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Viviana Zomosa-Signoret, Jacques-Damien Arnaud, Pascaline Fontes, Maria-Teresa Alvarez-Martinez, Jean-Pierre Liautard. Physiological role of the cellular prion protein. *Veterinary Research*, 2008, 39 (4), pp.1-16. 10.1051/vetres:2007048 . hal-00902908

HAL Id: hal-00902908

<https://hal.science/hal-00902908>

Submitted on 11 May 2020

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Physiological role of the cellular prion protein

Viviana ZOMOSA-SIGNORET¹, Jacques-Damien ARNAUD², Pascaline FONTES³,
Maria-Teresa ALVAREZ-MARTINEZ², Jean-Pierre LIAUTARD^{1,2*}

¹ CPBS, UMR 5236, CC 100, Université Montpellier, 2 Place E. Bataillon, 34095 Montpellier Cedex, France

² IFR-122, CC 100, Université Montpellier 2, Place E. Bataillon, 34095 Montpellier Cedex, France

³ CECEMA, Université Montpellier 2, Place E. Bataillon, 34095 Montpellier Cedex, France

(Received 10 May 2007; accepted 21 September 2007)

Abstract – The prion protein (PrP) plays a key role in the pathogenesis of prion diseases. However, the normal function of the protein remains unclear. The cellular isoform (PrP^C) is expressed most abundantly in the brain, but has also been detected in other non-neuronal tissues as diverse as lymphoid cells, lung, heart, kidney, gastrointestinal tract, muscle, and mammary glands. Cell biological studies of PrP contribute to our understanding of PrP^C function. Like other membrane proteins, PrP^C is post-translationally processed in the endoplasmic reticulum and Golgi on its way to the cell surface after synthesis. Cell surface PrP^C constitutively cycles between the plasma membrane and early endosomes via a clathrin-dependent mechanism, a pathway consistent with a suggested role for PrP^C in cellular trafficking of copper ions. Although PrP^{-/-} mice have been reported to have only minor alterations in immune function, PrP^C is up-regulated in T cell activation and may be expressed at higher levels by specialized classes of lymphocytes. Furthermore, antibody cross-linking of surface PrP^C modulates T cell activation and leads to rearrangements of lipid raft constituents and increased phosphorylation of signaling proteins. These findings appear to indicate an important but, as yet, ill-defined role in T cell function. Recent work has suggested that PrP^C is required for self-renewal of haematopoietic stem cells. PrP^C is highly expressed in the central nervous system, and since this is the major site of prion pathology, most interest has focused on defining the role of PrP^C in neurones. Although PrP^{-/-} mice have a grossly normal neurological phenotype, even when neuronal PrP^C is knocked out postnatally, they do have subtle abnormalities in synaptic transmission, hippocampal morphology, circadian rhythms, and cognition and seizure threshold. Other postulated neuronal roles for PrP^C include copper-binding, as an anti- and conversely, pro-apoptotic protein, as a signaling molecule, and in supporting neuronal morphology and adhesion. The prion protein may also function as a metal binding protein such as copper, yielding cellular antioxidant capacity suggesting a role in the oxidative stress homeostasis. Finally, recent observations on the role of PrP^C in long-term memory open a challenging field.

prion / PrP^C / physiology / biology

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* Corresponding author: liautard@univ-montp2.fr

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1. INTRODUCTION

Prion diseases are neurodegenerative disorders that affect animals and humans. In animals, the best known forms are scrapie in sheep and bovine spongiform encephalopathy in cattle, whereas the human variants include Creutzfeldt-Jakob disease, Gerstmann-Straussler-Scheinker disease, and fatal familial insomnia. The unique feature of these diseases is that, in addition to sporadic and inherited forms, they may be acquired by transmission of an infectious agent. According to the “protein-only” hypothesis, the infectious prion pathogen consists of an abnormal prion protein, PrP^{Sc} [84]. This protein is an altered isoform of a normal cellular protein, PrP^C, which is host-encoded by a chromosomal gene and abundantly expressed in mammalian cells [85]. However, the two prion protein isoforms have profoundly different physical properties. PrP^C exists as a monomer that is readily degradable by proteinase K, whereas PrP^{Sc} forms insoluble aggregates that show high resistance to proteinase K digestion and often have the characteristics of an amyloid [85]. The covalent structure of PrP^{Sc} is most likely identical with that of PrP^C [98]. PrP^C is a 209-residue glycoprotein that has a single disulfide bridge, two N-glycosylation sites, and a glycosyl phosphatidyl inositol anchor [98]. It is localized in cholesterol-rich membrane microdomains called rafts or caveolae-like domains [106]. However, cytosolic localized PrP has also been detected [66]. Besides its conservation among species and expression in most tissues, the role of the cellular prion protein is not completely understood [63]. PrP^C is expressed most abundantly in

the brain, but has also been detected in other non-neuronal tissues as diverse as lymphoid cells, lung, heart, kidney, gastrointestinal tract, muscle, and mammary glands [43]. In the last few years, some possible biological functions for the cellular prion protein have been described. It has been postulated that PrP^C may be involved in one or more of the following: neurotransmitter metabolism, immune cell activation [17, 64], cell adhesion, signal transduction, copper metabolism, antioxidant activity, or programmed cell death [46]. Writing a review of current knowledge of the role of PrP^C as a cellular protein appears to be a rather delicate task.

2. ROLE AND FUNCTION OF THE PRP^C IN THE CELL

2.1. Cellular localization and trafficking

Knowledge on cellular localization is essential to propose possible realistic functions to the PrP^C. A lot of papers have been published but a consensus emerges.

Most studies indicate that PrP^C associates with multi-molecular membrane complexes, which mediate a variety of functions in distinct cellular compartments [51]. PrP^C continuously cycles between the plasma membrane and endocytic compartments. This process is related to the availability of the protein at the cell surface, and involves internalization pathways that may be critical for PrP^C physiological functions [72]. Trafficking of PrP^C seems to be a complex cellular event and may involve more than one internalization mechanism. Results for PrP^C trafficking showed that the molecule traffics through the Golgi complex, plasma membrane, early recycling

endosomes, and that dynamin I, a GTPase involved in the scission of endocytic vesicles, participates in the initial steps of PrP^C cycling. These observations indicate that traffic of PrP^C is not determined predominantly by the GPI anchor and that, differently from other GPI-anchored proteins, PrP^C is delivered to classic endosomes after internalization [58]. PrP^C is enriched in caveolae-like (raft?) both at the transgolgi network and plasma membrane and in interconnecting chains of endocytic caveolae-like domains [81, 106]. It is delivered via caveolae-like domains to the pericentriolar region and via non-classical, caveolae-containing early endocytic structures to late endosomes/lysosomes, it seems to bypass the internalization pathway mediated by clathrin-coated vesicles but this work was performed using PrP-transfected CHO [81]. The distinctive feature of this trafficking is, however, that, while still on the cell surface, PrP^C leaves its raft environment to cross the non-raft membrane and then enter coated pits, where it is endocytosed. Endocytosed PrP^C-containing caveolae are not directed to the ER and Golgi complex but return to the surface [102]. It can be concluded that cell surface PrP^C constitutively cycles between the plasma membrane and early endosomes via a clathrin-dependent mechanism, a pathway consistent with a suggested role for PrP^C in cellular trafficking of copper ions [40].

2.2. Interaction of PrP^C with cellular proteins

Another approach to establish biological function in the cell is to identify protein partners. Indeed, PrP^C interacts with various macromolecules at the cell membrane, in endocytic compartments and in the secretory pathway.

The human 37-kDa laminin receptor precursor interacts with the prion protein [87]. It is a transmembrane protein that mediates internalization of PrP^C and directs the complex through clathrin coated pits [34]. The laminin receptor is able to mediate internalization of only 25% to 50% of the membrane-bound recombinant PrP^C, which is consistent with both the participation of other cell surface proteins, as well as other pathways of internalization.

Furthermore, PrP^C has also been reported as a saturable, specific, high-affinity receptor for laminin [36]. The interaction between PrP^C and laminin was related to rat memory consolidation [20]. In addition, PrP^C 173–192 peptide and anti-PrP^C or anti-laminin antibodies also inhibited the activation of hippocampal cAMP-dependent protein kinase A (PKA) and extracellular regulated kinases (ERK1/2), three kinases that mediate the up-regulation of signaling pathways needed for consolidation of inhibitory avoidance memory.

It has been demonstrated that PrP^C may associate with the microtubular cytoskeleton and its major component, tubulin [38]. The effect of full-length human recombinant PrP on tubulin polymerization was measured, and it was demonstrated that PrP^C induces rapid association of tubulin into oligomers thereby inhibiting microtubule formation. PrP^C seems to limit the number of tubulin heterodimers available for polymerization into microtubules, most probably by engaging them in the formation of oligomers. PrP^C acts as a tubulin-sequestering protein [73].

Stress inducible protein 1 (STI1) is a specific PrP^C ligand that promotes neuroprotection of retinal neurons. Biochemical approaches have demonstrated that PrP^C interacts specifically to this co-chaperone [114]. PrP^C binds to cellular STI1, and co-immunoprecipitation assays suggest that both proteins are associated in vivo. PrP^C interacts with STI1 through its highly conserved and hydrophobic domain, amino acids 113-129 [62]. PrP^C and STI1 interact in a high affinity manner with a K_d of 10^{-7} M. Cell surface binding and pull-down experiments showed that PrP^C and STI1 are highly expressed on the hippocampus and that their interaction induces neuritogenesis and neuroprotection in hippocampal neurons [56] (see above).

Many others ligands have been identified, using various approaches, as possibly interacting with PrP^C, among them : $\alpha\beta$ -crystalline, Na^+/K^+ -ATPase $\alpha 3$ subunit, CNPase, β -actin, α -spectrin and creatine kinase- β , synapsin Ib, the adaptator protein Grb2, and the prion interactor Pint1 [82]. It is, however, difficult to propose a specific role for such a number

of possibilities. Indeed, PrP^C is an inherently sticky protein and the meaning of the interaction with many intracellular proteins has to be questioned. A better knowledge of the physiological function is necessary. On the contrary, the activation of the non-receptor tyrosine kinase fyn, which is enriched in brain synaptosomes and has been implicated in long-term potentiation [37], could represent a cellular pathway through which PrP^C influences synaptic function [67].

The prion protein is a complex entity, although numerous binding partners have been found for the protein, its function still remains unclear. Each portion of the protein may have its own functional properties.

2.3. PrP^C as a copper ligand

It is well known that the prion protein binds copper *in vivo* [8]. The N-terminal domain of PrP^C contains octapeptide repeat motifs encompassing five Cu²⁺ ion binding sites. There is increasing evidence to support a functional role for PrP^C in copper metabolism and several groups have investigated the physiological meaning of this association [10]. PrP^C may be a principal copper-binding protein in brain membrane fractions and controls the activity of other membrane-associated copper-binding proteins. Prion protein expression alters copper uptake into cells and enhances copper incorporation into superoxide dismutase (SOD). Furthermore, the prion protein itself can act as a SOD [9]. Analysis of PrP related copper uptake by cells suggests that the K_m for PrP facilitated copper uptake is in the nanomolar range which would suggest an affinity of 10⁻⁹ M or less placing copper binding by the prion protein in an acceptable range. However, after a thorough analysis of the binding parameters, Wells et al. [112] have acutely argued for a physiological binding of Cu²⁺ to PrP^C in the brain only when concentration increases locally as for instance during depolarization.

Using mutants of PrP^C that lack either the octapeptide repeats or the N-terminal polybasic region, and a construct with a transmembrane anchor; Taylor and Hooper [103] showed that copper binding to octapeptide repeats promotes dissociation of PrP^C from lipid

rafts, whereas the N-terminal polybasic region mediates its interaction with a transmembrane adaptor protein that engages the clathrin endocytic machinery.

Cells deficient in PrP^C are less viable in culture compared with cells expressing wild-type PrP and are more susceptible to oxidative damage and toxicity caused by agents such as copper and hydrogen peroxide. PrP^C might play an important role in oxidative stress homeostasis, not only in the central nervous system, but also in other organs [52]. Recombinant chicken and mouse PrP^C, as well as PrP^C immunoprecipitated from brain tissue had superoxide dismutase (SOD) activity. The results suggest that PrP^C has an enzymatic function, dependent on copper incorporation, consistent with its cellular distribution and indicating its direct role in cellular resistance to oxidative stress [9]. Cleavage close to, or within the octapeptide region has been termed β -cleavage and appears to be mediated by reactive oxygen species (ROS); this cleavage is copper dependent [65]. β -cleavage of PrP^C could be a critical step whereby PrP^C protects cells against oxidative stress [108].

Reintroduction of PrP into PrP^{-/-} cells, rescues cells from apoptosis, upregulates SOD activity, enhances superoxide anion elimination, and inhibits caspase-3/9 activation. On the contrary, N-terminally truncated PrP^C enhanced apoptosis accompanied by potentiation of superoxide production and caspase-3/9 activation due to inhibition of SOD. PrP^C protects PrP^{-/-} cells from apoptosis via superoxide- and caspase-3/9 dependent pathways by upregulating SOD activity [90]. These results raise the possibility that some factors, which interact with PrP^C, may mediate copper transfer from PrP^C to Cu/Zn-SOD, and the existence of the octapeptide region of PrP^C may be indispensable for copper transfer and regulation of SOD activity. STI1 is a candidate for involvement in such interaction with PrP^C.

2.4. Signal transduction by the prion protein

PrP^C localization in lipid rafts, membrane microdomains enriched in sphingolipids and cholesterol, and the association with signal transduction mechanisms, indicate that PrP

may participate in cell signaling pathways [82]. Many signal transduction patterns have been uncovered during PrP^C function analysis.

Phosphatidylinositol 3-kinase (PI 3-kinase) is a protein kinase that plays a pivotal role in many cellular processes including cell survival and apoptosis. PI 3-kinase is known to be sensitive to redox signaling by superoxide and hydrogen peroxide and to be activated by copper ions. PI 3-kinase catalyzes the formation of D3-phosphorylated phosphoinositides, notably phosphatidylinositol 3,4,5-triphosphate (PIP₃). PIP₃ has several downstream target molecules, in particular the serine/threonine protein kinase B (PKB or Akt). The PI 3-kinase/Akt pathway suppresses cell death by regulating bcl-2 family member activity and mitochondrial function [12]. Both mouse neuroblastoma N2a cells and immortalized murine hippocampal neuronal cell lines expressing wild-type PrP^C had a significantly higher PI 3-kinase activity levels than their respective controls. PI 3-kinase activity was found to be elevated in brain lysates from wild-type mice, as compared to prion protein-knockout mice. Recruitment of PI 3-kinase by PrP^C was shown to contribute to cellular survival toward oxidative stress by using 3-morpholinosydnonimine (SIN-1) and serum deprivation. Moreover, both PI 3-kinase activation and cytoprotection by PrP^C appear to rely on copper binding to the N-terminal octapeptide of PrP^C. So the interaction of copper with the N-terminal domain of PrP^C could enable transduction of a signal to PI 3-kinase which, in turn, mediates downstream regulation of cell survival [105]. Due to its plasma membrane-localization, copper bound PrP^C that can act as a sensor for extracellular stress and transmit signals to the intracellular milieu, the PI 3-kinase cascade having a crucial role in activating neuroprotective mechanisms.

A caveolin-dependent coupling of PrP^C to the tyrosine kinase fyn was shown [68]. This coupling was evidenced using the murine 1C11 neuronal cell line. The 1C11 clones behave as a committed neuroepithelial progenitor that lack neuron-associated functions. On induction, nearly all 1C11 cells develop bipolar extensions, express neuronal markers,

such as neurofilaments, N-CAM, $\gamma\gamma$ -enolase, or synaptophysin, and convert into either 1C11^{5-HT} serotonergic or 1C11^{NE} noradrenergic cells. In the 1C11 progenitor and throughout differentiation, PrP^C, caveolin, and fyn are endogenously expressed [67]. Schneider et al. [93] investigated whether PrP^C might be coupled with a ROS production pathway in the 1C11 neuronal differentiation model. They identified NADPH oxidase, a major ROS generator in cells, and extracellular regulated kinases 1/2 (ERK 1/2), two mitogen-activated protein kinases (MAPK) as target of the PrP^C-mediated signaling. Similar responses were observed in neuronal and non-neuronal cells. Moreover, in 1C11 fully differentiated neuronal cells only, an involvement of the fyn kinase in the control of the PrP^C-induced transduction cascade was established, in agreement with the idea of some specificity of PrP^C signaling related to the expression of a complete serotonergic or noradrenergic phenotype. In fully differentiated 1C11 progenies, caveolin appears to be one of the protagonists involved in PrP^C-coupling to the tyrosine kinase fyn [68]. Some cell specificity of PrP^C signaling may be related to the onset of a PrP^C-caveolin-fyn complex. In these cells, the direct control of NADPH oxidase on PrP^C-induced ERK activation, within a single transduction pathway, may be accounted for by lack of a functional PrP^C-caveolin-fyn ternary complex. By mobilizing transduction cascades controlling the cellular redox state and the ERK1/2 kinases and by altering 5-HT receptor-mediated intracellular response, PrP^C takes part in the homeostasis of serotonergic neuronal cells. These findings may have implications for future research aiming at understanding the fate of serotonergic neurons in prion diseases [69].

2.5. Participation of PrP^C to the regulation of apoptosis

The mechanism by which PrP^C contributes to prion-induced neurotoxicity is unclear. One hypothesis is that PrP^C normally serves a neuroprotective function that is abolished or subverted by interaction with PrP^{Sc} [39]. In fact, several recent experiments have uncovered a cytoprotective activity of PrP^C [89].

PrP overexpression rescues cultured neurons, some mammalian cell lines, and yeasts from several kinds of death-inducing stimuli [49, 53, 89]. Limited similarity between the Bcl-2 homology domain (BH2) of the Bcl-2 protein family members and the octapeptide repeats in the N-terminal region of PrP suggest that PrP plays a role in survival or cell death. The BH2 domain was shown to mediate Bcl-2 interaction with Bax protein and also to be responsible for Bcl-2 protection against Bax-mediated cell death. Furthermore, the C-terminal end of Bcl-2 protein interacts with PrP in a yeast two-hybrid system [47,48]. Therefore, it is possible that PrP actually acts as a member of the Bcl-2 family. Effectively, PrP^C protects against Bax-mediated neuronal apoptosis and potently inhibits Bax-induced cell death in human primary neurons. Deletion of four octapeptide repeats of PrP as well as familial D178N and T183A PrP mutations completely or partially suppress the neuroprotective effect of PrP [7]. The molecular target of PrP is unknown. PrP could directly interact with and inhibit Bax or indirectly signal Bax inhibition [89]. The presence of PrP in the cytosol indicates that a direct interaction is possible. However, PrP^C interaction with STI1 induces neuroprotective signals that rescue cells from apoptosis [114]. STI1, whose interaction with PrP^C mediates neuroprotection through a cAMP/PKA signaling pathway [114] could be the receptor. Moreover, endogenous PrP has been found to protect cultured neurons against oxidative stress, and brain tissue against ischemia, hypoxia, or trauma in vivo [75, 97]. Nevertheless, how the putative neuroprotective activity of PrP^C might be altered during prion diseases to produce a neurotoxic effect remains unknown.

On the contrary, PrP overexpression has been reported to increase the susceptibility to the apoptotic inducer, staurosporine [76, 77]. PrP toxicity may be mediated through p53 in these cells [78]. These results are in contradiction with reports indicating that the absence of PrP sensitizes cells to apoptotic insults such as serum deprivation and oxidative stress. However, many explanations can be proposed to explain this discrepancy, all of them suppose

that overexpression results in a pathological form of the protein [89].

3. SPECIFIC ROLE OF PRP^C IN THE IMMUNE SYSTEM (IS)

The relationship between prions and the IS is complex. The lack of a clear immune response in prion disease is assumed to be due to tolerance to PrP^{Sc}. Further, the IS actually contributes to pathogenesis by amplifying prion “load” in lymphoid compartments thereby facilitating efficient neuroinvasion (reviewed in [1]). This process is dependent at least partly on expression of PrP^C by immune cells and pathogenesis is related to IS integrity and functionality [11]. Despite these results, the role of PrP^C in IS has been poorly understood.

3.1. Immune phenotype in animals deleted of PrP^C

PrP^C knockout in mice has not allowed to easily conclude on a particular role of the protein in IS as in the first studies no significant differences have been found in the immunological phenotype (number of cells in the different organs, sub-population percentage in lymphoid tissues and blood), in comparison with wild-type animals [13]. However, more recent analyses of the immune system in PrP^{-/-} mice reveal fine tuned regulations. PrP^C is expressed on the surface of long-term (LT) haematopoietic stem cells (HSC) and HSC from PrP^{-/-} mice exhibited impaired self-renewal in serial transplantation of lethally irradiated mouse recipients both in the presence and absence of competitors. Furthermore, when treated with a cell cycle-specific myelotoxic agent, the animals reconstituted with PrP^{-/-} HSC exhibit increased sensitivity to haematopoietic cell depletion. It can be concluded that PrP^C supports self-renewal HSC [115]. The same kind of finding has been done on neurons (see below). Besides this role in the development of the immune system, some results suggest a complex role in the function of the immune system. Comparison of lectin-induced mitogenesis and selected cell signaling pathways in splenocytes from wild-type BALB/c and PrP^{-/-} mice has revealed that activation was significantly reduced in PrP^{-/-}

splenocytes, most prominently early in activation [64]. Activation in PrP^{-/-} splenocytes is associated with differences in the phosphorylation patterns of protein kinase C and ERK1/2. However, phosphorylation profiles are similar in wild-type and PrP^{-/-} splenocytes following PMA treatment, indicating that the ability of these two enzymes to be phosphorylated is not impaired in the absence of PrP^C. The calcium induced fluxes are equivalent in PrP^{-/-} and PrP^{+/+} splenocytes, suggesting that calcium-dependent mechanisms are not directly implicated in the differential phosphorylation patterns or mitogenic responses. PrP^{-/-} splenocytes display defects in upstream or downstream mechanism(s) that modulate PKC phosphorylation, which in turn affects its capacity to regulate splenocyte mitosis, consistent with a role for PrP^C in immune function [64].

Based on work in PrP^{-/-} mice or cells derived from them, PrP^C has been proposed to down-regulate phagocytosis by macrophages [26] but also to be involved in the phagocytic machinery used by *Brucella abortus* to invade macrophages [107], although this finding has been clearly challenged [31].

3.2. Distribution expression of the PrP^C in the immune cells

Clues to the function of PrP^C may be gleaned by examination of cell-specific expression patterns. CD34+ haematopoietic stem cells express PrP^C, but, although lymphocytes and monocytes at least maintain PrP^C expression throughout their differentiation, PrP^C is downregulated upon differentiation along the granulocyte lineage [27]. In contrast, maturation of monocytes and dendritic cells (DC) leads to PrP^C up-regulation [15]. DC display the highest expression levels of PrP^C, in both humans and mice [15, 54]. PrP^C was found to be present on murine epidermis Langerhans cells [101], on DC in extrafollicular areas, including T cell zones, of the gut mucosa [32], and DC of the splenic white pulp [15]. PrP^C was found on the surface of bone marrow-derived human and mouse DC generated in vitro in the presence of GMCSF, at levels that increased with LPS stimulation and cor-

related with that of MHC II and costimulatory molecule CD86 [3, 15]. Higher PrP^C levels were found on the surface of spleen DC that are also CD8 α +; expression of this marker has been correlated with the secretion of IL-12 and IFN- α [61]. In lymph nodes, the highest PrP^C level was observed in the CD8^{int} subset, which are strong stimulators of Ag-dependent delayed-type hypersensitivity. This was interpreted as a possible involvement of PrP^C in T cell activation leading to Th1 responses [61]. However, the mechanisms involved in T cell activation related to PrP^C expressed on DC remain to be explored.

Studies in mice show a trend towards down-regulation of PrP^C with B and T cell maturation, and mature T lymphocyte expression during quiescence is low [55]. However, in humans and sheep PrP^C expression on mature blood and lymphoid cells remains high [54], with levels increasing further with ageing [83]. This discrepancy between mice and humans suggests that the results should be interpreted cautiously.

In mature immune cells, PrP^C has been detected on human T and B lymphocytes, natural killer (NK) cells, platelets, monocytes, dendritic cells, and follicular dendritic cells [2, 4, 15, 54, 55, 83]. CD8+ cells express slightly more PrP^C than CD4+ cells and PrP^C expression may be somewhat higher in peripheral blood T cells than in B lymphocytes [83]. PrP^C expression is also higher in CD45RO+ memory compared to CD45RA+ naive T lymphocytes [28, 54]. Furthermore, PrP^C expression increases during human NK cell differentiation, with particularly high levels on CD56+ CD3+ NK cells [28].

Gene expression microarrays have revealed murine *Pmp* to be up-regulated in certain types of T cell, via a Stat6-dependent mechanism after interleukin IL-4 treatment [18].

PrP^C expression by immune cells has been rarely studied in other mammalian species, probably because humans and mice are the better studied models of TSE pathogenesis. In the hamster, however, PrP^C has been observed, in very weak quantities on the diverse peripheral blood cell sub-populations [42].

3.3. Role of PrP^C in activation of immune cells

In regards to the necessity of the cellular prion protein expression for TSE pathogenesis, particularly during replication and multiplication phases of the infectious agent in secondary lymphoid organs, the role of PrP^C has been investigated in the different cells constituting immune tissues. In these cells, two main functions of PrP^C have been demonstrated: as a membrane receptor, and as a signaling molecule.

Despite the absence of evidence of a participation of T lymphocytes in TSE pathogenesis, studies on the immune role of PrP^C have focused on this cell sub-population after the results of Cashman et al. [17]. They first observed an up-regulation of PrP^C during mitogenic activation of human T cells isolated from peripheral blood as well as an implication of PrP^C crosslink by specific antibodies in the regulation of mitogenic activation of these cells. From this time, this result has been confirmed by others, nevertheless, no more investigations have been done in this way and the finding of such a role for the prion protein in mitogenic activation of T cells remained evasive [54].

In the absence of a natural ligand for PrP^C, spatial rapprochement of the membrane proteins by specific antibodies is currently used to mimic the “physiological” engagement of the prion protein. Due to its membrane localization, this subterfuge has been used with the aim to demonstrate a potential role of PrP^C as a membrane receptor. Crosslink of PrP^C on the surface of T cell lines has been associated with various cellular responses like intracellular calcium mobilization, Src and Erk kinase activation or capping of lipidic microdomains in a large membrane zone where PrP^C colocalize with Thy-1 or fyn kinase [44, 100]. The contribution of PrP^C to the classical T-lymphocyte activation process has been characterized by clustering the T-cell receptor component CD3ε as well as PrP with soluble and surface-immobilized antibodies, respectively. PrP^C is a component of signaling structures recently described as plasma membrane microclusters established during T-lymphocyte activation. The formation of immunological synapses,

however, did not depend on the presence of PrP^C as proven by siRNA knockdown experiments, indicating a fine physiological role of PrP in vivo within the immune system [74]. These findings underscore a very subtle role of PrP during the process of human T-lymphocyte activation. Although PrP seems to be dispensable during TCR-dependent induction of immune synapse formation, stimulation of cells with surface-immobilized anti-PrP antibodies resulted in the induction of complex Ca²⁺ signaling patterns. While the amplitude of these signals resembles the Ca²⁺ pulses obtained during TCR stimulation, the frequency as well as the pattern and the duration of these traces differed profoundly. Thus cross-linking of PrP^C with surface-immobilized antibodies is sufficient to initiate several different signaling cascades. PrP^C is very likely a component of signaling membrane microdomains. Furthermore, PrP^C has obvious in vivo consequences during the activation of the immune system by regulating the ability of APC to stimulate proliferative T-cell responses [3]. A modulating function of PrP^C during the fine tuning of the lymphocyte activation process is therefore very conceivable.

3.4. The immune system during infection in sheep

Significant differences in immune cell subsets and PrP expression occur between ARQ/ARQ and ARR/ARR Suffolk sheep in the preclinical phase of scrapie infection. Following challenge, significantly more CD8(+) and gamma-delta(+) T cells were detected in the PBMC of resistant sheep. However, CD21(+) cell expression was significantly higher in the lymph nodes of susceptible sheep. In contrast, more CD4(+) cells were detected in lymph nodes of resistant sheep at both time points [29].

4. SPECIFIC ROLE OF THE PRP^C IN THE CENTRAL NERVOUS SYSTEM (CNS)

PrP^C is highly expressed in CNS, and since this is the major site of prion pathology most interest has focused on defining the role of PrP^C in neurons.

4.1. Functions suggested by localization

The localization of PrP^C within the CNS is a key step to know the potential structures liable to be affected by the scrapie agent. Moreover, the study of the chemical nature of the PrP^C-containing neurons is fundamental to understand both the biology of PrP^C in vivo and the pathophysiology of PrP^{Sc}.

The fact that the protein is expressed in neurons at higher levels than in any other cell type suggests that PrP^C has special importance in neurons. Additionally, PrP^C is highly concentrated at the synapse [5] and there is evidence for intense localization not only at CNS synapses but also at endplates [33]. Furthermore PrP^C expression has been shown both presynaptically and postsynaptically [41, 91]. Evidence exists for specific axonal transport of different PrP^C glycoforms suggesting that perhaps one glycoform or another might be specifically presynaptic [88]. PrP^C can be isolated in detergent insoluble rafts, and there is evidence that such rafts might represent specialized areas of synaptic membrane that might in turn give rise to caveolae-like domains. The full details of the specialized association of PrP^C with the synaptic membrane have not yet emerged but it is probable that two forms of PrP^C exist in neurons: synaptic and non-synaptic.

Cellular prion protein is present in all cortico-cerebellar and deep nuclei neuronal cell types, as well as in all glial cell types of the rat cerebellum [50]. The protein is exclusively located on the outer cell membrane and in Golgi and endosomal intracytoplasmic organelles, with no cytoplasmic or synaptic vesicle labeling. Most importantly, the cellular prion protein is distributed on all portions of neurons, without any preferential synaptic targeting. The ubiquitous presence would support the notion that the prion protein has a generalized cellular function in brain tissue rather than a specialized role restricted to synaptic transmission. However, the labeling of glial cells is challenged by the careful study of Ford et al. [32].

4.2. A role for PrP^C at synapses?

Several experimental observations suggest that PrP^C could play a role in synaptic struc-

ture, function or maintenance. Light and electron microscopic studies indicate that PrP^C is preferentially concentrated along axons and in presynaptic terminals [66, 70]. In addition, PrP^C is subject to anterograde and retrograde axonal transport [6, 71]. Incubation of cultured hippocampal neurons with recombinant PrP induces rapid elaboration of axons and dendrites, and increases the number of synaptic contacts [45]. This result suggests that PrP^C could play a regulatory role in synapse formation. Electrophysiological recordings from brain slices of PrP^{-/-} mice also support a functional role for PrP^C in synaptic transmission. It has been reported, in hippocampal slices from PrP^{-/-} mice, that long term potentiation was impaired [23]. PrP^{-/-} mice have been reported to display several other neurobiological abnormalities that may also relate to the participation of PrP^C in synapse formation and function. These include alterations in nerve fiber organization [22], circadian rhythm [104], and spatial learning [24]. Conditional PrP knockout mice have shown that significant reduction of after hyperpolarization potentials in hippocampal cells was observed, suggesting a direct role for PrP in the modulation of neuronal excitability. These data provide new insight into PrP functions. Furthermore, studies have demonstrated a positive correlation between the expression level of PrP^C and the overall strength of glutamatergic transmission in the hippocampus, with PrP-over-expressing mice exhibiting supra-physiological responses [16]. At least part of this effect seems to result from more efficient recruitment of presynaptic fibers as the level of PrP^C increases, correlating with reduced after-hyperpolarization seen in hippocampal CA1 neurons of PrP^{-/-} mice [22]. Reduced after-hyperpolarization was observed in pyramidal neurons regardless of whether the PrP gene was deleted pre- or post-natally [59]. All these data are strong evidence for a functional role for PrP^C in modulating synaptic transmission.

4.3. Phenotype of animals KO for PrP^C

Attempts to deduce the function of PrP^C from the phenotypes of PrP-null mice had been long time unrewarding, since the lines of

these mice in which the adjacent Doppel (Dpl) gene is not artifactually upregulated display no major anatomical or developmental deficits [13, 60], but the mice are resistant to scrapie infection [14].

Nevertheless, recent findings show that mice devoid of the PrP gene present fine morphologic or phenotypic alterations. The PrP gene seems to play a role in sleep and in the circadian rhythms [104]. One must bear in mind that one of the inherited prion diseases consists of a profound alteration in sleep and the daily rhythms of many hormones [92].

Morphologic alterations have also been detected. Stained hippocampal sections from the PrP^{-/-} mice had more granules than the wild type in the following: the granule cell layer, the inner molecular layer of the dentate gyrus, and the infrapyramidal region [22]. This resembles the mossy fibre collateral and terminal sprouting seen in certain epilepsies. The abnormal connectivity might be predicted to promote epileptiform activity, but extracellular electrophysiological recordings from the granule cell layer reveals a reduced excitability in the PrP-null group, both with and without blockade of GABA receptor-mediated inhibition. It was proposed that reorganization of neuronal circuitry is a feature of PrP^{-/-} mice [22].

In one study [109, 110], infarct volumes were measured in wild-type and PrP^{-/-} mice that had been subjected to focal cerebral ischemia. It was found that PrP^{-/-} mice displayed significantly larger infarct volumes, demonstrating a protective role for PrP^C in response to brain injury.

Furthermore, it was demonstrated later on the Zurich PrP^{-/-} mice [13] that they develop an age-dependent impairment in memory consolidation which only becomes detectable in animals that are at least nine months old [19]. Criado et al. [24] observed that a second strain of PrP^{-/-} (the Npu mouse strain [60]), in either a pure 129/Ola background or a mixed 129/Ola×C57-Bl/10 background exhibited impaired hippocampal dependent spatial learning, while non-spatial learning remained intact. These deficits are rescued when PrP^C is selectively re-expressed in neurons, indicating that they are caused by PrP^C loss-of-

function in neurons [24]. In addition, as mentioned above, post-natal PrP^{-/-} mice exhibit reduced after-hyperpolarization potentials in hippocampal cells, a finding that suggests that PrP^C may directly modulate neuronal excitability [59]. Furthermore, we have seen in the first chapter that STI1 is a specific PrP^C ligand that promotes neuroprotection of retinal neurons through cAMP-dependent protein kinase A [114]. Both PrP^C and STI1 are abundantly expressed and highly colocalized in the hippocampus in situ, indicating that they can interact in vivo. Recombinant STI1 added to hippocampal cultures interacts with PrP^C at the neuronal surface and elicits neuritogenesis in wild-type neurons but not in PrP^C-null cells. This effect is abolished by antibodies against either PrP^C or STI1 and is dependent on the STI1 domain that binds PrP^C [56]. Binding of these proteins induced the phosphorylation/activation of the mitogen activated protein kinase, which is essential for STI1-promoted neuritogenesis. STI1, but not its counterpart lacking the PrP^C binding site, prevents cell death via PKA activation. These results demonstrate that two parallel effects of the PrP^C-STI1 interaction, neuritogenesis and neuroprotection, are mediated by distinct signaling pathways [56]. Furthermore, STI1 interaction with PrP^C affects short-term memory (STM) formation and long-term memory (LTM) consolidation [21]. Blockage of PrP^C-STI1 interaction with intra-hippocampal infusion of antibodies against PrP^C or STI1 immediately after training impaired both STM and LTM. Furthermore, infusion of PrP^C peptide 106-126, which competes for PrP^C-STI1 interaction, also inhibited both forms of memory. Remarkably, STI1 peptide 230-245, which includes the PrP^C binding site, had a potent enhancing effect on memory performance, which could be blocked by co-treatment with the competitive PrP^C peptide 106-126. These results demonstrate that PrP^C-STI1 interaction modulates both STM and LTM [21]. Since PrP^C interactions seem to be essential to many biological phenomena related to cell signaling, neural plasticity and memory consolidation, it is not surprising that alterations in this protein should affect cognitive processes. Indeed,

recent reports in humans indicate that PrP^C polymorphisms at codon 129, a site that is highly important for the protein structure [80, 94], affect cognition and LTM [79].

The diversity of phenotypes in PrP^{-/-} mice suggests either that PrP^C plays a widespread role in the normal function of membrane proteins, or that it affects events earlier in development, having a wide variety of non-lethal consequences. This noticing is corroborated by recent works on the role of PrP^C on the proliferation of neurons during development. Indeed, *in vivo* and *in vitro* studies have found that PrP^C is expressed in multipotent neural precursors and mature neurons. Loss and gain-of-function experiments demonstrate that PrP^C levels correlate with differentiation of multipotent neural precursors into mature neurons *in vitro* and that PrP^C levels positively influence neuronal differentiation in a dose-dependent manner. PrP^C also increases cellular proliferation *in vivo*. PrP^C overexpresser mice have more proliferating cells compared with wild-type or PrP^{-/-} mice implying that PrP^C plays an important role in neurogenesis and differentiation. However, because the final number of neurons produced is unchanged by PrP^C expression, other factors must control the ultimate fate of new neurons [99].

4.4. Neurotoxicity of partially deleted prion protein

Various PrP mutations have given rise to pathological phenotypes when overexpressed in mice. For example, PrP^{-/-} mice expressing PrP with deletions of the amino acid 121 or 134 develop severe ataxia and apoptosis of the cerebellar granule cell layer as early as 1–3 months of age [95]. Neurons in the cortex and elsewhere express truncated PrP at similar levels as granule cells but do not undergo cell death, arguing against an unspecific toxic effect [111]. The pathological phenotype is completely abolished by the introduction of a single wild-type *Prnp* allele. When the truncated PrP is specifically targeted to Purkinje cells of PrP^{-/-} mice, ataxia and Purkinje cell degeneration develop, while the cerebellar granule layer remains unaffected [30]. Mice expressing PrP with the octare-

peats (PrP Δ 23–88) deleted or even, in addition, lacking α -helix-1 and β -sheet-2 (Δ 141–176, “PrP106”) remained healthy. Wild-type mice transgenic for murine PrP23–230, which promotes accumulation of cytosolic PrP, develop severe ataxia, with cerebellar degeneration and gliosis [57] although it is not the case in culture cells [25]. The mice expressing a PrP Δ 105–125 spontaneously developed a severe neurodegenerative illness that is lethal within one week of birth in the absence of endogenous PrP [113]. This phenotype was reversed in a dose-dependent fashion by coexpression of wild-type PrP. This phenotype is reminiscent of those described in mice that express PrP harboring larger deletions.

The results have been explained by a model in which truncated PrP^C acts as dominant negative inhibitor of a functional homologue of PrP^C, with both competing for the same putative PrP^C ligand [95].

4.5. Phenotype of KO cattle

The ability to genetically engineer animals has become a standard laboratory tool for physiological, genetic, and biomedical research. However, mice represent the vast majority of transgenic animals produced to date. Additional animal models are also of critical importance for physiological and medical research because mice are not completely representative of livestock animals or human physiology. For example, the limited life span and small size of the mouse restrict its usefulness in studies requiring long-term evaluation of test subjects. Thus PrP knockout in animals naturally subject to prion disease represents a very interesting approach for the elucidation of PrP^C function and prion pathogenesis. In 2006, suppression of the prion protein in the goat thanks to RNA interference have been realised by Golding et al. [35] but the phenotype is not yet published. However, recently, generation of PrP^{-/-} cattle has been made [86] by a sequential gene-targeting system. PrP^{-/-} cattle do not present obvious abnormalities from birth to 20 months of age confirming in some ways the results obtained with mice. We do not yet know if the PrP^{-/-} cattle is resistant to the prion.

4.6. Other function observed in the CNS

Cross-linking of PrP^C in vivo with specific monoclonal antibodies was found to trigger rapid and extensive apoptosis in hippocampal and cerebellar neurons. These findings suggest that PrP^C functions in the control of neuronal survival and provides a model to explore whether cross-linking of PrP^C by oligomeric PrP^{Sc} can promote neuronal loss during prion infection [96].

5. CONCLUSION

Understanding the normal function of PrP^C has important implications for the therapy of prion disorders. At present, most therapeutic strategies are directed at inhibiting the formation of PrP^{Sc}. If alterations in PrP^C function play an important role in prion-induced pathology, then an alternative approach is to target the cellular pathways mediating the biological actions of PrP^C. In this regard, it might be possible to use the physiological activity of PrP^C to develop in vitro assays to screen for drugs that have therapeutic potential. In addition, if the toxicity of PrP^{Sc} is partly attributable to a loss of PrP function, then over-expression of wild-type PrP may represent a strategy for suppressing the disease phenotype. Conversely, reduction of PrP expression, a strategy that has been proposed for preventing or treating prion diseases, may have detrimental consequences due to loss of the neuroprotective activity of PrP^C [113].

Acknowledgements. This work was supported by grant FP6-023144 from the European Union.

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