are internalized along endocytic pathways and undergo sorting to distinct intracellular organelles prior to recycling or degradation. In this process, GSL metabolites act as second messengers or regulate the expression of cellular receptors in events like inflammation, apoptosis, and cell division. In addition, they can be reutilized for de novo biosynthesis.

In the biosynthetic pathway, sphingolipids form microdomains known as rafts (2). Rafts are instrumental in protein sorting and transport (3, 4) in both polarized and nonpolarized cells. As a result, these domains facilitate a variety of membrane (protein)-mediated functions, ranging from mating of yeast cells to signal transduction events. Here, we will highlight some recent developments in GSL research that are relevant to their role in the biogenesis and maintenance of plasma membrane polarity.

BIOSYNTHESIS AND LOCALIZATION OF SPHINGOLIPIDS

The hydrophobic core of (glyco)sphingolipids, ceramide (Fig. 1), is synthesized in the endoplasmic reticu-

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Abbreviations: CGT, UDP-galactose:ceramide galactosyltransferase; C_6NBD, 6-[*N*-(7-nitrobenz-2-oxa-1,3 diazol-4-yl)amino] hexanoyl; DRM, detergent-resistant membranes; GalCer, galactosylceramide; GlcCer, glucosylceramide; GPI, glycosylphosphatidylinositol; GSL, glycosphingolipid; pIgR, polymeric immunoglobulin receptor; PS, phosphatidylserine; SAC, subapical compartment; SM, sphingomyelin; TGN, *trans*-Golgi network.

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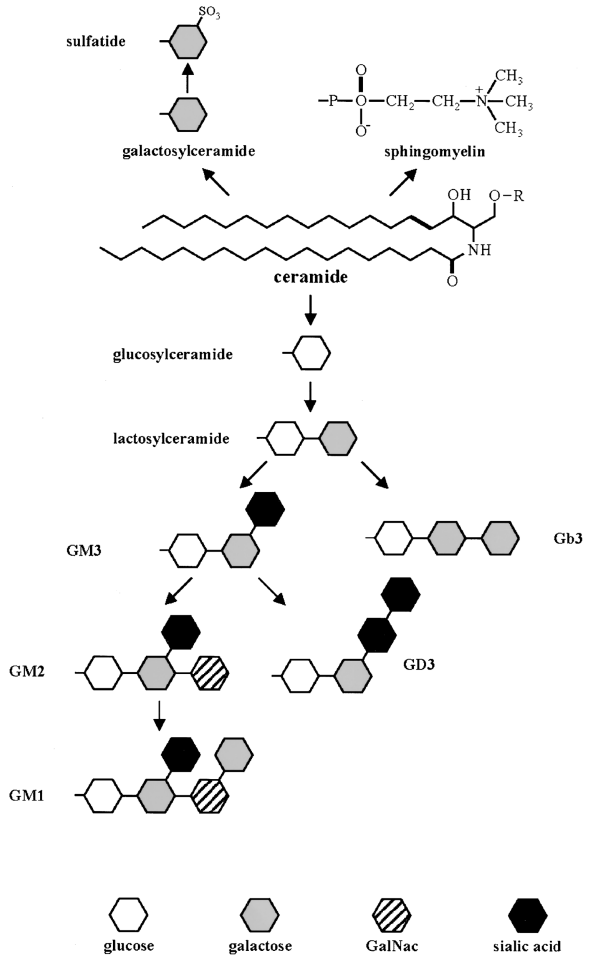

Fig. 1. Schematic representation of sphingolipid biosynthesis. Ceramide, the core structure of glycosphingolipid, is synthesized at the endoplasmic reticulum. Complex sphingolipids and sphingomyelin (SM) are synthesized in the Golgi lumen, and representative structures (R), referred to in the text, are shown.

lum (ER), as has been firmly established in both mammals (5) and yeast (6). Ceramide is transported to the Golgi by both vesicular and nonvesicular transport (7, 8). At the Golgi, glycosylation occurs at the cytosolic surface [in the case of glucosylceramide (GlcCer)] and within the Golgi lumen (in the case of complex GSL; ref. 9).

The predominant fraction of GSL is localized in the outer leaflet of the plasma membrane, which is reached via vesicular transport; however, a partly nonvesicular pathway for GlcCer is likely to exist (10). Presumably, lipid translocases like multidrug resistance (MDR) protein (Clifford Lingwood, personal communication) contribute to the translocation of GlcCer from the site of their synthesis to the opposite leaflet of the membrane. This is necessary for the biosynthesis of complex GSL within the Golgi lumen as well as at the plasma membrane (11) for surface expression.

Within the lumen of early Golgi compartments, ceramide serves as a substrate for the biosynthesis of SM. Recently, a minor ceramide synthesizing capacity (12) and glycosyltransferase activity (13) have been detected in a functional ER subcompartment called the mitochondria-associated membrane fraction that is closely linked with mitochondria (14). This observation may explain the presence of distinct GSL fractions in mitochondria (15). A cytosolic protein(s) functioning in conjunction with membrane contacts between organelles (7, 8, 16) could enable nonvesicular transfer of ceramide between the ER and the mitochondrion. This could explain the ability of ceramide to acquire access to target sites that trigger apoptotic events (see Discussion in 17).

LATERAL MEMBRANE ORGANIZATION OF SPHINGOLIPIDS: RAFTS

In membranes, GSLs often appear organized in clusters or microdomains called rafts (illustrated in **Fig. 2**). Cholesterol is also enriched in rafts where it fills up the voids between hydrocarbon chains caused by the bulky sugar head group. Raft formation presumably stems from the ability of GSL to readily self-associate and tightly pack in membranes. This occurs mainly because of interactions of their relatively long saturated acyl and alkyl chains that give rise to unusually high chain-melting temperatures.

With cholesterol and saturated phospholipids, GSLs form a unique liquid-ordered phase. Rough estimates suggest that most of the PM surface area is in the liquid-ordered state (18, 19, 20). Possibly, cholesterol may stabilize boundaries between the distinct domains. Indeed, reduction of cholesterol levels by metabolic means results in the merging of 70–100 nm domains (21) into domains of micrometer size (20). The latter data were derived from partitioning studies with relatively high concentrations of artificial lipid probes and therefore may not entirely reflect the physiological situation. Further proof awaits supportive data based upon the distribution of the natural lipids.

The preferential association between cholesterol and sphingolipids is dictated by sphingolipid-hydrogen bonding between the amide nitrogen of the sphingolipids and the 3-β hydroxyl group of cholesterol.

Lipids with saturated hydrocarbon chains, e.g., natural GSL including SM, display a higher affinity for cholesterol than unsaturated ones. In addition, there is a preferential association with cholesterol based on the head group of participating phospholipids in such domains *[SM>phosphatidylethanolamine>phosphatidylserine (PS)]*. This in turn may affect the rate and extent of cholesterol desorption from membranes (18), a property of relevance to the overall stability of these microdomains.

For domain stability, the relative proportions of both SM and cholesterol appear critical, since depletion of cholesterol or supplementation of the SM pool abolishes (2, 22) or (re)establishes, respectively, detergent insolubility of the domains and their sorting capacity (23, 24). As revealed in artificial membrane systems, cholesterol facilitates the formation of sphingolipid-containing microdomains, but is not absolutely required (25). When sufficient in concentration, GSL per se can form rafts. Provided that ceramide itself may also form rafts, it is not unlikely that raft assembly may already take place early after biosynthesis at the level of the ER (26).

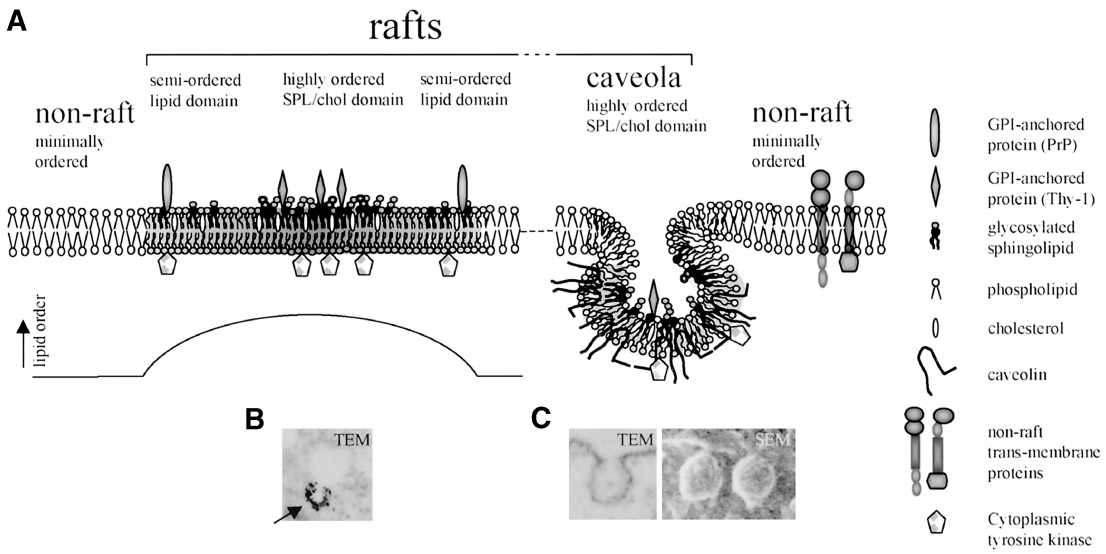

Fig. 2. Membrane domains classified by different lipid compositions. A: Diagram of membrane depicting a caveolar and noncaveolar raft enriched in (glyco)sphingolipids and cholesterol, and flanked by semiordered lipid domains that border minimally ordered (nonraft) membrane areas. Thy-1 localizes predominantly to the highly ordered lipid domains, while another glycosylphosphatidylinositol-anchored protein, PrP, prefers a semiordered lipid domain (see text). B: 6-[*N*-(7-nitrobenz-2-oxa-1,3 diazol-4-yl)amino]hexanoyl (C_6NBD)-labeled glucosylceramide (GlcCer) segregates into noncaveolar buds that appear to pinch off from recycling endosomal elements, visualized by electron microscopy and stained with diaminobenzidin (S. C. D. van IJzendoorn and D. Hoekstra, unpublished observations). C: Transmission and scanning electron microscopic images of caveolae. (Partly adapted from refs. 3 and 26). Figure partly adapted from Madore et al., 1999, and Simons and Ikonen, 1997.

Thus far, the effect of a coupling between inner and outer leaflet membrane organization has been largely unexplored. Potential transmembrane interactions between distinct PS and sphingolipid species have been described (27), as has a potential coordinated regulation of sterol, PS, and GlcCer biosynthesis (28).

MULTIPLE TYPES OF SPHINGOLIPID RAFTS

In terms of composition and related physical properties (e.g., lipid ordering), there are multiple types of sphingolipid-enriched domains in membranes (4, 18, 29; Fig. 2A). These domains display distinct physicochemical and structural parameters that provide a driving force for selective partitioning of membrane proteins.

The tight packing of sphingolipid-containing domains makes them relatively insoluble in certain nonionic detergents, and allows their isolation as insoluble domains [detergent-resistant membranes (DRMs) or detergent-insoluble GSL-enriched complexes]. Depending on the nature of the detergent, distinct domains in terms of protein composition can be distinguished. Differences in detergent solubility may arise for proteins with single as opposed to multiple membrane-spanning domains. The former are expected to fit better in tightly packed domains than the latter, which require more flexibility for proper membrane accommodation (29). Moreover, integration of a protein into a microdomain may affect the conformation of its transmembrane domain (30). Hence, lateral protein-lipid and protein-protein interactions will codetermine protein partitioning in sphingolipid-containing microdomains (31), and thereby the overall stability of such domains.

Distinct glycosylphosphatidylinositol (GPI)-linked proteins have been reported to partition into domains of different detergent solubility (Fig. 2A). For example, GPI-anchored protein Thy1 localizes predominantly to the highly ordered lipid domains, whereas another GPI-anchored protein, PrP, prefers a semi-ordered domain (19).

SPHINGOLIPIDS IN MEMBRANE TRAFFICKING AND POLARIZED TRANSPORT

The biogenesis of sphingolipid-enriched microdomains provides the cell with the possibility of localizing the molecular machinery involved in membrane-initiated cellular functions such as signal transduction or cell motility. In addition, glycolipids present on the apical surface of (intestinal) epithelial cells may be instrumental in transcytotic events that result in the delivery of pathogens like bacterial toxins and virions into cells (32–34). Lateral domain formation is also crucial in a variety of other transport events, including those involved in the targeting of proteins and lipids to basolateral and apical membrane domains during cell polarity development and in the transport of proteins to axonal and somatodendritic domains in neurons.

As reviewed elsewhere in this series (35), DRMs in the plasma membrane are implicated in numerous signaling processes by providing a platform for interactions between signaling molecules. Clustering of such molecules is

often not sufficient for signaling, indicating that the lipid environment and/or its ability to recruit additional molecular components are required for proper functioning. Raft formation may also regulate signaling events by influencing the transport of plasma membrane receptors. For example, activated B cell receptors are sequestered from rafts into clathrin-coated pits, thereby inducing their internalization (36). In addition, part of the increased signaling of the EGFR following cholesterol depletion may be due to the inhibition of its internalization (37). Finally, it was recently shown that the sphingolipid metabolites dihydrosphingosine and phytosphingosine are essential in endocytosis and are likely related to the need for a functional actin cytoskeleton and mechanistically mediated by activation of protein kinases (38).

Epithelial cell polarity and rafts

Because they simultaneously face different extracellular environments, epithelial cells acquire and maintain spatial and functional asymmetry of their plasma membrane. The apical (facing tissue lumen or blood) and basolateral (facing adjacent cells) membrane domains can be readily distinguished from each other. Tight junctions that separate the apical and basolateral membranes allow lateral diffusion in the inner, but not the outer, leaflet. Sphingolipid rafts are relatively enriched at the apical membrane surface but are also localized to the basolateral surface, although in lower abundance. These include caveolae (Fig. 2A, C) that are flask-like invaginations involved in endocytic internalization and signal transduction. Not all polarized cells contain caveolae. Although present in hepatocytes (39), they are absent in hepatoma HepG2 cells (40). Polarized oligodendrocytes (OLGs) also lack caveolae (41), although the major protein constituent of caveolae, caveolin, is expressed in these cells (42).

To maintain the distinct composition of apical and basolateral membrane domains, a sorting mechanism is required that mediates either delivery or retrieval of domain-specific compounds. Such sorting mechanisms operate in the biosynthetic, endocytic, and transcytotic pathways.

Sorting relies, at least in part, on the partitioning of proteins into sphingolipid-enriched domains that are generated in sorting organelles. Such domains have been identified in both the *trans*-Golgi network (TGN) and recycling endosome of MDCK cells (43, 44). These sphingolipid domains may display detergent-dependent differences in insolubility, implying that transport may involve the fusion of several domains into one (45) or the budding of distinct proteins in different transport vesicles (46).

The involvement of rafts as a direct means of polarized sorting of newly synthesized apical resident proteins from the TGN to the apical membrane is well established (3). Preventing the association of apical proteins with sphingolipid rafts perturbs the apical sorting of these proteins (4, 22).

The importance of rafts in the transcytotic sorting route is also becoming apparent, although some conflicting observations have been made. In enterocytes (47) and hepatocytes (48), the transcytosis of immunoglubulin A is mediated via a raft-containing compartment; however, a requirement for detergent-resistant microdomains was not observed for the transcytosis of the polymeric Ig receptor in MDCK and Fisher rat thyroid (FRT) cells (49). In other epithelial cells, e.g., FRT (50) and WIF-B cells (51), some apical resident proteins are first delivered to the basolateral membrane prior to apical delivery via transcytotic transport. More recently, direct evidence for sphingolipid-enriched microdomain-mediated transport to the basolateral membrane, and subsequently to the apical membrane via transcytosis, was obtained in polarized HepG2 cells (29). The domains in each pathway were characterized and could be distinguished by differences in detergent solubility (Lubrol WX versus Triton X-100) and differences in solubility in the cold. These findings suggest that segregation into a raft per se does not suffice for (apical) sorting. It will be of interest to determine the generality of raft-involvement in polarized trafficking in epithelia.

Polarized sorting and sphingolipid raft composition

The identification of trafficking pathways commonly relies on following the fate of distinct proteins and their eventual localization to rafts of different detergent solubilities. Knowledge of the lipid composition of these different rafts is scanty, but recent evidence suggests that the lipid compositions of rafts that exhibit different detergent solubilities can be different. The resistance of myelin proteins to CHAPS in OLGs depends on the extremely high concentrations of galactosylceramide (GalCer) and sulfatide in the myelin membrane (52). On the other hand, lactosylceramide has been shown to confer detergent-insolubility on a GPI-linked protein at concentrations much less than Glc- or GalCer. This effect was further enhanced by the presence of cholesterol (53). By contrast, preliminary data by Aït Slimane et al. (29) revealed no major differences in sphingolipid composition or cholesterol content in detergent-insoluble fractions carrying apical resident proteins directly or via transcytosis to the apical membrane in HepG2 cells. This suggests that the properties of the transmembrane domain of a sorted protein, and possibly lateral protein-protein interactions within the sphingolipid enriched domain, contribute to the sorting of proteins into rafts of specific detergent solubility properties. Further work is needed to better define domain-mediated transport.

Cholesterol appears to play a prominent role in raft-mediated trafficking and sorting. Cholesterol depletion with methyl-β-cyclodextrin causes dissociation of proteins from rafts and their disappearance from the floating fraction in density gradients in vitro. As a result, segregation of proteins into detergent-insoluble domains is diminished or no longer occurs, which may (22) or may not (45) affect apical sorting. Membrane domain formation appears to be involved in protein sorting in both biosynthetic and endocytotic pathways, but disruption of lipid raft structure affects trafficking of GPI-linked proteins in each pathway differently. Whereas cholesterol depletion impedes trafficking from the TGN to the apical mem-

brane (18), it accelerates transport out of the recycling endosome (54). Indirect sorting of apical resident proteins traveling via the basolateral membrane is not affected by cholesterol depletion (29). Interestingly, in liver hepatoma cells, missorting of MDR to the basolateral membrane occurs upon cholesterol depletion, but direct sorting to the apical membrane is reestablished upon cholesterol replenishment. Remarkably, the detergent-insolubility of the multispanning protein MDR1 was not affected following cholesterol depletion, suggesting that cholesterol depletion may have caused dissociation of a sorting factor from the raft.

Sphingolipids as markers for polarized transport and cell polarity

Since GSLs are highly enriched in the apical membrane of epithelial cells while SM is distributed about equally over both membrane domains, these sphingolipid classes have to be sorted from each other during transport. They can therefore be used as markers for the different trafficking pathways through which polarized membranes are generated and maintained. Fluorescently labeled 6-[*N*-(7-nitrobenz-2-oxa-1,3 diazol-4-yl)amino] hexanoyl (C_6NBD)-sphingolipid analogs have been applied to characterize these transport routes.

Polarized transport routes originate from the putative sorting organelles in membrane polarity development, i.e., the TGN in the biosynthetic pathway and a recycling endosome-like organelle in the endocytotic/transcytotic pathway, defined as subapical compartment (SAC) (55, 56) or common endosome (57). In fully polarized human hepatoma HepG2 cells, C_6NBD-GlcCer is recycling between the SAC and the apical bile canalicular membrane. By contrast, C_6NBD-SM initially accumulates in the SAC compartment but is ultimately transported to the basolateral membrane. It is apparent that C_6NBD-SM and C_6NBD-GlcCer must be segregated from each other in the SAC (Figs. 2B, 3B). Such separation could involve either a lateral segregation within in a single membrane domain or sorting via distinct SAC subcompartments (58). The existence of distinct sphingolipid domains is further supported by the observation that the calmodulin antagonist trifluoroperazin blocks the exit of SM from the SAC, but has no effect on recycling of GlcCer (59). Additional evidence for sphingolipid segregation has been obtained by demonstrating that both sphingolipids are released from the SAC in distinct transport vesicles (60).

Compared with Golgi, both the SAC and the apical membrane domain are relatively rich in cholesterol. The exit and delivery of cholesterol to the SAC predominantly involves vesicular transport (61). On the other hand, ATP depletion does not interfere with rapid sterol transport to the apical bile canalicular membrane or its redistribution to the basolateral membrane, implying that this occurs via a nonvesicular route. The latter presumably involves flip-flop and lateral diffusion via the inner leaflet. Alternatively, this could be accomplished via protein-mediated monomeric transport by a currently unidentified sterol carrier protein.

There is substantial evidence to suggest that cholesterol and SM levels are coordinately regulated. Changes in the level of one of these lipids alter the level of the other. In addition, it is known that SM levels regulate the capacity of membranes to absorb cholesterol and thereby control cholesterol flow. The recent work of Wüstner et al. (61) supports the notion of sphingolipid enrichment in SAC. It is therefore tempting to speculate that the sterol pool in SAC is tightly associated with that of sphingolipids and is necessary for the function of lipid raft domains in sorting in the SAC. Indeed, in nonpolarized cells, both sphingolipids and cholesterol are required for the slow export of GPI-linked proteins from the recycling endosome (54), indicating that their specific retention reflects a mechanism to segregate raft-associated proteins from basolaterally transported cargo.

Interestingly, protein kinase A (PKA)-activation induces a redistribution of C_6NBD-SM from the SAC to the apical membrane during the development of polarity in HepG2

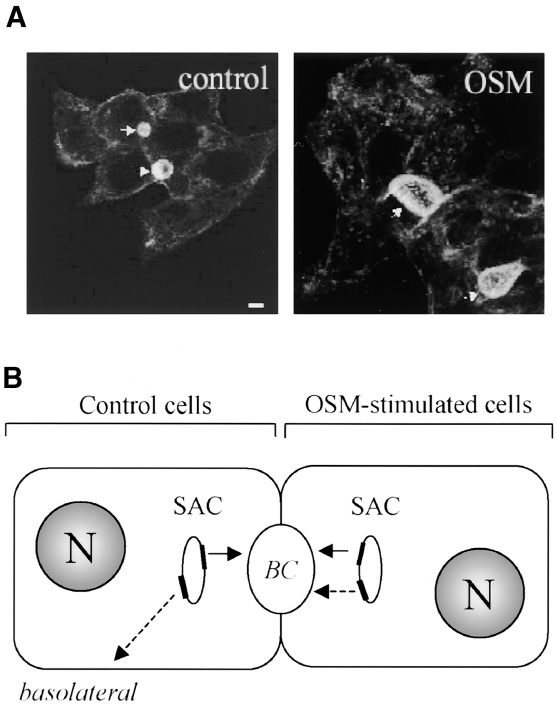

Fig. 3. The interleukin oncostatin M (OSM) and protein kinase A (PKA) stimulate polarity development and alter sorting of SM in the subapical compartment (SAC) of polarized HepG2 cells. A: HepG2 cells were cultured for 3 days in the presence of OSM. Afterwards, the cells were fixed and stained with phalloidin-TRITC to stain F-actin, which is abundantly present underneath the apical plasma membrane. Arrows point to bile canalicular (apical) structures; bar is 5 μm. B: Sphingolipids loaded in the SAC are sorted differently. In optimally polarized cells, SM is preferentially sorted to the basolateral domain, whereas GlcCer is directed to the apical, bile canalicular domain. Upon stimulation with OSM and/or activation of PKA, SM is targeted to the apical domain along a pathway that differs from that of GlcCer. Note that GlcCer and SM are sorted in different domains in the SAC. Given a subcompartmentalization of the SAC, localization of either lipid in different subcompartments cannot be excluded (for details see refs. 61, 66). Unbroken line, GlcCer trafficking; dashed line, SM trafficking.

cells (62). Apical SM transport is clearly distinguished from GlcCer (**Fig. 3B**) and proceeds along the same pathway as that of transcytosed pIgR. Upon triggering of the biogenesis of the apical membrane, SM is redistributed into a specific transcytotic apical membrane-directed route. Once optimal polarity is established, recycling of GlcCer-enriched domains may be sufficient to maintain the polarized phenotype. SM may be mainly restricted to a default pathway that is indistinguishable from trafficking of the basolateral membrane-localized transferrin receptor.

How redirection of SM during cell differentiation into a polarized phenotype is accomplished is largely unknown. Recent evidence suggests that signal transduction via interleukin (IL) 6-type cytokines at the basolateral plasma membrane supports polarity development of HepG2 cells. This occurs via an SM-marked apical membrane-directed transport pathway (Fig. 3A, B; ref. 63). PKA activation (62) appears to be involved in regulating apical-directed transport, with the SAC as a likely target for protein phosphorylation. For activation, the relevant IL receptors require recruitment into Triton X-100 rafts. This presumably enhances potential residence time, which may be accompanied by a reorganization of raft components, including sphingolipids (64). Cholesterol depletion abolishes the effect, although it should be noted that receptor clustering under such conditions might persist.

ROLE OF PROTEINS IN REGULATING RAFT TRANSPORT

There is substantial evidence that membrane domain formation is important for the segregation of multiple proteins into the apical transport pathway, yet it is unlikely that raft formation per se is sufficient for targeting to the apical membrane (29). Additional factors must be involved in the specificity of targeting.

Cholesterol appears to be relevant for the association of such a factor with rafts, and thus assists in determining direct apical targeting in polarized HepG2 cells (29). Sorting signals may also be present in the structure of the transported proteins and lipids, an example being their modification by *N*- and/or *O*-glycosylation in the TGN (65, 66). This suggests that lectins, such as VIP36, which recognize high mannose-type glycans, may be involved in targeting to the apical membrane (67). In this context, it is of interest to note that the (lipid) segregation of C_6NBD-SM, and in particular of C_6NBD-GalCer (basolateral) and C_6NBD-GlcCer (apical) in the SAC, clearly depends on distinct interactions with their head groups in the SAC lumen because the hydrophobic tails of the NBD-labeled lipid analogue are identical.

Additional protein families have been described that may play a general role in the specific delivery of cargo to the apical membrane. Although their exact role is not yet established, proteins of the MAL family are so far the best candidates for a ubiquitous role in the targeting of rafts to the apical membrane.

MAL is a 17 kDa nonglycosylated raft-associated protein that localizes in the Golgi (68) and is required for direct apical targeting in MDCK cells. MAL interacts with GSLs (69) and is required for overall transport from the TGN to the apical membrane in MDCK and FRT cells (70). Polarized HepG2 cells are devoid of MAL in the Golgi, but a MAL family member, MAL2, has been localized to SAC (48). The presence of MAL2 in rafts implicitly indicates that functional detergent-resistant microdomains must be present in this sorting compartment (58).

pIgR crosses the SAC on its way to the apical membrane. Apical trafficking of pIgR is blocked when endogenous MAL2 is depleted upon down-regulation of its expression by specific antisense oligonucleotides. Similarly, in MDCK cells, MAL depletion causes accumulation of apical cargo in the Golgi. This implicates MAL2 in the regulation of apical-directed transport via SAC (29, 56, 71).

It remains to be determined how MAL proteins direct rafts into the apical pathway. Although it is possible that MAL proteins interact with targeting proteins like SNAREs or the microtubule cytoskeleton, such an interaction has not been described. The involvement of microtubules in apical targeting of rafts (72, 73) is supported by the finding that certain kinesins bind preferentially to raft lipids or are localized in DRM domains (74).

MEMBRANE POLARITY IN NONEPITHELIAL CELLS

The development of specific antibodies against various sphingolipids and the finding that several bacterial toxins use gangliosides as receptors for cell attachment and internalization (32, 75, 76) has advanced our knowledge about the existence of distinct membrane domains in plasma membranes and their role in cellular function. Such studies emphasize that a nonrandom distribution of sphingolipids is not restricted to typical polarized epithelial cells. Migrating lymphocytes, neurons, and OLGs show a high degree of plasma membrane polarity, and in these cells, sphingolipid-rich membrane domains are important for such polarity development.

Migrating lymphocytes

In migrating lymphocytes, membrane polarity is acquired at the leading edge (front) and the uropode (rear end), revealing an enrichment of gangliosides GM1 and GM3, respectively (75). Cholesterol depletion abolished chemotaxis, suggesting a functional involvement of the principle of raft organization. Whether this treatment solubilized the raft or interfered with its lateral sorting by depleting essential sorting factors remains to be determined.

Neurons

SM synthesis is required for axonal outgrowth of developing neurons (24), a phenomenon that was linked to microdomain assembly and axonal-directed transport. This axonal domain-directed pathway bears similarity to that observed in the biogenesis of the apical membrane do-

main in hepatocytes. It should be noted, however, that in the latter case, the extent to which transport of fluorescently-tagged SM matches that of the natural counterpart has not yet been determined.

OLGs

OLGs are the myelin-forming cells of the central nervous system and depend on the biosynthesis of GalCer and sulfatide for polarity development. Both sphingolipids are highly enriched in myelin. In cultured OLGs, plasma membrane-inserted (BODIPY)-sulfatide is more efficiently internalized than lactosylceramide, which indicates that the two sphingolipid analogs are not homogeneously distributed in the plane of the membrane but rather are enriched in distinct membrane domains (77); however, the role of these sphingolipids in the formation of myelin is controversial.

The thickness of myelin sheaths of mice that are deficient in the gene for UDP-galactose:ceramide galactosyltransferase (CGT) is clearly reduced, and the paranodal regions are particularly affected. This suggests that normal sphingolipid synthesis is important in the formation of functional axo-glial junctions (78). Interestingly, the number of myelinating OLGs is substantially increased in CGT-knockout mice, even after incubation with antibodies against sulfatides. This suggests that sulfatides may play a negative role in OLG proliferation and differentiation.

Mating yeast

Polarization also occurs during mating in yeast and triggers polarized growth toward the mating partner (79). Proteins that have specific functions in the mating event are polarized to the tip of the mating projection. This does not occur in sphingolipid and ergosterol (the equivalent of cholesterol in mammalian cells) mutants, implying a role of rafts in the recruitment of proteins to the mating projections. Such polarized clustering in yeast does not require specific targeting of newly synthesized molecules to the tips of the mating projections. In contrast to the presence of tight junctions in epithelial cells to maintain membrane polarity, in yeast, rafts appear to provide a barrier that prevents the diffusion of proteins from the projections. In this regard, raft localization appears to impose a limit on the motional freedom of proteins, as evidenced by a very similar diffusion rate of raft-localized proteins irrespective of the nature of their membrane anchor (80).

CONCLUDING REMARKS

The ubiquitous role of sphingolipids in a variety of biologically important processes has become apparent in recent years. The best illustration of the significance of GSL is the observation that mutation of a glucosyltransferase that inhibits the biosynthesis of GlcCer and complex GSL is embryonic lethal.

While the biological significance of GSL is well recognized, the role and functioning of individual sphingolipid species is still poorly understood at the molecular level. Clearly, sphingolipids are instrumental in triggering lateral domain formation in membranes, and a multitude of such domains probably exist. Regulation of the biogenesis of these individual domains, their lifetimes, interactions with adjacent domains, and the molecular factors that determine their functioning as signaling platforms or transport entities are issues that are still largely unresolved.

The use of simple membrane model systems is likely to play a role in the continued elucidation of molecular interactions between distinct sphingolipids, phospholipids, and proteins; however, an important tool in further understanding the significance of distinct sphingolipid species in signaling and sorting events will be the exploitation of yeast as a model system. In *Saccharomyces cerevisiae*, essentially all genes involved in sphingolipid metabolism have now been identified (6). Use of this well-defined system will significantly aid the further understanding of the role of sphingolipids in the biology of eukaryotic cells.

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REFERENCES

1. Yamashita, T., R. Wada, T. Sasaki, C. Deng, U. Bierfreund, K. Sandhoff, and R. L. Proia. 1999. Avital role of glycosphingolipid synthesis during development and differentiation. *Proc. Natl. Acad. Sci. USA.* **96:** 9142–9147.
2. Brown, D. A., and E. London. 2000. Structure and function of sphingolipid- and cholesterol-rich membrane rafts. *J. Biol. Chem.* **275:** 17221–17224.
3. Simons, K., and E. Ikonen. 1997. Functional rafts in cell membranes. *Nature.* **387:** 569–572.
4. Röper, K., D. Corbeil, and W. B. Huttner. 2000. Retention of prominin in microvilli reveals distinct cholesterol-based lipid microdomains in the apical plasma membrane. *Nat. Cell Biol.* **2:** 582–592.
5. Van Echten, G., and K. Sandhoff. 1993. Ganglioside metabolism. Enzymology, topology and regulation. *J. Biol. Chem.* **268:** 5341–5344.
6. Dickson, R. C., and R. L. Lester. 2002. Sphingolipid functions in *Saccharomyces cerevisiae. Biochim. Biophys. Acta.* **1583:** 13–25.
7. Kok, J. W., T. Babia, K. Klappe, G. Egea, and D. Hoekstra. 1998. Ceramide transport from endoplasmic reticulum to Golgi apparatus is not vesicle-mediated. *Biochem. J.* **333:** 779–786.
8. Fukasawa, M., M. Nishijima, and K. Hanada. 1999. Genetic evidence for ATP-dependent endoplasmic reticulum-to-Golgi apparatus trafficking of ceramide for sphingomyelin synthesis in Chinese hamster ovary cells. *J. Cell Biol.* **144:** 673–685.
9. Kolter, T., R. L. Proia, and K. Sandhoff. 2002. Combinatorial ganglioside biosynthesis. *J. Biol. Chem.* **277:** 25859–25862.
10. Warnock, D. E., M. S. Lutz, W. A. Blackburn, W. W. Young, Jr., and J. U. Baenziger. 1994. Transport of newly synthesized glucosylceramide to the plasma membrane by a nonGolgi pathway. *Proc. Natl. Acad. Sci. USA.* **91:** 2708–2712.
11. Raggers, R. J., T. Pomorski, J. C. Holthuis, N. Kalin, and G. van Meer. 2000. Lipid traffic: the ABC of transbilayer movement. *Traffic.* **1:** 226–234.
12. Merrill, A. H., Jr. 2002. De novo sphingolipid biosynthesis: a necessary, but dangerous pathway. *J. Biol. Chem.* **277:** 25843–25846.
13. Vidugirienne, J., D. K. Sharma, T. K. Smith, N. A. Baumann, and A. K. Menon. 1999. Segregation of glycosylphosphatidylinositol biosynthetic reactions in a subcompartment of the endoplasmic reticulum. *J. Biol. Chem.* **274:** 15203–15212.

14. Rusinõl, A. E., Z. Cui, M. H. Chen, and J. E. Vance. 1994. A unique mitochondria-associated membrane fraction from rat liver has a high capacity for lipid synthesis and contains pre-Golgi secretory proteins including nascent lipoproteins. *J. Biol. Chem.* **269:** 27494–27502.
15. Garcia-Ruiz, C., A. Colell, A. Morales, M. Calvo, C. Enrich, and J. C. Fernandez-Checa. 2002. Trafficking of ganglioside GD3 to mitochondria by tumor necrosis factor-alpha. *J. Biol. Chem.* **277:** 36443–36448.
16. Funato, K., and H. Riezman. 2001. Vesicular and non vesicular transport of ceramide from the ER to the Golgi apparatus in yeast. *J. Cell Biol.* **155:** 949–959.
17. Hoekstra, D. 1999. Ceramide-mediated apoptosis of hepatocytes in vivo: A matter of the nucleus? *J. Hepatol.* **31:** 160–163.
18. Simons, K., and D. Toomre. 2000. Lipid rafts and signal transduction. *Nat. Rev. Mol. Cell Biol.* **1:** 31–39.
19. Madore, N., K. L. Smith, C. H. Graham, A. Jen, K. Brady, S. Hall, and R. Morris. 1999. Functionally different GPI proteins are organized in different domains on the neuronal surface. *EMBO J.* **18:** 6917–6926.
20. Hao, M., S. Mukherjee, and F. R. Maxfield. 2001. Cholesterol depletion induces large scale domain segregation in living cell membranes. *Proc. Natl. Acad. Sci. USA.* **98:** 13072–13077.
21. Varma, R., and S. Mayor. 1998. GPI-anchored proteins are organized in submicron domains at the cell surface. *Nature.* **394:** 798–801.
22. Keller, P., and K. Simons. 1998. Cholesterol is required for surface transport of influenza virus hemagglutinin. *J. Cell Biol.* **140:** 1357–1367.
23. Hoekstra, D., S. C. D. van IJzendoorn. 2000. Lipid trafficking and sorting: how cholesterol is filling gaps. *Curr. Opin. Cell Biol.* **12:** 496–502.
24. Ledesma, M. D., B. Brugger, C. Bunning, F. T. Wieland, and C. G. Dotti. 1999. Maturation of the axonal plasma membrane requires upregulation of sphingomyelin synthesis and formation of protein-lipid complexes. *EMBO J.* **18:** 1761–1771.
25. Brown, R. E. 1998. Sphingolipid organization in biomembranes: what physical studies of model membranes reveal. *J. Cell Sci.* **111:** 1–9.
26. Bagnat, M., S. Keranen, A. Shevchenko, A. Shevchenko, and K. Simons. 2000. Lipid rafts function in biosynthetic delivery of proteins to the cell surface in yeast. *Proc. Natl. Acad. Sci. USA.* **97:** 3254–3259.
27. Schneiter, R., B. Brugger, R. Sandhoff, G. Zellnig, A. Leber, M. Lampl, K. Athenstaedt, C. Hrastnik, S. Eder, G. Daum, F. Paltauf, F. T. Wieland, and S. D. Kohlwein. 1999. Electrospray ionization tandem mass spectrometry (ESI-MS/MS) analysis of the lipid molecular species composition of yeast subcellular membranes reveals acyl chain-based sorting/remodeling of distinct molecular species en route to the plasma membrane. *J. Cell Biol.* **146:** 741–754.
28. Hartmann, M-A., A-M. Perret, J-P. Carde, C. Cassagne, and P. Moreau. 2002. Inhibition of the sterol pathway in leek seedlings impairs phosphatidylserine and glucosylceramide synthesis but triggers an accumulation of triacylglycerols. *Biochim. Biophys. Acta.* **1583:** 285–296.
29. Aït Slimane, T., G. Trugnan, S. C. D. van IJzendoorn, and D. Hoekstra. 2003. Raft-mediated trafficking of apical resident proteins occurs in both direct and transcytotic pathways in polarized hepatic cells: role of distinct lipid microdomains. *Mol. Biol. Cell.* In press.
30. Chung, J., R. L. Lester, and R. C. Dickson. 2002. Vacuolar ATPase assembly requires sphingolipids. *Chem. Phys. Lipids.* **118:** 13.
31. Harder, T., P. Scheiffele, P. Verkade, and K. Simons. 1998. Lipid domain structure of the plasma membrane revealed by patching of membrane components. *J. Cell Biol.* **141:** 929–942.
32. Kovbasnjuk, O., M. Edidin, and M. Donowitz. 2002. Role of lipid rafts in Shiga toxin 1 interaction with the apical surface of Caco-2 cells. *J. Cell Sci.* **114:** 4025–4031.
33. Bomsel, M., and A. Alfsen. 2003. Entry of viruses through the epithelial barrier: pathogenic trickery. *Nat. Rev. Mol. Cell Biol.* **4:** 57–68.
34. Katagiri, Y. U., T. Mori, H. Nakajima, C. Katagiri, T. Taguchi, T. Takeda, N. Kiyokawa, and J. Fujimoto. 1999. Activation of Src family kinase Yes induced by Shiga toxin binding to globotriaosyl ceramide (/CD77) in low density, detergent-insoluble microdomains. *J. Biol. Chem.* **274:** 35278–35282.
35. Pike, L. J. 2003. Lipid rafts: bringing order to chaos. *J. Lipid Res.* **44:** 655–667.
36. Stoddart, A., M. L. Dykstra, B. K. Brown, W. Song, S. K. Pierce, and F. M. Brodsky. 2002. Lipid rafts unite signaling cascades with clathrin to regulate BCR internalization. *Immunity.* **17:** 451–462.
37. Pike, L. J., and L. Casey. 2002. Cholesterol levels modulate EGF receptor-mediated signaling by altering receptor function and trafficking. *Biochemistry.* **41:** 10315–10322.
38. Friant, S., R. Lombardi, T. Schmelze, M. N. Hall, and H. Riezman. 2001. Sphingoid base signaling via Pkh kinases is required for endocytosis in yeast. *EMBO J.* **20:** 6783–6792.
39. Calvo, M., F. Tebar, C. Lopez-Iglesias, and C. Enrich. 2001. Morphologic and functional characterization of caveolae in rat liver hepatocytes. *Hepatology.* **33:** 1259–1269.
40. Fujimoto, T., H. Kogo, R. Nomura, and T. Une. 2000. Isoforms of caveolin-1 and caveolar structure. *J. Cell Sci.* **113:** 3509–3517.
41. Wolburg, H. 1995. Orthogonal arrays of intramembranous particles: a review with special reference to astrocytes. *J. Hirnforsch.* **36:** 239–258.
42. Cameron, P. L., J. W. Ruffin, R. Bollag, H. Rasmussen, and R. S. Cameron. 1997. Identification of caveolin and caveolin-related proteins in the brain. *J. Neurosci.* **17:** 9520–9535.
43. Gkantiragas, I., B. Brugger, E. Stuven, D. Kaloyanova, X. Y. Li, K. Lohr, F. Lottspeich, F. T. Wieland, and J. B. Helms. 2001. Sphingomyelin-enriched microdomains at the Golgi complex. *Mol. Biol. Cell.* **12:** 1819–1833.
44. Gagescu, R., N. Demaurex, R. G. Parton, W. Hunziker, L. A. Huber, and J. Gruenberg. 2000. The recycling endosome of Madin-Darby canine kidney cells is a mildly acidic compartment rich in raft components. *Mol. Biol. Cell.* **11:** 2775–2791.
45. Jacob, R., and H. Y. Naim. 2001. Apical membrane proteins are transported in distinct vesicular carriers. *Curr. Biol.* **11:** 1444–1450.
46. Kaether, C., P. Skehel, and C. G. Dotti. 2000. Axonal membrane proteins are transported in distinct carriers: A two-color video microscopy study in cultured hippocampal neurons. *Mol. Biol. Cell.* **11:** 1213–1224.
47. Hansen, G. H., L. L. Niels-Christiansen, L. Immerdal, W. Hunziker, A. J. Kenny, and E. M. Danielsen. 1999. Transcytosis of immunoglobulin A in the mouse enterocyte occurs through glycolipid raft- and rab17-containing compartments. *Gastroenterology.* **116:** 610–622.
48. De Marco, M. C., F. Martín-Belmonte, L. Kremer, J. P. Albar, I. Correas, J. P. Vaerman, M. Marazuela, J. A. Byrne, and M. A. Alonso. 2002. MAL2, a novel raft protein of the MAL family, is an essential component of the machinery for transcytosis in hepatoma HepG2 cells. *J. Cell Biol.* **159:** 37–44.
49. Sarnataro, D., L. Nitsch, W. Hunziker, and C. Zurzolo. 2000. Detergent insoluble microdomains are not involved in transcytosis of polymeric Ig receptor in FRT and MDCK cells. *Traffic.* **1:** 794–802.
50. Lipardi, C., L. Nitsch, and C. Zurzolo. 2000. Detergent-insoluble GPI-anchored proteins are apically sorted in Fischer rat thyroid cells, but interference with cholesterol or sphingolipids differentially affects detergent insolubility and apical sorting. *Mol. Biol. Cell.* **11:** 531–542.
51. Bastaki, M., L. T. Braiterman, D. C. Johns, Y. H. Chen, and A. L. Hubbard. 2002. Absence of direct delivery for single transmembrane apical proteins or their "secretory" forms in polarized hepatic cells. *Mol. Biol. Cell.* **13:** 225–237.
52. Simons, M., E. M. Kramer, C. Thiel, W. Stoffel, and J. Trotter. 2000. Assembly of myelin by association of proteolipid protein with cholesterol- and galactosylceramide-rich membrane domains. *J. Cell Biol.* **151:** 143–154.
53. Parkin, E. T., A. J. Turner, and N. M. Hooper. 2001. Differential effects of glycosphingolipids on the detergent-insolubility of the glycosylphosphatidylinositol-anchored membrane dipeptidase. *Biochem. J.* **358:** 209–216.
54. Chatterjee, S., E. R. Smith, K. Hanada, V. L. Stevens, and S. Mayor. 2001. GPI anchoring leads to sphingolipid-dependent retention of endocytosed proteins in the recycling endosomal compartment. *EMBO J.* **20:** 1583–1592.
55. Van IJzendoorn, S. C. D., and D. Hoekstra. 1998. (Glyco)sphingolipids are sorted in sub-apical compartments in HepG2 cells: a role for nonGolgi-related intracellular sites in the polarized distribution of (glyco)sphingolipids. *J. Cell Biol.* **142:** 683–696.
56. Ihrke, G., G. V. Martin, M. R. Shanks, M. Schrader, T. A. Schroer, and A. L. Hubbard. 1998. Apical plasma membrane proteins and endolyn-78 travel through a subapical compartment in polarized WIF-B hepatocytes. *J. Cell Biol.* **141:** 115–133.
57. Mostov, K. E., M. Verges, and Y. Altschuler. 2000. Membrane traffic in polarized epithelial cells. *Curr. Opin. Cell Biol.* **12:** 483–490.

58. Van IJzendoorn, S. C. D., and D. Hoekstra. 1999. The sub-apical compartment: a novel sorting center? (Mini-review) *Trends Cell Biol.* **9:** 144–149.
59. Van IJzendoorn, S. C. D., and D. Hoekstra. 1999. Polarized sphingolipid transport from the subapical compartment: evidence for distinct sphingolipid domains. *Mol. Biol. Cell.* **10:** 3449–3461.
60. Maier, O., and D. Hoekstra. 2003. Trans-golgi network and subapical compartment of HepG2 cells display different properties in sorting and exiting of sphingolipids. *J. Biol. Chem.* **278:** 164–173.
61. Wüstner, D., A. Herrmann, M. Hao, and F. R. Maxfield. 2002. Rapid nonvesicular transport of sterol between the plasma membrane domains of polarized hepatic cells. *J. Biol. Chem.* **277:** 30325–30336.
62. Van IJzendoorn, S. C. D., and D. Hoekstra. 2000. Polarized sphingolipid transport from the sub-apical compartment changes during cell polarity development. *Mol. Biol. Cell.* **11:** 1093–1101.
63. Van der Wouden, J. M., S. C. D. van IJzendoorn, and D. Hoekstra. 2002. Oncostatin M regulates membrane traffic and stimulates bile canalicular membrane biogenesis in HepG2 cells. *EMBO J.* **21:** 6409–6418.
64. Holowka, D., E. D. Sheets, and B. Baird. 2000. Interactions between Fc(epsilon)RI and lipid raft components are regulated by the actin cytoskeleton. *J. Cell Sci.* **113:** 1009–1019.
65. Benting, J. H., A. G. Rietveld, and K. Simons. 1999. N-Glycans mediate the apical sorting of a GPI-anchored, raft-associated protein in Madin-Darby canine kidney cells. *J. Cell Biol.* **146:** 313–320.
66. Alfalah, M., R. Jacob, and H. Y. Naim. 2002. Intestinal dipeptidyl peptidase IV is efficiently sorted to the apical membrane through the concerted action of N- and O-glycans as well as association with lipid microdomains. *J. Biol. Chem.* **277:** 10683–10690.
67. Hara-Kuge, S., T. Ohkura, H. Ideo, O. Shimada, S. Atsumi, and K. Yamashita. 2002. Involvement of VIP36 in intracellular transport and secretion of glycoproteins in polarized Madin-Darby canine kidney (MDCK) cells. *J. Biol. Chem.* **277:** 16332–16339.
68. Puertollano, R., F. Martín-Belmonte, J. Millán, M. C. de Marco, J. P. Albar, L. Kremer, and M. A. Alonso. 1999. The MAL proteolipid is necessary for normal apical transport and accurate sorting of the influenza virus hemagglutinin in Madin-Darby canine kidney cells. *J. Cell Biol.* **145:** 141–145.
69. Kim, T., and S. E. Pfeiffer. 2002. Subcellular localization and detergent solubility of MVP17/rMAL, a lipid raft-associated protein in oligodendrocytes and myelin. *J. Neurosci. Res.* **69:** 217–226.
70. Martin-Belmonte, F., R. Puertollano, J. Millan, and M. A. Alonso. 2000. The MAL proteolipid is necessary for the overall apical delivery of membrane proteins in the polarized epithelial Madin-Darby canine kidney and Fischer rat thyroid cell lines. *Mol. Biol. Cell.* **11:** 2033–2045.
71. Maier, O., T. Aït Slimane, and D. Hoekstra. 2001. Membrane domains and polarized trafficking of sphingolipids. *Semin. Cell Dev. Biol.* **12:** 149–171.
72. Lafont, F., J. K. Burkhardt, and K. Simons. 1994. Involvement of microtubule motors in basolateral and apical transport in kidney cells. *Nature.* **372:** 801–803.
73. Zegers, M. M. P., K. J. M. Zaal, S. C. D. van IJzendoorn, K. Klappe, and D. Hoekstra. 1998. Actin and microtubules are involved in different membrane traffic pathways that transport sphingolipids to the apical surface of polarized HepG2 cells. *Mol. Biol. Cell.* **9:** 1939–1949.
74. Klopfenstein, D. R., M. Tomishige, N. Stuurman, and R. D. Vale. 2002. Role of phosphatidylinositol(4,5)bisphosphate organization in membrane transport by the Unc104 kinesin motor. *Cell.* **109:** 347–358.
75. Gomez-Mouton, C., J. L. Abad, E. Mira, R. A. Lacalle, E. Gallardo, S. Jimenez-Baranda, I. Illa, A. Bernad, S. Manes, and C. Martinez-A. 2001. Segregation of leading-edge and uropod components into specific lipid rafts during T cell polarization. *Proc. Natl. Acad. Sci. USA.* **98:** 9642–9647.
76. Wolf, A. A., Y. Fujinaga, and W. I. Lencer. 2002. Uncoupling of the cholera toxin-G(M1) ganglioside receptor complex from endocytosis, retrograde Golgi trafficking, and downstream signal transduction by depletion of membrane cholesterol. *J. Biol. Chem.* **277:** 16249–16256.
77. Watanabe, R., K. Asakura, M. Rodriguez, and R. E. Pagano. 1999. Internalization and sorting of plasma membrane sphingolipid analogues in differentiating oligodendrocytes. *J. Neurochem.* **73:** 1375–1383.
78. Marcus, J., and B. Popko. 2002. Galactolipids are molecular determinants of myelin development and axo-glial organization. *Biochim. Biophys. Acta.* **1573:** 406–413.
79. Bagnat, M., and K. Simons. 2002. Cell surface polarization during yeast mating. *Proc. Natl. Acad. Sci. USA.* **99:** 14183–14188.
80. Pralle, A., P. Keller, E. L. Florin, K. Simons, and J. K. H. Hörber. 2000. Sphingolipid-cholesterol rafts diffuse as small entities in the plasma membrane of mammalian cells. *J. Cell Biol.* **148:** 997–1008.