

# GM<sub>1</sub> and GM<sub>3</sub> gangliosides highlight distinct lipid microdomains within the apical domain of epithelial cells

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**Abstract** The apical domain of epithelial cells is composed of distinct subdomains such as microvilli, primary cilia and a non-protruding region. Using the cholesterol-binding protein prominin-1 as a specific marker of plasma membrane protrusions we have previously proposed the co-existence of different cholesterol-based lipid microdomains (lipid rafts) within the apical domain [Röper, K., Corbeil, D. and Huttner, W.B. (2000), Retention of prominin in microvilli reveals distinct cholesterol-based lipid microdomains in the apical plasma membrane. *Nat. Cell Biol.* 2, 582–592]. To substantiate the hypothesis that the microvillar plasma membrane subdomains contain a distinct set of lipids compared to the planar portion we have investigated the distribution of prominin-1 and two raft-associated gangliosides GM<sub>1</sub> and GM<sub>3</sub> by fluorescence microscopy. GM<sub>1</sub> was found to co-localize with prominin-1 on microvilli whereas GM<sub>3</sub> was segregated from there suggesting its localization in the planar region. Regarding the primary cilium, overlapping fluorescent signals of GM<sub>1</sub> or GM<sub>3</sub> and prominin-1 were observed. Thus, our data demonstrate that specific ganglioside-enriched rafts are found in different apical subdomains and reveal that two plasma membrane protrusions with different structural bases (actin for the microvillus and tubulin for the cilium) are composed of distinct types of lipid.

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**Keywords:** CD133; Epithelial cell; Apical domain; Microvillus; Cilium; Ganglioside

## 1. Introduction

The apical domain of polarized epithelial cells is composed of separate subdomains such as plasma membrane protrusions (microvillus and primary cilium) and a non-protruding region. It contains a variety of lipid species that have distinct physicochemical properties. Using the cholesterol-binding protein prominin-1 (CD133) as a specific marker of plasma membrane protrusions [1,2] (for reviews see [3–5]) we have previously proposed the co-existence of different cholesterol-based lipid microdomains (often referred to as “lipid- or membrane rafts” for an unified definition see Ref. [6]) within the apical plasma membrane [7]. Membrane rafts are viewed as liquid-ordered domains that are more tightly packed than the surrounding

“non-raft” phase of the bilayer. They are enriched in sterol and sphingolipids present in the exoplasmic leaflet membrane and contain a specific set of membrane and peripheral proteins [8]. Membrane rafts have been suggested to play a role in various cellular events including fission and membrane budding [9,10]. The classical biochemical method to determine the association of a given protein with such membrane rafts is based on their resistance to extraction with the non-ionic detergent Triton X-100 in the cold [11]. Proteins associated with Triton X-100 resistant membranes float in low-density fractions upon sucrose density gradient in a cholesterol-dependent manner [12].

How many types of membrane raft co-exist in a given plasma membrane? If they are more than one, how can we define them? We had previously observed that prominin-1, although completely soluble in Triton X-100, was insoluble in other non-ionic detergents (e.g. Lubrol WX, Brij 58) in a cholesterol-dependent manner [7]. Based in part on these biochemical features and on its segregation at the cell surface from placental alkaline phosphatase, which in contrast to prominin-1 is enriched in Triton X-100 resistant membranes, we had proposed that distinct types of membrane rafts could co-exist within the apical plasma membrane domain [7]. However, concerns about the ability of these mild detergents to selectively solubilize membrane proteins and thus discriminate between those associated or not with membrane rafts have been raised [13,14]. Moreover, given that some detergents, e.g. Triton X-100, might create ordered domains in a homogeneous fluid membrane, it appeared that detergent-resistant membranes should not be assumed to resemble to biological membrane rafts [15].

Here, we have re-investigated the issue whether the microvillar plasma membrane contains a distinct set of lipids compared to the planar, non-protruding portion of the apical domain by analyzing the distribution of prominin-1 and the raft-associated gangliosides GM<sub>1</sub> and GM<sub>3</sub>. The fluorescence microscopy analyses demonstrate that specific ganglioside-enriched rafts are found in different apical subdomains.

## 2. Materials and methods

### 2.1. Cell culture

MDCK cells (strain II) stably transfected with mouse prominin-1 [16] were cultured in a humidified incubator at 37 °C under a 5% CO<sub>2</sub> atmosphere in Minimal Essential Medium supplemented with 10% fetal calf serum, 10 mM HEPES pH 7.2, 1% non-essential amino acids, 2 mM L-glutamine, 100 U/ml penicillin and 100 µg/ml streptomycin. For fluorescence microscopy, cells were grown on glass coverslips.

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## 2.2. Fluorescence and confocal microscopy

**2.2.1. Cell-surface fluorescence of living cells.** Double labelling cell-surface fluorescence of prominin-1-transfected MDCK cells was performed as described [16], except that the cells were incubated at 4 °C first with the primary antibodies, i.e. rat mAb 13A4 against prominin-1 (10 µg ml<sup>-1</sup>, Ref. [1]) and mouse mAb anti-GM<sub>3</sub> (1:100, clone GMR6, Seikagaku Corporation), for 30 min, and then with the appropriate secondary antibodies (Cy5- or Cy2-conjugated goat anti-rat IgG, Jackson Immuno Research; Alexa488- or Alexa546-conjugated goat anti-mouse IgM, Molecular Probes) and/or Alexa488- or Alexa546-coupled cholera toxin B subunit (1:1000, Molecular Probes) for 30 min. Labelled cells were fixed with 4% paraformaldehyde (PFA) in PBS for 30 min at room temperature, quenched with 50 mM NH<sub>4</sub>Cl in PBS for 10 min and then sequentially washed in PBS and distilled water, and finally mounted in Mowiol 4.88 (Calbiochem-Behring GmbH). Cells were observed with a Zeiss 510 Meta confocal laser-scanning microscope.

**2.2.2. Fluorescence of fixed cells.** Prior to labelling cells were fixed according to one of the following three protocols. (i) Cells were fixed with 4% PFA in PBS for 30 min at room temperature. The fixative was removed by three washes with PBS, and the residual formaldehyde was quenched with 50 mM NH<sub>4</sub>Cl in PBS for 10 min. Fixed cells were blocked and permeabilized with 0.2% saponin/0.2% gelatin in PBS for 30 min, and then incubated with the appropriate antibodies and when necessary with cholera toxin B subunit for 30 min at room temperature. Acetylated tubulin was detected using mouse mAb anti-acetylated-tubulin (1:1000, clone 6-11B-1, Sigma) followed by Alexa488- or Alexa546-conjugated goat anti-mouse IgG2b (1:1000, Molecular Probes). (ii) Cells were fixed using a PFA/methanol-fixation protocol as described [7]. Briefly, cells were washed with ice-cold PBS and fixed for 5 min with 4% PFA in PBS at 4 °C, followed by a 5 min fixation in methanol at -20 °C. Following quenching with 50 mM NH<sub>4</sub>Cl, fixed cells were permeabilized and double-labelled as described above. (iii) Cells were fixed according to a methanol/acetone-fixation protocol. Briefly, cells were fixed for 2 min with methanol at -20 °C and then 1 min in acetone at -20 °C prior to labelling. Irrespective of the protocol used, labelled cells were sequentially washed in PBS containing 0.2% gelatine, PBS and distilled water, mounted in Mowiol 4.88 and observed with a confocal laser-scanning microscope.

The confocal microscope settings were such that the photomultipliers were within their linear range. The images shown were prepared from confocal data files using LSM 5 Image Browser software.

## 3. Results and discussion

The ganglioside composition of MDCK cells is known to be dependent on their state of differentiation, i.e. cell density, and passage number [17]. In keeping with this report, we have observed, using 5-day-old post-confluent MDCK cells stably transfected with mouse prominin-1 [16] that GM<sub>3</sub> was the major ganglioside expressed on their surface whereas GM<sub>1</sub> was present in a low amount and only in a limited number of cells (data not shown). It was extremely rare to find cells that express simultaneously both gangliosides on their surface. In double-positive cells, GM<sub>1</sub> was mostly located inside the cells in contrast to GM<sub>3</sub> (data not shown).

### 3.1. GM<sub>1</sub>, but not GM<sub>3</sub>, is found in microvilli

The subcellular localization of GM<sub>1</sub> and GM<sub>3</sub> present at the apical domain of polarized epithelial cells was then investigated by confocal laser scanning microscopy analysis. Double cell surface labelling of living 5-day-old post-confluent MDCK cells with cholera toxin B subunit, which binds GM<sub>1</sub>, and anti-prominin-1 antibody showed that within the apical plasma membrane GM<sub>1</sub> was selectively concentrated on microvilli as revealed by its co-localization with prominin-1 (Fig. 1A, subpanel a, see inset in merge). On the other hand GM<sub>3</sub> appeared to be excluded from there (Fig. 1A, subpanel b, inset). The

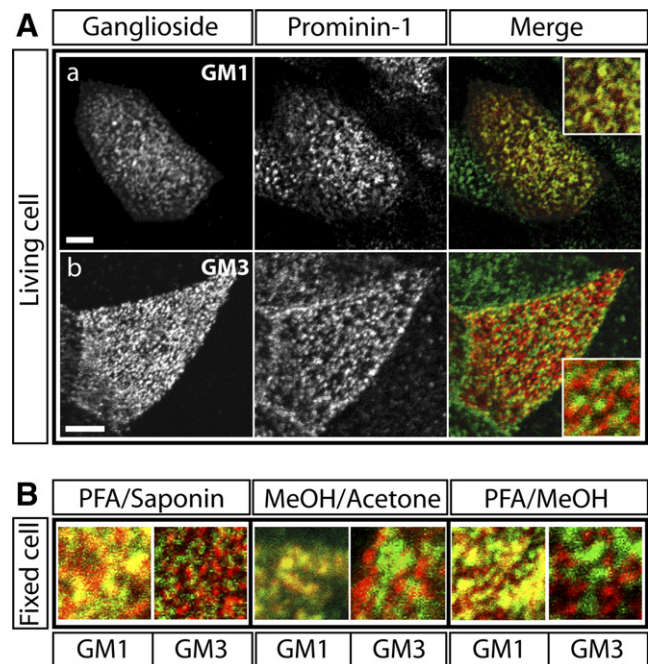


Fig. 1. Distinct ganglioside-enriched microdomains within the apical plasma membrane domain. (A) Living prominin-1-transfected MDCK cells were double-labelled for either GM<sub>1</sub> or GM<sub>3</sub> (left and right panels, red) and the microvillar marker prominin-1 (middle and right panels, green) and observed by confocal laser scanning microscopy. Cells were first labelled in the cold with anti-GM<sub>3</sub> and/or anti-prominin-1 primary antibodies followed by the appropriate secondary antibodies or cholera toxin subunit B coupled to fluorescent dyes, which recognizes GM<sub>1</sub>, and then 4% PFA fixed. (B) MDCK cells were fixed prior labelling according to three different protocols. Cells were fixed either with 4% PFA at room temperature for 30 min following by a 0.2% saponin/PBS permeabilization treatment (left panels) or methanol for 2 min followed by acetone for 1 min both at -20 °C (middle panels) or with 4% PFA at 4 °C and methanol at -20 °C for 5 min each (right panels) and then incubated with the above mentioned antibodies and toxin. Gangliosides are pseudocolored in red whereas prominin-1 in green. All pictures show a single optical xy-section at the apex of the cells with a high magnification view in the corresponding inset (panel A, merge). (See also Fig. 1 in Supplementary materials for a low magnification of data presented in the panel B.) Irrespective of the labelling and fixation protocols, GM<sub>1</sub> was found in microvilli as revealed by prominin-1 staining whereas GM<sub>3</sub> was segregated from there. Scale bars, 5 µm.

exclusion of GM<sub>3</sub> from the prominin-1-containing microvillus was also observed when the cells were fixed with paraformaldehyde (PFA) after the incubation with the primary antibodies and prior to the addition of the secondary antibodies (data not shown).

When MDCK cells were fixed and permeabilized with either PFA/saponin or methanol/acetone or PFA/methanol before the entire labelling procedure (for experimental detail see Section 2) to preclude potential clustering of the distinct components particularly prominin-1 inside a specific membrane raft due to primary/secondary antibody binding, GM<sub>1</sub> also co-localized with prominin-1 independently of the fixation protocol (Fig. 1B, see also Fig. 1 in Supplementary materials). Double immunofluorescence analysis of GM<sub>3</sub> and prominin-1 confirmed the mosaic staining within the apical surface (Fig. 1B, Fig. 1 in Supplementary materials). The exclusion of GM<sub>3</sub> from microvillar-associated prominin-1-positive microdomains particularly upon cell surface staining of living

cells (Fig. 1A) suggest its localization in the more planar regions of the apical domain. Chigorno et al. have demonstrated by electron microscopy using immunogold labelling indeed that GM<sub>3</sub> is found in the planar areas of MDCK plasma membrane [18].

### 3.2. Both gangliosides GM<sub>1</sub> and GM<sub>3</sub> are associated with the primary cilium

We extended our analysis to another plasma membrane protrusion emerging from the apical plasma membrane domain, i.e. the primary cilium, where prominin-1 is concentrated as well [19,20] (see also Fig. 2, top panels). Surprisingly, both GM<sub>1</sub> and GM<sub>3</sub> were detected in the primary cilium as revealed by their co-localization with acetylated tubulin (Fig. 2, middle and bottom panels, respectively). [Note that the method of fixation does not interfere with the conclusion (data not shown; see also Fig. 3; Fig. 2 in Supplementary materials)]. Thus, GM<sub>3</sub> appears to be enriched in the primary cilium (Fig. 3, sections a and b; see also Fig. 2 in Supplementary materials), but not in microvilli (Fig. 3, sections b and c; Fig. 2 in Supplementary materials), although both membrane protrusions contain prominin-1 (Fig. 3, sections a, b, c; Fig. 2 in Supplementary materials). In the cilium, GM<sub>3</sub> is found at the base up to the tip (Fig. 3, arrowheads and arrows, respectively; Fig. 2 in Supplementary materials). Together, these observations reveal a complex distribution of distinct membrane microdomains within the apical domain of polarized epithelial cells. Interestingly, Vieira and colleagues have drawn recently a similar conclusion based on the subcellular localization of various apical proteins [21] (see also commentary in Ref. [22]).

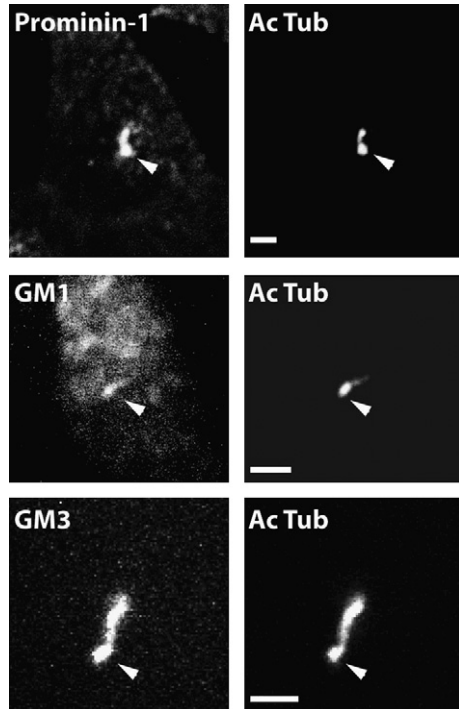


Fig. 2. Both gangliosides GM<sub>1</sub> and GM<sub>3</sub> are found in the primary cilium like prominin-1. Double fluorescence analysis of PFA – (top and bottom panels) or PFA/methanol – (middle panels) fixed, saponin-permeabilized prominin-1-transfected MDCK cells shows the presence of prominin-1, GM<sub>1</sub> and GM<sub>3</sub> in primary cilium as revealed by their co-localization with acetylated tubulin (Ac Tub). An optical *xy*-section is presented. Scale bars, 2  $\mu$ m.

The observation that the primary cilium, in contrast to the microvillus, could host both types of gangliosides is highly intriguing and might reveal a new complexity of cilium function(s). Whether both gangliosides reside inside the same microdomain or are part of two distinct entities needs to be solved, and novel techniques such as atomic force microscopy will be needed to investigate this issue.

### 3.3. Ganglioside distribution in non-epithelial cells

The segregation of GM<sub>3</sub> from microvillar structures positive for prominin-1 is not unique to epithelial cells since a similar result was observed with primary mesenchymal stem cells transfected with a prominin-1 expression plasmid (data not shown). This indicates that the microvillar exclusion of this particular ganglioside is a general phenomenon observed not only in epithelial cells but also in non-epithelial cells. These observations are in line with the asymmetrical distribution of membrane raft markers (lipids and proteins) observed in migrating lymphocytes [23] and hematopoietic progenitors [24] where they acquire a polarized cell morphology with the formation of a leading edge in the front and a uropod at the rear pole. Membrane microdomains of the leading edge have been reported as being enriched in GM<sub>3</sub> whereas the uropod contained GM<sub>1</sub>-enriched microdomain [23]. It is not a coincidence that prominin-1 is concentrated as well in uropods [24,25].

### 3.4. Physiological considerations

The molecular mechanism underlying the selective association of the pentaspan membrane glycoprotein prominin-1 with plasma membrane protrusions as well as its incorporation into specific membrane microdomain are currently unknown. However, two scenarios, not mutually exclusive, can be envisioned. First, the prominin-1 transmembrane domains, of which three are unusually long (26–28 amino acids) might increase by their interaction with the surrounding lipids, e.g. GM<sub>1</sub>, the thickness of the plasma membrane, and consequently modify locally the general organization of the lipid bilayer. Second, the large prominin-1 extracellular loops carrying glycan moieties might induce the self-aggregation of prominin-1 by homotypic interaction, which in turn would drive the clustering of small anisotropic membrane inclusions and, hence, potentially change the membrane curvature. Such theoretical considerations have been recently postulated [26,27]. Physiologically, the coalescence of small membrane inclusions into a large membrane microdomain [3,10] particularly at the edge of the microvillus, might create a phase separation with regard to the surrounding “non-raft” microenvironment leading to the budding of small raft-associated membrane particles [4]. In this particular context, it is important to note that small membrane particles (50–80 nm) containing prominin-1 are indeed released into the MDCK culture medium [19] as well as into various physiological extracellular fluids, e.g. urine, tear, saliva and seminal fluid [20,28]. Remarkably, prominin-1 molecules associated with these particles exhibited the same detergent solubility/insolubility and cholesterol dependence (Marzesco A.-M., Wiltsch-Bräuninger M., Janich P., Huttner W.B. and Corbeil D., manuscript in preparation) as those found within the apical plasma membrane protrusions (see Section 1 Ref.[7]).

Finally, it is important to note that neither prominin-1 nor any gangliosides studied here are essential for the formation



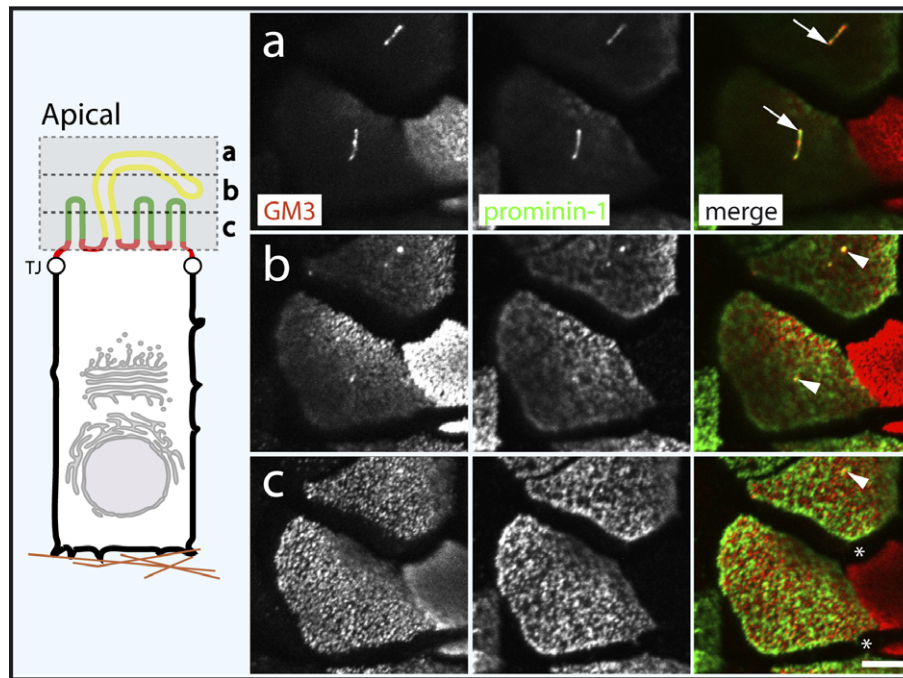


Fig. 3. Differential subcellular localization of GM<sub>3</sub> and prominin-1 within the apical plasma membrane. PFA/methanol-fixed prominin-1-transfected MDCK cells were double-labelled with anti-GM<sub>3</sub> (red) and prominin-1 (green) antibodies and observed by confocal laser scanning microscopy. Three consecutive single optical *x*-*y*-plane sections (1  $\mu$ m each) at the level of the primary cilium (a,b) and microvilli (b and c), as outlined in the cartoon, are shown. Note the presence of GM<sub>3</sub> in the primary cilium (a,b), but not in microvilli (b,c), although both membrane protrusions contain prominin-1. Arrows (a) and arrowheads (b,c) indicate the tip and base of cilia, respectively. Asterisks mark an intercellular space artefact created upon PFA/methanol fixation. TJ, tight junction. Scale bar, 5  $\mu$ m.

and/or maintenance of plasma membrane protrusions within the apical domain given that such membrane structures exist without them (data not shown). Nevertheless, their presence might confer some specific properties to these protrusions. Clearly, further investigations are needed to determine the complete composition and/or organization of various microdomains within the apical plasma membrane of polarized epithelial cells.

#### 4. Conclusions

In conclusion, the present results substantiate the hypothesis that the microvillar plasma membrane subdomains of the apical domain are composed of lipid microdomains that differ in composition, e.g. ganglioside species, from those found in the non-protruding region, and reveal surprisingly that two apical plasma membrane protrusions with different structural bases (actin for the microvilli and tubulin for the primary cilium) contain distinct lipids.

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#### Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.febslet.2007.03.065.

#### References

- [1] Weigmann, A., Corbeil, D., Hellwig, A. and Huttner, W.B. (1997) Prominin, a novel microvilli-specific polytopic membrane protein of the apical surface of epithelial cells, is targeted to plasmalemmal protrusions of non-epithelial cells. *Proc. Natl. Acad. Sci. USA* 94, 12425–12430.
- [2] Corbeil, D., Röper, K., Hellwig, A., Tavian, M., Miraglia, S., Watt, S.M., Simmons, P.J., Peault, B., Buck, D.W. and Huttner, W.B. (2000) The human AC133 hematopoietic stem cell antigen is also expressed in epithelial cells and targeted to plasma membrane protrusions. *J. Biol. Chem.* 275, 5512–5520.
- [3] Corbeil, D., Röper, K., Fargeas, C.A., Joester, A. and Huttner, W.B. (2001) Prominin: a story of cholesterol, plasma membrane protrusions and human pathology. *Traffic* 2, 82–91.
- [4] Fargeas, C.A., Fonseca, A.V., Huttner, W.B. and Corbeil, D. (2006) Prominin-1 (CD133): from progenitor cells to human diseases. *Future Lipidol.* 1, 213–225.
- [5] Jászai, J., Fargeas, C.A., Florek, M., Huttner, W.B., and Corbeil, D. (2006). Focus on Molecules: Prominin-1 (CD133). *Exp. Eye Res.* Epub ahead of print; PMID: 16733052.
- [6] Pike, L. (2006) Rafts defined: a report on the Keystone symposium on lipid rafts and cell function. *J. Lipid Res.* 47, 1597–1598.
- [7] Röper, K., Corbeil, D. and Huttner, W.B. (2000) Retention of prominin in microvilli reveals distinct cholesterol-based lipid microdomains in the apical plasma membrane. *Nat. Cell Biol.* 2, 582–592.
- [8] Simons, K. and Toomre, D. (2000) Lipid rafts and signal transduction. *Nat. Rev. Mol. Cell Biol.* 1, 31–39.
- [9] Sharma, P., Sabharanjak, S. and Mayor, S. (2002) Endocytosis of lipid rafts: an identity crisis. *Semin. Cell Dev. Biol.* 13, 205–214.
- [10] Huttner, W.B. and Zimmerberg, J. (2001) Implications of lipid microdomains for membrane curvature, budding and fission. *Curr. Opin. Cell Biol.* 13, 478–484.
- [11] Brown, D. and Rose, J. (1992) Sorting of GPI-anchored proteins to glycolipid-enriched membrane subdomains during transport to the apical cell surface. *Cell* 68, 533–544.

- [12] Brown, D.A. and London, E. (2000) Structure and function of sphingolipid- and cholesterol-rich membrane rafts. *J. Biol. Chem.* 275, 17221–17224.
- [13] Schuck, S., Honsho, M., Ekroos, K., Shevchenko, A. and Simons, K. (2003) Resistance of cell membrane to different detergents. *Proc. Natl. Acad. Sci. USA* 100, 5795–5800.
- [14] Shogomori, H. and Brown, D.A. (2003) Use of detergents to study membrane rafts: the good, the bad, and the ugly. *Biol. Chem.* 384, 1259–1293.
- [15] Heerklotz, H. (2002) Triton promotes domain formation in lipid raft mixtures. *Biophys. J.* 83, 2693–2701.
- [16] Corbeil, D., Röper, K., Hannah, M.J., Hellwig, A. and Huttner, W.B. (1999) Selective localization of the polytopic membrane protein prominin in microvilli of epithelial cells – a combination of apical sorting and retention in plasma membrane protrusions. *J. Cell Sci.* 112, 1023–1033.
- [17] Markwell, M., Fredman, P. and Svennerholm, L. (1984) Receptor ganglioside content of three hosts for Sendai virus. MDBK, HeLa, and MDCK cells. *Biochim. Biophys. Acta* 775, 7–16.
- [18] Chigorno, V., Palestini, P., Sciannamblo, M., Dolo, V., Pavan, A., Tettamanti, G. and Sonnino, S. (2000) Evidence that ganglioside enriched domains are distinct from caveolae in MDCK II and human fibroblast cells in culture. *Eur. J. Biochem.* 267, 4187–4197.
- [19] Florek, M., Bauer, N., Janich, P., Wilsch-Bräuninger, M., Fargeas, C.A., Marzesco, A.-M., Ehninger, G., Thiede, C., Huttner, W.B. and Corbeil, D. (2007) Prominin-2 is a cholesterol-binding protein associated with apical and basolateral plasmalemmal protrusions in polarized epithelial cells and released into the urine. *Cell Tissue Res.* 328, 31–47.
- [20] Dubreuil, V., Marzesco, A.-M., Corbeil, D., Huttner, W.B. and Wilsch-Bräuninger, M. (2007) Midbody and primary cilium of neural progenitors release extracellular membrane particles enriched in the stem cell marker prominin-1. *J. Cell Biol.* 176, 483–495.
- [21] Vieira, O.V., Gaus, K., Verkade, P., Fullekrug, J., Vaz, W.L. and Simons, K. (2006) FAPP2, cilium formation, and compartmentalization of the apical membrane in polarized Madin-Darby canine kidney (MDCK) cells. *Proc. Natl. Acad. Sci. USA* 103, 18556–18561.
- [22] Reiter, J.F. and Mostov, K. (2006) Vesicle transport, cilium formation, and membrane specialization: the origins of a sensory organelle. *Proc. Natl. Acad. Sci. USA* 103, 18383–18384.
- [23] Gomez-Mouton, C., Abad, J.L., Mira, E., Lacalle, R.A., Gallardo, E., Jimenez-Baranda, S., Illa, I., Bernad, A., Manes, S. and Martinez-A, C. (2001) Segregation of leading-edge and uropod components into specific lipid rafts during T cell polarization. *Proc. Natl. Acad. Sci. USA* 98, 9642–9647.
- [24] Giebel, B., Corbeil, D., Beckmann, J., Höhn, J., Freund, D., Giesen, K., Fischer, J., Kögler, G. and Wernet, P. (2004) Segregation of lipid raft markers including CD133 in polarized human hematopoietic stem and progenitor cells. *Blood* 104, 2332–2338.
- [25] Freund, D., Bauer, N., Boxberger, S., Feldmann, S., Streller, U., Ehninger, G., Werner, C., Bornhauser, M., Oswald, J. and Corbeil, D. (2006) Polarization of human hematopoietic progenitors during contact with multipotent mesenchymal stromal cells: effects on proliferation and clonogenicity. *Stem Cells Dev.* 15, 815–829.
- [26] Iglic, A., Hagerstrand, H., Veranic, P., Plemenitas, A. and Kralj-Iglic, V. (2006) Curvature-induced accumulation of anisotropic membrane components and raft formation in cylindrical membrane protrusions. *J. Theor. Biol.* 240, 368–373.
- [27] Hagerstrand, H., Mrowczynska, L., Salzer, U., Prohaska, R., Michelsen, K., Kralj-Iglic, V. and Iglic, A. (2006) Curvature-dependent lateral distribution of raft markers in the human erythrocyte membrane. *Mol. Membr. Biol.* 23, 277–288.
- [28] Marzesco, A.-M., Janich, P., Wilsch-Bräuninger, M., Dubreuil, V., Langenfeld, K., Corbeil, D. and Huttner, W.B. (2005) Release of extracellular membrane particles carrying the stem cell marker prominin-1 (CD133) from neural progenitors and other epithelial cells. *J. Cell Sci.* 118, 2849–2858.