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Review

Scavenger, transducer, RNA chaperone? What ligands of the prion protein teach us about its function

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Abstract. Prion protein, a misfolded isoform of which is the essential component of the agent of prion diseases, still remains an enigmatic protein whose physiological functions are at best hypothetical. To gain a better insight into its putative role, many studies were undertaken to look for molecules that bind prion protein, and have notably identified divalent metal ions, several proteins, and nucleic acids. At first sight, the diversity of prion protein's ligands seems of little help to infer a plausible function. However, the intrinsically disordered property of its N-terminal tail and the potential of the protein to adopt a transmembrane topology, can both be taken into account to predict its different states during its cellular cycle and its possible functions, of which the most promising correspond to a general scavenger, a sensor or adaptor in a signaling cascade, and an RNA chaperone.

Keywords. Prion protein, ligands, copper, transmissible spongiform encephalopathy, prion function.

Introduction

Transmissible spongiform encephalopathies (TSE) are a peculiar class of transmissible diseases, which notably include scrapie of small ruminants, bovine spongiform encephalopathy, chronic wasting disease of wild and captive ruminants, kuru and Creutzfeldt-Jakob disease in humans. Prion protein (PrP) was identified as the major component of the purified infectious agent, in the form of a protease-resistant polypeptide with an apparent molecular size of 27–30 kDa [1]. It is chromosomally encoded as a cellular, non-pathological protein PrP^{C}, while the infectious agent essentially contains its misfolded "scrapie" isoform PrP^{Sc}. According to the prion hypothesis, all the information characterizing the agent is contained in the pathological three-dimensional structure of PrP^{Sc} [2, 3]. This model is supported by a large body of data [4, 5], although it does not yet provide a complete understanding of the infection process, strain diversity and adaptive properties of the agent [6].

PrP^{C}, a ~255-residue protein (Fig. 1), is expressed predominantly in neurons and to a lesser extent in some non-neuronal tissues [7, 8]. It is synthesized at the rough endoplasmic reticulum (ER) and traffics through the Golgi to the outer cellular membrane surface, to which it is anchored by a glycosylphosphatidylinositol (GPI) moiety [9]. Its N-terminal signal peptide is co-translationally cleaved during its translocation to the ER lumen, while a 20-residue C-terminal peptide is removed during the post-translational addition of the GPI anchor at serine 231 [10]. Two oligosaccharide groups are linked to asparagine residues 181 and 197 (mouse PrP numbering), and a disulfide bridge is established between cysteines 179

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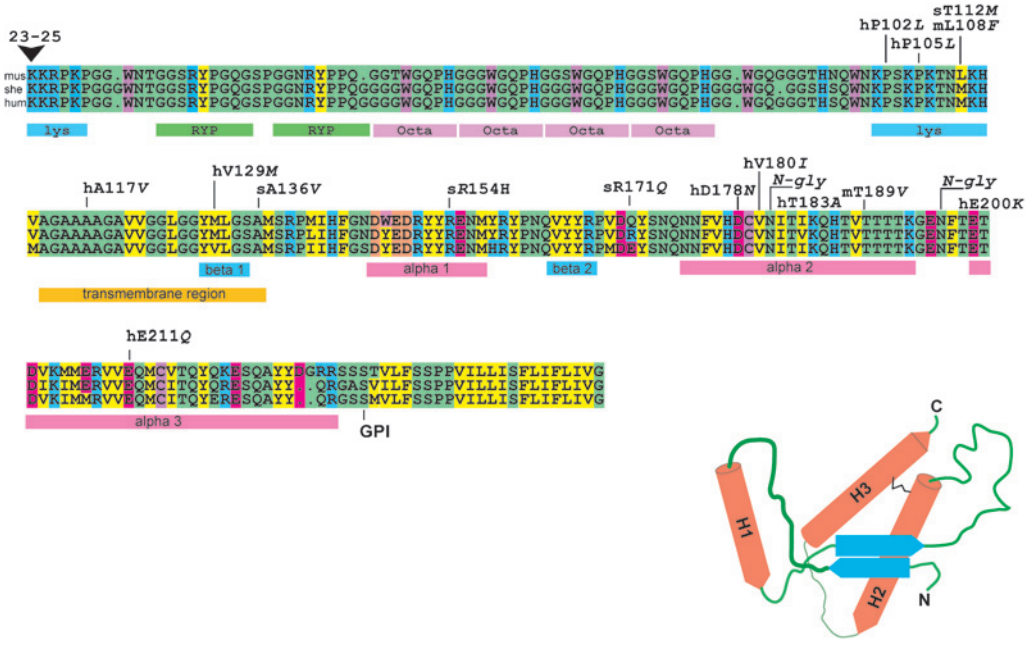

Figure 1. Color-coded alignment of prion proteins (PrP). Peptide sequences of murine (top), sheep ARR (middle) and human Val129 prion proteins were aligned, to emphasize the high sequence conservation. N-terminal signal peptides were omitted. Amino acids are color-coded as follows: small, uncharged, polar and proline residues are green (G, A, N, S, T, Q, P), hydrophobic residues are yellow (Y, F, V, I, L, M), basic residues are blue (R, K, H), acidic residues are red (E, D), and rare residues purple (W, C) [151]. The high proportion of green residues in the N-terminal part is a hallmark of intrinsically unstructured regions. Color boxes underneath correspond to characteristic repeated modules (first line) or secondary structure elements: lysine clusters, nonapeptide repeats, octapeptide repeats, β-strands and α-helices. Polymorphic residues associated with familial diseases or susceptibility to prion diseases are indicated above the alignment. Since numbering varies between species, these are denoted as follows: the first letter (lowercase), indicating the species, is followed by the common amino acid at the given position, followed by the susceptibility-linked residue (thus, hP102L is proline 102 in human PrP, which is replaced by leucine in some familial prion diseases). *N*-Gly are the two *N*-glycosylation sites. Right corner: structure of recombinant PrP [173, 174]: the 121-231 peptide sequence folds in three α-helices and a short anti-parallel β-sheet.

and 214, the only two cysteine residues of the mature protein.

At the cellular membrane, PrP^C, together with other GPI-anchored proteins, is localized in specialized domains known as lipid rafts, which are rich in cholesterol and sphingomyelin [11–13]. Membrane-bound PrP^C can undergo internalization and recycling through the endosomal pathway [14]. Alternatively, it can be degraded in the lysosomes after internalization [15], or shed from the cell surface as a soluble molecule after cleavage of its GPI moiety [9, 16]. Depending on the cell type in which it was studied, PrP^C has an observed half-life of 3–6 h [16–18]. These are thought to be the major steps in the metabolism of PrP (reviewed in [19, 20]). However, beside the major GPI-anchored, membrane-bound form, a fraction of the PrP molecules may be found in distinct topologies, notably transmembrane [21–23] and cytosolic forms [24]. A hydrophobic segment of PrP encompassing residues A113–S135 (mouse PrP) is a potential membrane-spanning domain that accounts for the synthesis of distinct topological isoforms of PrP at the ER: in Ctm-PrP, the C-terminal half is luminal and the N-terminal end is cytosolic, while the reverse is true of Ntm-PrP [25].

Knowledge of the function of PrP would certainly help in understanding the processes involved in both the multiplication of the infectious agent and the neuronal damage leading to the neurodegeneration observed in TSEs. Since the lack of PrP^C in $PrP^{0/0}$ animals does not lead to obvious physiological defects that could provide clues as to its role, knowing its ligands could hopefully shed some light on its possible functions.

Our goals in the present review are (i) to list all the published PrP ligands, together with the rationale of the studies and the methods used for their identification and confirmation, (ii) to identify the effects of the interaction for both PrP and its ligand, and (iii) to take into account the properties of PrP^C and its topology, to propose possible functions for the PrP^C.

Copper(II) ions, and other metal ligands of the PrP

Based on the observation that the octapeptide repeat of PrP shares with histidine-rich glycoprotein (HRG) the tripeptide motif PHG, it was proposed that PrP, like HRG, might bind copper [26]. Indeed, peptides composed of three or four octapepetide repeats of the PrP were shown to bind various divalent metal ions

(zinc, manganese, cobalt, nickel, copper), with a strong preference for copper and a K_d close to 5 μM for that metal. Similar properties were observed with recombinant Syrian Hamster PrP (recShaPrP), which was shown to bind about two copper(II) ions per molecule with micromolar affinity [27]. Copper binding to PrP^C *in vivo* was also indicated by measurements of the weight content of copper and other metals in tissues from wild-type and PrP-knockout mice [28].

PrP also binds nickel, zinc or manganese, albeit with a much reduced affinity [29–31]. In addition, some authors found that the metal occupancy of PrP^{Sc} by copper, zinc or manganese might be strain specific [32, 33]. Besides binding of PrP to soluble metal ions, it has also been observed that prion infectivity adsorbs strongly to stainless steel [34].

Affinity, stoichiometry and binding site

Because both recPrP and PrP-derived peptides have been used in interaction studies using various monitoring methods, a wide range of values has been obtained for the affinity and stoichiometry of the binding reaction [35–37]. However, the most recent studies indicate affinities in the femto- to nanomolar range, depending on the binding site, and four to six copper atoms per protein, *in vitro,* depending on the pH [37, 38]. As for the binding site, the prevailing view is that copper binds primarily to the histidine residues within octapeptide repeats [37]. However, histidine residues 111 and 96, outside the octarepeat region, were also shown to bind copper with high affinity [31, 38, 39]. The Cu^{2+} ion might be held in place through multiple modes of coordination with the imidazole nitrogen atom from one or more histidine residues [39, 40], amide nitrogen atoms from the main chain and possibly the indole nitrogen atom from tryptophan residues within octarepeats [36]. Binding of the first copper ions induces structural organization of the PrP, which in turn facilitates binding of additional copper ions, and results in cooperative binding [36].

Metal-induced structural changes

Binding of copper induced structural modifications in synthetic peptides corresponding to various parts of the 57–115 region of PrP [26, 39]. Similarly, copper binding to the N terminus of PrP was found to trigger its structural organization [36], or facilitate the temperature-induced formation of β-sheet structures in recombinant ShaPrP [27]. It has been observed that different metal ions induce distinct structural changes in PrP [29, 41]. For instance, some authors found that manganese, but not copper, induced polymerization of PrP into fibrils [42, 43] or rendered it partially resistant to proteinase K digestion [29]. Furthermore, occupancy of the protein by distinct metal ions was also shown to determine strain-specific conformations of PrP^{Sc} [32, 33]. These data, combined to the observation of an altered metal-ion metabolism in the course of the disease [33, 44], suggest that metal-ion occupancy of PrP plays a central role in both the pathogenesis and phenotypic diversity of prion diseases. However, the hypothesis that was once proposed, of a link between scrapie incidence and the levels of trace elements in soil was refuted by a careful analysis, which failed to evidence any such correlation [45].

Proposed functions linked to the metal-binding properties

Copper and zinc transport. Transport of copper across the intestinal epithelium, in blood, and finally into cells, involves numerous specialized proteins, which include hCtr1, hCtr2 and Nramp2 for intestinal absorption, ceruloplasmin and transcuprein for transport, ATP7A and ATP7B for subcellular distribution (reviewed in [46]). Similarly, more than 20 specialized zinc transporters of the ZnT and ZIP families have been described in human cells [47]. In addition, many other nonspecific metal chelators also operate in various cellular or extracellular compartments.

The observation that PrP binds cooperatively to copper ions within their physiological range of concentration suggests that it could play a specialized function in the metabolism of copper and other metal ions. This is also supported by the changes in metal balance that were observed in tissues from diseased animals or from patients with Creutzfeldt-Jakob disease [33, 44, 48]. Since it is primarily expressed in the central nervous system (CNS), PrP^C has been proposed to act in copper or zinc homeostasis in that tissue [49]. Indeed, both copper and zinc, at high micromolar concentrations (100–250 μM) were found to induce endocytosis of PrP^C to early endosomes and Golgi compartments of cultured neuroblastoma cells [49–51]. Copper uptake was also observed in cultured astrocytes expressing PrP [52]. However, this activity may depend on the cell type used, or require additional factors, since overexpression of PrP in non-neuronal cultured cells failed to demonstrate a direct role for PrP in copper uptake and homeostasis [53, 54]. More specifically, PrP^C was proposed to regulate copper level at the synapse [55, 56], for instance by sequestering the copper and zinc ions in excess. This could have biological relevance in some cells or subcellular compartments with high concentrations of the two metal ions. Notably, copper and zinc are concentrated at the presynaptic terminals of glutamatergic synapses, from which they are released after depolarization, transiently reaching values as high as

100 μM or more in the synaptic cleft [57]. Therefore, a membrane-bound, synaptically localized PrP^C [58, 59], could capture copper and zinc ions in the synaptic cleft and take part in their recycling through endocytosis, guiding them towards specialized transmembrane transporters. Release of copper ions and their transfer to specialized transporters could occur in acidic endosomes, based on the pH sensitivity of copper binding by PrP, and it has been proposed that PrP could reduce captured Cu(II) ions and transfer them to Cu(I)-specific intracellular copper trafficking proteins [60]. However, this scenario still awaits experimental confirmation, and a recent study found no relation between PrP^C and copper uptake in isolated mouse synaptosomes [61].

Copper-dependent enzyme activity. Copper and zinc are essential cofactors for many enzymatic activities, including cytochrome c oxidase, Cu/Zn superoxide dismutase (SOD), metallothioneins, ceruloplasmin, tyrosinase and numerous metalloproteinases [46, 62]. Both recombinant and tissue-purified PrP were observed to exhibit an SOD activity that was relative to the amount of bound copper [63, 64], suggesting that impairment of an SOD function may contribute to neurodegeneration in prion diseases. Appealing though it may be, this view was challenged by independent studies that failed to evidence any PrP-specific SOD activity [65, 66].

Copper sensing. With K_d values in the femto- to nanomolar range associated with its four to six Cu-binding sites [30, 37, 38], PrP has the potential to respond to a large range of copper concentrations, and could therefore act as a sensor for divalent metal ions. The latter could act by inducing structuration of the N-terminal part, or by controlling its self association [39]. This in turn could trigger a cascade of events, which could include binding to, and activation of, other PrP ligands, or assembly of a molecular complex, somewhat similar to the zinc-dependent scaffold assembly that was recently described at the postsynaptic density [67]. On the other hand, PrP^C might play an indirect role by linking copper metabolism with other cellular functions, similar to the recently described function of X-linked inhibitor of apoptosis (XIAP). Binding of copper to XIAP was shown to induce structural changes in the protein and favor its degradation. This in turn rendered cells more susceptible to apoptosis, thus linking copper homeostasis to the regulation of cell death [68].

Protein partners of PrP^C

Protein ligands of PrP were found through various methods, from screening of cDNA expression libraries to cross-linking methods and affinity pull-down assays (Table 1). A fraction of them have been further characterized or confirmed by complementary methods. Among these protein ligands, there is no recognizable linear sequence pattern that could help in building a PrP-interaction network [69]. Candidates include both intra- and extracellular proteins.

Membrane-anchored extracellular proteins and secreted proteins

Beside its capacity to self-associate and form dimers or various oligomers [70, 71], PrP was found to bind several membrane-associated proteins.

N-CAMs, caveolin. Transcardiac perfusion of paraformaldehyde was used to cross-link PrP^C-interacting proteins in the brains of mice [72], and identified GPI-anchored proteins and neural cell adhesion molecules (N-CAM). A substantial fraction of these were found to harbor fibronectin type III or immunoglobulin C2 domains, to which recombinant mouse PrP was shown to bind *in vitro* [73]. Interaction of PrP with N-CAM was shown to involve its N-terminal part and first helix (residues 144–154), while its carbohydrate moieties did not seem to contribute significantly [73]. It is likely that the GPI anchor dictates the distribution of PrP^C in specialized lipid rafts, leading it to interact with only a subset of GPI-anchored proteins and other raft-associated proteins [72]. A similar co-localization is also probably responsible for the interaction of PrP^C with caveolin, allowing the caveolin-1-dependent coupling of PrP^C to Fyn, a membrane-anchored tyrosine kinase [74].

Laminin receptor. The 37-kDa LRP, the precursor of the 67-kDa laminin receptor (LR) and also a receptor for Sindbis virus on mammalian cells, was identified through a yeast-two hybrid (Y2H) screen of a HeLa cell cDNA library [75]. The same system, using a LexA-GST-PrP fusion as a bait, had previously identified Hsp60 [76]. Interaction of PrP with LRP was confirmed in transfected COS-7 cells expressing the two proteins. The 67-kDa LR is expressed in the cytoplasm and at the plasma membrane of most neurons and in some glial cells, while its 37-kDa precursor is only expressed by a subpopulation of interneurons [77]. PrP residues 143–179 interact directly with LRP, while its octarepeats (residues 53–93) constitute a second domain to which LRP binding is dependent upon the presence of heparan sulfate proteoglycans (HSPG) [78]. Reciprocally, LRP/LR

Table 1. Protein ligands of prion proteins (PrP). The methods, as well as the models, tissues and cell lines used in the different studies are listed. When appropriate, binding sites are indicated, either as amino acid residues, or as structural regions

PrP partner	Cell, tissue, organism	Method	Binding site, PrP	Reference (no.)
N-CAMs	N2a neuroblastoma cells	Formaldehyde crosslinking	N terminus, helix I, loop	73
N-CAMs, and other CAMS	Mouse brain	Formaldehyde crosslinking (transcardiac perfusion)		72
14-3-3	Differentiated neurons	Protein overlay, MS	23–137	90
Hsp60	HeLa cell cDNA library	Yeast two-hybrid (LexA-GST-PrP)	180–210	76
37-kDa laminin receptor precursor	HeLa cell cDNA library	Yeast two-hybrid (LexA-GST-PrP)	143–179 (LRPbd1), and 53–93 (LRPbd2)	75, 78, 79
Laminin		*In vitro*, purified proteins		172
NF-E2 related factor 2 Amyloid precursor-like protein 1 Organ of Corti protein 1 Four putative PrP ligands (Pli3-5 and 8)	Mouse brain cDNA library	Library screen with PrP-alkaline phosphatase fusion protein		105
Synapsin Ib Grb2 Pint1	Mouse brain cDNA library	Yeast two-hybrid (Gal4-PrP)	N- and C- terminus N- and C- terminus	102
Stress-inducible protein 1		Complementary hydropathy	113–128	94, 95, 96
Bcl-2	Mouse cerebellum cDNA library	Yeast two-hybrid (LexA-Bcl-2)		97
Dystroglycan	Hamster brain homogenate	Co-immunoprecipitation		81
BiP	Transfected human neuroblastoma cell line M17	Co-immunoprecipitation		91
NRAGE	Rat brain cDNA library	Yeast two hybrid (Gal4-PrP)		100
Plasminogen	Serum	Pull-down assay	N-terminal (lysine clusters)	83, 86
Apolipoprotein B	Human plasma	PrPsc capture		111
GFAP (Pli45) Pli 110	Hamster brain homogenate	Ligand blot		104
hnRNP A2/B1 Aldolase C	Mouse brain homogenate	Far-Western immunoblot and MALDI-TOF		106

was also found to harbor both a direct PrP-binding site (residues 161–179) and a HSPG-dependent PrP binding site (residues 101–160 or 181–285). LRP/LR was shown to act as a receptor for both PrP^{C} [79] and PrP^{Sc} [80].

Dystroglycan. PrP^{C} was observed to co-localize with neuronal nitric oxide synthase (nNOS) in the molecular layer of hamster cerebellum, as a result of its strong association with β-dystroglycan [81]. This raft-localized transmembrane protein is part of the dystrophin-glycoprotein complex, which provides a connection between the extracellular matrix and the cytoskeleton (reviewed in [82]). It may therefore serve as a link between PrP^{C} and intracellular proteins. These include dystrophin Dp71, syntrophin, nNOS, α-tubulin, glutamic acid decarboxylase and presynaptic protein synaptophysin, which were all found to co-immunoprecipitate with PrP^{C} under mild conditions that preserved the integrity of lipid rafts.

Plasminogen. Several blood proteins, notably fibrinogen, plasminogen, antithrombin III and factor IX were found to bind specifically to PrP^{Sc} or PrP 27–30 and not to PrP^{C} [83]. Of these, only plasminogen was able to bind full-length PrP^{Sc}, as well as prion infectivity, in the absence of Ca^{2+} ions. Plasminogen is thought to bind through its kringle domains to the N-terminal region of PrP [84]. Kringle domains, of which plasminogen contains five repeating units, are 80-residue peptides that are responsible for interactions of plasminogen and plasmin with substrates, inhibitors and regulators of plasminogen activation [85], which notably include chloride ions and lysine-rich peptides. Binding of plasminogen to PrP^{Sc} was proposed to depend on the highly ordered aggregates

of the disease-associated isoform, since it was no longer observed under denaturing conditions [83, 86]. This property was subsequently used to selectively immunoprecipitate PrP^{Sc} and facilitate TSE diagnosis [86]. However, the specificity towards PrP^{Sc} was challenged by subsequent studies in which both recombinant PrP and tissue-purified full-length PrP^{C} were shown to bind plasminogen *in vitro* [87, 88]. Furthermore, the proposal that binding of plasminogen to PrP^{Sc} might play a role in pathogenesis [83] was questioned by the observation that this interaction required specific combinations of detergents [88].

Intracellular proteins

Chaperones: Hsp60, BiP and STI1. PrP-binding partners comprise at least three protein chaperones. Hsp60 was identified as the major PrP target in a Y2H screen of a HeLa cell cDNA library [76]. The interaction was verified through an *in vitro* pull-down assay using the two recombinant proteins. In what subcellular compartment this interaction could take place *in vivo* was not identified, and is still unclear: although minor fractions of Hsp60 were observed at the cell membrane or associated with the ER, this chaperone is mainly localized to mitochondria [89]. Interestingly, both Hsp60 and PrP^{C} were found to co-immunoprecipitate with the 14-3-3 protein from a brain protein extract [90], suggesting that the three proteins interact under physiological conditions.

BiP is a resident chaperone of the ER. In the ER, it was found to specifically sequester a mutant form of PrP^{C} (Q217R) that lacked the GPI anchor, until it was degraded in the proteasome [91]. This is probably part of the ER quality control system; the finding was corroborated by an independent study showing that mutated forms of PrP^{C} are retained in the ER [92]. A third chaperone candidate was found through application of the complementary hydropathy theory, which states that complementary DNA strands encode peptide segments that have the potential to associate because of their inverted and complementary hydropathy profiles [93]. Based on that theory, Martins et al. [94] generated a hypothetical peptide complementary to prion peptide 114–129. A serum raised against this peptide recognized a 66-kDa protein that bound PrP^{C} and was later characterized as stress-inducible protein 1 (STI1, STIP1), a heat shock protein [95]. The interaction sites were mapped to PrP residues 113–128, *i.e.*, its transmembrane domain, and STI1 residues 230–245. Although STI1 is mostly cytoplasmic, a fraction of it is also present at the cell membrane, where the interaction with PrP^{C} is thought to occur. Moreover, both STI1 and a peptide derived from it exhibited a neuroprotective effect that was dependent upon PrP^{C} expression [95]. In a model of murine neuronal cells, Sakudo et al. [96] demonstrated that PrP^{C} was associated with STI1 *in vivo*, and showed that this interaction up-regulated an SOD activity. These authors proposed that this up-regulation accounted for the anti-apoptotic effect of PrP^{C} observed in some cell lines.

Other molecules. Of the following candidates, Bcl2, NRAGE, Grb2, Pint1 and synapsin 1b were isolated through various Y2H systems (see Table 1), while the others were mostly found *in vitro* by the ligand blot or related methods. They were not subjected to further investigations, but could nevertheless have biological relevance.

Interaction of PrP with Bcl-2 was proposed to support the neuroprotective effect of this oncoprotein [97]. However, Rambold et al. [98] showed that the observed toxicity of cytosolic PrP [99] resulted from coaggregation of the cytosolic, misfolded PrP with anti-apoptotic molecule Bcl-2. Increased expression of chaperones Hsp70 and Hsp40 prevented the formation of PrP/Bcl-2 coaggregates and interfered with PrP-induced apoptosis. In a similar manner, PrP^{C} was shown to interact with a repeat region within the neurotrophin receptor interacting MAGE homolog (NRAGE), and it was suggested that an excess of cytosolic PrP^{C} could be toxic to some neuronal cells, by preventing the normal interaction of NRAGE with its many physiological partners [100]. The toxicity of cytosolic PrP^{C} probably depends on the cellular context, since it was not always observed. Artificial targeting of mutated PrP to the cytosol [98], or treatment of cells with a proteasome inhibitor [99], both resulted in the accumulation of a toxic cytosolic PrP. However, this toxicity was not observed in other studies [101], and cytosolic PrP^{C} has also been described in some neuronal cells under physiological conditions [24]. Murine growth factor receptor-bound protein 2 (Grb2), synapsin Ib, and a previously unknown prion interactor 1 (Pint1) were identified through a Y2H screen and were found to co-immunoprecipitate with PrP when expressed in mammalian cells [102]. Their subcellular co-localization in neuronal cells, together with the fact that synapsin I is a known ligand of Grb2 [103], gives weight to the possibility of their physiological interaction *in vivo*. This suggests a possible role of PrP^{C} in a signaling pathway, since synapsin I and Grb2 are involved in extracellular and intracellular signaling, respectively. In the ligand blot technique used by Oesch et al. [104], proteins from a hamster brain homogenate were separated through a two-dimensional gel electropho-

resis and probed with a detergent-solubilized radioiodinated PrP^{Sc}. The two major ligands thus identified were glial fibrillary acidic protein (GFAP), and an uncharacterized Pli 110. Six other "Plis" (Pli3–Pli8) were identified [105] through screening a mouse brain cDNA library with an alkaline phosphatase-PrP fusion protein. Three of the six candidates contained partial open reading frames from annotated proteins: organ of Corti protein 1, mouse p45 NF-E2-related factor and amyloid precursor-like protein 1. Interaction of PrP with these putative ligands was not confirmed *in vivo* or *in vitro*. A conceptually similar "far-Western" method allowed the identification in the mouse brain of heterogeneous nuclear ribonucleoprotein A2/B1 (hnRNPA2/B1), aldolase C [106] and casein kinase 2 [107], of which the latter had its *in vitro* activity enhanced by full-length recombinant PrP.

Lipids

Although lipids are not considered as PrP ligands, PrP^{C} is mostly associated with membranes, from its synthesis to its subcellular localization. First, its GPI anchor dictates the localization of PrP^{C} to membrane micro-domains known as detergent resistant membranes (DRMs), or lipid rafts [108]. Studies in cultured rat astrocytes showed that the majority of cellular PrP is associated with a detergent-insoluble sphingolipid-enriched membrane fraction, together with other DRM-localized proteins such as Thy-1, Lyn and Fyn [108]. In addition to the properties conferred to PrP^{C} by its GPI anchor, other regions of PrP^{C} also show an intrinsic affinity for lipids. Bacterially expressed recombinant PrP (Syrian Hamster PrP 90–231) was shown to bind to vesicles made of synthetic lipids [109], resulting in structural modifications of the protein [110]. Binding was even better after PrP had been refolded to a β-sheet-rich isoform. Reduced binding in the presence of NaCl suggested that it was largely due to charge interaction between PrP and the polar group of lipids. Therefore, both charge attraction and the GPI anchor probably contribute to the strong association of extracellular PrP^{C} with the membrane. It is also probably through its lipophilic nature that PrP^{Sc} binds to apoB, the major protein component of low-density lipoproteins [111].

In addition to this surface interaction, PrP^{C} also has the potential to cross the membrane, by inserting its transmembrane segment into the lipid bilayer. This transmembrane domain was mapped to residues 110–135 [25], thus encompassing the first strand of the short β-sheet. It contains the conserved AGAAAAGA palindrome, which is immediately followed by an extended glycine zipper [112]. Glycine zippers consist of nonapeptide motifs (GxxxGxxxG) that were recently identified as a common feature of homo-oligomeric transmembrane proteins. Both PrP and human amyloid β peptide belong to a limited set of proteins that contain extended glycine zippers (GxxxGxxxGxxxG, residues 119–131 of human PrP). Interestingly, these extended motifs are found in a number of pore-forming proteins that are known to insert themselves post-translationally into membranes as homo-oligomeric pores, *e.g.*, influenza virus hemagglutinin and *Helicobacter* vacuolating cytotoxin precursor. In the case of PrP, this could account for the observed cytotoxicity of prion peptide 106–126 [113–115]. Although there are no data relative to the structural organization of PrP^{C}, electron micrographs of two-dimensional crystals formed by PrP 27–30 are consistent with the existence of PrP oligomers made up from either three or six PrP molecules [116, 117]. Whether these may reflect the existence of similar structures for PrP^{C} remains to be established, but a number of independent studies have clearly demonstrated that PrP is prone to oligomerization [70, 71].

Although transmembrane PrP^{C} is thought to insert itself into the membrane co-translationally, one may ask whether a surface-anchored PrP^{C} could translocate to a transmembrane form, as has been described for some model peptides [118]. For instance, copper binding to the N-terminal half of PrP could induce a major structural ordering, which in turn could lead to its translocation across the membrane and oligomerization.

Glycosaminoglycans

Earlier studies in the 1980s had shown that polyanions could lengthen scrapie incubation [119], and reduce PrP^{Sc} accumulation [120]. A number of studies have since shown that PrP binds polyanions and glycosaminoglycans (GAG) [121–123]. Both recombinant and brain-derived PrP^{C} were observed to bind *in vitro* to many distinct GAG – heparin, chondroitin sulfates A and B, and hyaluronic acid [123]. Binding primarily involved the N-terminal lysine cluster (residues 25–35) [123] and was stabilized by interactions with histidine residues from the octarepeats, possibly involving bridging Cu^{2+} ions [121, 124].

Nucleic acids

The exact biochemical nature of the scrapie agent is still a matter of debate, and purified preparations of infectious material contain residual amounts of appa-

rently nonspecific nucleic acids [5, 125]. Interaction of PrP with nucleic acids *in vitro* was first studied using DNA and prion peptide 106–126 [126]. Both recombinant protein and prion peptide 106–126 were found to polymerize upon interaction with nucleic acids [127, 128]. A number of studies have since shown that PrP interacts more or less specifically with various nucleic acids or nucleic acid-like molecules [129–132]. Using the SELEX method, both DNA [133] and RNA aptamers [134–137] that specifically bind to PrP or PrP^{Sc} [135, 138] were isolated in different laboratories. Sequence comparisons among these aptamers and other PrP-binding nucleic acids do not identify any obviously shared sequence motif. Whereas most of the aptamers isolated by the Famulok laboratory [134, 136] contained putative guanine quadruplex structures, this feature was not found in other PrP aptamers [135, 138]. However, we identified two shared motifs between the unmodified RNA aptamers that we raised against the ovine PrP [137] and other prion aptamers that were independently obtained in other laboratories, suggesting that PrP preferentially binds nucleic acids containing defined patterns.

Most studies have located the nucleic acid-binding site on PrP to its N-terminal half. Using a series of recombinant proteins in which GST was fused to successive parts of PrP, Weiss et al. [134] mapped the major site to PrP residues 23–52. This site is probably one of low specificity and high affinity primarily based on charge interaction [135], and likely coincides with the N-terminal lysine cluster that was also identified as the dominant heparin-binding site of PrP (see above, [123]). Other less well delimited nucleic acid binding sites map downstream of residue 90. Notably, some RNA aptamers have been raised against peptide 90–129 from human PrP [136], based on the consideration that this peptide belongs to a region known to be involved in the PrP^{C} to PrP^{Sc} conversion. Our own work with a panel of nonapeptides derived from the ovine PrP allowed us to identify the two lysine clusters contained in the N-terminal part of PrP as its main nucleic acid-binding sites [137]. Interestingly, the alternating proline and basic residues that are invariantly found in both lysine clusters are reminiscent of the binding motifs found in proteins that are known to interact with nucleotides and nucleic acids [139]. In addition, Rhie et al. [135] suggested the presence of a conformation-specific "aptatope" downstream of residue 110, to account for the fact that aptamer SAF93 bound to PrP 110–230 only if the latter was in a β-folded isoform. This is corroborated by studies based on small angle X-ray scattering and nuclear magnetic resonance spectroscopy [130], which showed that both the globular and unstructured domains of PrP are involved in its interaction with nucleic acids.

The biological significance, if any, of PrP binding to nucleic acids is perhaps suggested by the modifications induced in both partners as a result of their interaction. Nucleic acids rendered PrP partially resistant to proteinase K digestion and favored its oligomerization [127, 131, 140], or, in contrast, reduced PrP^{Sc} accumulation [135, 136] or exhibited anti-scrapie activities *in vivo* [129]. On the other hand, it was found that specific DNA sequences could induce conversion of recombinant PrP to a soluble β-sheet isoform [132]. Reciprocally, PrP also modified the nucleic acid: in a manner strikingly similar to the nucleoprotein Ncp7 of HIV, recombinant PrP was observed to favor the dimerization of HIV RNA *in vitro*, and to enhance the efficiency its reverse transcription [141]. This RNA chaperone activity required the N-terminal part of PrP, since it was not supported by a N-terminally truncated protein.

What could be the physiological meaning of PrP binding to nucleic acids? This depends primarily upon the subcellular topology of PrP. If it is exclusively located at the outer face of the cell membrane, the probability of encountering nucleic acids is low, except for nucleotide neurotransmitters [142]. On the other hand, both the cytosolic [24] and transmembrane Ctm (cytosolic N terminus, [143]) forms of PrP are likely to interact with cellular nucleic acids. In spite of the scarcity of data showing that PrP^{C} physiologically interacts *in vivo* with nucleic acids [144], it is likely that the RNA-chaperone activity that was observed *in vitro* with recombinant PrP [141, 145] has a counterpart *in vivo*. For instance, PrP^{C} may assist the proper folding of some RNA molecules through facilitating their structural changes, or it could participate in the many steps of RNA-mediated regulations, *i.e.*, translation, transport, and silencing [146, 147].

Ligands as clues to a PrP function?

The collection of known PrP ligands could seem at first sight too disparate to infer a function to the cellular protein. However, one can notice that a majority of these ligands, including PrP itself, are localized at the extracellular membrane surface (lipids, GAG, N-CAM, LRP/LR, dystroglycan, *etc.*). Their association with PrP seems therefore biologically relevant and fits well with the model of a surface-anchored PrP^{C} that traffics through the cell by endocytosis and recycling to the cell surface [12, 19, 148]. This is reinforced by the fact that these ligands were identified *in vivo* (N-CAM) or because the interaction and its consequences have been fully characterized (LR). On the other hand, the topology and topogenesis of PrP^{C} do not always obey that

model, but instead vary depending upon cell-specific factors [149]. Intracellular and transmembrane forms of PrP, which comprise only minor fractions of total PrP at any given time, may well represent key features of the protein, or play important functions in some cell types. We therefore consider two different situations in relation with the subcellular topology of the protein.

Membrane-anchored PrP and its extracellular ligands

It has been proposed that PrP^C might be part of a scavenger receptor complex [12]. This indeed fits well with the peculiar nature of the N-terminal tail of PrP, to which most of PrP ligands bind (notably divalent metal ions, plasminogen, chaperones, LRP/LR, GAG). This N-terminal tail is an intrinsically unstructured (or natively disordered) domain. It can be viewed (Fig. 1) as a succession of short modules: a first N-terminal lysine cluster, followed by two nonapeptides [GG(S/N)RYP(G/P)QG], four octapeptides, a second lysine cluster, and the transmembrane domain containing the AGAAAAGA palindrome. It is also particularly rich in amino acids that confer a high propensity to native disorder, *i.e.*, small residues, prolines and uncharged hydrophilic residues (Gly, Ala, Asn, Gln, Ser, Thr, Pro, which are all labeled in green in Fig. 1). This biased composition and the presence of repeats are both hallmarks of intrinsically unstructured polypeptides [150], which are increasingly being recognized in various proteins [151]. If indeed they remain unstructured in the crowded macromolecular environment of a cell [152, 153], these can be considered as ligand-seeking domains, because their extended conformation allows them to probe a large volume and thereby increases the probability of encountering their ligand through a "fly-casting" mechanism [154]. They can undergo conditional folding (coupled to ligand binding) or act as flexible linkers that allow the assembly of macromolecular structures. Our favored view is therefore that the function of PrP^C is dependent upon its binding of ligand(s) and its subsequent folding within a macromolecular complex. Folding or dimerization of the N-terminal flexible tail of PrP could be controlled by the concentration of copper ions [39] in the synaptic cleft, or through binding to other small molecules and neurotransmitters, which to our knowledge have not been investigated. Alternatively, this conditional folding may also be triggered by variations in the environmental pH [155]. PrP^C could thereby participate in a signaling cascade [156] by facilitating or strengthening protein-protein interactions at the cell surface (Fig. 2). These interactions may also include homophilic interactions of PrP with itself that could mediate intercellular adhesion, for instance across the synaptic cleft [155, 157]. PrP could thus function as a sensor by integrating diverse signals, for instance fluctuations in pH or copper concentration. Many reports have demonstrated the neuroprotective properties of PrP^C [158–160]. Several signaling pathways probably contribute to that effect [161], involving the participation of PrP^C in distinct macromolecular complexes. Thus, in 1C11 serotonergic neurons, PrP^C was shown to function as a signaling receptor that is coupled to tyrosine kinase Fyn through its association with caveolin-1 [74, 156, 162]. PrP^C was also proposed to operate within a complex formed with STI1 and laminin [95]. Beside these proposed structures, the raft-localized dystrophin–glycoprotein complex with which PrP was found to interact *in vivo* [81] could also constitute one of the platforms allowing extracellular signals to activate intracellular cascades. Signaling through PrP^C has been shown to involve various intracellular cascades, notably NADPH oxidase and kinases ERK1/2 [162], phosphatidylinositol 3-kinase and Akt [161, 163] and the cAMP-dependent protein kinase PKA [159]. Interestingly, Grb2, one of the intracellular ligands of PrP^C, also participates in these pathways [161, 162]. How these cascades promote neuron survival is not fully understood, but this seems to result from both the activation of pathways involved in cell proliferation or differentiation, and the prevention of cell death, notably through control of the redox equilibrium [159, 162]. Involvement of PrP^C in signaling cascades is not limited to neurons, since it was also documented in cells of the lymphoreticular system [162, 163].

Acting as a sensor is compatible with the recycling of PrP^C, and there are several published examples of receptor internalization following ligand binding. For instance, upon activation by its natural ligand 5-HT, serotonin receptor 2A was shown to undergo desensitization and internalization, followed by its recycling to the plasma membrane [164]. In neurons, internalization of PrP^C occurs through clathrin-mediated endocytosis and requires prior egress of the protein from the raft domains, possibly with the aid of a transmembrane protein [12, 148]. Notwithstanding a possible role towards other unidentified ligands [12], PrP^C could play a specialized function in the poorly understood homeostasis of copper in the CNS [165], thus providing a connection between copper homeostasis, neurodegeneration and the neuroprotective activity of PrP^C.

Transmembrane and intracellular PrP

Observations of intracellular PrP^C under physiological conditions include the cytosolic PrP that was observed in some neurons of the hippocampus, neocortex, and thalamus [24], and a nuclear form that was

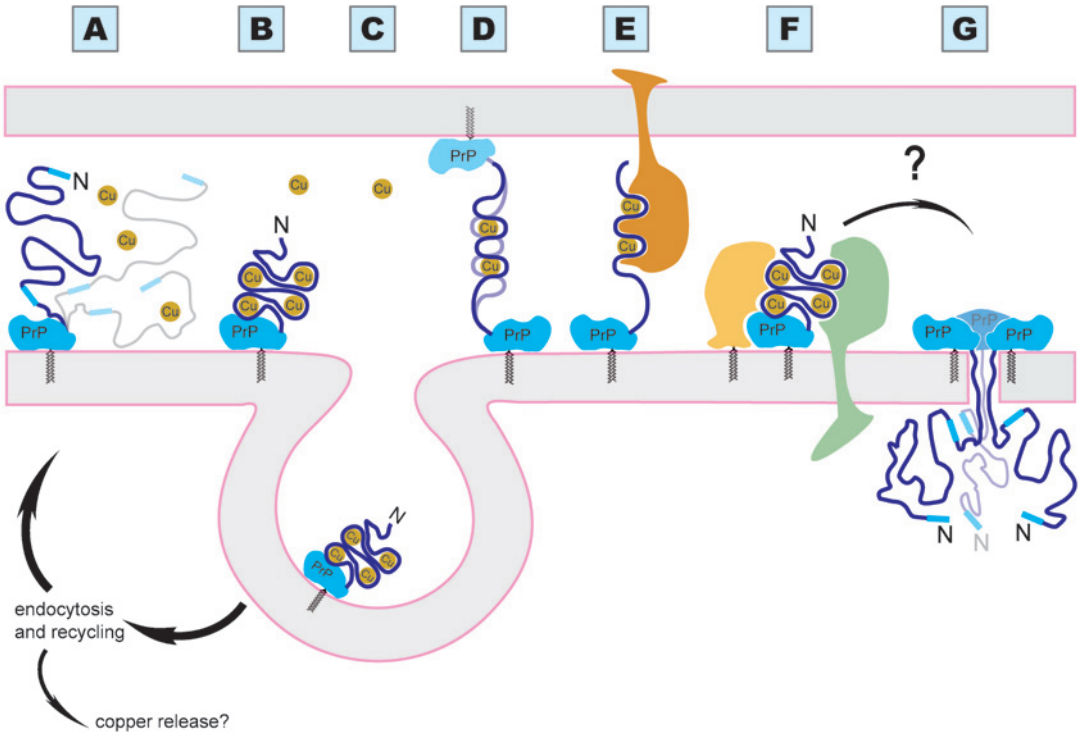

Figure 2. Cartoon model of PrP. The structured core of PrP (blue shape) is tethered to the external surface of the cell membrane via its GPI anchor (A). The N-terminal unstructured region, with its two lysine clusters (blue boxes) freely " explores " a semispherical volume with a radius up to 20–25 nm. (B) Binding of a ligand (here copper ions, yellow spheres) induces structuration of the flexible tail. Ligand-bound PrP can then become internalized (C), and thus recycle the bound copper ions. Alternatively, PrP could interact with itself (D) or with other protein partners (E, F) in a copper- or pH-dependent manner, and participate in a signaling cascade. A fraction of PrP adopts a transmembrane topology. We have drawn a model of a transmembrane PrP (G) with the Ctm topology (the unstructured N terminus is cytoplasmic). In addition, we have represented it as a trimer that potentially could form a pore channel through the membrane (G). Additionally, the now cytoplasmic N-terminal tail has the potential to bind RNA or other cytoplasmic nucleic acids. One could also imagine a ligand-induced transition from F to G, perhaps with the aid of other proteins.

found to interact with lectin CBP70 in the nucleus of a human promyelocytic leukemia cell line [166]. Transmembrane isoforms of PrP^C have also been observed [25]. In spite of the scarce data on the fraction of PrP^C adopting that topology, the presence of an extended glycine-zipper in its transmembrane domain also suggests that PrP has the potential to form transmembrane oligomers. In all these situations in which the N-terminal tail of PrP^C is localized in the cytosol or in the nucleus, PrP is likely to interact with its putative intracellular ligands, which notably include nucleic acids. The RNA-chaperone property of recombinant PrP *in vitro* [141], which is also consistent with the intrinsic disorder of its N-terminal tail [167], likely reflects a similar activity *in vivo*, that PrP^C could exert in some specialized cells or subcellular compartments.

Conclusion

It is likely that the present list of PrP ligands is incomplete, and it probably lacks proteins with which PrP may interact transiently and reversibly. Yet, we have the feeling that the distinct topologies of the protein could correspond to distinct functions, in relation with the known ligands. An increasing body of data support the view that in its extracellular form, PrP^C acts as a sensor within a macromolecular complex in a signaling cascade, whereby it could perceive extracellular stress and promote cell survival, in both neurons and non-neuronal cells. This function could be linked to some aspects of copper metabolism. In contrast, its intracellular and transmembrane Ctm isoforms could exert an RNA-chaperone activity. In both cases, it is likely that interaction of PrP^C with its partners mostly relies on its intrinsically unstructured N-terminal domain. Intrinsic disorder, by enabling both reversible binding and conformational plasticity in the geometry of binding [168–170], could allow PrP^C to interact differently with distinct ligands and to

function in multiple pathways like a hub protein within a complex interaction network.

This conformational plasticity could also explain the ability of PrP to polymerize into distinct pathological isoforms which, unlike the mere lack of PrP, could perturb distinct pathways and thus account for the diversity of phenotypes [171] observed in prion diseases.

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