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Review

Lipid rafts in epithelial brush borders: atypical membrane microdomains with specialized functions

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Abstract

Epithelial cells that fulfil high-throughput digestive/absorptive functions, such as small intestinal enterocytes and kidney proximal tubule cells, are endowed with a dense apical brush border. It has long been recognized that the microvillar surface of the brush border is organized in cholesterol/sphingolipid-enriched membrane microdomains commonly known as lipid rafts. More recent studies indicate that microvillar rafts, in particular those of enterocytes, have some unusual properties in comparison with rafts present on the surface of other cell types. Thus, microvillar rafts are stable rather than transient/dynamic, and their core components include glycolipids and the divalent lectin galectin-4, which together can be isolated as ''superrafts'', i.e., membrane microdomains resisting solubilization with Triton X-100 at physiological temperature. These glycolipid/lectin-based rafts serve as platforms for recruitment of GPI-linked and transmembrane digestive enzymes, most likely as an economizing effort to secure and prolong their digestive capability at the microvillar surface. However, in addition to microvilli, the brush border surface also consists of membrane invaginations between adjacent microvilli, which are the only part of the apical surface sterically accessible for membrane fusion/budding events. Many of these invaginations appear as pleiomorphic, deep apical tubules that extend up to 0.5–1 μm into the underlying terminal web region. Their sensitivity to methyl-β-cyclodextrin suggests them to contain cholesterol-dependent lipid rafts of a different type from the glycolipid-based rafts at the microvillar surface. The brush border is thus an example of a complex membrane system that harbours at least two different types of lipid raft microdomains, each suited to fulfil specialized functions. This conclusion is in line with an emerging, more varied view of lipid rafts being pluripotent microdomains capable of adapting in size, shape, and content to specific cellular functions.

Keywords: Brush border; Lipid raft; Epithelial cell; Membrane microdomain; Enterocyte

1. The brush border: a highly specialized design for apical cell surfaces on epithelial cells

Brush borders are specialized apical cell surface domains of epithelial cells heavily engaged in high-throughput absorptive/secretory functions of vital importance for the organism [1,2]. Morphologically, they are composed of numerous microvilli (up to 3000/cell), which are cylindrical projections of the apical plasma membrane about 1-μm long and 0.1 μm in diameter (Fig. 1). The most prominent examples of brush border-bearing cells are the small intestinal enterocyte, the kidney proximal tubule cell, and the placental syncytiotrophoblast, but lesser degrees of organization are found on the exposed surfaces of many other cell types, including cells of the pancreas, the liver, and a number of commonly used epithelial cell lines (e.g. MDCK and Caco-2). The brush border architecture is defined by a longitudinal, actin-based cytoskeleton in the core of the microvillus and short, actin-binding cross filaments that are connected transversely with the inner leaflet of the microvillar membrane and the actin core filaments [3]. The latter radiate vertically as microvillar rootlets into the so-called terminal web region, a myosin-rich filamentous structure that extends up to 0.5–1 μm into the cell. Historically, brush borders were recognized microscopically more than 160 years ago as refractive lamella of intestinal epithelial cells, containing fine striations [4], but the unique structural organization of the brush border has continued to attract the interest of cell biologists, and today we have a comprehensive picture of its morphogenesis and molecular composition [5].

From a functional point of view, the brush border membrane can de divided into two parts. One—the larger by far—

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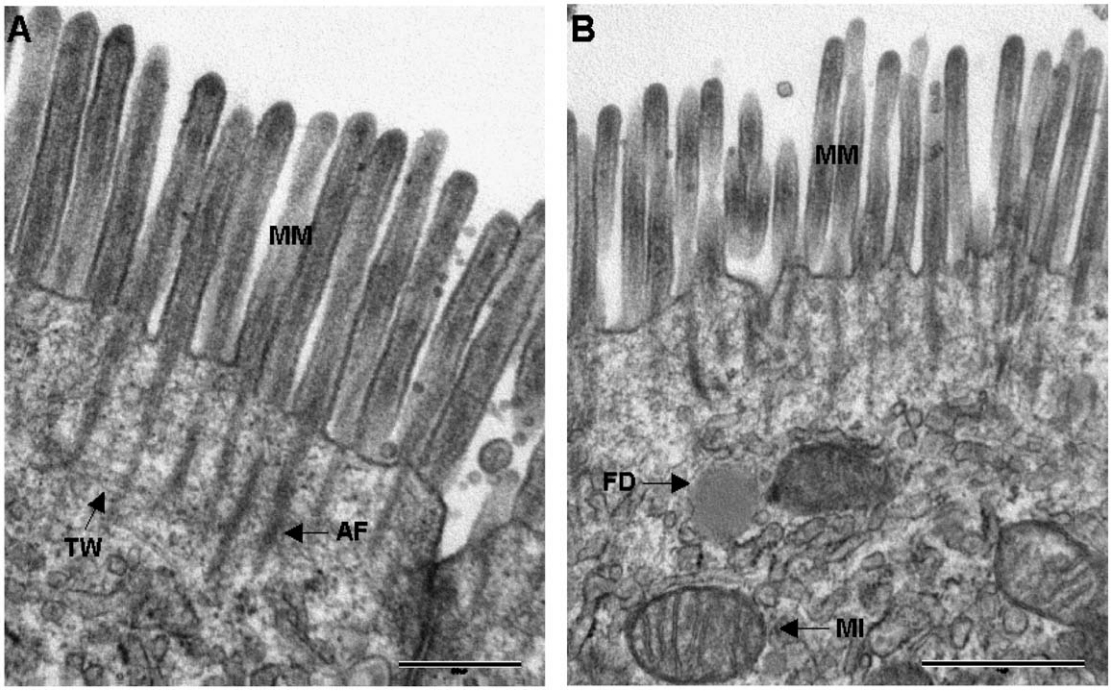

Fig. 1. Ultrastructure of the brush border and terminal web region of a small intestinal enterocyte. (A–B) Electron micrographs showing the dense apical microvillar membrane (MM). Notice the actin filaments (AF) that descend from each microvillus deep into the underlying terminal web (TW) region (A). The dense terminal web sterically excludes organelles, including fat droplets (FD), mitochondria (MI), and membranes of the endosomal subapical compartment (SAC) and the rough endoplasmic reticulum from the uppermost 0.5–1 μm of the apical cytoplasm. Bars: (A) 0.5 μm; (B) 1.0 μm.

is the microvillar surface, which is stationary in the sense that it is unlikely to engage in exocytic or endocytic membrane traffic. The second, much smaller part is defined by the numerous invaginations of cell surface membrane situated between adjacent microvilli. With regard to membrane trafficking, this part represents the dynamic section of the brush border membrane where transport vesicles are able to either bud or fuse.

2. Lipid raft membrane microdomains

Within the wider field of membrane biology, cholesterol/sphingolipid-rich, liquid-ordered (l_o phase) membrane microdomains, commonly known as lipid rafts [6], have attracted increasing interest from researchers studying the structure/function relationships that relate to complex events taking place at the surface of cells. Thus, ‘‘raftology’’ has become fashionable, and over the past few years, work from many laboratories has focused on lipid rafts in the brush border of epithelial cells. Here, we will review the data that have emerged on this subject and try to construct a coherent picture of our current knowledge with regard to lipid raft microdomains of epithelial brush borders.

The general existence of lipid raft microdomains in cell membranes [7,8] is now widely accepted among cell biologists as an important refinement of the classic fluid mosaic model of cell membranes [9]. It is no exaggeration to state that the lipid raft concept has revolutionized the way we view complex events occurring at cellular membranes. Thus, the functional diversity of the different types of membrane lipids is now being fully recognized by most cell biologists, and it is fair to state that membrane lipids have been promoted from the role of merely fulfilling a passive barrier function to molecular players of equal importance to proteins. In recent years, lipid raft discoveries have flourished particularly within the fields of signal transduction [10–14], pathogen invasion [15–19], drug targeting [20], and cholesterol transport [21,22], but it is worth recalling that the lipid raft or ‘‘membrane cluster’’ hypothesis originally emerged from studies on membrane traffic in epithelial cells (MDCK), where lipid rafts were proposed to function in apical exocytosis [23]. Through their ability to act as lateral sorting platforms, lipid rafts were thought to assemble apically destined cargo in transport vesicles originating from the *trans*-Golgi network (TGN) [24]. Since its proposal, the membrane cluster hypothesis has proved fertile in inspiring scores of researchers to discover and characterize lipid rafts in various organisms and cell types. With few exceptions, rafts are commonly defined biochemically as membrane complexes insoluble in nonionic detergents (most often Triton X-100) at low temperature [25]. Probably because of this operational definition, lipid rafts have acquired a confusing array of acronyms over the years, including DIGs (detergent-insoluble glycosphingolipids), DICs (detergent-insoluble

complexes), DRMs (detergent-resistant membranes), to name but a few.

Despite its popularity, the raft hypothesis has also attracted considerable skepticism along the way, and basic questions particularly concerning the size, let alone existence, of lipid rafts in vivo, are still being debated [26–29]. However, studies performed in vitro on model membranes, using mixtures of synthetic and natural lipids, have corroborated the notion that raft lipids do indeed possess the ability to aggregate laterally in biomembranes to form ordered structures [30].

Morphologically, the only regular and well-defined membrane structures occurring in vivo that have so far been identified with lipid raft microdomains are caveolae [31–35]. These small flask-shaped membrane invaginations seen in many cell types were once considered the main morphological manifestation of lipid raft microdomains, but it has been realized for some time now that rafts may occur in other, less-defined shapes as well [31]. A popular view now seems to be that noncaveolar lipid raft microdomains may be quite small entities (diameter 50–70 nm) [36,37]. They may even be as small as 7 nm and be composed of only a single membrane protein surrounded by ~ 80 raft lipid molecules [38]. However, such "shells" or small dormant rafts are dynamic and may increase in size in response to specific stimuli, as exemplified by the formation of a "signalosome" during T-cell receptor signaling [13].

3. Lipid rafts in brush borders

3.1. The brush border, a cell membrane rich in lipid rafts

Brush borders on kidney and intestinal epithelial cells are coated with a broad variety of ectoenzymes that enable these membranes to act as digestive/absorptive surfaces [39–41]. Much of the pioneering work on differential detergent solubility of brush border proteins was performed by Nigel Hooper and his colleagues who, studying enzymes of microvillar membrane vesicles isolated from kidney proximal tubule cells, observed that GPI-linked enzymes, for instance membrane dipeptidase and aminopeptidase P, generally resist detergent solubilization, whereas transmembrane-anchored peptidases, including aminopeptidases N and A and dipeptidyl peptidase IV, are predominantly detergent soluble [42,43]. The membrane lipids constituting rafts of kidney proximal tubule cell microvilli are mainly cholesterol and sphingomyelin, which together comprise about 65% of the lipid in detergent resistant membranes, whereas glycolipids represent less than 10% [44]. In contrast, brush border membranes isolated as microvillar vesicles from small intestinal enterocytes are particularly rich in glycolipids [45–47]. Thus, mono-, di-, and pentohexosylceramides account for >30% of the total lipid of the pig enterocyte brush border, and in rafts, they may comprise about half the total amount of lipid [48]. This significant difference in lipid composition between kidney and intestinal microvillar lipid rafts is the most likely reason why several of the major transmembrane peptidases common to both kidney and intestine, including aminopeptidases N and A, and dipeptidyl peptidase IV, are mainly raft-associated in intestinal microvilli despite being detergent soluble when extracted from similar preparations of kidney microvilli [49–52]. In addition to the major digestive enzymes of the brush border, including sucrase–isomaltase, several other types of membrane proteins have been shown to reside in microvillar rafts. These include peripheral membrane proteins such as annexin A2 [53] and galectin-4 [54], as well as integral membrane proteins like the Na^+–H^+ exchanger 3 [55], the epithelial sodium channel [56], melanotransferrin, a GPI-linked iron-receptor [57], prominin [58], and stomatin [59]. Of equal importance, it is also worth pointing out that some intestinal microvillar proteins are absent from rafts. Examples of such prominent "nonraft" proteins are the glycosidases lactase, which is virtually excluded from rafts, and maltase-glucoamylase, which is predominantly (80%) detergent soluble [50].

3.2. Intestinal microvillar rafts are stable, glycolipid-based microdomains

As mentioned above, lipid rafts often present as caveolae at the surface of many cell types. However, in a brush border densely packed with microvilli, morphologically defined caveolae are rarely seen. Caveolin-1 is the main structural coat protein of caveolae [60], and the morphological absence of caveolae undoubtedly relates to the fact that caveolin-1 is only modestly expressed in the intestinal brush border [61] and cannot be detected in kidney microvilli [44]. In addition, the shape of microvilli defines a lipid surface with the opposite curvature of caveolae, i.e. characterized by a positive curvature of the membrane as opposed to the saddle-like curvature at the base of the microvilli where they emerge from the planar part of the plasma membrane, which has no, or even opposite, curvature [62]. Based on sterical considerations, it seems likely that a high percentage of bulky-headed glycolipids in the outer leaflet of the membrane favors the stable positive microvillar curvature, but it is not known whether the microvillar lipid composition adapts to alterations in the membrane curvature at the microvillar base.

In an attempt to characterize such "noncaveolar" microvillar lipid rafts, we observed that a raft marker, galectin-4, and a marker for "nonraft" membranes, lactase, were distributed heterogeneously on subpopulations of microvillar vesicles, indicating that stable lipid rafts exist in this membrane despite the lack of morphologically identifiable microdomains [63]. This study did not determine the actual size of intestinal microvillar rafts, but indicated them to be too big to be randomly distributed on microvillar vesicles (which have a diameter of about 100 nm). Surprisingly, cholesterol,

although present, is not crucial for the stability of the intestinal microvillar lipid rafts, which relies instead on a high content of glycolipids. This atypical lipid composition is probably part of the structural basis for the preparation of the superrafts described below.

The glycolipid-based rafts described above are not the only type of rafts reported to exist in microvilli. In fact, a view is now emerging that more than one type of lipid raft microdomains may be present at the same cell surface. Thus, the apical pentaspanning protein prominin resides in microvillar lipid rafts from MDCK cells that are soluble in cold Triton X-100, but insoluble in another nonionic detergent, Lubrol WX, and physically separated from "normal" rafts containing the GPI-anchored alkaline phosphatase that occupy the planar parts of the apical surface of this cell type [58]. A similar, differential sensitivity to Triton X-100 and Brij 58 was recently observed for the EGF receptor [64], also suggesting the existence of lipid rafts with lipid–protein interactions too weak to be detected by the conventional criterion of Triton X-100 insolubility. In polarized hepatic cells, two types of lipid rafts seem to be operating in exocytotic trafficking of apical resident proteins [65]. Thus, rafts of the indirect transcytotic pathway via the basolateral surface, taken by GPI-linked proteins, are both Lubrol WX- and Triton X-100-insoluble, in contrast to rafts of the direct Golgi to apical pathway that are only insoluble in the former detergent. Furthermore, whereas cholesterol depletion alters raft-detergent insolubility in the indirect pathway without affecting apical sorting, protein missorting occurs in the direct pathway without affecting raft insolubility. Undoubtedly, more reports describing lipid raft diversities will appear in the future when a broader spectrum of detergents have been employed to discriminate better between strong, cohesive rafts, and more loosely assembled microdomains.

3.3. Microvillar lipid rafts can be isolated at physiological temperature

Until recently, biochemical isolation of lipid rafts using detergent invariably entailed a membrane extraction performed at low temperature (typically on ice or at 4 °C). Hypothetically, raft microdomains could be a low-temperature phenomenon if the lipid–lipid and lipid–protein interactions involved are too weak to be of functional importance at physiological temperature. If this were to be the case, "raftology" would indeed be of marginal importance to membrane biology. Recently, a detergent of the Brij series, Brij 98, was used to isolate lipid rafts harbouring functional T cell receptors at 37 °C from T cells [66]. Brij 98 extraction of intestinal microvillar membrane vesicles at 37 °C was also able to define rafts similar, but not identical, to those obtained using Triton X-100 extraction on ice [48]. In this study, the lectin galectin-4, the GPI-linked alkaline phosphatase and the transmembrane aminopeptidase N were all contained in rafts, isolated by Brij 98 at 37 °C, whereas lactase behaved as a "nonraft" protein. The main difference between the two types of raft was morphological. Whereas microvillar rafts, prepared by cold Triton X-100 extraction appeared as closed, spherical vesicle-like structures with an average diameter in the range of 200–300 nm, rafts prepared by Brij 98 extraction at physiological temperature were mainly seen as nonvesiculated, pleiomorphic membrane sheets with an approximate length of 200–300 nm (Fig. 2). The fact that lipid rafts can be isolated at 37 °C from two membrane systems as different as T cells and microvillar membranes is strong evidence favouring their bona fide existence in living cells.

3.4. "Superraft" analysis of microvillar membrane microdomains

Although preparations of intestinal microvillar membrane vesicles, like other cell membranes, are fully soluble in Triton X-100 at 37 °C, an insoluble membrane fraction (termed "superrafts") was prepared by sequential extraction at 0, 20, and 37 °C [48]. The membrane composition of these superrafts revealed that the raft-forming lipids, cholesterol and in particular glycolipids like asialo-GM_1, were even more enriched relative to the phospholipids than in "normal" lipid rafts. The superraft analysis provided a way to determine the relative strength of the interactions between different raft-associated proteins. Thus, galectin-4 was seen as the most prominent protein in intestinal microvillar superrafts. As a divalent lectin with affinity towards β-galactosyl residues [67], it appears that galectin-4 acts as a stabilizer/organizer of microvillar rafts by virtue of its ability to form lattices containing both glycolipids and glycoproteins [68]. Other more loosely associated proteins, such as alkaline phosphatase and aminopeptidase N, are "glued" to the rafts by the divalent lectin. Undoubtedly, one function of galectin-4 is to salvage the digestive enzymes at the brush border that would otherwise have been lost to the gut lumen by the proteolytic/lipolytic actions of the pancreatic secretions during the digestive process. This galectin-4/glycolipid based organization may also explain the apparent cholesterol independence of intestinal microvillar rafts and also why a cholesterol binding protein like caveolin-1 in microvillar membranes is largely excluded from the rafts [63]. Interestingly, another cholesterol binding protein, the scavenger receptor class B type I (SR-BI), which is present in the intestinal brush border [69], likewise localizes in "nonraft" membranes of intestinal microvilli (Hansen et al., unpublished data), despite the fact that it behaves as a raft protein in other cell types [70,71]. Furthermore, during fat absorption, microvillar SR-BI is endocytosed via clathrin-coated pits, whereas in other cell types, the receptor seems to operate by a nonendocytic mechanism involving caveolae [72,73]. To conclude, microvillar superrafts support the notion that membrane microdomains do exist in the intestinal brush border, and that these are rather different from the prevail-

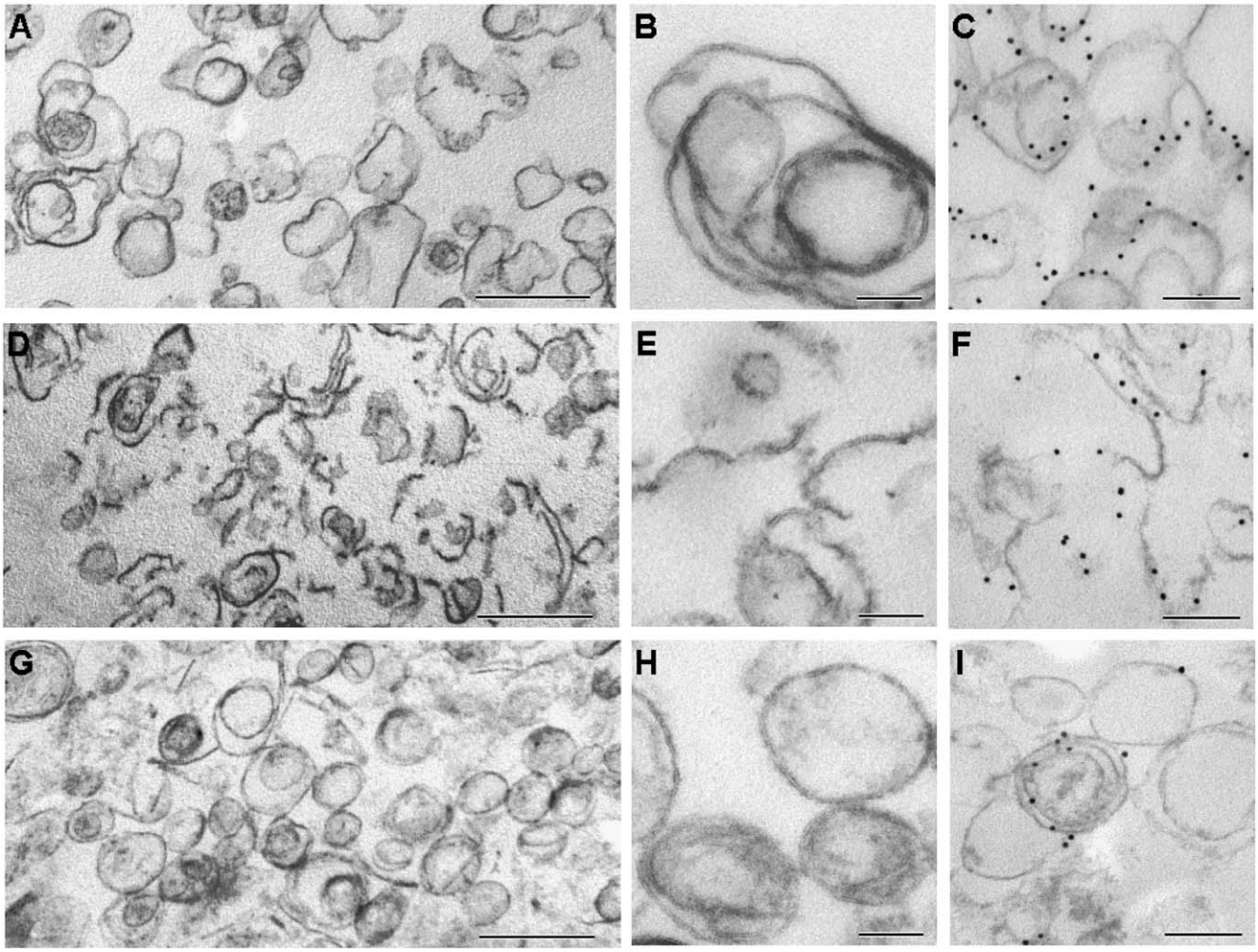

Fig. 2. Ultrastructure of lipid rafts and "superrafts" isolated from small intestinal microvillar membranes. Electron micrographs showing lipid rafts and "superrafts" prepared by detergent extraction and sucrose density gradient centrifugation as described in Ref. [48]. (A–C) Lipid rafts prepared using extraction with Triton X-100 on ice. This detergent generates vesicle-like rafts with a diameter in the range of 200–300 nm (A), but in addition, some multilamellar structures are seen (B). The rafts are intensely immunogold-labeled using a primary antibody to aminopeptidase N (C). (D–F) Rafts prepared at 37 °C using Brij 98 as detergent. Here, pleiomorphic membrane sheets with an approximate length of 200–300 nm are seen with only few vesicle-like structures present (D–E). Also, these structures are aminopeptidase N-positive (F). (G–I) Superrafts prepared by sequential extraction with Triton X-100 at increasing temperature. The superrafts are vesicle-like and often multilamellar structures with a diameter of about 150–200 nm. They are immunogold-labeled for aminopeptidase N (I), but more sparsely so than the "normal" lipid rafts (C, F). Notice that the cell membrane bilayer architecture is well preserved in all types of rafts (B, E, H). Bars: (A, D, G) 0.5 μm; (B, E, H) 0.1 μm; (C, F, I) 0.2 μm.

ing concept of rafts being small, cholesterol-dependent, transient, and dynamic structures [38].

4. Deep apical tubules, an unrecognized part of the brush border membrane

Simple calculations reveal that the organization of the cell membrane into a brush border may increase the apical surface of an epithelial cell about 40 times [74], thus endowing the cell with a huge capacity for digestive and absorptive processes at the surface. However, the brush border architecture, including the terminal web region, poses logistical problems for the apical membrane trafficking required to maintain a fully functional apical surface. First, the filamentous terminal web generally excludes organelles the size of endosomes from achieving close contact with the apical cell surface (Fig. 1). Second, the bulky microvillar core- and cross filaments will prevent any reasonable size of transport vesicle (min. 40–50 nm in diameter) from obtaining direct access to the microvillar surface. Theoretically, this leaves only the relatively small areas of nonmicrovillar surface membrane situated between adjacent microvilli available for vesicle fusion and budding. In the case of the small intestinal enterocyte, this restricted access to the luminal surface could potentially be a bottleneck for the intense exocytic membrane traffic needed to supply the developing brush border with membrane and to replenish enzymes lost during the digestive process. In particular, enzyme loss is crucial since most of the major peptidases and glycosidases

are easily solubilized by pancreatic proteases and typically have residence times in the brush border in the range of 5–10 h [75]. In addition to this constitutive exocytotic membrane traffic of newly synthesized brush border enzymes, the ongoing transcytosis and apical secretion of IgA required for the local immune defense of the gut must be added. The solution to this logistical problem of apical membrane trafficking seems to be the design of structures most adequately described as deep apical tubules [76]. Not easily detected in normal Epon- or ultracryo-sections by electron microscopy, their existence is readily revealed by exposure of mucosal tissue to the nonpermeable surface marker Ruthenium Red. As shown in the panel of Fig. 3, the deep apical tubules appear as a pleiomorphic part of the apical surface of the enterocyte. In some cases, they can be seen penetrating up to 0.5–1 μm into the cytoplasm, a distance sufficient to reach across the terminal web and obtain a close proximity to the subapical compartment (SAC), which is unlabeled by Ruthenium Red. Vesicle-like structures in the terminal web region are frequently labeled, and serial sectioning reveals them to be part of larger, tubular structures (Fig. 3).

Caveolin-1, a frequently used marker for lipid rafts and caveolae [31], as well as the glycolipid GM_1 [77], distinctly localizes to deep apical tubules, and cholesterol extraction with methyl-β-cyclodextrin, which inhibits apical membrane trafficking [78], essentially causes the tubules to disappear from the apical surface, indicating that they are composed of lipid raft microdomains [76]. Annexin A2, another protein known to be associated with lipid rafts [53,77] and proposed to be involved in membrane trafficking events [79,80], is also present in deep apical tubules (Danielsen et al., unpublished data). Annexin A2 is capable of interacting with actin and actin-binding proteins such as α-actinin, ezrin, and moesin, and thus to function as an interface between lipid raft membranes and the actin cytoskeleton [81,82]. It is also a known substrate for protein kinases, including protein kinase C [79]. Given the close proximity of the deep apical tubules to the microvillar core actin rootlets, an annexin A2-dependent interaction with microvillar actin rootlets might provide the tubules with a dynamic capability, as suggested by their pleiomorphic appearance.

IgA and the polymeric immunoglobulin receptor are prominent examples of a ligand–receptor complex undergoing basolateral-to-apical transcytosis in epithelial cells [83,84], and both have been localized to deep apical tubules [76]. This particular transcytotic trafficking route has been studied intensively for many years by several groups, and work conducted mainly with MDCK cells, has mapped in close detail the intracellular transport events leading from internalization into basolateral early endosomes, via a common endosome, to an apical recycling endosome before secretion from the apical surface [85–87]. The apical recycling endosome, also referred to as the subapical compart-

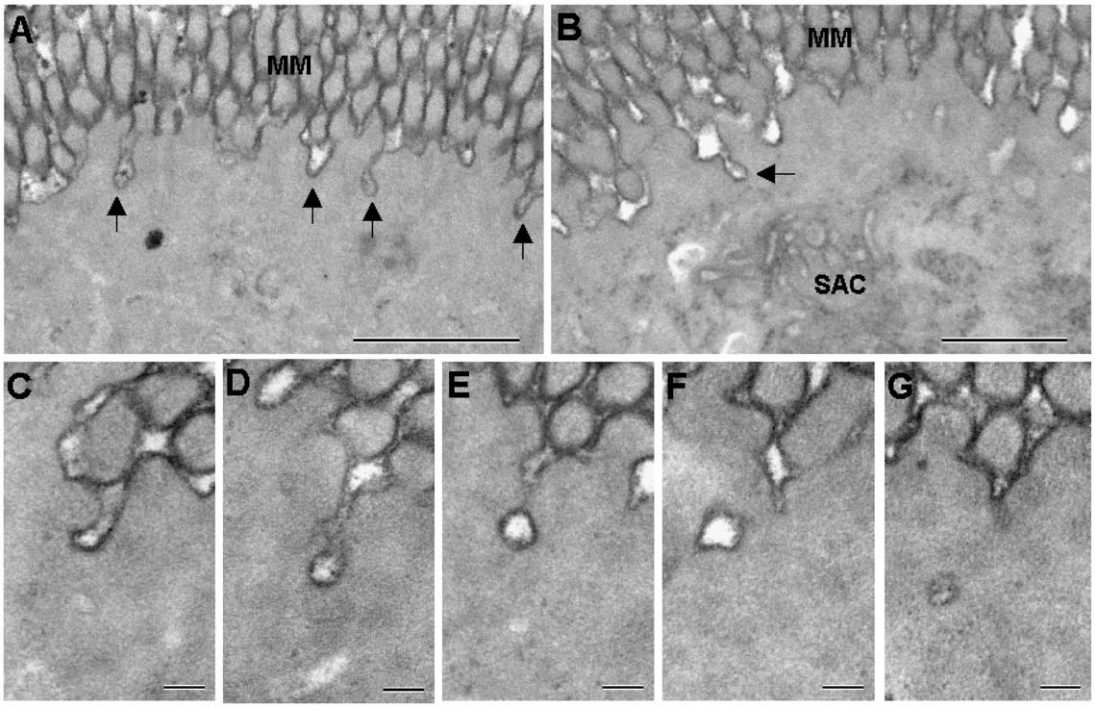

Fig. 3. Deep apical tubules in the apical cell membrane of a small intestinal enterocyte. Electron micrographs of the apical region of enterocytes, treated with the membrane-impermeable dye Ruthenium Red [76]. (A–B) In addition to the microvillar membrane (MM), pleiomorphic, tubular invaginations (termed deep apical tubules) are stained by the dye (arrows). They extend up to 0.5–1 μm into the terminal web region and are often seen in close proximity to the subapical compartment (SAC). (C–G) Serial sectioning of a deep apical tubule. This series of electron micrographs show how an apical tubule (C–E) is connected with a vesicle-like structure seemingly disconnected from the cell surface (F, G). Bars: (A) 1.0 μm; (B) 0.5 μm; (C–G) 0.1 μm.

ment or "SAC", has attracted particular interest in recent years because of its crucial role in sorting of both proteins and lipids in dynamic transit between the basolateral and apical plasma membrane domains [88–90] The SAC is the last known stage in the basolateral-to-apical transcytotic pathway [85,87], and in the enterocyte, it is typically found in the apical cytoplasm just beneath the terminal web region [91]. Deep apical tubules are the only part of the brush border surface directly accessible to membrane traffic from the SAC and are often seen in very close proximity to this endosomal compartment, lending support to the notion that elements of the SAC may fuse directly with the cell surface [90]. Such a type of transfer is indicated by videomicroscopic studies of exocytotic movements of GFP-tagged membrane proteins, which seem to occur in quite large transport containers rather than in small vesicles [92]. Alternatively, the final stage of transport could be vesicle-mediated, but regardless of the mechanism, the function of deep apical tubules is that of a hub in the final exocytotic stage of transcytosis, a process that has previously been shown to occur through lipid raft-containing compartments in enterocytes [91].

Like the SAC, deep apical tubules also harbour the resident brush border enzyme aminopeptidase N, suggesting that the constitutive biosynthetic membrane traffic that maintains and develops the brush border also employs the tubules as a hub [76].

5. Conclusion: lipid rafts are pluripotent and adaptable membrane microdomains

Previous controversies about the size, shape, and stability of lipid rafts should now be replaced by a more flexible and pluralistic view on rafts. While cholesterol and sphingolipids, including glycolipids, remain the common building blocks of rafts in all types of cells, their relative amounts and importance vary, and it now seems that raft microdomains may attain very different structures, depending on the function(s) they fulfil in a given type of cell. For instance, rafts that act as scaffolds for assembly of receptor signaling complexes, such as the T-cell receptor, need to be small and inactive in the resting state but with the ability to expand rapidly upon receptor activation. This type of raft probably fits the recently proposed description as being small, dynamic "shells" [38]. At the other extreme, intestinal microvillar rafts, capable of forming superrafts, appear to be stationary microdomains containing clusters that include several proteins and glycolipids. This type of raft seems particularly well suited as a mechanism for maintaining a permanent digestive capability at the brush border. They have the opposite curvature (i.e. positive vs. negative) of caveolae, and although cholesterol is present, this lipid is not essential for the integrity of intestinal microvillar rafts as it is for caveolae. The minor importance of cholesterol is also implied by the fact that caveolin-1, a marker for other types of raft, is excluded from microvillar rafts. But in addition to these glycolipid-based rafts, the brush border also seems to harbour a separate type of microdomains, as defined by the Lubrol WX insolubility of prominin. The solubility of these rafts in Triton X-100 and sensitivity to cholesterol depletion suggest them to be less stable and more like lipid rafts, as seen in other types of cell membranes. Like the deep apical tubules, these rafts could predominate in the areas of the apical cell surface engaged in exo- or endocytic membrane trafficking.

Recently, a number of different detergents were compared with regard to the lipid and protein composition of detergent-resistant membranes (DRMs) they generated from two types of cell membranes (MDCK and Jurkat) [93]. It was concluded that different detergents emphasize different aspects of membrane organization and complexity. As a consequence, care should always be taken in equating DRMs with membrane microdomains at the surface of a living cell. In addition, this work underscored the need for more sophisticated analytical tools in our work to unravel the architecture of these membrane microdomains that have now for sure become a reality of life but one in need of deeper understanding.

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