Deep-apical tubules: dynamic lipid-raft microdomains in the brush-border region of enterocytes¹

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The brush border of small intestinal enterocytes is highly enriched in cholesterol- and glycosphingolipid-containing membrane microdomains, commonly termed as lipid ‘rafts’. Functionally, transcytosis of IgA and exocytosis of newly made brush-border proteins in enterocytes occur through apical lipid raft-containing compartments, but little is otherwise known about these raft microdomains. We therefore studied in closer detail apical lipid-raft compartments in enterocytes by immunogold electron microscopy and biochemical analyses. Novel membrane structures, deep-apical tubules, were visualized by the non-permeable surface marker Ruthenium Red in the brush-border region of the cells. The surface-connected tubules were labelled by antibodies to caveolin-1 and the glycolipid asialo G_{M1}, and they were sensitive to cholesterol depletion by methyl- β -cyclodextrin, indicating the presence of raft microdomains. Deep-apical tubules

were positioned close to the actin rootlets of adjacent microvilli in the terminal web region, which had a diameter of 50–100 nm, and penetrated up to 1 μ m into the cytoplasm. Markers for transcytosis, IgA and the polymeric immunoglobulin receptor, as well as the resident brush-border enzyme aminopeptidase N, were present in these deep-apical tubules. We propose that deep-apical tubules are a specialized lipid-raft microdomain in the brush-border region functioning as a hub in membrane trafficking at the brush border. In addition, the sensitivity to cholesterol depletion suggests that deep-apical tubules function as a cell-surface membrane reservoir for cholesterol and for rapid adaptive changes in the size of microvilli at the brush border.

Key words: deep-apical tubule, lipid raft, membrane trafficking, small intestine, transcytosis.

INTRODUCTION

Epithelial cells face the dual task of maintaining cell polarity and at the same time allowing transcytosis of proteins and membrane lipids between the two cell-surface domains. A number of endosomal compartments taking part in transcytotic sorting and transport have been identified so that today, a fairly detailed and comprehensive map of the trafficking route has been drawn [1–4]. In particular, the transcytosis of dimeric IgA has proved a valuable model in the characterization of the various apical endosomal compartments involved. After ligand binding to its cognate polymeric immunoglobulin receptor, IgA is taken up into basolateral early endosomes before being transported to a ‘common’ endosome. From here, receptor-bound IgA is transferred to an apical recycling endosome, which is the last known stage in the transcytotic pathway in Madin–Darby canine kidney (MDCK) cells [1,4].

Unlike MDCK cells and most other epithelial cell lines, intestinal enterocytes are endowed with a highly ordered apical brush border, and are designed specifically for providing the enterocyte with a maximal absorptive capacity for nutrient assimilation. Each of the 1000–2000 microvilli of a fully mature brush border is supported by a bundle of actin filaments, the basal end of which descends below the membrane up to 1 μ m into the apical cytoplasm as a rootlet. Here, a dense and complex meshwork of filaments in this so-called terminal web region interdigitates between the rootlets and serves probably to stabilize the overall brush-border architecture and possibly endow it with a contractile capacity [5]. The terminal web excludes major organelles such as mitochondria, endoplasmic reticulum and endosomal compartments from this apical-most

region of the enterocyte. Presumably, it also sterically restricts the membrane traffic to the apical-cell surface, but little is known about membrane transport through the terminal web region of brush-border-endowed epithelial cells.

Cholesterol- and glycolipid-enriched microdomains, commonly known as lipid ‘rafts’, have been implicated in a number of cell functions, including membrane sorting and trafficking, receptor signalling and cholesterol homeostasis [6–10]. The enterocyte microvillar membrane contains lipid-raft microdomains that harbour several of the major digestive enzymes of the brush border [11], and transcytosis of IgA occurs through apical lipid raft-containing compartments [12]. In other cell types, raft microdomains often reflect the presence of caveolae, small flask-shaped membrane invaginations [13,14], but caveolae are not seen commonly at the apical surface of enterocytes. Furthermore, only a small amount of the main structural protein of this type of membrane invaginations, caveolin-1, is expressed in enterocytes [15]. This finding, together with the complex architecture of the brush border described above, raises the question of how lipid raft-based membrane trafficking occurs in the apical-most region of enterocytes.

In the present study, we characterized in closer detail the apical surface of enterocytes. This led to the identification of a novel lipid raft-containing structure, which morphologically appeared as surface-connected deep-apical tubules in the terminal web region. The tubules, positioned between actin rootlets of adjacent microvilli, contained lipid-raft microdomains as judged by their labelling for caveolin-1 and the glycolipid asialo G_{M1}, as well as their sensitivity to cholesterol depletion. In addition, they were labelled distinctly by antibodies to IgA, the polymeric immunoglobulin receptor and the brush-border

Abbreviations used: MDCK, Madin–Darby canine kidney; SAC, subapical compartment.

¹ Dedicated to the memory of John Kenny, E. M. D.’s longtime friend and mentor, who died on 25 November 2002.

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Figure 1 Visualization of deep-apical tubules in Ruthenium Red-exposed enterocytes

(A) The dye was seen along the entire apical microvillar (MV) surface and in deep-apical tubules (arrows) of enterocytes. (B–G) Closer views of the pleiomorphic apical tubules (arrows). They often appear as narrow tubules up to 1 µm long with vesicular protrusions at the tip (B–D). Some appear as Ruthenium Red-labelled, closed vesicle-like structures in the terminal web region (E–G). Scale bars: (A), 0.5 and (B–G), 0.1 µm.

enzyme aminopeptidase N. The existence of this tubular part of the brush border reveals that membrane trafficking in epithelial cells of native tissues is more complex than that inferred from studies on commonly used epithelial cell lines, such as MDCK cells.

EXPERIMENTAL

Materials

Rabbit polyclonal and mouse monoclonal antibodies to human caveolin-1, and mouse monoclonal antibodies to bovine annexin A2 were from BD Transduction Laboratories (Lexington, KY, U.S.A.). Rabbit antibodies to human IgA and secretory component, horseradish peroxidase-coupled swine anti-rabbit IgG and horseradish peroxidase-coupled rabbit anti-mouse IgG were from Dako (Glostrup, Denmark). Affinity-purified rabbit anti-mouse IgA (α -chain specific) antibodies and rabbit anti-actin antibodies were from Zymed Laboratories (San Francisco, CA, U.S.A.). Rabbit anti-asialo G_{M1} was from Biotrend (Cologne, Germany). The rabbit antibodies to pig small intestinal aminopeptidase N were as described previously [16]. Gold-labelled goat anti-rabbit IgG was from Amersham Pharmacia Biotech (Hørsholm, Denmark) and methyl- β -cyclodextrin and Ruthenium Red were obtained from Sigma. Segments of pig small intestine were kindly provided by Letty Klarskov and Mette Olesen from the Department of Experimental Medicine, The Panum Institute, Copenhagen, Denmark.

Figure 2 Immunogold labelling of aminopeptidase N in Ruthenium Red-exposed enterocytes

The labelling was performed using an antibody to aminopeptidase N that gave an intense labelling of ultrathin Epon sections. (A) Overview showing labelling of the SAC, deep-apical tubules (arrows), and the microvillar (MV) membrane. (B–G) Closer views of deep-apical tubules (arrows). EN, endosome-like structure. Scale bars: (A), 0.5 and (B–G), 0.1 µm.

Organ culture of mucosal explants

After a rinse of the small intestine in Hanks buffered salt solution, mucosal explants were excised and cultured in Trowell's T-8 medium [17]. In experiments with cholesterol depletion, explants were cultured for 1 h in the presence of 2% (w/v) methyl- β -cyclodextrin.

Preparation and raft analysis of mucosal-membrane fractions

Microvillar and non-microvillar (Mg^{2+} -precipitated) membranes were prepared from intestinal mucosa by the bivalent cation precipitation method [18]. The protease inhibitors leupeptin and aprotinin were included in all buffers (10 µg/ml). Lipid-raft analysis of microvillar and non-microvillar membranes was performed by 1% Triton X-100 extraction on ice followed by sucrose-gradient centrifugation [19] as described previously [11], with the modification that the extracts were placed in a 60% sucrose cushion with a 50–25% sucrose gradient layered on top.

Electrophoresis and immunoblotting

SDS/PAGE was performed in 10 or 15% (w/v) polyacrylamide gels [20]. After electrophoresis and transfer on to ImmobilonTM membranes, immunoblotting was performed with antibodies to caveolin-1, annexin A2, actin or IgA. Blots were developed by an electrochemiluminescence detection reagent according to the manufacturer's instructions (Amersham Pharmacia Biotech).

Electron microscopy

For surface staining with Ruthenium Red, mucosal segments were fixed in 3% (w/v) glutaraldehyde and 2% (w/v)

Figure 3 Localization of caveolin-1 in ultracryo sections of enterocytes

(A) Labelling for caveolin-1 is seen over microvilli (MV). (B–D) Caveolin-1 labelling of tubular and vesicular structures in the terminal web region. Scale bars: (A), 1.0 and (B–D), 0.2 μm .

paraformaldehyde for 20 h in the presence of 0.2 % Ruthenium Red, followed by post-fixation for 2 h in 1 % osmium tetroxide and 0.3 % Ruthenium Red, as described previously [21]. Microvillar lipid rafts were fixed in 2.5 % glutaraldehyde for 2 h at 4 °C. Ruthenium Red-treated mucosal segments and lipid rafts were embedded in Epon according to the standard procedure.

Post-embedding immunogold labelling of ultrathin Epon sections, using antibodies to caveolin-1, IgA, secretory component, asialo G_{M1} or aminopeptidase N, was performed as described previously [22].

For immunogold labelling of ultracryo sections, mucosal segments were fixed in either 4% paraformaldehyde (for labelling of caveolin-1) or in 2% paraformaldehyde/0.1% glutaraldehyde (for labelling of asialo G_{M1}). Ultracryo sectioning and immunogold labelling were performed as described previously [23]. In all labelling experiments, controls were performed by omission of the primary antibody. The sections were examined in a Zeiss EM 900 electron microscope equipped with a Mega View II camera system.

RESULTS

Characterization of deep-apical tubules in the brush border of enterocytes

The terminal web region of the enterocyte brush border extends up to approx. 1 μm into the apical cytoplasm, and the actin rootlets of microvilli together with a dense interdigitating meshwork of filaments excludes major membranous organelles from entering this apical-most part of the cytoplasm [5]. Unfortunately, the membrane morphology of this filament-rich region is difficult to visualize clearly. To overcome this problem, we used Ruthenium

Figure 4 Localization of caveolin-1 in Epon sections of Ruthenium Red-exposed enterocytes

(A–D) Labelling for caveolin-1 in membranes of deep-apical tubules. (E, F) Caveolin-1 labelling of the tubulovesicular membranes of the SAC. Note that unlike the deep-apical tubules, the SAC is unlabelled by Ruthenium Red. In (F), an arrow indicates the close proximity between a deep-apical tubule and the SAC. Scale bars: (A–D), 0.1 and (E, F), 0.5 μm .

Red in the present study to provide an enhanced view of the ultrastructure of the apical-cell surface. Ruthenium Red is a convenient membrane-impermeable cell-surface marker for electron microscopy used previously with mucosal explants to assess cell viability [21] and, as shown in Figure 1, it labelled the entire microvillar surface of the enterocyte brush border. Surprisingly, it also labelled a non-microvillar part of the apical surface in the shape of deep-apical tubules extending into the terminal web region. The apical tubules, which had a diameter of approx. 50–100 nm, were positioned between the narrow spaces of adjacent microvillar actin rootlets and were up to 1 μm in length. Like the microvillar part of the brush border, they were conspicuously immunogold-labelled by an antibody to the resident brush-border enzyme aminopeptidase N (Figure 2). Vesicular profiles were often seen at the tips of the deep-apical tubules (Figures 2B and 2C). Also, some apparently disconnected, vesicular structures located up to 1 μm from the cell surface were stained by the dye and thus surface-connected (Figures 1F and 1G; Figures 2F and 2G). The apical recycling compartment, also known as the subapical compartment (SAC) [24], was located deeper into the apical cytoplasm and devoid of any staining by the dye, showing its complete separation from the cell surface (Figures 2A and 2D).

Although classical caveolae are not observed generally at the brush-border surface of enterocytes, a faint but caveolin-1-specific expression in the apical membrane of human jejunal enterocytes has been described previously [15], raising the possibility that caveolin-1 in enterocytes might be associated

Figure 5 Localization of asialo G_{M1} in the brush-border region and in lipid rafts

(A) Immunogold labelling of an ultracryosection for asialo G_{M1} . Labelling is seen over microvilli (MV) and in the terminal web region. MB, multivesicular body (B) Immunogold labelling of asialo G_{M1} in an Epon section from a Ruthenium Red-exposed explant showing the glycolipid at the membrane of a deep-apical tubule (arrow). (C) Immunogold labelling of an Epon section of lipid rafts, isolated by sucrose-density centrifugation as described in the Experimental section, showing asialo G_{M1} at the raft membranes. Scale bars: (A), 0.2 and (B, C), 0.1 μm .

with non-caveolar membranes. By immunogold labelling of ultracryo sections, caveolin-1 was localized in the apical part of the enterocyte where it was seen over the microvilli as well as in tubular/vesicular structures in the terminal web region just beneath the microvilli (Figure 3). In Epon sections of Ruthenium Red-treated explants, this labelling for caveolin-1 was seen distinctly over the membranes of deep-apical tubules (Figures 4A–4D). In addition, caveolin-1 labelling was also seen in the tubulo/vesicular structures of the SAC in the apical cytoplasm (Figures 4E and 4F).

Glycolipids are known to be enriched in lipid rafts and have been shown to be present in lipid-raft microdomains of enterocytes [25]. As shown in Figure 5(A), an antibody to asialo G_{M1} conspicuously labelled the microvillar membrane as well as the underlying terminal web region of ultracryo sections. Figure 5(B) is an Epon section of a Ruthenium Red-exposed explant showing labelling over the membrane of deep-apical tubules. A microvillar raft localization of asialo G_{M1} was also indicated by a dense immunogold labelling of an Epon section of lipid rafts, isolated by density-gradient centrifugation (Figure 5C).

Figure 6 Localization of IgA and the polymeric immunoglobulin receptor in Epon sections of Ruthenium Red-exposed enterocytes

Both IgA (A, B) and the polymeric immunoglobulin receptor (C, D) were distinctly seen in the deep-apical tubules, some of which appeared as vesicle-like structures. In addition, both proteins could be found at the basolateral plasma membrane (BM). Scale bar, 0.2 μm .

IgA was localized previously in the SAC of the enterocyte [12], a compartment known to be the last stage in the transcytotic pathway in MDCK cells before secretion from the apical surface [4]. Figure 6 shows that IgA as well as the polymeric immunoglobulin receptor were also present in the deep-apical tubules of the brush border. The proximity of deep-apical tubules to the SAC suggests that the former membrane structures might function directly as focal sites for apical externalization of transcytosed IgA.

Differential raft association and molecular forms of microvillar and non-microvillar caveolin-1 in enterocytes

Caveolin-1 is a frequently used marker for lipid rafts in many cell types [13], but in intestinal epithelial cells, lipid rafts isolated as detergent-resistant membranes were reported heterogeneous with regard to their content of caveolin-1 [15]. In the present study, mucosal caveolin-1 was seen mainly in either non-microvillar (Mg^{2+} -precipitated) or microvillar membranes, whereas the soluble fraction harboured only minute amounts of the protein (results not shown). However, caveolin-1 from the two membrane fractions behaved very differently when subjected to extraction with Triton X-100 at low temperature and subsequent flotation analysis by sucrose-gradient centrifugation (Figure 7A). Thus microvillar caveolin-1 remained in the non-floating fractions of the gradient, as described previously [25], indicating its absence from lipid-raft membranes. The distribution in the same gradient of annexin A_2 , a peripheral membrane protein known to be associated with lipid rafts [26], is shown for comparison (Figure 7A). In contrast, caveolin-1 from simultaneously prepared non-microvillar membranes (including the deep-apical tubules and the SAC) was seen largely in the floating fractions like annexin A_2 , indicating a 'normal' association with lipid-raft membrane microdomains (Figure 7B).

Together, these results indicate heterogeneity in the lipid-raft association of enterocyte caveolin-1, depending on its subcellular localization, and they may suggest that the membrane interaction of the protein is modulated during trafficking between microvillar and non-microvillar membranes.

Figure 7 Analysis of lipid-raft localization of enterocyte caveolin-1

Lipid-raft analysis of enterocyte microvillar (A) and non-microvillar (Mg^{2+} -precipitated) (B) membranes prepared from the same mucosal homogenate by sucrose-gradient centrifugation. The membranes were prepared, extracted with 1% ice-cold Triton X-100 and subjected to sucrose-gradient centrifugation as described in the Experimental section. Samples of the gradient fractions were analysed by SDS/PAGE and immunoblotted for the distribution of caveolin-1 (21 and 15 kDa), annexin A2 (36 kDa) and total protein, visualized by staining with Coomassie Brilliant Blue. Gradient fractions containing raft membranes and soluble protein are indicated. (In this experiment, the 21 and 15 kDa bands were detected with a polyclonal antibody (C13630)

Figure 8 Proteolytic processing of mucosal caveolin-1

Non-microvillar (Mg^{2+}) and microvillar (Mic) membranes prepared simultaneously from the same homogenate were suspended in 25 mM Hepes/HCl, 150 mM NaCl (pH 7.1), and incubated at 37 °C. At the indicated periods of time, samples were collected and subjected to SDS/PAGE. After electrophoresis, the 21 and 15 kDa bands of caveolin-1 were visualized by immunoblotting.

As also apparent from Figure 7, microvillar and non-microvillar caveolin-1 differed in molecular composition. Whereas the latter was seen predominantly as a 21 kDa polypeptide, microvillar caveolin-1 also contained about equal amounts of a 15 kDa polypeptide. Caveolin-1 is known to exist in two isoforms generated from distinct mRNAs by alternative transcription initiation sites [27], and we initially took the 15 kDa form to represent the β -isomer which is the smaller of the two by 31 amino acids [28]. However, when non-microvillar membranes were incubated at 37 °C for periods of up to 6 h, a precursor-product relationship between the 21 and 15 kDa forms was seen in that the predominant 21 kDa form disappeared gradually, whereas the amounts of the minor 15 kDa form increased significantly by 3 h and became the dominant form by 6 h (Figure 8). Microvillar membranes, prepared from the same subcellular fractionation, contained about equal amounts of the 21 and 15 kDa molecular forms, but as shown in Figure 8, no great amount of degradation/conversion took place during incubation of this fraction for 6 h.

Taken together, these results suggest that the 15 kDa form is generated quite probably from the full-length 21 kDa form by proteolysis. Although some proteolysis occurring in the non-microvillar fraction may have been caused by released lysosomal enzymes, at least some processing must take place *in vivo* since the 15 kDa form was always seen in the microvillar fraction also without auto-incubation. In fact, since the 15 kDa form was relatively abundant in the microvillar fraction, but not generated there, the site for proteolytic processing may be in close proximity to the microvillar membrane. The SAC or the deep-apical tubules would be a possible site for this processing.

Deep-apical tubules are sensitive to cholesterol extraction by methyl- β -cyclodextrin

Many membrane structures composed of lipid-raft microdomains are known to be sensitive to cholesterol extraction [29,30], and we have previously used methyl- β -cyclodextrin to extract > 50% of cholesterol from mucosal explants [25,31]. As shown in Figures 9(A) and 9(B), a brief exposure to methyl- β -cyclodextrin reduced significantly the number of deep-apical tubules in the terminal web region, indicating that their extended morphology relies on the presence of cholesterol. The virtual absence of deep-apical tubules made the exposed microvillar actin rootlets appear as the only prominent structure immediately underneath

to caveolin-1, but both bands also reacted with a monoclonal antibody (C37120). We take the 15 kDa band to represent a proteolytic fragment of the 21 kDa species because a precursor-product relationship between the two molecular forms was observed in auto-incubation experiments with mucosal membranes (Figure 8).

Figure 9 Deep-apical tubules are sensitive to cholesterol depletion with methyl- β -cyclodextrin

Electron micrographs of Epon sections of mucosal explants cultured for 1 h in the absence (A) or presence (B) of methyl- β -cyclodextrin and exposed to Ruthenium Red as described in the Experimental section. Few, if any, tubules (arrows) were observed between the exposed microvillar actin rootlets (arrowheads) after cholesterol depletion. In addition, treatment with methyl- β -cyclodextrin reduced the length of the microvilli (MV), as described previously [31]. (C–E) Higher magnification micrographs showing how deep-apical tubules and microvillar actin rootlets in control explants are often seen in direct contact. Scale bars: (A, B), 0.5 and (C–E), 0.05 μ m.

the microvilli. Taken together with the caveolin-1 and G_{M1} localization, the sensitivity of deep-apical tubules to methyl- β -cyclodextrin is a strong indicator of the presence of lipid-raft microdomains. In addition, Figure 9 also shows that methyl- β -cyclodextrin caused a shortening of the microvilli. This microvillar shortening and other effects of cholesterol depletion were described previously [31].

Proteins associated with lipid raft are often released from these microdomains on cholesterol depletion [30]. As shown in Figure 10(A), caveolin-1 from the non-microvillar fraction shifted partially from raft fractions to the soluble fractions of the density gradient after treatment with methyl- β -cyclodextrin, indicating a partial dissociation from lipid rafts. Annexin A2 was likewise released only partially from the raft fractions. More specifically, the release of both caveolin-1 and annexin A2 was seen primarily from the raft fractions of lowest density, suggesting heterogeneity

Figure 10 Raft association of caveolin-1, annexin A₂, IgA and actin is sensitive to treatment with methyl- β -cyclodextrin

Non-microvillar membranes were subjected to lipid-raft analysis as described in the legend to Figure 7. Before extraction with Triton X-100, the membranes were incubated for 30 min on ice in the absence or presence of 1% methyl- β -cyclodextrin (m β CD). Gradient fractions were analysed by SDS/PAGE, followed by immunoblotting. (A) Caveolin-1 and annexin A₂; (B) IgA and actin.

of the lipid rafts with regard to cholesterol, but the partial release could also reflect the fact that cholesterol is only extracted partially by methyl- β -cyclodextrin. Previously, we have applied a similar cholesterol depletion treatment method to fibroblast-like synoviocytes and observed a near-complete disruption of caveolae together with only a partial release of caveolin-1 from lipid rafts [14].

As shown in Figures 9(C)–9(E), the microvillar actin rootlets in the terminal web region interdigitate closely between the deep-apical tubules and frequently appear to be in direct contact with them. A physical association between the actin cytoskeleton

and lipid rafts was also indicated by the presence of actin in the floating fractions of the density gradient (Figure 10B). Interestingly, the raft association of actin was eliminated completely by treatment with methyl- β -cyclodextrin, suggesting that intact raft microdomains are required for membrane–cytoskeletal interactions.

Finally, Figure 10(B) shows that a fraction of the soluble protein IgA was also raft-associated, as reported previously [25], and that cholesterol depletion reduced markedly the relative amounts of raft-associated IgA.

DISCUSSION

The main finding of the present study was the identification of a tubular membrane structure in the terminal web region of enterocytes. Not visualized easily in normal ultracryo sections, this pleiomorphic structure was viewed readily in ultrathin Epon sections of Ruthenium Red-treated mucosal segments. The accessibility of the deep-apical tubules to the exogenous dye proved them to be part of the apical surface and thus clearly distinguished them from the underlying endosomal compartment, the latter often being referred to as the 'apical recycling endosome' or 'SAC' of epithelial cells. The sensitivity of the caveolin-1-positive, deep-apical tubules to cholesterol depletion by methyl- β -cyclodextrin was similar to that observed for caveolae seen in other cell types. However, although pit-like indentations in the brush-border surface of enterocytes [32] and kidney proximal tubule cells [33] are known, membrane invaginations resembling the classical flask shape of caveolae [13] are not observed. A likely explanation could be that formation of caveolae requires a much higher level of caveolin-1 expression than that seen in enterocytes, as judged by the modest immunogold labelling observed in the present study and also described previously by others [15].

Caveolin-1 in the intestinal epithelial cells is unusual in being heterogeneous with regard to lipid-raft microdomains [15]. In line with this, we have observed previously that microvillar caveolin-1 is not raft-associated, probably because these rafts are mainly glycolipid-based and relatively cholesterol-independent [25]. The membrane topology of caveolin-1 is presumed generally to be dependent on a central hydrophobic stretch forming a hairpin loop within the membrane, leaving both N- and C-termini on the cytoplasmic side [34,35]. Its C-terminal domain is lipid-modified by palmitoylation on multiple cysteine residues [36], a modification required for cholesterol binding and transport [37], suggesting that this region of the polypeptide may be part of the interaction with the raft microdomains. Speculatively, the proteolytic processing of the 21 kDa form to that of 15 kDa may be related to the shift in raft association, observed for caveolin-1 in microvillar as opposed to non-microvillar membranes. At present, however, we are uncertain about the function of enterocyte caveolin-1. However, the uptake of lipids, including dietary/biliary cholesterol, takes place across the enterocyte brush border by a mechanism that is not yet understood fully. Thus it may involve a protein-based absorption system [38] of which caveolin-1 could be included [39]. In this context, it is of interest to note that several members of the caveolin family can be targeted to lipid storage droplets [40], possibly as a key component involved in cellular cholesterol balance and lipid transport [41–43]. However, further work is obviously needed to clarify if microvillar caveolin-1 plays a role in cholesterol absorption and whether its presence in the deep-apical tubules may be related to such a function.

Labelling by antibodies to IgA as well as the polymeric immunoglobulin receptor indicates that the deep-apical tubules

quite probably function in membrane trafficking to the brush border in enterocytes. In previous studies of IgA transcytosis in MDCK cells, the apical recycling compartment is the last known stage before release from the apical surface [4], and to our knowledge, no membrane structure like the deep-apical tubules has been described for transcytosis in this commonly used model cell type. From their unique position in the terminal web, a region of the enterocyte inaccessible to other endosomal compartments, the deep-apical tubules quite probably serve as a hub, connecting the apical surface to the network of intracellular trafficking routes, including the transcytotic pathway [2–4,44]. They may very well be a special feature of epithelial cells possessing a fully developed apical brush border, because the dense architecture of the actin rootlets poses an obvious steric hindrance for docking/budding of vesicles at the small non-microvillar patches of the apical surface. Tubular membrane structures that extend these patches through the filamentous terminal web region seem well-suited focal points for membrane traffic connecting the brush border with the vesicular transport pathways of the cell. Enterocytes require a massive net flow of membrane to the brush border to sustain its dramatic development in size during their short lifespan of approx. 3 days, and we have earlier observed that cholesterol depletion by methyl- β -cyclodextrin inhibits newly synthesized aminopeptidase N in reaching the microvillar membrane [31]. The lipid-raft dependency of apical transport correlates well with the sensitivity of the deep-apical tubules to methyl- β -cyclodextrin and is suggestive of their involvement in exocytotic transport.

Conceivably, another role of deep-apical tubules could be to act as an apical membrane reservoir, enabling the enterocyte to adjust rapidly to the microvillar length, and thus absorptive capacity, in response to changing needs that occur when the gut is active during digestion and absorption of various dietary nutrients. The microvillar length is known to increase very rapidly in response to stimulation with epidermal growth factor, a mechanism whereby glucose transport [45], salt absorption [46] and possibly other absorptive functions of the gut epithelium may be regulated under physiological conditions. In addition, the virtual disappearance of the deep-apical tubules following cholesterol depletion with methyl- β -cyclodextrin suggests that they may act as a cholesterol reservoir during lipid absorption. In conclusion, the pleiomorphic appearance of these tubules certainly suggests them to be dynamic structures, but a more detailed account of their functions must await further studies.

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