Lipid Rafts Exist as Stable Cholesterol-independent Microdomains in the Brush Border Membrane of Enterocytes*

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Glycosphingolipid/cholesterol-rich membranes ("rafts") can be isolated from many types of cells, but their existence as stable microdomains in the cell membrane has been elusive. Addressing this problem, we studied the distribution of galectin-4, a raft marker, and lactase, a protein excluded from rafts, on microvillar vesicles from the enterocyte brush border membrane. Magnetic beads coated with either anti-galectin-4 or anti-lactase antibodies were used for immunosolubilization of vesicles followed by double immunogold labeling of the two proteins. A morphometric analysis revealed subpopulations of raft-rich and raft-poor vesicles by the following criteria: 1) the lactase/galectin-4 labeling ratio/vesicle captured by the anti-lactase beads was significantly higher (p < 0.01) than that of vesicles captured by anti-galectin-4 beads, 2) subpopulations of vesicles labeled by only one of the two antibodies were preferentially captured by beads coated with the respective antibody (p < 0.01), 3) the average diameter of "galectin-4 positive only" vesicles was smaller than that of vesicles labeled for lactase. Surprisingly, pretreatment with methyl-β-cyclodextrin, which removed >70% of microvillar cholesterol, did not affect the microdomain localization of galectin-4. We conclude that stable, cholesterol-independent raft microdomains exist in the enterocyte brush border.

Ever since the "membrane cluster" hypothesis was proposed well over a decade ago (1), the biological relevance of glycosphingolipid/cholesterol-rich "raft" microdomains has been debated. The potential to serve as lateral platforms for selective subsets of peripheral and integral membrane proteins made rafts attractive candidates as actors in membrane trafficking, particularly in polarized cells, and in signaling events at the cell surface (recent reviews: Refs. 2–9). Biochemically, lipid rafts are characterized by their resistance to detergent extraction at low temperature combined with the ability to float during density gradient centrifugation (10), and studies on model membranes have indicated that cholesterol and sphingolipids are capable of forming ordered (l1 phase) subdomains that resemble rafts (11–13). However, raft microdomains have been difficult to visualize directly by microscopy in cell membranes without prior clustering of protein components, for instance by use of antibodies, toxins, or chemical cross-linkers (14–17), begging the question if rafts as such, however small, exist as stable, functional entities in the membrane (4, 18–19).

The problem concerning the size, if not the existence, of rafts has been tackled in several investigations during the past few years. Unfortunately, the use of fluorescence resonance energy transfer technique has given contradicting answers. Thus, by comparing the GPI-anchored folate receptor with a transmembrane isoform, Varma and Mayor (20) proposed the former to reside in raft domains with a size of up to 70 nm in diameter. However, studying the folate receptor as well as other GPI-anchored proteins (5'-nucleotidase and CD59) and a glycosphingolipid by fluorescence resonance energy transfer, it has also been argued that rafts are either only transient structures or, if stable, comprise only a minor fraction of the cell surface (21–23). Recently, a novel technique measuring the viscosity of the lipid environment around single particles was used to calculate a size of rafts that remained stable for several minutes to about 26 nm in radius (24). Studying raft localization of aminopeptidase N/CD13 in fibroblast-like synoviocytes by immunogold labeling of fixed cells, we observed the protein in 60–160 nm flat patches at the cell surface as well as in caveolae (25). Nevertheless, more studies are clearly needed to visualize raft microdomains in membranes before their functional significance can be fully assessed.

The small intestinal brush border is a highly organized membrane system with a high content of glycosphingolipid and cholesterol (26). Accordingly, many of its major digestive enzymes partially resist extraction at low temperature with Triton X-100 (27). Upon tissue homogenization brush borders spontaneously form uniform, right side out spherical microvillar vesicles that can be easily isolated from other cell membranes by the divalent cation precipitation method (28–29). In the present work, we made use of this property to study whether raft microdomains about the size previously suggested by others (20, 24) exist in the brush border membrane, or alternatively, whether stable rafts are non-existent or too small to act as lateral sorting platforms (23). We took advantage of previous observations that lactase is virtually absent from the biochemical defined raft fraction (27), whereas galectin-4 essentially is confined to this fraction (30). A morphometric analysis by immunogold double labeling of microvillar vesicles, isolated onto magnetic beads coated with antibodies to either of these two marker proteins, revealed the existence of subpopulations of relatively "raft-rich" and "raft-poor" microvillar vesicles, strongly arguing that stable raft microdomains do exist at

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1 The abbreviations used are: GPI, glycosylphosphatidylinositol; PB, phosphate buffer; PBS, phosphate-buffered solution; BSA, bovine serum albumin; TBS, Tris-buffered saline; PAGE, polyacrylamide gel electrophoresis.
the microvillar surface of small intestinal brush borders. Surprisingly, these microvillar rafts do not depend on the presence of cholesterol.

**EXPERIMENTAL PROCEDURES**

**Reagents and Antibodies**—Dynabeads™ M-500 Subcellular were purchased from Dynal (Oslo, Norway). The rabbit antibodies to pig small intestinal lactase and galectin-4 used in this work were described previously (30–31). A rabbit antibody to caveolin 1 was purchased from Transduction Laboratories (Lexington, KY) and horseradish peroxidase-coupled goat anti-rabbit IgG were purchased from DAKO (Glostrup, Denmark). Methyl-β-cyclodextrin was obtained from Sigma.

**Organ Culture of Mucosal Explants**—Pig small intestines were kindly given by the Dept. of Experimental Medicine, the Panum Institute. After a rinse of the small intestinal segments in ice-cold Hank’s buffered salt solution, mucosal explants were excised and cultured in Trowell’s T-8 medium for 2 h in the presence or absence of 2% (w/v) methyl-β-cyclodextrin as described previously (32–33). After culture, the explants were frozen quickly at −20 °C.

**Anti-galectin-4 and Anti-lactase Magnetic Beads**—Antibodies were coupled to magnetic beads using the protocol supplied by the manufacturer. Briefly, the beads were first coated with a linker antibody (swine anti-rabbit IgG, using about 0.1 mg of antibody to 8 × 10⁶ beads) in a volume of 0.5 ml of 0.05 M Na₂B₄O₇, pH 9.5. After washing the beads four times, they were mixed with either anti-galectin-4 or anti-lactase using about 0.5 mg of antibody to 2 × 10² beads in a volume of 0.15 ml of 0.01 M sodium phosphate, 0.15 M NaCl, and 0.1% bovine serum albumin, pH 7.4. After washing the beads four times, they were resuspended in the above buffer containing 0.02% NaN₃ and kept at 4 °C until use.

**Immunolocalization of Microvillar Vesicles**—Right side out microvillar vesicles were prepared from control and cholesterol-depleted mucosal explants by the divalent cation precipitation method (28). For subsequent immunolocalization on magnetic beads, 0.3 ml of a microvillar suspension (about 1 mg of protein/ml) in 0.01 M sodium phosphate, 0.15 M NaCl, 2 mM EDTA, and 5% bovine serum albumin, pH 7.4, was cleared by centrifugation at 10,000 × g for 5 min and mixed with 0.7 ml of antibody-coated magnetic beads (about 2 × 10⁷ beads) in the same buffer. After incubation overnight at 4 °C with continuous mixing, the magnetic beads were washed four times in 0.01 M sodium phosphate, 0.15 M NaCl, and 2 mM EDTA, pH 7.4.

**Lipid Raft Analysis**—A lipid raft analysis of native and methyl-β-cyclodextrin-treated microvillar vesicles was performed by Triton X-100 extraction (1%) on ice followed by sucrose gradient centrifugation according to Brown and Rosenthal (10) as previously described (27) with the modification that the extracts were placed in a 60% sucrose cushion with a 50–25% sucrose gradient layer on top.

**Electron Microscopy**—Microvillar membrane vesicles from native and cholesterol-depleted mucosal explants captured onto antibody-coated magnetic beads were washed three times in 0.1 M sodium phosphate buffer (PB), pH 7.2 and then fixed in 2% paraformaldehyde/0.1% glutaraldehyde in PB for 1 h at 4 °C. The vesicles were then washed in PB (3 × 10 min), PBS (3 × 5 min), 37°C PBS (1 × 5 min) and finally treated with 7.5% gelatin in PBS for 30 min at 37 °C and overnight for 4°C. The pellet of magnetic beads now embedded in gelatin was transferred to PB and then treated with 1% osmium tetroxide in PB for 15 min at 4 °C. The magnetic beads were further treated with 1% uranyl acetate in water for 1 h at room temperature and then hydrated in ethanol and embedded in Epon as previously described (34). Ultrathin sections were cut using an LKB ultrotome 8800 III, collected on nickel grids and stained in lead citrate. The sections were examined in a Zeiss EM 900 electron microscope operated at 80 kV.

Preembedding immunogold double labeling of native and cholesterol-depleted microvillar vesicle preparations and vesicles, immunolocalized on magnetic beads from these preparations, was performed as follows: vesicles were washed three times in PB before fixation in 2% paraformaldehyde/0.1% glutaraldehyde in PB for 1 h at 4 °C. After a rinse in PB (3 × 10 min) the magnetic beads were treated with 3% BSA for 30 min and incubated in rabbit anti-pig galectin-4 overnight at 4 °C and for 1 h at room temperature. After a rinse in TBS containing 0.25% BSA (3 × 10 min), the vesicles/magnetic beads were then washed in TBS (3 × 10 min) and PB (2 × 5 min) before fixation in 2% paraformaldehyde/0.1% glutaraldehyde in PB for 1 h (this treatment destroys the antigenicity in the antibody complex). After a rinse in PB (3 × 5 min) and TBS (2 × 5 min) the vesicles/magnetic beads were treated with 3% BSA in TBS (30 min) at 4 °C before incubation in rabbit anti-pig lactase for 1 h. After a wash in TBS containing 0.25% BSA (3 × 10 min), the vesicles/magnetic beads...
were incubated in sheep anti-rabbit IgG conjugated to 13 nm of large gold particles for 1 h. The vesicles/magnetic beads were washed in TBS (3 × 10 min) and PB (2 × 5 min) before being fixed in 2.5% glutaraldehyde in PB for 5 min at 4 °C. The vesicles/magnetic beads were then washed in PB (2 × 5 min), PBS (three times), and PBS at 37 °C (5 min) and finally treated with 7.5% gelatin in PBS for 30 min at 37 °C and overnight at 4 °C. The pellet of vesicles/magnetic beads now embedded in gelatin was processed for ultrastructural analysis as described above.

For ultracytosectioning of pig intestinal enterocytes, small pieces of pig small intestinal mucosa were fixed in 4% paraformaldehyde in PB for 2 h at 4 °C. After a rinse in PB (3 × 10 min) the tissue pieces were infused with 2.3 M sucrose in PB for 30 min, then mounted on top of a metal pin, and subsequently frozen in liquid nitrogen. Ultracytosectioning and immunogold double labeling were performed as described previously (36).

**Morphometric Analysis of Immunosolated Microvillar Vesicles**—The morphometric analysis was performed on a monitor using a MTI CCP 72 video camera system. The labeling density of lactase and galectin-4 on immunosolated native and cholesterol-depleted microvillar vesicles was determined by counting the number of gold particles on 250 randomly chosen labeled vesicles from each immunoisolation (a total of 1000 vesicles).

In another analysis, 250 randomly chosen labeled immunosolated vesicles from each immunoisolation (a total of 1000 vesicles) were examined and grouped into three categories according to their labeling as follows: 1) lactase- and galectin-4-negative (those vesicles labeled by at least one gold particle for each of the two markers), 2) ‘galectin-4-positive only’, and 3) ‘lactase-positive only’. The mean diameter of the vesicles in the above categories was determined by measuring the diameter of 50 randomly chosen vesicles from each category. Statistic analysis of the data was performed using the $\chi^2$-test.

**Electrophoresis and Western Blotting**—SDS/PAGE in 15% polyacrylamide gel was performed according to Laemmli (37). After electrophoresis and transfer onto Immobilon™, Western blotting was performed with rabbit antibodies to lactase (31), galectin-4 (30), and cavelin-1. Blots were developed by electrochemiluminescence (ECL) detection reagents according to the protocol supplied by the manufacturer (Amersham Pharmacia Biotech).

**Lipid Analysis**—The cholesterol concentration in native and cholesterol-depleted microvillar vesicle preparations and sucrose gradient fractions was determined spectrophotometrically by a cholesterol oxidase/peroxidase assay (38). Protein concentration was determined by the method of Bradford (39). Chloroform/methanol extraction of total lipid in native and methyl-β-cyclodextrin-treated microvillar vesicle membranes was carried out as described previously (26, 40). Aliquots of the lipid extracts (120–130 μg of lipid) were subjected to thin layer chromatography analysis together with lipid standards on 0.25 mm silica gel 60 plates (Merck). Cholesterol was separated from other neutral lipids in petroleumsether/diethylether/acetic acid (50:50:1, v/v/v). Glycolipids and phospholipids were separated in chloroform/methanol/water (64:24:4, v/v/v). After separation, cholesterol was detected with a CuSO$_4$/H$_2$PO$_4$ reagent and glycolipids were detected with a α-naphthol spray reagent.

**RESULTS AND DISCUSSION**

**Galectin-4 and Lactase Are Markers for Brush Border Raft and Non-raft Microdomains, Respectively**—From a theoretical viewpoint, rafts should be of sufficient size to accommodate several proteins at a time if they are likely to be of functional significance in complex protein-protein and protein-lipid interactions taking place during sorting and signal transduction events (4, 18–19). In the present work, we tried to address the problem whether lipid rafts are able to exist in a cell membrane as stable domains of a size large enough to be detectable as clusters of marker proteins by immunogold electron microscopy. As a model membrane, we chose to study the enterocyte brush border membrane, which is known to contain high amounts of cholesterol and glycolipids (26) and is a rich source of lipid rafts defined by biochemical criteria (27). In addition, because endocytosis/exocytosis does not take place along the microvillar surface but at the ‘microcrypts’ between adjacent microvilli, clustering of proteins by forces acting in coated pit formation (41) would not be likely to obscure the study.

Galectin-4, a member of the galectin family of β-galactoside-binding proteins (42–44), was almost exclusively present in the Triton X-100-resistant fraction of microvillar vesicles and is thus a suitable marker for raft membrane microdomains (30, Fig. 1). Members of the galectin family of β-galactoside-binding proteins generally lack a signal peptide for cotranslational membrane translocation but are instead secreted by a poorly understood ‘nonclassical’ mechanism (45). In accordance with this, we have previously localized a substantial fraction of the protein on the ectoplasmic side of microvilli (30). In contrast to galectin-4, lactase almost exclusively resided in the “non-raft” fraction (Fig. 1) as previously reported (27), and it is therefore a suitable marker protein for non-raft membrane domains in the microvillar membrane. Surprisingly, the major part of microvillar caveolin 1, a commonly used raft marker in other cell types (5), was solubilized by Triton X-100 (Fig. 1). We have no explanation for this unusual observation but speculate that it might be related to the fact that morphologically recognizable caveolae are not normally present in the intestinal brush border membrane (46–47).
entire length of the microvilli, but although galectin-4 labeling frequently appeared in clusters, it is not possible from this type of data alone to decide whether or not the two markers are truly restricted to separate microdomains. We therefore chose to study the two raft and non-raft markers by an analysis of their distribution on microvillar vesicles prepared from small intestinal mucosa by the divalent cation precipitation method (28). As shown in Fig. 3, such a preparation predominantly consisted of dark-filled spherical vesicles of about 100 nm in diameter, characteristic of right side out-vesiculated microvilli with elements of the actin cytoskeleton entrapped inside (29). Labeling for both galectin-4 and lactase was seen widespread over the microvillar vesicle population with many vesicles being positive for only one of the two markers, suggesting that raft and non-raft domains are distributed over most of the brush border membrane. The labeling for galectin-4 frequently appeared in clusters, as was also seen with intact microvilli. Methyl-$
abla$H$
abla$-cyclodextrin is an agent commonly used for disruption of rafts by specifically depleting membranes of cholesterol, and we have previously used this agent to reduce the cholesterol content of mucosal explants by $>50\%$, a treatment that perturbs the Golgi complex morphology and apical membrane trafficking (33). However, the labeling pattern observed for methyl-$
abla$H$
abla$-cyclodextrin-treated microvillar vesicles appeared similar to that of control vesicles (Fig. 3B).

Despite the frequently observed clustering of galectin-4, the fact that a significant proportion of vesicles were positive for both markers is suggestive that raft microdomains, if they exist, are likely to be smaller than a 100-nm spherical vesicle. As indicated by Fig. 1, the biochemically defined raft fraction contained somewhat less than half the total amount of protein of intestinal microvilli. Given a total surface area of about 31,000 nm$^2$ for a 100-nm spherical microvillar vesicle and assuming a similar density in the membrane of raft and non-raft proteins, respectively, this implies that 15,000–16,000 nm$^2$/vesicle on average is occupied by raft microdomains. If a circular raft patch as reported (24) has a radius of 26 nm and thus occupies $\approx 2100$ nm$^2$, it follows that a microvillar vesicle on average should harbor $\approx7$–$8$ separate rafts. In a large population, however, such an average number would probably reflect a broad distribution of raft-rich and raft-poor vesicles as illustrated by Fig. 4A. If, on the other hand, raft microdomains...
are very much smaller than assumed in the above calculation, then all microvillar vesicles in a large population should have roughly similar contents of raft and non-raft markers as illustrated in Fig. 4B. To test experimentally the two rivaling raft models depicted in Fig. 4, we isolated microvillar vesicles on antibody-coated magnetic beads and analyzed them by double immunogold labeling. If vesicles thus immunoisolated showed the same relative distribution of galectin-4 and lactase on their surface regardless of which antibody was used for their capture, then raft membrane domains most likely must be of a very small size as indicated in Fig. 4B. If, on the other hand, microvillar vesicles captured by anti-galectin-4 beads and anti-lactase beads differed in their relative contents of galectin-4 and lactase, raft membrane microdomains of a larger size (Fig. 4A) should indeed exist.

Table I

| Calculation                                      | Native vesicles captured by Anti-galectin-4 | Anti-lactase | Methylβ-cyclodextrin-treated vesicles captured by Anti-galectin-4 | Anti-lactase |
|-------------------------------------------------|-------------------------------------------|--------------|-----------------------------------------------------------------|--------------|
| Lactase/galectin-4 immunogold Labeling ratio     | 0.82 (295/359)                            | 1.56 (382/245) | 0.63 (260/411)                                                   | 1.79 (376/210)|
| Lactase and galectin-4 positive                 |                                           |              |                                                                 |              |
| Fraction of all vesicles:                       | 17.5%                                     | 19.8%        | 22.0%                                                           | 24.8%        |
| Average vesicle diameter:                       | 89 nm                                     | 83 nm        | 82 nm                                                            | 86 nm        |
| Galectin-4 positive only                        |                                           |              |                                                                 |              |
| Fraction of all vesicles:                       | 42.0%                                     | 26.6%        | 46.0%                                                           | 22.4%        |
| Average vesicle diameter:                       | 79 nm                                     | 72 nm        | 73 nm                                                            | 75 nm        |
| Lactase-positive only                           |                                           |              |                                                                 |              |
| Fraction of all vesicles:                       | 40.5%                                     | 53.5%        | 32.0%                                                           | 52.8%        |
| Average vesicle diameter:                       | 91 nm                                     | 77 nm        | 90 nm                                                            | 91 nm        |

As shown in Fig. 5, spherical microvillar vesicles adhered both to beads coated with anti-galectin-4 and anti-lactase but not to control beads containing only linker antibody, indicating a specific immunoadsorption of vesicles to both the anti-galectin-4 and anti-lactase beads. Fig. 6 shows a panel of immunoisolated vesicles at higher magnification after a subsequent double immunogold labeling for lactase (large gold particles) and galectin-4 (small gold particles). Some vesicles captured by anti-lactase or anti-galectin-4 beads were labeled by both antibodies, but vesicles labeled for only one of the two markers were frequently observed. This labeling heterogeneity resembles the pattern seen in Fig. 3 and shows that both types of immunobeads captured vesicles representative of the whole population of microvillar vesicles. The heterogeneity with respect to labeling could potentially reflect the existence of genuine subpopulations of raft-rich and raft-poor microvillar ves-
Fig. 7. Raft analysis of microvillar membranes by sucrose gradient centrifugation. Native and methyl-β-cyclodextrin treated microvillar vesicles were extracted with ice-cold 1% Triton X-100 and subjected to sucrose gradient centrifugation as described under “Experimental Procedures.” A, samples of the gradient fractions were analyzed by SDS/PAGE and blotting for the distribution of lactase (160 kDa), galectin-4 (36 kDa), and caveolin 1 (22/18 kDa). Arrows indicate the positions in the gradient of floating raft fractions and fractions containing soluble protein. Notice that galectin-4, in contrast to caveolin 1, largely remained in the floating fractions after the methyl-β-cyclodextrin treatment. B, cholesterol concentration in the same gradient fractions of native (61) and methyl-β-cyclodextrin-treated (53) vesicles. Notice that very little cholesterol remained in the floating fractions (1–9) after the treatment.

Fig. 8. Lipid analysis of microvillar vesicles and rafts. The lipid composition of native and methyl-β-cyclodextrin-treated (MβCD) microvillar vesicles (Mic., ves.) and rafts prepared from these fractions were analyzed by thin layer chromatography as described under “Experimental Procedures.” Chol, cholesterol; GSL’s, glycosphingolipids; PE, phosphatidylethanolamine; PC, phosphatidylcholine; PS, phosphatidylserine.

icles, but it might also simply be caused by an inconsistent labeling of the vesicles. However, we have previously shown that immunogold labeling of microvillar vesicles is a quantitative method (35), so a true vesicle heterogeneity should be revealed by a closer comparison of anti-galectin-4 and anti-lactase-captured vesicles as the two types of antibody-beads have complementary affinities for such vesicle subpopulations. Therefore, a morphometric analysis was performed to characterize immunoisolated vesicles with regard to labeling density and size (diameter), and the data obtained are summarized in Table I. First, the average lactase/galectin-4 labeling ratio (ratio of number of large and small gold particles) was significantly higher (p = 0.01) on vesicles captured by the anti-lactase beads, implying that the population of microvillar vesicles is indeed heterogeneous with respect to the two markers. To explore this difference further, the immunoisolated and double immunogold labeled vesicles were next grouped according to their labeling as either being: 1) “lactase- and galectin-4-positive” (being labeled by at least one gold particle of each type), 2) “galectin-4-positive only”, or 3) “lactase-positive only”. For both types of beads, a minor fraction of captured vesicles were both lactase- and galectin-4-positive. However, anti-galectin-4 and anti-lactase beads captured the latter two vesicle subpopulations in significantly different relative proportions (p = 0.01), with vesicles positive for only one of the two markers being captured most frequently by beads coated with antibodies to the respective marker. Finally, a measurement of diameters of the three categories of vesicles indicated that galectin-4-positive only vesicles on average are somewhat smaller than those labeled by anti-lactase gold particles, irrespective of the type of beads used for the vesicle capture. The latter observation may reflect differences in the physical properties of raft-rich and raft-poor membrane microdomains (11).

As shown in Table I, the above morphometric analysis was also performed on microvillar vesicles prepared from mucusol explants treated with methyl-β-cyclodextrin. Surprisingly, the morphological analysis of cholesterol-depleted vesicles did not indicate a disruption of microdomains by the treatment. Thus, the difference in lactase/galectin-4 labeling ratio of vesicles captured by the two types of beads was increased rather than decreased relative to native vesicles, and the percentage of lactase- and galectin-4-positive vesicles was only marginally increased (Table I). In agreement with this, a raft analysis by sucrose gradient centrifugation showed that galectin-4 largely remained in the floating part of the gradient also after a treatment with methyl-β-cyclodextrin, which extracted >70% of the microvillar cholesterol in the experiment shown (Fig. 7). This “cyclodextrin resistance” of microvillar rafts was studied further by an analysis of the lipid composition of native and cholesterol-depleted microvillar vesicles and rafts. As shown in Fig. 8, methyl-β-cyclodextrin removed the major part of the microvillar cholesterol without affecting the membrane contents of phospholipids and glycosphingolipids. Rafts prepared from native and cholesterol-depleted microvillar vesicles had comparable amounts of glycosphingolipids (and phospholipids) regardless of their differing contents of cholesterol, demonstrating that cholesterol is not essential for maintaining the raft microdomain stability.

In summary, the above results taken together strongly indicate that microvillar vesicles are not uniform with respect to their contents of raft and non-raft domains, and consequently
argue in favor of a microdomain organization according to the model depicted in Fig. 4A. Because the microvillar vesicles were prepared by a detergent-free protocol this implies that rafts most likely are also able to exist as stable microdomains in the brush border membrane. Surprisingly, cholesterol-depletion had little, if any effect on the stability of microvillar rafts. Instead they seem to rely on the presence of glycosphingolipids. The lipid composition of pig intestinal microvillar vesicles has previously been determined (26), showing that cholesterol comprises 12% and glycosphingolipids about 30% of the total lipid. Di- and pentahexosylceramides containing fucose, galactose, glucose, N-acetylgalcosamine and N-acetylgalactosamine were identified as the major glycosphingolipids. Although the latter have long been acknowledged as raft components, cholesterol has generally been considered the major lipid molecule for maintaining raft stability, probably because its removal effectively disrupts caveolae and renders caveolin 1 detergent-soluble in various types of cells. However, cholesterol is not indispensable for raft stability. Thus, in a study on model liposomal membranes, a high concentration (33 mol%) of sphingolipid was shown to allow ordered phase formation in the absence of cholesterol (12). In vivo, stable, cholesterol-independent rafts may well be a specialty of apical membranes of epithelial cells that seem to lack caveolae. In the enterocyte, as in other epithelial cells, raft domains form during intracellular transport and are likely to play a key role in the apical sorting of newly synthesized brush border proteins as well as in transluminal membrane trafficking of IgA (27, 36). Methyl-β-cyclodextrin treatment affects both the Golgi complex morphology and apical membrane trafficking, indicating the importance of cholesterol for normal functioning of the exocytic pathway in this cell type (33). However, after exocytosis at the brush border membrane, clustering in stable, cholesterol-independent microdomains may be a mechanism to increase local concentrations (creating catalytic ‘hot spots’) of certain proteins, maybe to facilitate a more efficient assimilation of nutrients. Interestingly, prominin, a microvillar protein when expressed in Madin-Darby canine kidney cells, was recently shown to reside in rafts segregated from other raft microdomains harboring alkaline phosphatase, implying that the raft concept may cover a more heterogeneous variety of subdomains than previously acknowledged (48).

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