

Review

Prominin: A Story of Cholesterol, Plasma Membrane Protrusions and Human Pathology

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Prominin is the first identified member of a novel family of polytopic membrane proteins conserved throughout the animal kingdom. It has an unusual membrane topology, containing five transmembrane domains and two large glycosylated extracellular loops. In mammals, prominin is expressed in various embryonic and adult epithelial cells, as well as in nonepithelial cells, such as hematopoietic stem cells. At the subcellular level, prominin is selectively localized in microvilli and other plasma membrane protrusions, irrespective of cell type. At the molecular level, prominin specifically interacts with membrane cholesterol and is a marker of a novel type of cholesterol-based lipid 'raft'. A frameshift mutation in the human prominin gene, which results in a truncated protein that is no longer transported to the cell surface, is associated with retinal degeneration. Given that prominin is concentrated in the plasma membrane evaginations at the base of the outer segment of rod photoreceptor cells, which are essential precursor structures in the biogenesis of photoreceptive disks, it is proposed that prominin has a role in the generation of plasma membrane protrusions, their lipid composition and organization and their membrane-to-membrane interactions.

Key words: AC133 antigen, cholesterol, epithelial cell, membrane microdomain, microvilli, prominin, retinal degeneration, rod photoreceptor cell

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The generation and maintenance of structurally and functionally distinct domains of the plasma membrane is of fundamental importance for the function of eukaryotic cells and their interaction in the organism. These processes involve the organization of the principal membrane constituents (proteins and lipids) into specific microdomains. The outgrowth of the plasma membrane into either relatively stable structures (microvilli in epithelial cells, axons and dendrites in neurons, myelin in oligodendrocytes and Schwann cells) or

transient, dynamic structures (filopodia in migrating fibroblasts, membrane evaginations in photoreceptor cells) could be seen as examples of specific plasma membrane domains. The generation and, when applicable, the maintenance of such plasma membrane protrusions involves not only a reorganization of cytoskeletal elements (1–4) but also the delivery of the relevant membrane constituents (5,6). So far most studies have addressed the mechanisms of transport of membrane constituents to the plasma membrane (7–9), whereas the mechanisms underlying the retention of membrane constituents in the various types of plasma membrane protrusions remain largely unknown (10–13). This review focuses on the recently identified polytopic membrane protein, prominin, which is specifically localized to various plasma membrane protrusions, describes the role of lipid microdomains in its retention in these protrusions and discusses prominin's potential role as an organizer of these specific plasma membrane domains.

Discovery of Prominin

Prominin was identified in two independent studies, both being reported in 1997, which concerned murine neuroepithelial cells (14) and human hematopoietic stem and progenitor cells (15).

Neuroepithelial cells

In the vertebrate central nervous system, all neurons and macroglial cells derive from a common precursor cell population, the neuroepithelial cells of the ventricular zone (16,17). Our group of researchers has been studying the cell biological basis of neurogenesis, specifically the switch of neuroepithelial cells from symmetric proliferative division to asymmetric neuron-generating division (18,19). For this purpose, we have been searching for novel markers by raising rat monoclonal antibodies (mAb) against mouse neuroepithelial cells. One such antibody (mAb 13A4) was found to specifically label the apical plasma membrane of neuroepithelial cells (14). Within the apical plasma membrane, the 13A4 antigen was found to be selectively associated with microvilli and absent from the planar regions of the membrane (14). In view of this remarkably specific localization, the 13A4 antigen, which upon molecular cloning turned out to be a novel membrane protein, was named 'prominin' (14).

Hematopoietic stem and progenitor cells

In the hematopoietic system, several distinct cell lineages arise from rare pluripotent stem cells. The developmental potential of hematopoietic stem cells is of major importance for medical therapy. Since the total bone marrow-derived cell population is heterogeneous and the pluripotent hematopoietic stem cells constitute only a small fraction of this population, numerous efforts have been made towards their

efficient isolation. Thus, Buck and colleagues have generated a panel of mouse mAbs against human hematopoietic progenitor cells. One of these antibodies, mAb AC133, led to the identification and molecular characterization of the protein bearing the AC133 epitope (CD133) (15,20). Interestingly, as will be described below, the human protein bearing the AC133 epitope is highly related to murine prominin in sequence, membrane topology, tissue distribution and sub-cellular localization (21,22), and therefore highly likely to be the human orthologue of murine prominin (23). The human protein bearing the AC133 epitope has been referred to as prominin (mouse)-like 1 (PROML1) (23), given that the existence of a human gene product even more closely related to murine prominin cannot yet be excluded. However, in anticipation that no such gene will be found in the complete human genome, and for the sake of simplicity, we will use here the term 'human prominin' rather than 'human PROML1'. In this context, it is important to point out that the AC133 epitope (CD133) depends on glycosylation (15) and is detected on human prominin only in certain states of cellular differentiation (23). Hence, the term 'AC133 antigen' should not be used synonymously with 'human prominin' or 'human PROML1'.

The Prominin Family

Membrane topology

Prominin was originally cloned by immunological screening of a mouse kidney cDNA library using the rat mAb 13A4 (14). Murine kidney prominin is an 858 amino acid protein with a predicted molecular weight of 96222. Its apparent molecular mass of ca. 115 kDa observed upon sodium dodecyl-polyacrylamide gel electrophoresis (SDS-PAGE) is explained by the presence of N-linked glycans (14). Prominin from adult brain has a lesser apparent molecular mass (ca. 100 kDa) which, at least in part, reflects a shorter polypeptide chain (24; Joester A, Corbeil D, Huttner, WB. Unpublished data. See 'Prominin isoforms'). Prominin does not show obvious sequence homology to other proteins (except for prominin-related proteins, see below) nor does its sequence reveal motifs that could provide clues as to its physiological role. Prominin is a polytopic membrane protein containing five putative transmembrane segments and two large extracellular loops with more than 250 amino acid residues each, which is a unique overall structure (Figure 1A). A human prominin cDNA has been isolated from a retinoblastoma cell line (15). The predicted 865 amino acid protein shows an average of 60% amino acid identity to murine prominin (21,22), with the intracellular and transmembrane domains exhibiting a greater degree of conservation (64–85%) than the extracellular domains (48–57%) (for details see Figure 1B). Like murine prominin (Figure 1A), human prominin (not illustrated) contains eight potential N-glycosylation sites (15). Both human and murine prominin contain a cysteine-rich region of as yet unknown function, which is located at the transition of the first transmembrane domain and the first cytoplasmic loop (14,15).

Prominin isoforms

So far, four alternatively spliced transcripts of murine prominin have been identified (Figure 1A). One has been found in brain (22), the others in testis (Fargeas CA, Huttner WB, Corbeil D. Unpublished data). (In the following, all isoforms will be designated by a capital letter that refers to the tissue in which they were first observed, followed by a number that reflects their order of identification.) By comparison with murine kidney prominin (14), the murine brain prominin isoform *B1* contains an additional block of nine amino acid residues within its first extracellular domain (22) (Figure 1A). The human prominin from retinoblastoma cells (15) corresponds to the murine *B1* isoform. The exon structure of human prominin (25; Fargeas CA, Huttner WB, Corbeil D. Unpublished data) reveals that the block of nine amino acids is encoded by one exon (exon 3). The *T1*, *T2* and *T3* isoforms of murine prominin identified in testis display a distinct, shorter cytoplasmic C-terminal domain (I3 domain, see Figure 1A). In addition, the *T2* and *T3* isoforms bear two and one in-frame deletion(s), respectively, in the extracellular domain(s) (for details see Figure 1A). The existence of prominin splice variants has at least two implications. First, the splice variants may well be the underlying cause of some of the heterogeneity of prominin as observed upon SDS-PAGE (see, for example, figure 3, lane 4, in Reference (14)) and also may explain the faster electrophoretic mobility of adult brain-derived prominin with SDS-PAGE. Second, the lack of prominin immunoreactivity in tissues containing prominin mRNA, as has been observed for human prominin in differentiated Caco-2 cells (23) (see below), may reflect not only a change in glycosylation of a given splice variant but also the expression of a splice variant lacking the peptide epitope or N-glycosylation consensus sequence.

Tissue distribution of prominin

The expression of human and mouse prominin in various embryonic and adult tissues has been studied by analysis of the presence of prominin mRNA as well as AC133 (human) and 13A4 (mouse) immunoreactivity (Table 1). Prominin expression is not restricted to the neuroepithelial and hematopoietic stem/progenitor cells in which it was originally observed, but extends to several epithelial and non-epithelial cell types. Thus, with regard to mouse prominin, 13A4 immunoreactivity has also been detected in non-epithelial, bone marrow-derived cells and, with regard to human prominin, AC133 immunoreactivity has also been detected in the epithelial cell line Caco-2. As to the tissue distribution, in embryonic mouse, 13A4 immunoreactivity has been observed at the apical surface of epithelia of all three germinal layers. With respect to endoderm, 13A4 immunostaining has been detected in the lung buds and the gut (14), including Rathke's pouch (26). The urethra buds, a mesoderm-derived epithelium, have also been found to show 13A4 immunoreactivity (14). In developing kidney tubules, 13A4 immunoreactivity appears concurrently with the transition of the mesenchymal cells to epithelial cells (26). With respect to ectoderm, 13A4 immunoreactivity has been detected not only in the neuroepithelium (14), but also in non-neural ectoderm of 8-day-old

mouse embryos, from which, however, it disappears at later developmental stages, except for the invaginating otic vesicles and olfactory epithelium (26). Likewise, for human embryonic epithelia, AC133 immunoreactivity has been detected on the apical surface of the neural tube, gut and mesonephros (23).

Expression of AC133 and 13A4 immunoreactivity in adult tissues appears to be less widespread than in embryonic tissues (Table 1). This, however, reflects the above-mentioned glycosylation-dependence of the AC133 epitope (15)

and, presumably, expression of splice variants lacking the 13A4 epitope (which does not involve N-glycosylation (14)). In the central nervous system, murine prominin has been detected at the apical side of the ependymal layer (14) and, recently, in myelin (24). In addition, murine prominin has been found in rod photoreceptor cells (25) (see below). With regard to epithelial tissues, murine prominin has been observed at the brush border membrane of kidney proximal tubules (14). However, 13A4 immunoreactivity has not been detected in other adult epithelial tissues, such as the gut and lung. This is surprising since a prominin transcript is de-

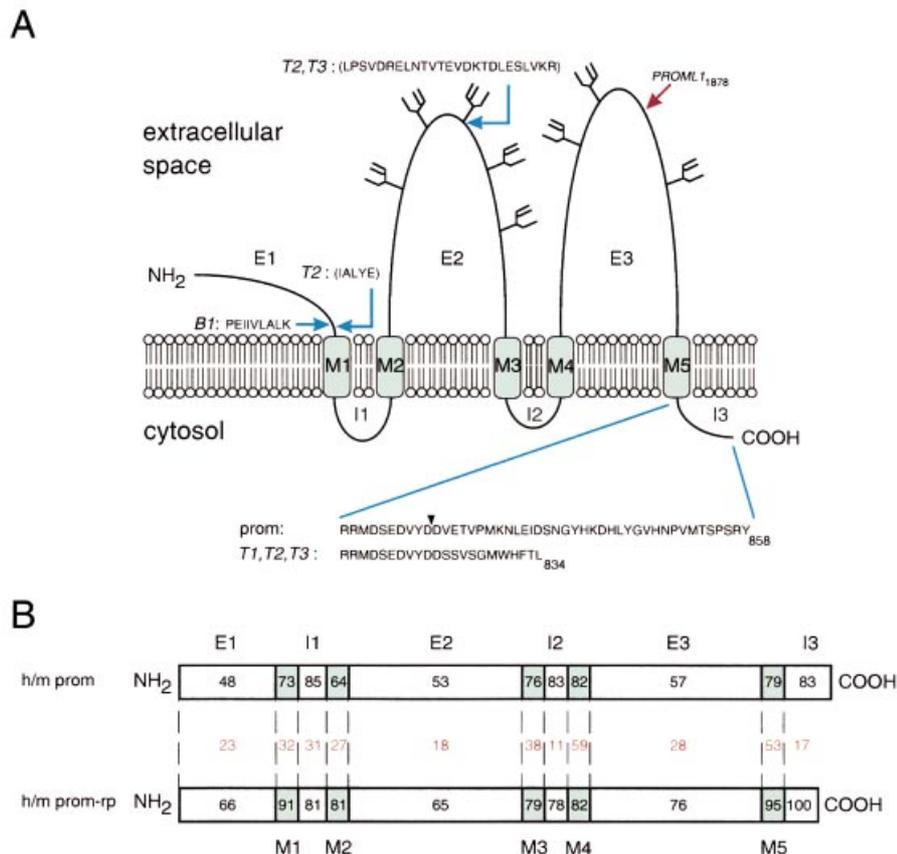


Figure 1: Membrane topology of prominin and prominin-related protein. A. Murine prominin. After cleavage of the signal sequence, prominin is predicted to consist of an extracellular N-terminal domain (E1), five membrane-spanning domains (M1–M5) separating two small intracellular domains (I1 and I2) and two large glycosylated extracellular domains (E2 and E3) and a cytoplasmic C-terminal domain (I3). The extracellular location of the E2 and E3 domains, which contain all eight potential N-glycosylation sites (forks) and the cytoplasmic location of the I3 domain have been confirmed by antibody accessibility and epitope insertion analyses (14,25) (Corbeil D, Adrien C, Huttner WB. Unpublished data). The brain isoform *B1* (22) contains an insertion of nine amino acid residues in the E1 domain between the glutamic acid residue 93 and the isoleucine residue 94 (14). The C-terminal sequence of the I3 domain of the testis isoforms *T1*, *T2* and *T3* (Fargeas CA, Huttner WB, Corbeil D. Unpublished data) is different from that of the originally described (14), kidney-derived prominin (prom); the triangle indicates an exon boundary in human prominin. In addition, the *T2* isoform shows two deletions (sequences in parentheses) in the E1 and E2 domains, whereas the *T3* isoform shows only the deletion in the E2 domain. The red arrow indicates the position of the premature stop codon introduced in human prominin by the deletion of nucleotide 1878 in the *PROML1* gene in retinal degeneration (25). B. Comparison between prominin and prominin-related protein. Black numbers in boxes indicate the amino acid identity (in percent) between the various domains of either human (h) (15) and murine (m) (14) prominin (prom) or human and murine prominin-related protein (prom-rp) (Fargeas CA, Huttner WB, Corbeil D. Unpublished data). Red numbers indicate the amino acid identity between the various domains of human prominin (15) and human prominin-related protein (Fargeas CA, Huttner WB, Corbeil D. Unpublished data). Similar degrees of identity are found between murine prominin (14) and murine prominin-related protein (not shown) (Fargeas CA, Huttner WB, Corbeil D. Unpublished data).

Table 1: Tissue distribution of prominin

Tissue	Human		Mouse	
	mRNA	AC133	mRNA	13A4
Embryonic				
Brain	n.d.	+	n.d.	+
Gut	n.d.	+	n.d.	+
Kidney	n.d.	+	n.d.	+
Stomach	n.d.	n.d.	n.d.	+
Adult				
Bone marrow	+	+	n.d.	+
Brain	+	-	+	+
Colon	+	-	+	-
Heart	+	-	-	-
Kidney	+	-	+	+
Liver	+	-	-	-
Lung	+	-	+	-
Pancreas	+	-	n.d.	n.d.
Placenta	+	-	n.d.	n.d.
Skeletal muscle	(+)	-	+	n.d.
Small intestine	+	n.d.	+	-
Spleen	-	n.d.	-	-
Testis	n.d.	-	+	n.d.

Data are compiled from References (14,15,21–23,26). RNA data are from Northern blot analyses. AC133 and 13A4 data refer to immunodetection analyses of human and mouse prominin using mAb AC133 (20) and mAb 13A4 (14), respectively. n.d., not determined.

tectable in these tissues by Northern blot analysis (21,22) and would be consistent with the expression of a prominin splice variant lacking the 13A4 epitope. Immunodetection of human prominin in adult tissues other than bone marrow is not documented. This is presumably due to the fact that the mAb AC133 binds to a glycosylation-dependent epitope (15) and that the processing of asparagine-linked oligosaccharides may vary depending on the tissue (27) and the state of cell differentiation (28), which limits the use of mAb AC133 for studies of the tissue distribution of human prominin. In this context, our observations with the human intestine-derived epithelial cell line Caco-2 are instructive. In the non-differentiated subconfluent state, all Caco-2 cells exhibited AC133 immunoreactivity, whereas in the differentiated postconfluent state, < 20% of the cells displayed AC133 immunoreactivity, despite the upregulation of a prominin transcript (23).

The data so far available on the tissue distribution of prominin and its expression in various cell types do not provide obvious clues as to its physiological role. It appears that a common denominator of prominin's expression in a given cell type is the presence of plasma membrane protrusions, reflecting its subcellular localization (see below).

Prominin-related proteins

Since the identification and molecular cloning of mammalian prominins (14,15), a second membrane protein, which is highly related to prominin and referred to as prominin-related protein (PROM-RP), has been identified *in silico* and cloned

from both human and murine kidney (Fargeas CA, Huttner WB, Corbeil D. Unpublished results). Like prominin, PROM-RP contains five putative transmembrane segments (M1–M5) and two large loops between M2 and M3 and between M4 and M5 segments (Figure 1B). However, the amino acid sequence identity between PROM-RP and prominin is relatively low (26 and 29% for the human and murine proteins, respectively) (for details see Figure 1B). PROM-RP shows a higher degree of interspecies conservation (73%) than prominin (60%) (for details see Figure 1B). The human PROM-RP mRNA originates from 24 exons of a gene located on chromosome 2 (Fargeas CA, Huttner WB, Corbeil D. Unpublished results), which shows a striking similarity with regard to exon–intron organization to the human prominin (PROML1) gene located on chromosome 4 (25). This strongly suggests that prominin and PROM-RP are derived from a common ancestral gene.

Phylogenetic conservation

cDNAs that predict proteins obviously related in membrane topology to mammalian prominin and PROM-RP are found in various vertebrates and invertebrates, including the zebrafish *Danio rerio* (GenBank No. AF160970), *Drosophila melanogaster* (GenBank No. AF127935) and *Caenorhabditis elegans* (14). In spite of their similar membrane topology, the invertebrate prominin-like proteins show relatively little amino acid sequence identity (ca. 20%) to their vertebrate counterparts. In addition to the above zebrafish and *C. elegans* homologues, a zebrafish EST (clone fk58e12, GenBank No. BE015742) and at least two open reading frames (M28.9 and M28.8, GenBank No. Z49911) in the worm genome predict additional proteins related to prominin and PROM-RP. By contrast, analysis of the yeast genome does not reveal the existence of proteins with an obvious relationship to prominin and PROM-RP. It remains to be established whether the presence of prominin and PROM-RP throughout the animal kingdom but their apparent absence in yeast reflect a phylogenetically conserved, albeit animal-specific, physiological role of these proteins, or, given the specific localization of prominin in plasma membrane protrusions (see below), their absence in species with a cell wall, which limits the occurrence of plasma membrane protrusions.

Prominin and Plasma Membrane Protrusions

Prominin has a remarkable subcellular localization. Being a plasma membrane protein, it is confined to subdomains of the cell surface, which, although distinct in structure in various cell types, have one feature in common, i.e. to protrude from the planar regions of the plasmalemma. In epithelial cells, both in tissues and in cell lines expressing endogenous or transfected prominin, prominin is exclusively located in microvilli and not detected on the planar regions of the apical domain (14,23,29) (Figure 2A). This presumably explains why prominin retains its apical-specific localization when epithelial cells lose their tight junctions (14,29), which prevent the lateral diffusion of apical transmembrane proteins into the lateral plasma membrane (30,31). In non-epithelial cells, such

as hematopoietic progenitor cells, prominin is also enriched in plasma membrane protrusions (23) (Figure 2B). In rod photoreceptor cells, prominin is concentrated in the plasma membrane evaginations present at the base of the outer segment (Figure 2C) (25) (see below). In the optic nerve,

prominin is observed in the myelin sheath (Joester A, Corbeil D, Hellwig A, Huttner WB. Unpublished data) (Figure 2D), which represents a specialized domain of the oligodendrocyte plasma membrane. Together these observations show that prominin exhibits a profound preference for protrusions

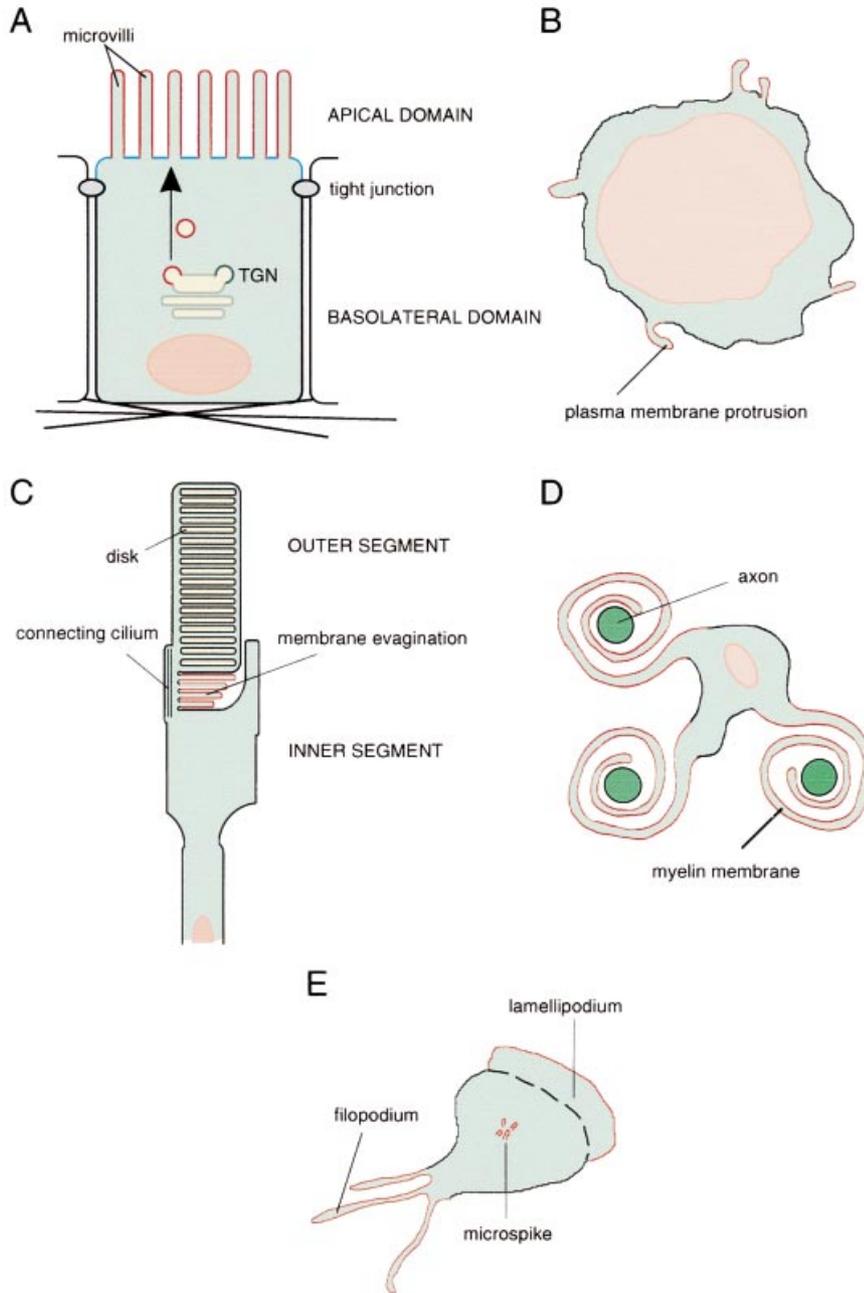


Figure 2: Prominin's preference for plasma membrane protrusions in various cell types. A. In epithelial cells, newly synthesized prominin is directly targeted (arrow) from the trans-Golgi network (TGN) to the apical plasma membrane domain. Within this domain, prominin is selectively associated with microvilli (red) rather than the planar subdomain (blue) of the plasma membrane (14,29). B. In hematopoietic progenitor cells, prominin is enriched in the sparse, small plasma membrane protrusions of these cells (red) (23). C. In rod photoreceptor cells, prominin is concentrated in the plasma membrane evaginations (red) at the base of the outer segment (25). D. In oligodendrocytes, prominin appears to be enriched in the plasma membrane protrusions forming the myelin sheath (red) (Joester A, Corbeil D, Hellwig A, Huttner WB. Unpublished observation). E. In transfected fibroblasts, prominin is preferentially localized in microspikes, filopodia and at the leading edge of lamellipodia (red) (14,23).

rather than the planar regions of the plasma membrane, irrespective of the cell type-specific features of these protrusions. This conclusion is further supported by results obtained with fibroblasts expressing transfected prominin, in which prominin is concentrated in microspikes, filopodia and the leading edge of lamellipodia (14,23) (Figure 2E).

The selective localization of prominin in distinct types of plasma membrane protrusions probably reflects a cell type-specific adaptation of a membrane transport process common to most, if not all eukaryotic cells. If so, this would be of interest given that some of the cytoskeletal components that organize plasma membrane protrusions differ between various cell types. For example, the actin-bundling protein villin occurs only in microvilli of epithelial cells, in which it has a key organizing role in the morphology of microvilli (32,33). Yet profound alteration of the cytoskeletal architecture of microvilli, as observed in Caco-2 expressing a villin anti-sense cDNA (34), does not abolish the preferential localization of prominin in microvilli, which in these cells are reduced in number (35). Furthermore, deletion of the C-terminal cytoplasmic domain of prominin, which is the most likely candidate to mediate a potential interaction with the cytoskeleton, does not impair prominin's concentration in microvilli (29). It appears, therefore, that in contrast to what has been observed for other microvillar membrane proteins (13,36), the primary cause of the selective localization of prominin in plasma membrane protrusions is not a direct interaction with the subplasmalemmal actin-based cytoskeleton.

Prominin-containing Plasma Membrane Protrusions – A New Cholesterol-based Lipid Microdomain

If a direct interaction with the cytoskeleton is not responsible for prominin's selective localization in plasma membrane protrusions, which other mechanism could be involved? Insight has come from morphological and biochemical studies comparing prominin with another apical membrane protein, placental alkaline phosphatase (PLAP), in transfected epithelial cells (MDCK). The localization within the plasma membrane of PLAP, a glycosylphosphatidylinositol (GPI)-anchored protein, was found to be distinct from that of prominin, i.e. PLAP was largely excluded from microvilli and predominantly found in a 'floe-like' pattern on the planar regions of the cell surface (35) (Figure 3). This is in line with previous observations (37) showing that within the apical plasma membrane, the microvillar and nonmicrovillar subdomains contain distinct sets of membrane proteins. The exclusion of PLAP from microvilli is not unique to this protein nor to transfected MDCK cells, but is also observed for other GPI-anchored cell surface proteins in distinct cellular systems (38,39).

Remarkably, a mild cholesterol depletion of the cell surface leads to a redistribution of prominin from microvilli over the entire apical plasma membrane, whereas the distribution of PLAP is largely unaffected (35). Since most of the cell surface molecules stained by wheat germ agglutinin behave

similar to prominin (35), it appears that prominin and other microvillar glycoproteins (as well as glycolipids) are constituents of membrane microdomains, which are more sensitive to cholesterol removal than the microdomains found in the planar region. Indeed, the cell surface form of prominin interacts directly and specifically with cholesterol (35), as shown by using a photoactivatable analogue of cholesterol (40). Surprisingly, however, prominin is not a constituent of the 'classical' cholesterol-sphingolipid 'rafts', which are characterized by their insolubility in the non-ionic detergent Triton X-100 in the cold (41), because it was found to be completely soluble in this detergent, in contrast to PLAP (35). Rather, prominin is a constituent of a distinct type of raft that is dissolved by Triton X-100 but can be preserved using another non-ionic detergent, Lubrol WX (35). These novel detergent-insoluble complexes (referred to as 'Lubrol rafts') exhibit several characteristics previously reported for the Triton X-100-insoluble complexes (42,43) (referred to as 'Triton rafts'), such as (i) floatation in sucrose density gradients along with the majority of the cellular cholesterol and glycolipids, and (ii) loss of floatation upon cholesterol depletion (35). The Lubrol rafts containing prominin are likely to be the biochemical correlates of a novel type of membrane microdomain that serves as a building unit to construct the plasma membrane subdomain of the microvillus (Figure 3, red segments), and that is distinct from the microdomains in the planar part of the apical plasma membrane containing Triton rafts with GPI-linked proteins (Figure 3, green segments). The differential sensitivity to detergent and behaviour upon cholesterol depletion of Lubrol rafts and Triton rafts indicate that the lipid-protein interactions in these two types of rafts, and hence between microvilli and planar regions of the apical plasma membrane, differ significantly. These differences may reflect a distinct composition of these rafts not only with regard to proteins but also to lipids.

It therefore appears that a novel cholesterol-based lipid microdomain is involved in the retention of prominin in microvilli and, presumably, in other plasma membrane protrusions as well. This in turn raises further questions. Where are the prominin-containing Lubrol rafts assembled? How are they delivered to the apical plasma membrane and into the microvillus? How are they retained in microvilli? Some answers to these questions have been obtained by studying the intracellular transport of prominin, as a marker of the Lubrol raft, in prominin-transfected MDCK cells (29,35). Prominin is transported from the endoplasmic reticulum to, and through, the Golgi complex in Lubrol-soluble form, and is incorporated in the *trans*-Golgi/TGN into relatively small Lubrol rafts (as defined by their sedimentation after 1 h centrifugation at 100000 *g* but lack of sedimentation after 10 min centrifugation at 17000 *g* (35)). Concomitant with the formation of the post-Golgi carriers that deliver prominin directly from the TGN to the apical cell surface (29) (Figure 2A), the small Lubrol rafts cluster to form large Lubrol rafts (as defined by their sedimentation already after 10 min centrifugation at 17000 *g* (35)). Lubrol rafts and Triton rafts are delivered to the apical cell surface either separately in two distinct trans-

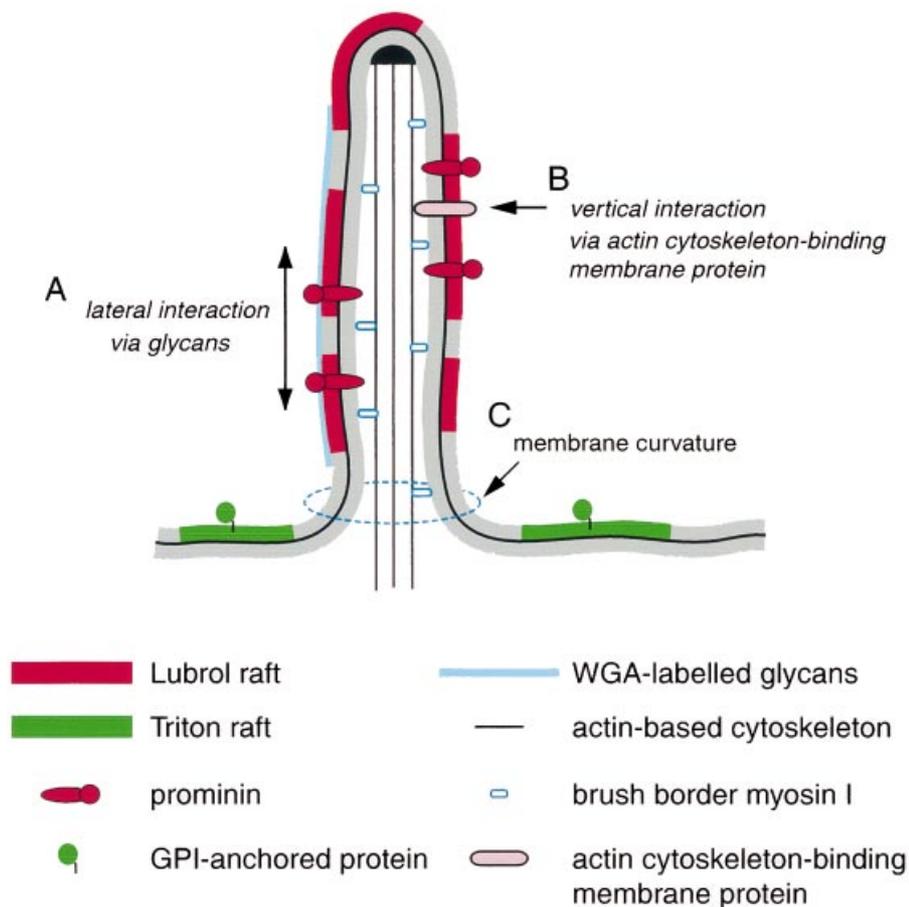


Figure 3: Illustration of three hypothetical mechanisms underlying the retention of prominin-containing Lubrol rafts in the microvillar plasma membrane. **A.** Retention due to lateral interactions of multiple rafts involving the glycan moieties of their glycoproteins and glycolipids WGA, wheat germ agglutinin. **B.** Retention due to vertical interaction with the actin-based cytoskeleton of a Lubrol-insoluble transmembrane protein distinct from prominin. **C.** Retention due to the negative membrane curvature which exists in the external membrane leaflet at the transition of the microvillus to the planar region of the plasma membrane and which may constrain the lateral mobility of prominin-containing Lubrol rafts.

port containers or together in a common membrane carrier. It is currently unknown whether the apical membrane carrier(s) containing Lubrol rafts fuse at the base of the microvillus, randomly over the apical plasma membrane, or in regions close to cell–cell contact (44), followed by lateral movement of the Lubrol rafts into the microvillus. In this context, it has been proposed (45) that brush border myosin 1 (a linker protein between actin filaments and the microvillar membrane), which is located in microvilli (Figure 3) as well as in post-Golgi vesicles (46), could play an important role in the insertion of these vesicles at the base of the microvillus.

At least three mechanisms, which are not mutually exclusive, can be hypothesized to explain the specific retention of the prominin-containing Lubrol rafts in the microvillar plasma membrane (Figure 3). First, Lubrol rafts may be linked to each other by numerous weak interactions involving the glycan moieties of their glycoproteins and glycolipids, thereby forming a stable structure (Figure 3A). This hypothesis is supported by the observation that the membrane con-

stituents that bind wheat germ agglutinin are enriched in microvilli and behave like prominin upon cholesterol depletion (35). It is tempting to speculate that glycan-mediated interactions within and between Lubrol rafts could also play a role in their packaging into membrane carriers budding from the TGN. A similar proposal has been made for the apical sorting of GPI-anchored proteins present in Triton rafts (47). Second, Lubrol rafts may be linked to the underlying actin cytoskeleton via a transmembrane protein other than prominin and which is a constituent of these rafts (Figure 3B). Such a link has been proposed to explain the reduced lateral mobility of CD44-containing Triton rafts in the basolateral membrane of epithelial cells (48). Third, membrane curvature at the base of the microvillus may physically, by functioning like a fence, constrain the lateral diffusion of the prominin-containing Lubrol rafts and hence prevent their escape from the microvillus (Figure 3C). If the latter mechanism exists, this would imply that the apical transport vesicles carrying the Lubrol rafts are directly targeted to the base of the microvillus.

A Frameshift Mutation in the Prominin Gene is Associated with Retinal Degeneration – Clues to its Function?

Is the selective localization of prominin in plasma membrane protrusions and its association with the novel (Triton-soluble but Lubrol-insoluble) cholesterol-based lipid microdomain of physiological relevance? An answer has come from studying the gene mutation associated with inherited, autosomal recessive, retinal degeneration in a human pedigree (25). The affected individuals were found to be homozygous for a single-nucleotide deletion (nt 1878) in the human prominin (*PROML1*) gene (25). This deletion is predicted to cause a frameshift starting at codon 614, resulting in premature termination of translation. The truncated prominin would lack about half of the second extracellular loop, the fifth transmembrane segment and the cytoplasmic C-terminal domain (Figure 1A). Expression in fibroblasts of a deletion mutant of murine prominin corresponding to the predicted deletion mutant of human prominin shows that the truncated prominin does not reach the cell surface (25).

Within the retina, prominin is expressed in rod photoreceptor cells, where it is concentrated in the plasma membrane evaginations at the base of the outer segment (25) (Figure 2C). These plasma membrane evaginations are essential precursor membrane structures in the biogenesis of the photoreceptive disk membranes, which are constantly renewed throughout adult life (49,50). This biogenesis involves a complex process of plasma membrane outgrowth: first, flattened evaginations are being generated; further outgrowth of the plasma membrane between the rims of two flattened evaginations then leads to their merging at their rims; finally, fusion of the leading edges of these two plasma membrane outgrowths renders the nascent disk membrane internal to the rod outer segment plasma membrane (49,51). This process of plasma membrane outgrowth, whose underlying mechanism is poorly understood (52), is associated with complex changes in membrane curvature (both negative and positive membrane curvature).

Given the general preference of prominin for plasma membrane protrusions (14,23), its ability to specifically interact with cholesterol (35) and the retinal degeneration associated with a mutation preventing its cell surface appearance (25), the concentration of prominin in the plasma membrane outgrowths at the base of the rod photoreceptor outer segment is highly intriguing and provides clues as to prominin's physiological role. First, prominin may be required for the formation of the plasma membrane outgrowths at the base of the rod outer segment. Second, prominin may endow these plasma membrane outgrowths with an appropriate lipid composition, notably with respect to cholesterol, which is known to be enriched in newly formed photoreceptive disks (53,54). Third, prominin, perhaps via its two large extracellular loops, may be involved in the interaction between the plasma membranes at the rims of two flattened evaginations during the merging process of the rims. It should be noted that these

hypothetical roles of prominin are not mutually exclusive, nor are they restricted to the plasma membrane outgrowth process underlying disk biogenesis, but may hold true also for the generation and lipid composition of other types of plasma membrane protrusions (Figure 2) and their membrane-to-membrane interactions.

Future Perspectives

Current knowledge about the prominin family suggests the following directions of future research on these proteins. First, we need more 'descriptive' data: a complete catalogue of the members of the prominin family, including splice variants, their tissue distribution and subcellular localization. Second, we need 'functional' data, which fall into two categories: molecular and physiological. As to prominin's molecular function, it needs to be determined whether cholesterol is its only specific interaction partner or whether other lipids and proteins engage dynamically or stably with it. Also, the molecular composition of the prominin-containing Lubrol raft would be of interest. As to prominin's physiological role, the available sequence information and cell surface expression offer several loss-of-function and gain-of-function approaches in cells in culture as well as in experimental organisms. These should be complemented by further studies whether alterations in prominin structure and expression are a cause of, as yet unexplained, human pathology. Finally, irrespective of prominin's physiological role, given its expression on hematopoietic and neuroepithelial stem cells, prominin, in line with previous studies (15,20,55,56), may well become an established tool not only for medical diagnosis but also for therapy.

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