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Distinct Lipid Microdomains within the Apical Plasma Membrane of Polarized Epithelial Cells

A potential role of the lipid bilayer in the retention of prominin in microvilli

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Introduction

One interesting field of cell biology concerns the molecular mechanisms underlying the sorting of membrane proteins to, and their retention in, specific subcellular organelles. It becomes more and more evident that the plasma membrane is subdivided into distinct regions, which might constitute specific "organelle-like" functional compartments. A classical example is the plasma membrane of epithelial cells, which is characterized by two functionally and structurally distinct domains, the apical and basolateral membrane. Both membrane domains differ from each other in protein and lipid composition, and are spatially separated by the presence of tight junctions [1-3].

Asymmetric distribution of plasma membrane proteins in polarized epithelial cells is achieved by three major mechanisms, which are not mutually exclusive [4-6]. First, newly synthesized membrane components are sorted at the level of the trans-Golgi network (TGN) or the basolateral endosome into carrier vesicles which are targeted to the appropriate membrane domain [7-11]. Second, the selective proteolytic processing of randomly delivered membrane proteins at one specific cell surface leads to their enrichment in the opposite domain [12]. Third, certain integral membrane proteins are selectively retained in the appropriate plasma membrane domain, whereas they are endocytosed from the other membrane domain and become quickly degraded [13-16].

Mechanisms underlying the sorting of transmembrane proteins to the apical and basolateral plasma membrane domains have been the subject of intense studies during the last twenty years leading to the identification and characterization of several sorting signals (for a review, see ref. [72]). For instance, short cytoplasmic amino acid stretches consisting of either a tyrosine or a di-leucine motif have been found to constitute basolateral sorting signals

[17,18]. N- and possibly O-linked glycans [19,20], as well as association with specific cholesterol/glycosphingolipid-rich membrane microdomains termed rafts [21], mediated by either a GPI-moiety or a transmembrane segment, have been identified as potential apical sorting signals [22-24]. Recently, cytoplasmic amino acid-based motifs have also been proposed to act as apical sorting signals [25-27]. Finally, several studies using chimerae and/or truncated membrane proteins have shown that a single membrane protein could contain more than one type of sorting information, one acting dominant over the other [28-30].

Once targeted to the appropriate plasma membrane domain, certain integral membrane proteins are specifically retained and concentrated in subdomains of either the apical or basolateral plasma membrane [31-33]. In contrast to sorting information, very little is known regarding the mechanisms involved in this retention and concentration (see below). Such specific retention and concentration could provide a general explanation for plasma membrane compartmentalization, and this not only in epithelial cells which contain tight junctions, but also in non-epithelial cells [34]. Furthermore, it has been shown in epithelial cells that the polarized distribution of several apical cell surface glycoproteins does not depend on the presence of functional tight junctions [5,35-37], suggesting that other fence mechanisms can operate in this plasma membrane domain [38].

Our group has been studying the apical-basal polarity of neuroepithelial cells, which constitute the inner layer of the neural tube of the vertebrate embryo and give rise to all neurons and most glial cells of the central nervous system [39]. Interestingly, during the transition from the neural plate stage to the neural tube stage, neuroepithelial cells lose functional tight junctions [40] and polarity of certain plasma membrane proteins [41]. However, after neural tube closure, not all plasma membrane proteins of neuroepithelial cells become randomly distributed over the apical and basolateral plasma membranes. One of these plasma membrane proteins is prominin [42] (see below). Remarkably, prominin is exclusively associated with microvilli present at the apical domain of neuroepithelial cells which suggests that entry into, and retention in, microvilli may play an important role in the maintenance of apical-basal polarity of epithelial cells in the absence of tight junctions.

Prominin, a specific marker of plasma membrane protrusions

Prominin is a polytopic membrane protein that is present on the apical surface of several mouse embryonic epithelia including neuroepithelial cells, and on the brush border membrane of epithelial cells lining the adult kidney proximal tubules [42]. It was first identified using the monoclonal antibody (mAb) 13A4 generated against E12 mouse telencephalic neuroepithelium, and its cDNA was cloned from mouse kidney [42]. This novel 115-kD glycoprotein contains five putative transmembrane domains with an N-terminal extracellular domain and a cytoplasmic C-terminal domain (Fig. 1).

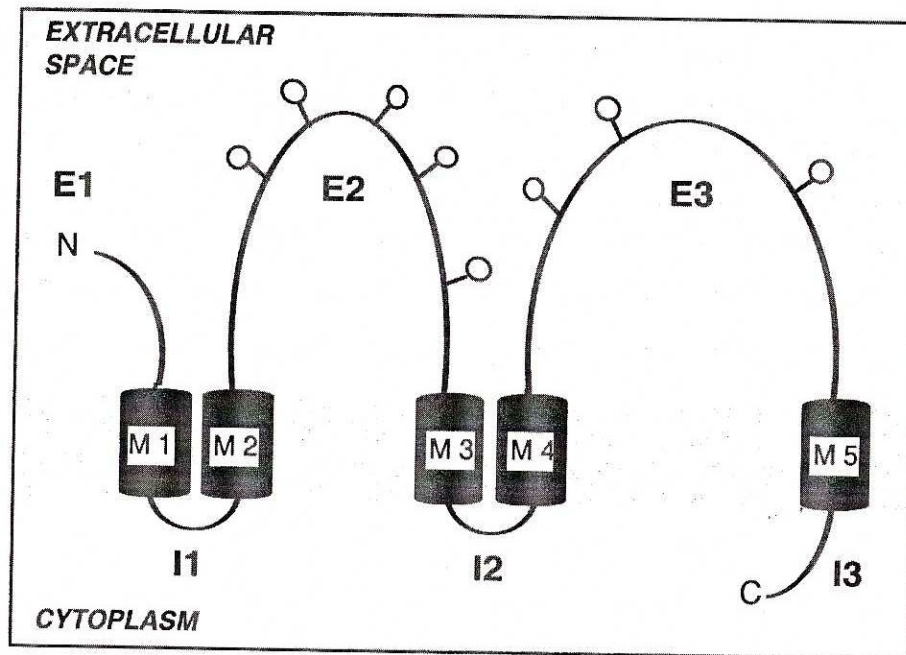


Figure 1. Topological model of prominin. M1-M5, putative transmembrane segments; E1-E3, extracellular domains; I1-I3, cytoplasmic domains. The two large extracellular loops (domains E2 and E3) contain all eight potential N-glycosylation sites (lollipops). Modified from Corbeil et al., 1999.

Its human orthologue [43,44], prominin (mouse)-like-1 (originally referred to as AC133 antigen), recently found to be expressed in epithelial cells (D.C., K.R., A. Hellwig, M. Tavian, S. Miraglia, S.M. Watt, P.J. Simmons, B. Peault, D.W. Buck, W.B.H., manuscript in preparation), was initially described in non-epithelial cells, i.e. hematopoietic progenitor cells [45,46]. Although the function of prominin is unknown, the occurrence of orthologues/paralogues in zebrafish (accession number: AF160970), *Drosophila melanogaster* (accession number: AF127935) and *C. elegans* [42-44] suggests that this polytopic membrane protein plays an important role from invertebrates to vertebrates.

One particularly interesting feature of prominin in epithelial cells is its selective occurrence in specific subdomains of the apical plasma membrane. In epithelial cells with a brush border, two structurally and functionally distinct subdomains of the apical plasmalemma can be distinguished; (i) the microvilli, which enlarge the apical surface; and (ii) an extended system of clathrin coated pits located at the base of the microvilli [47-50]. In kidney proximal tubules, prominin is restricted to the brush border membrane, where it appears to be concentrated at the tips of the microvilli, and is largely excluded from the intermicrovillar plasma membrane regions [42]. In the apical plasma membrane of neuroepithelial cells, which contains less microvilli than the kidney brush border, prominin is associated with microvilli and other small plasma membrane protrusions rather than the large planar subdomain [42]. Furthermore, this protrusion-specific localization of prominin does not depend on an epithelial state of the cell. When prominin is ectopically expressed in fibroblasts such as CHO cells, it is

also selectively found in plasma membrane protrusions such as microspikes, filopodia, membrane ruffles and the leading edge of lamellipodia [42] (unpublished data).

Although the general mechanisms underlying the retention of membrane proteins in plasma membrane protrusions has not been elucidated (see below), expression and localization of prominin in epithelial and non-epithelial cells, as revealed that this mechanism is highly conserved between these types of cells and may probably reflect a cell type-specific adaptation of a process common to all eukaryotic cells.

Prominin contains dual targeting information

Recently, we could show that the enrichment of prominin in plasma membrane protrusions, i.e. microvilli at the apical surface of polarized epithelial MDCK cells, is due to both sorting to the apical membrane domain at the TGN level and subsequent retention in the microvillar subdomain [5]. This dual "targeting" does not require the cytoplasmic tail of prominin and, more interestingly, the selective retention of prominin in microvilli was maintained when prominin-transfected MDCK cells were cultured in low-calcium medium [5], i.e. in the absence of tight junctions [51]. These *in vitro* data resemble the *in vivo* situation observed in the neuroepithelium (see Introduction). They suggest a potential role of the microvillar retention of certain plasma membrane proteins in maintaining their polarized distribution even without functional tight junctions, which would be achieved by preventing their lateral movement.

Retention of integral membrane proteins in the apical or basolateral domain

The retention of membrane proteins inside a specific plasmalemmal subdomain can be obtained by direct protein-protein interactions occurring in that particular domain. For example, selective anchoring of plasma membrane proteins to submembraneous cytoskeletal elements, and/or specific interaction with other membrane proteins or extracellular components, are possible alternative retention mechanisms.

Cytoskeletal interactions have been reported to explain the polarized distribution of the Na⁺/K⁺-ATPase [14,52], the Na⁺/H⁺-exchanger [53] and the amiloride-sensitive Na⁺ channel [54] in epithelial cells, the latter two being captured on the apical side via association of their C-terminal proline-rich domain with the SH3-domain of α -spectrin. In contrast, the Na⁺/K⁺-ATPase is maintained on the (baso)lateral side via binding to an ankyrin/fodrin/uvomorulin complex in MDCK cells [14], whereas in the retinal pigment epithelium, where the polarity of some cytoskeletal components is reversed, a similar association with ankyrin restricts it to the apical side [52].

As shown more recently, several GABA-transporter subtypes contain a PDZ-interacting domain, which is necessary for their retention, and hence polarized localization, on either the basolateral membrane domain (as in the case of BGT-1 [6]) or the apical membrane domain (as in the case of GAT-3 [26]). PDZ domain-containing proteins are known to cluster interacting proteins at specific functional sites of the cell such as synapses or tight junctions in order to allow these proteins to exert their function.

Retention of prominin in microvilli: facts and a hypothesis

Microvilli are a characteristic subdomain of the plasma membrane present not only in epithelial cells [47], but also in non-epithelial cells, as for instance leukocytes. Physiological functions of microvilli are reflected by the specific membrane proteins found therein; cell adhesion molecules in the case of leukocytes; brush border enzymes and transporters in the case of resorptive epithelia. The molecular mechanisms underlying the retention of integral membrane proteins in microvilli and other membrane protrusions are poorly understood [55-58]. To our knowledge, it is not clear whether all microvillar membrane proteins interact with constituents of the subplasmalemmal actin network such as brush-border myosin I, ezrin or other members of the ERM (ezrin-radixin-moesin) family, which are known to serve as general plasma membrane protrusion-actin microfilament linkers [59,60], although some clearly do [61,62]. For certain membrane proteins such as sucrase-isomaltase [63], the presence of "intact microvilli" appears to be essential for their cell surface expression and polarized distribution.

In order to elucidate a possible association of prominin with components of the microvillar cytoskeleton, prominin-transfected MDCK cells were solubilized in Triton X-100 in the cold and fractionated into detergent-soluble and -insoluble material. Prominin appeared to be completely soluble in Triton X-100 [5], as reported already for several brush border enzymes, such as lactase-phlorizin hydrolase [64,65]. Interestingly, this brush-border hydrolase, like prominin, has been found to be specifically associated with microvilli [66]. One interpretation of these data is that prominin does not bind to cytoskeletal components. However, an alternative possibility is that prominin is anchored to the cytoskeleton via a labile interaction which may have been disrupted under our experimental conditions. To further investigate a putative interaction of prominin with cellular components, we decided to explore other solubilization conditions that might prevent such dissociation. Interestingly, the cell surface, microvilli-associated form of prominin was found to be insoluble in cold Lubrol WX, another nonionic detergent [67,68], whereas the ER form was completely soluble (manuscript in preparation).

In principle, two modes of interaction could cause the Lubrol WX insolubility of prominin, (i) binding to cytoskeletal or extracellular matrix (ECM) constituents (referred to as vertical interaction), and/or (ii) association with other transmembrane proteins or certain lipids (referred to as lateral interaction). The latter type of interaction has been proposed to explain the microvillar presentation of integrins in leukocytes. In this particular case, it was postulated that

certain tetraspan proteins and other integrin-associated molecules form multimolecular complexes with the integrins, and this complex formation may have a role in targeting/retention of integrins in microvilli [57].

Further biochemical analysis of the microvilli-associated, Lubrol WX insoluble prominin suggested a lateral interaction. Interestingly, this insoluble complex showed several characteristics of detergent resistant membrane microdomains (DRMMs) [22], including (i) floatation to lower density fractions in sucrose density gradients; (ii) loss of insolubility at 37°C; and (iii) a high content of cholesterol and glycolipids. In addition, removal of plasma membrane cholesterol by means of methyl- β -cyclodextrin (m- β -CD) treatment led to the complete solubilization of prominin in Lubrol WX at 4°C and to loss of floatation. Remarkably, m- β -CD treatment resulted in the redistribution of prominin, as observed by cell surface immunofluorescence, from an exclusively microvillar localization to a more homogeneous distribution over the apical surface. The effects of cholesterol depletion were reversed by refeeding the cells with cholesterol.

Taken together, these data suggest that a certain lipid composition and/or organization of the lipid bilayer of microvilli and other plasma membrane protrusions is necessary (and perhaps sufficient) to maintain prominin in its proper location. In analogy to the role of the length of transmembrane segments in membrane protein retention in, vs. exit from, the Golgi complex [69,70], a certain length of prominin's transmembrane segments is possibly required for its lateral diffusion into, and concentration in, plasma membrane protrusions. In this context, it is interesting to note that four of the five predicted transmembrane segments of prominin contain >25 amino acid residues and thus exceed the average length of the transmembrane segment of plasma membrane proteins [69].

Distinct lipid microdomains as building blocks of the apical plasma membrane: a model

The specific subcellular localization of prominin in the apical microvillar subdomain and its complete solubilization in Triton X-100 but not Lubrol WX strongly suggest that the lipid bilayer of this microdomain is sensitive to extraction by the former detergent but is preserved by Lubrol WX. This differential detergent extraction may reflect the existence of at least two distinct lipid microdomains at the level of the apical plasma membrane, characteristic of either the microvillar plasmalemma (Triton X-100 soluble and Lubrol WX insoluble) or the planar, intermicrovillar plasmalemma (Triton X-100 and Lubrol WX insoluble) (Fig. 2).

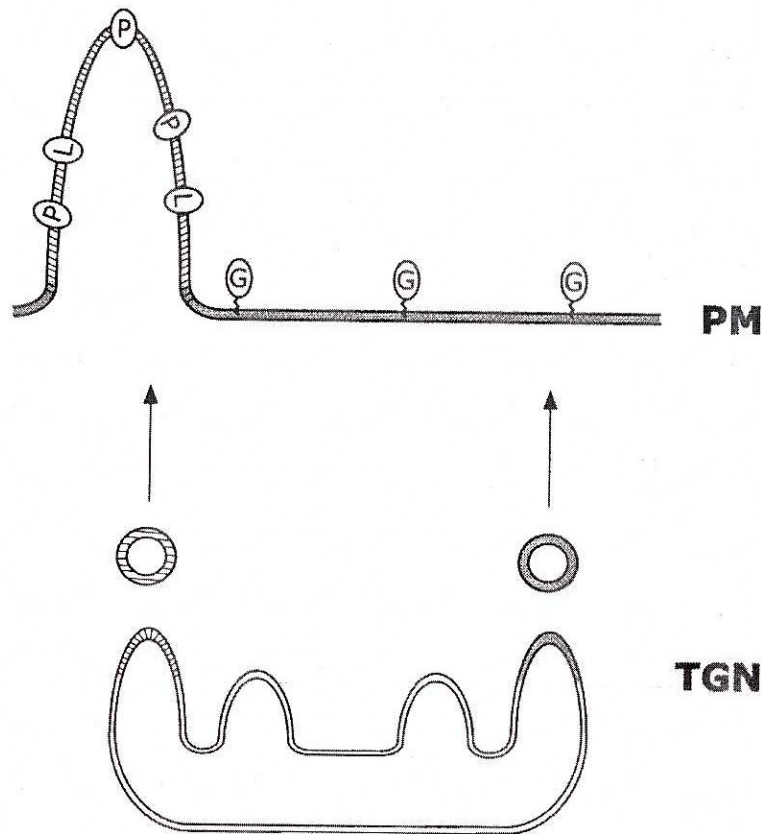


Fig.2 Formation of distinct lipid microdomains in the apical plasma membrane. The differential detergent extraction properties of prominin and other apical plasma membrane protein constituents could reflect the existence of at least two distinct lipid microdomains at the level of the apical plasma membrane. Striped area, Lubrol WX insoluble and Triton X-100 soluble membrane; grey area, Lubrol WX and Triton X-100 insoluble membrane). These microdomains could serve a role in retaining proteins at sites of their action, and/or, as both domains seem to be formed already at the level of the TGN, in sorting of apical plasma membrane components. PM: plasma membrane; TGN: trans-Golgi network; P: prominin; L: lactase-phlorizin hydrolase; G: GPI-linked proteins.

Consistent with this proposal, Danielsen and van Deurs (1995) have observed that a GPI-linked protein, the transferrin-like GPI-linked iron-binding protein (which was found to be insoluble in Triton X-100 like other GPI-anchored proteins) was mainly localized in patches of flat or invaginated apical membrane subdomains rather than at the surface of microvilli (see figure 3 in [50]). Thus, the “affinity” of certain plasma membrane proteins to particular plasma membrane lipids, as defined here by their solubility in different detergents (Triton X-100 vs. Lubrol WX), could target these molecules to, and maintain them in, spatially distinct microdomains. It remains to be established whether microvillar association of transmembrane proteins is generally reflected by an insolubility in Lubrol WX (and solubility in Triton X-100), as demonstrated here for prominin.

It has been proposed that the Triton X 100 insoluble raft has a function in sorting of newly synthesized plasma membrane components from the TGN to the cell surface, via the formation of lipidic platforms that incorporate the cargo proteins [71] (see also Introduction). As prominin achieves its Lubrol WX-insolubility during transport to the cell surface (manuscript in preparation), it is tempting to speculate that the Lubrol WX-insoluble/Triton X-100-soluble microdomain could also function in sorting (Fig. 2). Quantitative analysis of the lipid composition of Lubrol WX-insoluble complexes as well as a comprehensive analysis of its protein composition may reveal further details about the mechanisms involved in either sorting to the apical domain or retention in a specific apical subdomain via this type of DRMM.

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