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Roles of the cellular prion protein in the regulation of cell-cell junctions and barrier function

Constance S.V. Petit,[1,2,3] Laura Besnier,[1,2,3] Etienne Morel,[1,2,3] Monique Rousset[1,2,3] and Sophie Thenet[1,2,3,4,*]

[1]Centre de Recherche des Cordeliers; Université Pierre et Marie Curie; Paris, France; [2]INSERM; Paris, France; [3]Université Paris Descartes; Paris, France; [4]Ecole Pratique des Hautes Etudes; Laboratoire de Pharmacologie Cellulaire et Moléculaire; Paris, France

In memory of Caroline Clair

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Abbreviations: AJ, adherens junction; APP, amyloid precursor protein; Bcl-2, B-cell lymphoma-2; COX-IV, cytochrome c oxydase IV; DSS, dextran sodium sulfate; E-, N-, VE-cadherin, epithelial-, neural-, vascular endothelial-cadherin; EGF/EGFR, epidermal growth factor/receptor; Erk, extracellular signal-regulated kinase; FAK, focal adhesion kinase; GFAP, glial fibrillary acidic protein; GPI, glycosylphosphatidylinositol; Grb2, growth factor receptor-bound protein 2; GTPase, guanosine triphosphate hydrolase; HGF, hepatocyte growth factor; HSPG, heparan sulfate proteoglycan; LIMK, LIM kinase; LRP/LR, laminin receptor precursor/laminin receptor; MAPK, mitogen-activated protein kinase; N-CAM, neural cell adhesion molecule; NF-kappaB, nuclear factor kappa B; PECAM-1, platelet endothelial cell adhesion molecule-1; PI3K/AKT, phosphoinositide 3-kinase/protein kinase B; Pint 1, prion interactor 1; PKA, protein kinase A; PrP^C, cellular prion protein; PrP^{Sc}, scrapie prion protein; RhoA, Ras homolog gene family, member A; ROS, reactive oxygen species; TJ, tight junction; TSE, transmissible spongiform encephalopathy; VEGF, vascular endothelium growth factor; VLA-4, very late antigen-4; ZO-1, zonula occludens-1

The cellular prion protein was historically characterized owing to its misfolding in prion disease. Although its physiological role remains incompletely understood, PrP^C has emerged as an evolutionary conserved, multifaceted protein involved in a wide-range of biological processes. PrP^C is a GPI-anchored protein targeted to the plasma membrane, in raft microdomains, where its interaction with a repertoire of binding partners, which differ depending on cell models, mediates its functions. Among identified PrP^C partners are cell adhesion molecules. This review will focus on the multiple implications of PrP^C in cell adhesion processes, mainly the regulation of cell-cell junctions in epithelial and endothelial cells and the consequences on barrier properties. We will show how recent findings argue for a role of PrP^C in the recruitment of signaling molecules, which in turn control the targeting or the stability of adhesion complexes at the plasma membrane.

Introduction

The cellular prion protein (PrP^C) is the normal isoform of the pathogenic scrapie protein (PrP^{Sc}), which is involved in transmissible spongiform encephalopathy (TSE). The expression of PrP^C is necessary for the development of these pathologies,[1] which, according to Prusiner's theory, is linked to the transconformation of PrP^C by direct contact with PrP^{Sc}.[2] Consequently, investigations about prion pathogenesis led to an impressive number of studies on the biology of PrP^C, which focused essentially on the nervous system, even if it appeared rapidly that this protein is expressed ubiquitously.

The *prnp* gene encoding PrP^C is located on chromosome 20 in humans and on chromosome 2 in mice.[3] The gene has been well conserved throughout evolution in mammals and paralogue genes have been described in chicken, amphibians, reptiles and fish.[4] In mammalian species, the *prnp* gene comprises two or three exons, but the complete PrP^C protein is encoded by the terminal exon in all cases. Traduction product of *prnp* is a 209-residue protein that contains two glycosylation sites and a GPI anchor.[5] It has a long flexible N-terminal tail, which comprises series of four or five repeats of eight amino acids (PHGGGWGQ), a central domain, which includes a hydrophobic region, and three α-helices and two β-sheets (**Fig. 1**). PrP^C is processed post-translationally to remove an N-terminal signal peptide and a C-terminal peptide, which directs addition of the GPI anchor. Mature PrP^C is targeted to the plasma membrane, where it is inserted in the outer leaflet through its GPI anchor, within detergent-resistant raft domains (for a review, see ref. 4).

PrP^C is particularly abundant in brain, but it is also expressed in muscle, heart, lymphoid tissues, kidney, digestive tract, skin, mammary gland and endothelia. In order to decipher PrP^C physiological function(s), several lines of PrP^C knockout mice have

*Correspondence to: Sophie Thenet; Email: sophie.thenet@crc.jussieu.fr
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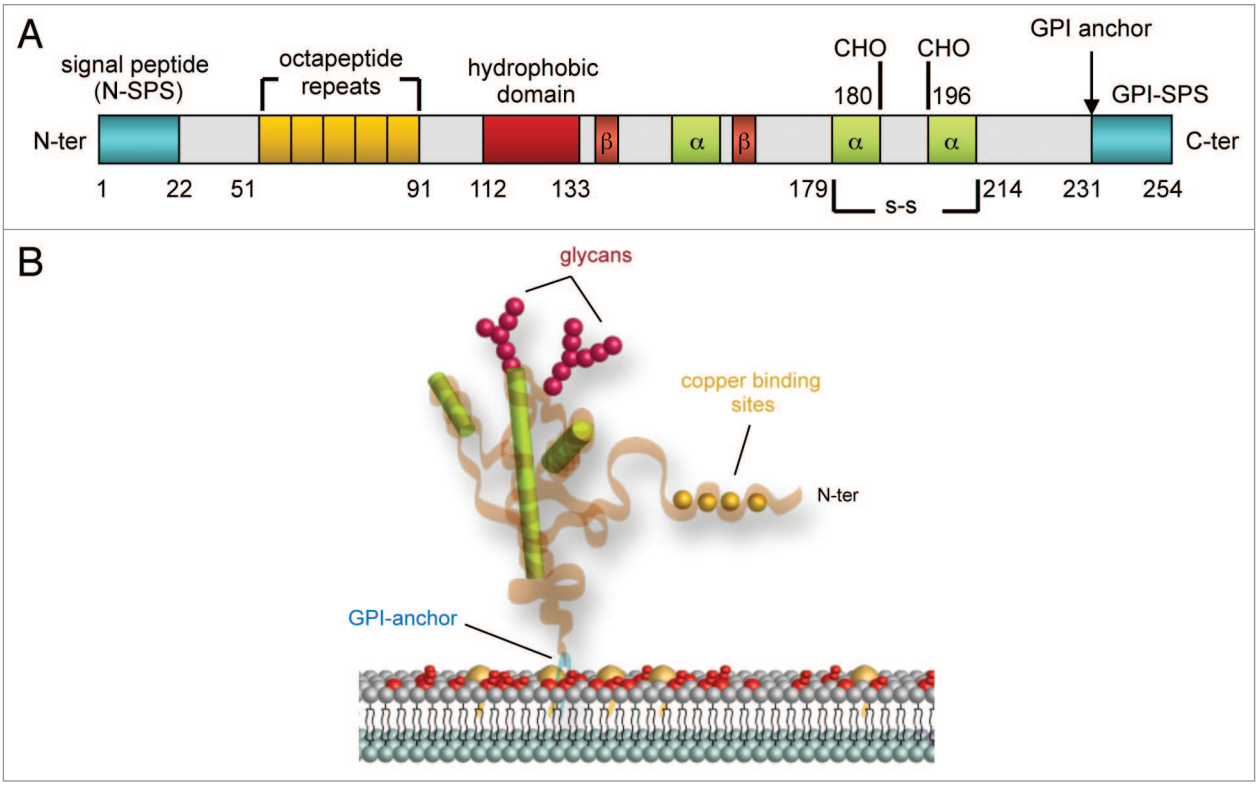

Figure 1. Schematic representation of PrP^C primary structure and topology. (**A**) Major identified domains are shown in color (amino acid residue numbers refer to the mouse PrP^C).[9] The position of the two β sheets and the three α helices is presented. Note also the presence of glycosylation sites (CHO), of one hydrophobic domain, of octapeptide repeats and of a signal peptide sequence in the N-ter domain (N-SPS). Removal of the C-ter GPI-SPS allows the attachment of the GPI anchor. (**B**) The mature protein is inserted in the outer leaflet of the plasma membrane through the GPI anchor, within raft domains. Copper binding sites in the octapeptide repeat region and glycans are shown.

been generated. These mice are viable, fertile and display no severe phenotype, except for one case, which was attributed to the artifactual upregulation of the adjacent *doppel* gene.[6] The absence of developmental or anatomical gross abnormality led temporarily to the conclusion that PrP^C does not fulfill any important biological function. However, studies that focused essentially on the nervous system revealed subtle neurologic alterations in these mice, including dysfunctions of circadian rhythm, memory, cognition, synaptic transmission or olfaction, as well as immunologic alterations. Increased levels of nuclear factor NF-kappaB, Mn superoxide dismutase and COX-IV, decreased levels of Cu/Zn superoxide dismutase activity and p53, and altered melatonin levels were observed in PrP^C knockout mice. Additionally, cultured cells from these mice were more sensitive to oxidative stress.[7] These perturbations have been extensively described in previous reviews[4,8,9] and it is worth noting that most of these alterations occur upon various stresses rather than in basal conditions. Emerging and controversing roles of PrP^C have been proposed recently in amyloid-β peptide production from amyloid precursor protein (APP),[10] in mediating effects of amyloid-β soluble oligomers on synapse alteration[11] and Alzheimer disease progression (reviewed in refs. 4 and 12). The ability of PrP^C to modulate cell proliferation and apoptosis has been linked to a possible role on cancer development, tumor progression and response to therapy (reviewed in ref. 13). Accordingly, PrP^C level is increased in some cancer types including gastric cancer,[14] colorectal cancer[15] and its increased expression has been associated with adenoma-to-carcinoma progression.[16] Controversial results have been reported on its involvement in metastatic processes.[17,18]

An important insight in the appreciation of PrP^C biological importance came from recent studies of Claudia Stuermer's group, which showed that downregulation of the *PrP-1* gene in the zebrafish embryo led to a letal phenotype with loss of embryonic cell adhesion and arrested gastrulation.[19] Partial rescue of the developmental arrest by zebrafish or mouse PrP^C mRNAs was a strong argument for conserved PrP^C functions.[19]

In parallel of knockout studies in whole organisms, cell biology approaches revealed that PrP^C participates to many fundamental biological processes including cell signaling, proliferation, survival, and differentiation.[20] PrP^C interacts with proteins involved in signal transduction (Fyn, Grb2), in neuronal architecture and function (synapsin Ib, GFAP) and with nuclear proteins (Pint 1) (for reviews, see refs. 9 and 21). PrP^C may link the anti-apoptotic Bcl-2 protein[22] and was involved in neurite outgrowth and neurone survival.[23] A well-known function of PrP^C is its ability to selectively bind copper ions.[24] Many reports showed a protective role of PrP^C against oxidative damage, conferring redox properties to PrP^C (reviewed in ref. 9). Reactive oxygen species (ROS) lead to cell damage. Free Cu^{2+} is highly cytotoxic and must be minimized, because redox reactions of Cu^{2+} generate ROS. The

antioxidant activity of PrP^C has been attributed to copper binding to the octarepeat region.[25,26] It has also been reported that PrP^C, via interaction with phosphorylated Fyn,[27] participates in cell redox homeostasis through ROS production.[28] Many other partners of PrP^C were identified, but the functional role of these interactions often remains to be elucidated.[9,29]

Among the different partners of PrP^C are proteins involved in cell adhesion. PrP^C interacts with cell adhesion molecules or extracellular matrix proteins to mediate different biological processes. For example, PrP^C was demonstrated to interact with laminin, thereby contributing to the regulation of neuritogenesis.[30,31] An interaction of PrP^C with the laminin receptor LRP/LR was demonstrated in the context of prion infection, but no link with adhesion processes was reported in these studies[32-36] and the role of this complex is still subject to debate.[37] PrP^C was shown interacting also with vitronectin[38] and with the neural cell adhesion molecule N-CAM.[39] Most of the studies dealing with these aspects were conducted in nerve cells and have been extensively discussed in a recent review.[37] More recently, PrP^C was shown to be targeted to cell-cell junctions in epithelial, endothelial and embryonic cells. In the present review, a survey of PrP^C involvement in cell-cell junctions and barrier function is presented and the underlying mechanisms, which might be regulated by PrP^C to control such interactions, are discussed.

PrP^C in Cell-Cell Junctions and Barriers

The possible implication of PrP^C in cell-cell adhesion came from the pioneering observation that PrP^C overexpression increased calcium-independent aggregation of N2a neuroblastoma cells.[40] The same N2a neuroblastoma cells were used to show the accumulation at cell-cell contacts of the heterologous zebrafish PrP-1.[19] Intrinsic calcium independent adhesive properties of mouse, chick, xenopus and zebrafish PrP^C were further demonstrated by aggregation assays after overexpression in non-adhesive *Drosophila* S2 cells.[19]

The conclusive demonstration of a biological role of PrP^C in cell-cell adhesion was obtained from results with endogenous PrP^C and the identification of its partners, which will be developed below (**Table 1**).

PrP^C is a component of desmosomes in intestinal epithelium and an important contributor of its barrier function. The expression and subcellular localization of PrP^C was initially investigated in the intestine considering that this epithelium could potentially explain contamination of certain TSE by an oral route. Many possible entry points, by which prions could enter the intestinal epithelium, have been discussed (for a review, see ref. 41) with possible roles of M cells,[42] Peyer patches[43] and enteroendocrine cells.[41] The passage of prions through enterocytes, which are the most abundant cells in this epithelium, must also be considered, as cultured enterocytes can internalize bovine prion particles by endocytosis involving the apical brush-border-associated laminin receptor LRP/LR (37 kDa/67 kDa).[35,36]

The *prnp* gene is expressed all along the intestine.[44-46] In the epithelial compartment, greater amounts of mRNA and proteins are found in the colon compared with the small intestine in mice.[47] PrP^C is expressed in various amounts in different human colon adenocarcinoma cell lines such as Caco-2/TC7,[48] HT29,[46] LS174T,[49] SW480 and HCT116 (unpublished data). In Caco-2/TC7 enterocytes, PrP^C is localized in lateral junction domains, in lipid rafts.[48,50] This localization at junctions was also found in intestine in vivo, in epithelial cells of villi and at the top of crypts,[48] as well as in colon.[47] Usually, GPI-anchored proteins are considered targeted to the apical membrane of polarized cells, although the localization of a GPI-anchored protein in junctional domains has already been observed for T-cadherin, a cell-cell binding signaling protein.[51] Basolateral targeting of transfected PrP^C was also shown in MDCK and FRT epithelial cells[52] and was later confirmed for the endogeneous protein in endothelial cells (see below).[53] Moreover, PrP^C exhibits the same localization in normal human keratinocytes as in enterocytes, i.e., at the junctions between adjacent cells (unpublished results).

In Caco-2/TC7 enterocytes, PrP^C co-localizes with E-cadherin along the lateral membrane in lipid rafts, but the two proteins do not interact.[48] Instead, PrP^C interacts with several desmosomal proteins, i.e., the desmosomal cadherin desmoglein-2, plakoglobin (γ-catenin) and plakophilin, from the catenin protein family, and the plakin desmoplakin.[50] In raft microdomains, PrP^C was demonstrated interacting with Src kinase,[48] which, as Fyn, belongs to the Src-related kinase family. Src is cytoplasmic or anchored in the inner plasma membrane leaflet whereas PrP^C is anchored in the outer one, leading to propose desmoglein-2 as an intermediary transmembrane protein between PrP^C and Src (**Fig. 2**). Accordingly, Src, PrP^C, but not E-cadherin can be co-immunoprecipitated with Desmoglein-2 in raft extracts from Caco-2/TC7 cells.[50] Therefore, PrP^C and Src kinase must be now considered as components of the desmosomal complexes in the intestinal epithelium, and it remains to be determined whether this can be extended to other tissues. Desmosomal junctions, which are known for their mechanical resistance properties, have been studied mainly in skin and cardiac muscle and numerous desmosomal pathologies have been described in these tissues.[54] Intriguingly, only very rare studies have addressed the role of desmosomal proteins in the intestinal epithelium,[55-57] which is subjected periodically to mechanical stresses through peristaltic movements. Ultrastructural analysis of the intestinal epithelium of $PrP^{C-/-}$ mice revealed shorter desmosomes than in wild type mice,[50] small desmosomes being usually considered to be less mature.[54]

PrP^C downregulation in Caco-2/TC7 enterocytes led to a notable alteration in the subcellular distribution of its partners desmoglein, plakoglobin, desmoplakin and Src.[50] Strikingly, absence of PrP^C was associated with alterations in the three types of intercellular junctions, namely desmosomes, adherens (AJ) and tight junctions (TJ) and in the organization of the monolayer.[47] For numerous junctional proteins (i.e. E-cadherin, desmoplakin, plakoglobin, occludin, ZO-1 and tricellulin), imaging studies revealed a loss of intensity in cell-cell contact areas and an increased intracellular signal. A loss of the apical restriction for TJ proteins was observed. These defects, which were not accompanied by modification of mRNA or protein levels, suggest that PrP^C may contribute to mechanisms that control the targeting,

Table 1. PrP^C-associated cell adhesion processes

Tissue or cell models	Adhesion or junctional proteins found in complexes with PrP^C (or colocalized with PrP^C)	Adhesion or junctional proteins regulated by PrP^C	Signaling proteins involved in PrP^C effects or modulated by PrP^C engagement	Biological processes	Refs.
Intestinal epithelium, enterocytic cell line: Caco-2/TC7	desmoglein-2 plakoglobin plakophilin-2a desmoplakin	E-cadherin occludin tricellulin ZO-1 desmoglein-2 plakoglobin desmoplakin actin	Src	Cell-cell adhesion (desmosomes, AJ and TJ); intestinal barrier	47, 48, 50
Epithelial cell lines: A431, MCF7, HeLa	Not determined	E-cadherin, β-catenin	reggie-1, EGF-R	Cell-cell adhesion (AJ)	76
Brain endothelial cells, Endothelial cell lines: RBE4, hCMEC/D3	(PECAM-1)	Not determined	Not determined	Endothelial barrier, monocyte trans-endothelial migration	53
Zebrafish embryo (PrP-1)	Not determined	E-cadherin β-catenin actin	Fyn	Cell-cell adhesion (AJ, not TJ), gastrulation, epiboly/radial intercalation	19
Neuroblastoma cell line: N2a, brain, hippocampal neurons	N-CAM	N-CAM	Fyn	Neurite outgrowth	39, 78
Neuronal cell line: PC12	β1-integrin	β1-integrin	Fyn, caveolin-1, FAK, Erk1/2	neuritogenesis	79
Neuroectodermal cell line: 1C11	Not determined	fibronectin, β1-integrin, paxillin, cofilin, actin	FAK, Src, Rho A, ROCK, LIMK	Focal adhesion, neurite formation	80
Drosophila S2, neuroblastoma cell line: N2a, HeLa, (PrP^C overexpression)	(β3-integrin)	paxillin, vinculin, β3-integrin	reggie-1, Src, FAK	Focal adhesion, filopodia formation	86
Brain, hippocampal neurons	(N-cadherin)	N-cadherin	reggies, Fyn, MAPK, TC10 GTPase	Neurite outgrowth	85

In the second column, interactions between PrP^C and adhesion proteins established by co-immunoprecipitation studies are reported. When only co-localizations were demonstrated, they are indicated between brackets.

the stability and/or the turnover of proteins at cell-cell junctions. Therefore, in enterocytes, PrP^C is required for the proper assembly or the stability of cell-cell junctions, not only of desmosomes, but also of AJ and TJ.

Cell-cell junctions are key actors in the organization and integrity of tissue barriers. PrP^C clearly contributes to the barrier function of the intestinal epithelium since paracellular permeability of the intestinal epithelium was found significantly increased in $PrP^{C-/-}$ mice compared with wild type mice.[47] Tight junctions, which play a key role in the control of paracellular permeability, were found irregular in their ultrastructure in intestinal cells of $PrP^{C-/-}$ mice, in concordance with the defects observed in PrP^C knockdown Caco-2/TC7 enterocytes.[47]

Intestinal barrier dysfunction is associated with inflammatory bowel diseases, such as Crohn's disease.[58] Whether intestinal barrier dysfunction precedes or follows inflammation in such pathologies is controversial but several mouse models with intestinal barrier defects clearly display increased propensity to develop intestinal disease.[59-63] The intestinal mucosa of $PrP^{C-/-}$ mice displayed no histological indication of inflammation but a slight increase in the amount of mucosal pro-inflammatory cytokines was observed. Although healthy in basal conditions, $PrP^{C-/-}$ mice were more sensitive to colitis induced by dextran sodium sulfate (DSS).[47] Accordingly, PrP^C overexpression in transgenic mice was shown to attenuate the severity of the DSS-induced colitis, but no difference in basal intestinal permeability was detected between wild type, PrP^C overexpressing and PrP^C knockout mice in this latter study.[64] PrP^C deficiency was not associated with increased tissue damage in response to DSS, but an increased pro-inflammatory cytokine expression was observed, in accordance with our results.

To conclude and summarize, the intestinal barrier function is impaired in the absence of PrP^C. Consistent with many studies on the physiological function of this protein,[8,9,65,66] the associated defects have visible functional consequences only when animals are subjected to stress challenges. Importantly, the organization of cell-cell junctions, the paracellular permeability and the trans-epithelial resistance, which are the main parameters reflecting a functional epithelial barrier, were found compromised in PrP^C knockdown Caco-2/TC7 enterocytes,

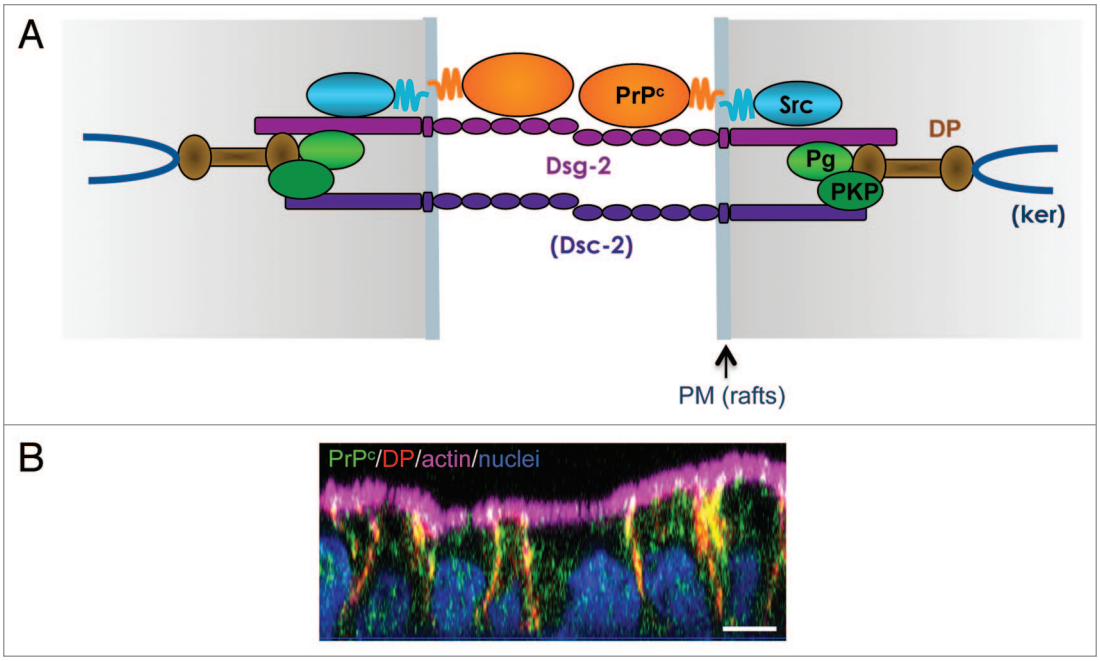

Figure 2. PrP^C is a component of desmosomes in intestinal epithelial cells. (**A**) Proposed model for PrP^C and Src interaction with the desmosomal complex within raft microdomains of the plasma membrane (PM). PrP^C interacts with Src kinase[48] and with the desmosomal proteins desmoglein-2 (Dsg-2), plakoglobin/γ-catenin (Pg), plakophilin-2 (PKP), and desmoplakin (DP).[50] The other desmosome components desmocollin-2 (Dsc-2) and keratins (ker), which were not detected in PrP^C immunoprecipitates, are shown between brackets. Note that PrP^C-PrP^C trans-interaction is still hypothetical (see text). (**B**) Confocal imaging (XZ view) showing colocalization of PrP^C with the desmosomal protein desmoplakin (DP) along the lateral membrane of highly polarized Caco-2/TC7 enterocytes. Actin was stained by phalloidin and nuclei by DAPI. Scale bar = 10 μm.

showing that the function of PrP^C in intestinal barrier is related to the protein expressed in epithelial cells, and is not a consequence of the effect that it could exert in non-epithelial tissues, like the immune system.[47] In this context, it is worth noting that the levels of PrP^C were decreased at cell-cell junctions in colonic epithelia from patients with Crohn's disease or ulcerative colitis, with an accumulation of the protein in intracellular compartments.[47]

As already shown for several junctional proteins,[67] a pool of PrP^C can be found in the nucleus. This nuclear targeting is observed when Caco-2/TC7 enterocytes are not engaged in cell-cell contacts and/or proliferate[48,50] as well as in the lower part of intestinal crypts in vivo[50] or in proliferating keratinocytes (unpublished data). While the nuclear localization of certain truncated forms of PrP^C or the scrapie form had already been described, only a few examples of nuclear localization of the full-length normal form of the protein had been reported,[68-70] without clear associated putative function. It can be envisaged that PrP^C, in interaction with nuclear protein partners, regulates other biological processes than cell adhesion and that an equilibrium between the different pools of this protein contributes to tissue homeostasis as shown, for example, for β-catenin.[71]

PrP^C is targeted toward endothelial junctions. PrP^C was found at cell-cell junctions in mouse brain endothelial primary cells as well as in rat (RBE4) and human (hCMEC/D3) brain endothelial cell lines.[53] This junctional targeting was dependent on PrP^C expression by the two cells involved in this contact. Indeed, in mixed endothelial primary cultures from wild type and PrP^C knockout mice, PrP^C was addressed at the junction between two wild type cells, but not between a wild type and a knockout cell, which are able yet to form cell-cell contacts delineated by β-catenin.[53] PrP^C colocalized with the platelet endothelial cell adhesion molecule-1 (PECAM-1) in raft caveolin-rich microdomains,[53] but no evidence for an interaction between the two proteins in junctional complexes could be established in this study. Interestingly, PrP^C is also expressed at the surface of monocytic cell lines, as is PECAM-1. Whereas anti-PrP^C or anti PECAM-1 antibodies had no effect on cytokine-induced adhesion of monocytes to endothelial cells, contrary to anti-VLA-4 antibodies, they strongly inhibited their trans-endothelial migration. Therefore, although the underlying mechanisms are still unknown, these results suggested that PrP^C could play a role in the regulation of junctions between endothelial cells, in vascular permeability and in extravasation of leukocytes. The recent observation that PrP^C downregulation in the brain microvascular endothelial cell line bEND.3 suppresses migration in a scratch-wound assay suggests that PrP^C could be necessary for junction remodeling in this cell type.[72]

It is also tempting to confront these results with data showing that the prion peptide PrP 106–126, which corresponds to the amyloidogenic region of PrP^C, with biochemical properties resembling the infectious form of prion protein, alters barrier function of brain capillary endothelial cells, as shown by decreased trans-endothelial electrical resistance and redistribution of TJ and AJ proteins, such as occludin, claudin-5 and VE-cadherin, from cell-cell contacts to the cytosol.[73] However, it is difficult to

link such effects with the endogenous PrP^C since its interaction with the peptide was not characterized in this study. Moreover, the peptide PrP 106–126 induced important cell toxicity, making difficult to ascertain that junction alterations were not secondary to cell death.

PrP-1 is required for cell-cell adhesion in zebrafish embryo. In the zebrafish *Danio rerio*, gene duplication gave rise to PrP1 and PrP2,[74] which exhibit different patterns of embryonic expression.[19] PrP-1 is early and ubiquitously expressed whereas expression of PrP-2 is restricted to the developing nervous system. In zebrafish embryos, morpholino-induced downregulation of PrP-1, but not PrP-2, induced gastrulation arrest, which was due to loss of embryonic cell-cell adhesion.[19] As described above for transfected PrP^C, endogenous PrP-1 was shown to mediate calcium independent aggregation in fish embryonic cells. Moreover, calcium-dependent cell adhesion is affected by absence of PrP-1 in morphant embryos, assessed by intracellular accumulation of E-cadherin and β-catenin, with actin disorganization at cell-cell contacts. Increased colocalization between E-cadherin and the recycling endosome marker Rab11, together with a slight increase of the 140-kDa E-cadherin immature form, could evoke a role of PrP-1 in E-cadherin trafficking toward or from the plasma membrane.[19,75] Consistently, E-cadherin, β-catenin and Fyn tyrosine kinase accumulated at cell-cell contacts between blastomeres dissociated from control embryos and allowed to reaggregate in culture, but not between PrP-1 morphant blastomeres. The role of PrP^C in E-cadherin-mediated cell-cell contact formation was confirmed in several human epithelial cell lines (A431, MCF7 and HeLa),[76] including enterocytes, as described above.[47] The proposed underlying mechanisms will be discussed in the next section.

How PrP^C might Regulate Junctional Complexes

Is PrP^C a trans interacting adhesion molecule? An important issue to understand the role of PrP^C in cell-cell adhesion regulation is whether PrP^C molecules on adjacent cells are engaged in homophilic interactions. In vitro modeling of PrP^C oligomerization suggested trans-dimerization through α-helices in a "head-to-tail conformation."[77] In enterocytes, PrP^C is addressed at the plasma membrane only when this membrane portion is in contact with an adjacent cell.[48] Accordingly, as mentioned above, mixed primary cultures of brain endothelial cells from wild type and PrP^C knockout mice showed that PrP^C is not addressed at intercellular junctions when PrP^C is missing on the adjacent cell.[53] In the same way, the zebrafish PrP-1 transfected in mouse N2a cells or in *Drosophila* S2 cells accumulates at cell-cell contact only when both cells forming the contact express the PrP-1 construct.[19] Finally, PrP-1 morphant cells transplanted in control embryos are unable to establish cell contacts.[19] All these observations are concordant to suggest that PrP^C of opposing cell membranes may interact in *trans* and that this interaction is important for cell adhesion. However, the lack of transmembrane and cytoplasmic domain in the PrP^C protein renders unlikely its contribution to strong adhesion processes. On the other hand, its ability to interact with signaling proteins, such as Src-related kinases, argues for a role in adhesion regulatory processes by the modulation of signaling pathways.

Role of PrP^C in signal transduction. *PrP^C is able to modulate several signaling pathways in neurons.* The recruitment of Src and Fyn kinases at adherens junctions[19] or desmosomes[47] is probably crucial to mediate the influence of PrP^C on these junctions (**Table 1**). Underlying mechanisms for such regulation have not been unraveled yet, but results on the PrP^C-mediated modulation of signaling cascades in the context of cell-matrix interactions during neuritogenesis could provide working hypotheses (**Table 1**). Fyn kinase was one of the first signaling proteins that was shown activated by PrP^C in a line of precursor neuroectodermic cells, using a cross-linking antibody.[27] This activation was dependent on caveolin and induced a signaling cascade leading to the stimulation of NADPH-oxidase, Erk1/2 and production of ROS.[28] Involvement of Fyn activation in PrP^C-mediated neurite outgrowth, along with the implication of PI3K/AKT, cAMP-dependent PKA and MAPK signaling in neuron survival, were further confirmed using PrP-Fc fusion protein.[23] While the use of antibodies or soluble PrP-Fc to artificially cluster the PrP^C protein present at the cell surface is a fruitful approach to decipher the involved signaling pathways, it gives no information on the physiological ligands of the protein. The adhesion molecule N-CAM could be such a ligand; in fact, cis- and trans-interactions between N-CAM and PrP^C at the neuronal surface induce recruitment of N-CAM in lipid rafts to regulate activation of Fyn kinase, which is involved in N-CAM-mediated signaling, and to promote neurite outgrowth.[78] Neurite growth is also stimulated in the neuronal cell line PC12 in response to activation by anti-PrP^C antibodies in a signaling cascade involving Fyn and Ras-Raf-Erk1/2 pathway. This cascade is initiated by the translocation of Fyn and FAK caveolae-like domains in a manner that is dependent on the engagement of integrins.[79] PrP^C function in neuritogenesis was also demonstrated to rely on its capacity to negatively regulate the clustering, activation, and signaling activity of β1 integrins at the plasma membrane. After PrP^C depletion in 1C11 neuroectodermal cell line, β1 integrin aggregation triggers overactivation of the RhoA-Rho kinase-LIMK-cofilin pathway, which, in turn, alters the turnover of focal adhesions, increases the stability of actin microfilaments, and, finally, impairs neurite formation. An increased secretion of fibronectin is observed around PrP^C-depleted cells, which may sustain β1 integrin signaling overactivation and contribute to neuritogenesis defect.[80]

Role of PrP^C in raft microdomain signaling platforms. Several studies demonstrated overlapping functions of PrP^C and reggie/flotillin proteins. Reggies/flotillins reside at the cytoplasmic face of the plasma membrane and on intracellular compartments. Reggie oligomers form clusters at the cell surface where GPI-anchored proteins, such as Thy-1 and PrP^C, as well as Src-family tyrosine kinases co-cluster (reviewed in refs. 81–83). Reggies have been involved in various cell processes, such as axon regeneration in neurons, insulin-induced membrane targeting of the glucose transporter Glut-4 in adipocytes, T cell receptor recruitment in T lymphocytes.[81,83] Most of these processes were associated to the regulation of cell-matrix or cell-cell contacts, suggesting a connection between reggies and cell adhesion processes.

Several observations strongly argue for an important role of reggies in mediating effects of PrP^C engagement. PrP^C and reggies can be co-immunoprecipitated in T cells[84] and in brain extracts.[85] In hippocampal neurons, PrP^C "activation" by anti-PrP antibodies or by PrP-Fc chimera proteins was shown to promote PrP^C-reggie co-clusterisation, to increase Fyn and MAPK activity in reggie-immunoprecipitates and was associated with increased neurite length.[85] Moreover, PrP-Fc promoted delivery of N-cadherin into reggie microdomains. N-cadherin recruitment to reggie was inhibited in the presence of a dominant negative form of the GTPase TC10, suggesting a role of PrP^C and reggies in exocyst-dependent polarized delivery of membrane proteins.[85] Such mechanisms could explain the abnormally small growth cones of PrP^C knockout hippocampal neurons.[85] Reggies are also required for PrP^C-dependent formation of focal adhesion structures through activation of Src and FAK in drosophila S2, neuroblastoma N2a or HeLa cells.[86]

Concerning E-cadherin-mediated contacts, similar defects of AJ formation were induced by reggie-1 and PrP^C downregulation in A431 epithelial cells.[76] Such defects were associated with decreased endocytosis of EGF receptor upon EGF stimulation. Reggie-1 downregulation caused a reduction in EGFR phosphorylation, modifying downstream signals such as Src, Erk1/2, PI3K, Akt and p38 MAPK, the role of PrP^C in these signaling events remaining to be determined. Absence of Reggie-1 or PrP^C were both shown to promote macropinocytosis, which could be responsible for E-cadherin recycling since AJ phenotype of shReggie-1 was rescued by several macropinocytosis blockers.[76] PrP^C is therefore proposed to act, in collaboration with reggies/flottilins, as a cell-surface platform regulator facilitating the recruitment of signaling molecules, among which Src-related kinases and GTPases, which participate in diverse processes, such as cell-cell junction assembly, T-cell receptor complex gathering in the cap, neurite elongation and guidance through the regulation of membrane protein trafficking.[83]

Although precise mechanisms remain to be deciphered in most systems, it seems obvious that the localization of PrP^C in lipid rafts is important to regulate cell-cell junctions. The interaction of PrP^C with desmosomal proteins was demonstrated in raft preparations,[50] suggesting for the first time that at least some of the desmosomal complexes are localized within these membrane microdomains. Similarly, as described above, PrP^C was found in raft-like junction membrane microdomains with the platelet adhesion protein PECAM-1 in brain endothelial cells.[53] The relationship between cell-cell junctions and lipid rafts is a relatively unexplored field of research. It has been clearly shown that complexes forming TJ are organized within raft-like microdomains rich in cholesterol and caveolin.[87] With respect to AJ proteins, the presence of E-cadherin and the Met receptor for factor HGF at sites of lipid rafts plays a determinant role in permitting entry of *Listeria monocytogenes* into the host cell.[88] In myogenic cells, the association of $p120^{Ctn}$ to N-cadherin occurs specifically and exclusively at sites of raft microdomains rich in cholesterol; this localization is essential for stabilization of the complex to cell-cell contacts and activation of RhoA and myogenesis.[89] Similarly, in enterocytic cells, lipid rafts are required for the recruitment of $p120^{Ctn}$ to E-cadherin and downregulation of flotillin-1 (reggie-2) delays enterocytic differentiation in HT29 cell line.[90] It has also been shown that annexin A2, a scaffolding protein that participates in membrane and F-actin dynamics and is involved in the organization of lipid-microdomains, contributes to the architecture of mature AJ of endothelial cells by simultaneously binding to actin and to the VE-cadherin complex and by recruiting this complex to rafts.[91] Destabilization of VE-cadherin junctions by the growth factor VEGF correlates with uncoupling of the VE-cadherin/annexin A2 complex.[91] Interestingly, one of the PrP^C junctional partners identified in enterocytes is annexin 2.[50] The role of PrP^C in the distribution of junctional complexes between raft and non-raft membrane domains remain to be determined, as well as the possible consequences for the recruitment/activation of associated signaling proteins such as Src-related kinases. Likewise, the role of PrP^C partners such as reggies and annexin 2, which are important actors of lipid platform organization, will have to be determined clearly in these regulations.

It seems probable that according to cell types and culture conditions or differentiation state, PrP^C associates to different partners within microdomains, which in turn can recruit different signaling entities. Thus, it has been proposed that PrP^C rather than being a major actor in a given pathway, could be considered as a "scaffolding" protein for the assembly of different signaling complexes.[9] PrP^C being confined to the external leaflet of the lipid bilayer, the existence of transmembrane partners could be considered necessary to transduce signals. Such proteins have been proposed for some, but not all, systems in which PrP^C was shown to mediate adhesion (**Table 1**). Some hypothetical models were proposed in which allosteric function of PrP^C modifies the conformation of its transmembrane partners and the resulting downstream signals.[92] Alternatively, it cannot be excluded that co-clusters of proteins in the outer and inner leaflets are sufficient to transduce signals into cells.[82]

Conclusion

The fate of cells strictly depends on signal integration coming from their environment including cell-matrix and cell-cell interactions. These signals must be finely tuned during embryonic development but also to maintain tissue homeostasis in adult. Moreover, integrity of epithelial and endothelial barriers depends also on a multiplicity of regulatory events, in physiology as well as in pathological situations, through the remodeling of cell-cell junctions. It appears clearly that PrP^C, whose contribution to cell adhesion in neural cells has been extensively documented, acts also as an important regulator of cell-cell junctions and barrier function. The basis of this function likely relies on the ability of PrP^C to interact in junctional complexes with signaling proteins. The mechanisms, through which PrP^C may regulate their activity and downstream targets, have now to be discovered.

Disclosure of Potential Conflicts of Interest

The authors have no conflicts of interest.

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