

## Hypothesis

# Prion Protein PRNP: A New Player in Innate Immunity? The A $\beta$ Connection

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**Abstract.** The prion protein PRNP has been centrally implicated in the transmissible spongiform encephalopathies (TSEs), but its normal physiological role remains obscure. We highlight emerging evidence that PRNP displays antimicrobial activity, inhibiting the replication of multiple viruses, and also interacts directly with Alzheimer's disease (AD) amyloid- $\beta$  (A $\beta$ ) peptide whose own antimicrobial role is now increasingly secure. PRNP and A $\beta$  share membrane-penetrating, nucleic acid-binding, and antiviral properties with classical antimicrobial peptides such as LL-37. We discuss findings that binding of abnormal nucleic acids to PRNP leads to oligomerization of the protein, and suggest that this may be an entrapment and sequestration process that contributes to its antimicrobial activity. Some antimicrobial peptides are known to be exploited by infectious agents, and we cover evidence that PRNP is usurped by herpes simplex virus (HSV-1) that has evolved a virus-encoded 'anti-PRNP' function. These findings suggest that PRNP, like LL-37 and A $\beta$ , is likely to be a component of the innate immune system, with implications for the pathoetiology of both AD and TSE.

**Keywords:** Alzheimer's disease, amyloid- $\beta$  peptide, herpes simplex, innate immunity, PRNP, spongiform encephalopathy

## INTRODUCTION

Several brain conditions, including Alzheimer's disease (AD) and the transmissible spongiform encephalopathies (TSEs)—Creutzfeldt–Jakob disease (CJD) in human, scrapie in sheep, and bovine spongiform encephalopathy (BSE) in cattle—are associated with the presence of abnormal protein deposits in brain. For years it was thought that the amyloid- $\beta$  (A $\beta$ ) in AD brain might cause the disease,

but emerging evidence argues that A $\beta$  is an antimicrobial defense peptide induced in response to infection [1–5], raising the prospect that AD might be associated with brain infection (e.g., [6]). Indeed, it has been argued, notably by Kagan and colleagues, that amyloid peptides in general may have antimicrobial properties [7, 8]. This paper addresses whether PRNP, the central component of the proteinaceous 'prion' deposits found in the TSE disease brain, might also have antimicrobial activity.

Starting with a brief overview of prion theory, this article examines findings that PRNP associates directly with the antimicrobial peptide A $\beta$ , that PRNP and A $\beta$  are codeposited in disease brain, and that PRNP (like A $\beta$ ) binds to both membranes and nucleic acids and displays antiviral activity. We then examine

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in more depth how herpes simplex virus (HSV-1) usurps PRNP protein. We conclude by examining the nucleic acid-binding properties of PRNP, and suggest that oligomerization of the protein in response to abnormal nucleic acids may represent an entrapment and sequestration mechanism as a central component of PRNP antimicrobial activity.

## PRION DISEASE

TSEs include diverse neurodegenerative diseases, including scrapie in sheep, BSE in cattle, and CJD in human. For more than 40 years it has been known that brain tissues from individuals with TSE diseases contain aggregated protein deposits [9–11]. These fractions are infectious upon reinoculation into a new host, and infectious fractions are enriched in protease-resistant aggregates of the host protein 'PrP' [12,13], a processing product of the native precursor protein, PRNP, encoded by the *PRNP* gene in human, *Prnp* in mouse.

The basic tenet of the prion theory [14, 15] is that, in disease, a cellular form of PRNP, dubbed PrP<sup>C</sup>, undergoes a conformation change, generating the 'scrapie-specific' form PrP<sup>Sc</sup>. In turn, PrP<sup>Sc</sup> binds to PrP<sup>C</sup> and promotes PrP<sup>C</sup> → PrP<sup>Sc</sup> conversion, leading to amplification of (supposedly neurotoxic) PrP<sup>Sc</sup> and disease ([16–18] for review).

In support of the protein-only hypothesis, no agent other than PRNP protein has been routinely detected in purified infectious fractions from disease brain. Moreover, the agent is resistant to some treatments that normally inactivate nucleic acids, and any prospective nucleic acid that might be associated with infectious PRNP must be short [19], thereby excluding a conventional virus. However, this has been widely debated (e.g., [20–23] and references therein) and, although innate immune cells and molecules including interferons and interleukins have been implicated in TSE disease progression (reviewed in [24]), the precise pathoetiology of TSEs and the normal role of PRNP remain enigmatic.

## PRNP IS WIDELY DISTRIBUTED IN INTRACELLULAR AND EXTRACELLULAR COMPARTMENTS

PRNP is generally thought of as a cell-surface molecule that is attached to the membrane by a glycosyl phosphatidylinositol (GPI) anchor. However, common PRNP isoforms lacking the GPI anchor are

widely distributed across multiple intracellular compartments, including the nucleus, as well as in the extracellular milieu. The human, mouse, hamster, and sheep PRNP coding sequences contain methionine triplets downstream of the usual initiation codon, and 10–15% of PRNP polypeptides in human and hamster are generated by translation initiation at these downstream sites [25], generating an intracellular protein. Cell-surface PRNP is also cleaved by  $\alpha$ -secretase and other cellular proteases to liberate extracellular fragments (e.g., [26–28]) or is released from the membrane by the action of phospholipases; furthermore, cell-surface and extracellular PRNP can be re-internalized by several routes including direct endocytosis (e.g., [29–31]).

Expressed widely in multiple tissues including the immune system, the true biological role of PRNP has remained elusive, and laboratory pathogen-free *Prnp* knockout mice display only subtle and irreproducible deficits in parameters such as synaptic plasticity (not reviewed). As we will see, binding partners of PRNP may cast light on its role.

## PRNP BINDS TO A $\beta$

It has recently emerged that the AD peptide A $\beta$  is a potent antimicrobial molecule that mediates broad-spectrum resistance against a variety of infectious agents including viruses, bacteria, and yeasts [1–5]. An interaction between PRNP and A $\beta$  could argue that the two polypeptides are components of the same pathway.

The first indication that PRNP interacts with APP-derived molecules came from investigation of PRNP binding partners. Schmitt-Ulms et al. performed *in vivo* crosslinking of normal mouse brain proteins with formaldehyde; stable complexes were retrieved using anti-PRNP antibody and bound proteins were analyzed by mass spectrometry. APP fragments were among the major binding partners of PRNP [32], indicating that the two proteins are in close proximity in the absence of disease.

Lauren et al. [33] subsequently performed a screen of 225 000 mouse cDNA clones for polypeptides binding specifically to A $\beta$ : only two positive clones were retrieved, and both corresponded to PRNP. Binding did not require the disease-specific PrP<sup>Sc</sup> configuration. High-affinity binding has been confirmed for human PRNP [34–36]; these studies report two A $\beta$  binding sites within PRNP, one within a central segment (residues 95–110) and a second

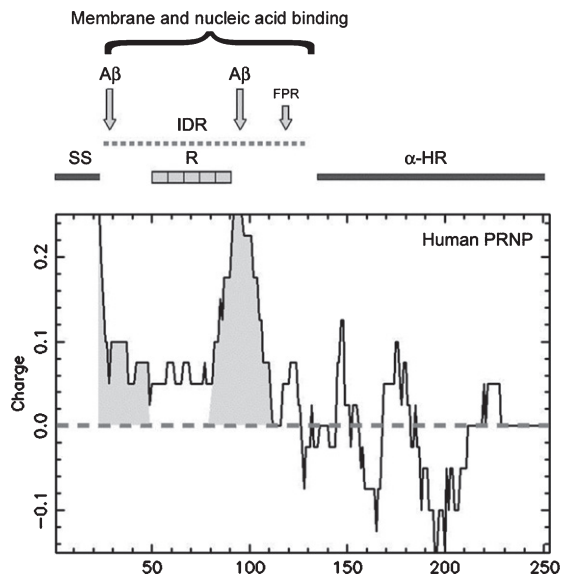


Fig. 1. Domains of Human PRNP Protein. The graph plots the charge of the mature protein (residues 23–253) analyzed with EMBOSS (<http://www.bioinformatics.nl/cgi-bin/emboss/charge>; window size = 20). Grey, basic regions. Abbreviations: A $\beta$ , binding sites for A $\beta$  peptide; FPR, formyl peptide receptor binding site;  $\alpha$ -HR,  $\alpha$ -helical region; IDR, intrinsically disordered region; R, octapeptide repeats; SS, signal sequence.

close to the N-terminus (residues 23–27) (Fig. 1). A direct interaction between A $\beta$  and PRNP has now been demonstrated by multiple biochemical and biophysical techniques including immunoprecipitation, tagged recombinant proteins, surface plasmon resonance, dissociation-enhanced lanthanide fluorescence immunoassay, and fluorescence polarization (e.g., [37, 38]).

Interestingly, in the study of Schmitt-Ulm et al. [32], other proteins binding to PRNP in this assay included the APP-like protein APLP2 and APOE; this latter is notable because APOE is an immune mediator [39] and mutations in *APOE* govern susceptibility to AD (reviewed in [40]). Indeed, APOE also binds to A $\beta$ , and *APOE* alleles also modulate susceptibility to several different microbes (e.g., *Chlamydomyphila*, *Klebsiella*, malaria, hepatitis C, HIV, HSV; not reviewed), as well as to CJD (e.g., [41]).

#### *PRNP and A $\beta$ are codeposited in disease brain*

Do PRNP and A $\beta$  associate in disease brain *in vivo*? Esiri and colleagues [42] screened brain samples from unselected postmortem cases, and reported that in 42% of cases low levels of PRNP immunoreactivity were associated with AD-type plaques.

A similar finding of punctate PRNP immunoreactivity in AD plaques was reported by Ferrer et al. [43], whereas Kovacs found colocalization with tau aggregates in AD [44], another signature of the disease. More recently it has been reported that A $\beta$  predominantly interacts with aggregated forms of PRNP in AD brain [36, 45]. Zou et al. reported that A $\beta$  in AD brain forms coaggregates with a PrP<sup>Sc</sup>-like form of the molecule, termed PrP<sup>I</sup>, that copurifies with A $\beta$  on gel filtration, and the two proteins coimmunoprecipitate with either anti-A $\beta$  or anti-PRNP antibody from extracts of human AD brain [36].

Similar interactions are reported in TSE. Hainfellner et al. [46] analyzed brain samples from individuals with confirmed CJD and non-CJD controls. AD-type pathology was seen in 10–20% of both groups, which the authors ascribed to age-related changes, but it was observed that PRNP deposits frequently accumulate at the periphery of A $\beta$  plaques.

Colocalization has also been reported in mice. For example, in double *Prnp/App* transgenic mice—expressing AD-associated mutant APP as well as hamster PRNP—extensive brain amyloid deposition was seen, and A $\beta$  and PRNP colocalized in almost all plaques [47].

On balance it appears that, when PRNP amyloid is present, in both AD and TSE, it tends to colocalize with A $\beta$  plaques, providing evidence that the interaction detected *in vitro* is reiterated *in vivo*. This supports the view that the two polypeptides are part of the same biological pathway.

#### *PRNP promotes the generation and fibrillization of A $\beta$ in disease*

In further support of an interaction, Parkin et al. [48] studied the effect of PRNP on the release of A $\beta$  peptide from its precursor protein APP. In AD, two specific protease cleavages are required to release A $\beta$ —one at the N-terminus, the so-called  $\beta$ -secretase site, and a second at the C-terminus of the molecule, the  $\gamma$ -secretase site. Downregulation of PRNP by antisense or knockout led to increased levels of A $\beta$  processing; the authors inferred that PRNP normally inhibits  $\beta$ -secretase cleavage and A $\beta$  release [48]. It was suggested that PrP<sup>C</sup>  $\rightarrow$  PrP<sup>Sc</sup> conversion may release inhibition, leading to dual upregulation of PrP<sup>Sc</sup> and A $\beta$  in disease, consistent with a synergistic role for the two peptides. Indeed, A $\beta$  levels are increased several-fold in TSE infection [48, 49]. In addition, there is evidence from knockout cells

that PRNP not only promotes A $\beta$  release but also fibrillization of the molecule [50].

#### *PRNP alleles constitute a risk factor for AD*

If AD-related A $\beta$  interacts with PRNP, then different allelic variants of *PRNP* might be anticipated to modulate A $\beta$  function. An association between *PRNP* mutations and AD has been documented. Several studies have addressed the specific linkage between the common codon 129 polymorphism (a risk factor for CJD) and AD, but failed to find an association (e.g., [51]). However, when the influence of *APOE* alleles was taken into account, *PRNP* variants were reported as a significant contributing factor to AD (e.g., [52, 53]). Different meta-analyses of *PRNP* variants have reported a positive association between *PRNP* with AD [54–56], and indeed carriers of some *PRNP* mutations are primarily diagnosed as AD (e.g., [57]). In short, *PRNP* genotype is a modifier of AD risk, arguing again that the *in vitro* interaction between PRNP and A $\beta$ /APP is functional *in vivo*.

### **EVIDENCE THAT PRNP IS ITSELF A DEFENSE MOLECULE**

The above studies demonstrate that PRNP is a primary binding partner of the antimicrobial peptide A $\beta$ , both *in vitro* and *in vivo*. This opens the possibility that PRNP may also be a component of the innate immune system.

#### *Antimicrobial peptides*

Antimicrobial peptides are a large and diverse group of evolutionarily ancient proteins that pre-date the adaptive immune system. For example, the A $\beta$  sequence is conserved between humans and primitive fish [5]. They have potent activity against a wide range of bacteria, viruses, and yeasts, and classically exert their actions in multiple ways (reviewed in [58–60]). First, by physical association with membranes that can lead to membrane penetration and/or antimicrobial activity via receptor blockade. Second, by recruiting immunomodulators to the site of infection. Third, by the formation of extensive protein networks (amyloids) that act as traps for pathogens, sequestering them into an insoluble fraction from which they cannot escape. Other mechanisms include the local generation of toxic reactive oxygen species, the induction of cell death ‘beneficial suicide’

pathways (e.g., [61]), and modulation of viral nucleic acid synthesis.

The crucial importance of these broad-specificity anti-infection mechanisms is illustrated by the antimicrobial peptide cathelicidin LL-37; if untreated, genetic deficiency in human leads to death from infection in the first year of life [62].

#### *PRNP resembles an antimicrobial peptide*

PRNP displays many of these properties. First, like A $\beta$ , PRNP is substantially conserved through evolution, and homologs can be traced back to frogs and fish [63]. Second, PRNP shares the membrane-binding/inserting [64–69] and nucleic acid-binding properties of classical antimicrobial peptides (discussed in more detail below). Third, PRNP is a powerful immunomodulator (reviewed in [70]) and, like both the classical antimicrobial peptide LL-37 and A $\beta$ , PRNP is a ligand for formyl peptide receptors (reviewed in [71]), key components of the innate immune system.

Centrally, the formation of polymeric aggregates by PRNP reiterates the aggregation and pathogen entrapment mechanisms attributed to A $\beta$  and classical antimicrobial peptides. It is plausible to suggest that the PrP<sup>c</sup> to PrP<sup>sc</sup> conversion might be part of a pathway paralleling the conversion of APP to A $\beta$  and subsequent aggregation and entrapment activity (discussed further below), perhaps in direct physical association with A $\beta$ .

In addition, for several antimicrobial peptides, copper binding permits the generation of reactive oxygen species that contribute to the inactivation of bound pathogens. Like the recently uncovered antimicrobial peptide A $\beta$ , PRNP binds tightly to copper ion [72, 73]. It is not yet known whether copper binding contributes to the immune defense properties of PRNP, and further work in this direction is warranted.

Finally, like other innate immune molecules, PRNP expression is upregulated by infection. Cellular infection with HIV-1 leads to increased PRNP mRNA levels [74]. Upregulation was reported following infection with, among others, vesicular stomatitis virus and murine leukemia virus [75], adenovirus 5 [76, 77], hepatitis C virus [78, 79], Epstein–Barr virus [80], and *Mycobacterium bovis* [81]. Similar findings have been reported *in vivo*, and PRNP was upregulated in brain of individuals infected with HIV-1 as well as in cases of simian immunodeficiency virus encephalitis in macaques [82]. Interestingly, Voigtländer et al. reported striking

Table 1  
Antiviral Effects of PRNP<sup>a</sup>

Virus inhibited	Virus type	Ref.
Adenovirus 5	dsDNA virus, non-enveloped	[77]
Coxsackievirus B3	ss(+)RNA virus, non-enveloped	[85]
HIV-1	Retrovirus, ss(+)RNA, enveloped	[84]
HSV-1 virus (del68)	dsDNA virus, enveloped	[89]
Murine leukemia virus	Retrovirus, ss(+)RNA, enveloped	[87]
Poliovirus type 1	Picornavirus, ss(+) RNA, non-enveloped	[86]

<sup>a</sup>(+), positive sense genome; ds, double-stranded; ss, single-stranded.

upregulation of PRNP in AD [83], a condition now increasingly linked to infection [6]. Notably, the formation of the PrP<sup>Sc</sup> form is induced by HIV-1 infection [84].

#### Antimicrobial activity of PRNP

There is direct evidence that PRNP plays a direct protective role in defense against virus infection (the special case of wild-type HSV is examined in the next section). In cell culture, titers of coxsackievirus B3 were 30–100-fold higher in *Prnp* knockout cells, and cells could be rescued by expression of PRNP. Protection corresponded to increased interferon production and elevated apoptotic cell death as assessed by DNA fragmentation [85]. Expression of PRNP decreased HIV-1 gene expression and virus production was reduced by eightfold [84]. Similar findings have been reported for poliovirus type 1, where the titer of virus was increased by a factor of 10<sup>2</sup> to 10<sup>4</sup> in *Prnp* knockout cells, and virus replication was blocked by re-expression of PRNP [86]. *Prnp* knockout was associated with a fivefold increase in expression of murine leukemia virus [87]. Also in cell culture, antisense blockade of PRNP expression increased adenovirus 5 mRNA and DNA content by up to 10-fold [77].

*In vivo*, although titers of encephalomyocarditis virus were not significantly different between *Prnp* wild-type and knockout mice following intracerebral inoculation, wild-type mice had higher levels of brain inflammation consistent with a more active response [88]. Convincingly, however, *in vivo* titers of a mutant HSV-1 virus (del68) were reduced by a factor of 600 in wild-type versus *Prnp* knockout mice ([89], discussed further below).

Although more *in vivo* studies are required, together these data indicate that PRNP, that is itself upregulated in response to infection, leads to a dramatic fall (in the range 10<sup>1</sup> to 10<sup>4</sup>) in the proliferation of a diverse range of viruses (Table 1); one may reliably conclude that PRNP is a defense molecule.

#### HSV-1 HIGHLIGHTS PRNP-DEPENDENT AUTOPHAGY AS A MECHANISM OF ANTIVIRAL DEFENSE

As noted earlier, antimicrobial peptides typically have multiple modes of action that centrally involve membrane association. However, there is one area in which we are beginning to understand the mechanism of action of PRNP in antiviral defense, and studies on HSV-1 have been crucial. Indeed, the very first studies on PRNP and virus resistance employed HSV-1, but at that time it was not known that HSV-1 deploys an ‘anti-PRNP’ strategy which has been highly informative.

Titers of the HSV-1 mutant del68 (ICP34.5) are reduced 600-fold in wild-type versus *Prnp* knockout cells [89], as noted earlier. *In vivo*, all wild-type animals infected with the mutant virus survived, whereas the majority of *Prnp* knockout animals died following infection, demonstrating the protective role of PRNP. By contrast, the wild-type virus displays an entirely different pattern (next section).

It now emerges that wild-type HSV-1 virus has a specific ‘anti-PRNP’ function. Orvedahl et al. [90] reported that the virus function in question, ICP34.5 (infected cell polypeptide 34.5 kDa, that is altered in the del68 mutant), binds to BECLIN1, a protein involved in autophagy – a process in which a section of the cytoplasm is enclosed in an isolation membrane to generate ‘autophagosomes’, that then fuse with lysosomes to permit degradation of the contents, including infectious agents (reviewed in [91]).

Using a HSV ICP34.5 mutant selectively deficient in BECLIN1 binding, Korom et al. [89] demonstrated that PRNP normally blocks the neurovirulence of the virus by targeting the virus for autophagosome-mediated degradation. In the absence of ICP34.5, a PRNP-dependent process is set in train that blocks virus proliferation by autophagic mechanisms. However, in wild-type virus (isolate 17) ICP34.5 stops this process in its tracks, permitting virus proliferation [89].

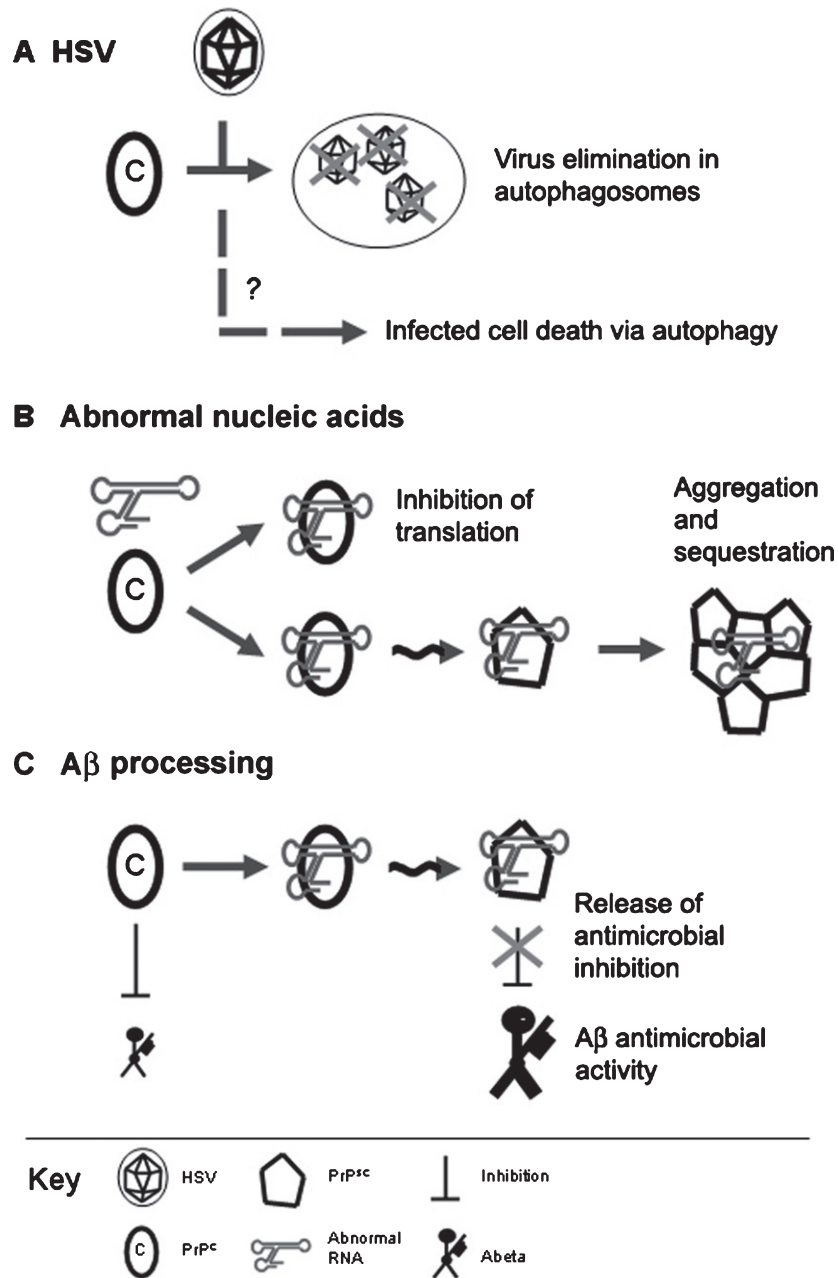


Fig. 2. Antimicrobial Activities of PRNP. In addition to the listed categories, other mechanisms are likely to include the generation of reactive oxygen species, and binding to immunomodulatory molecules including APOE and formyl peptide receptors is likely to direct the recruitment of other actors in innate immunity. Direct binding to Aβ (not depicted) and potential interactions with other antimicrobial peptides that bind to Aβ (e.g., LL-37, α-synuclein) add a further dimension. Abbreviation: HSV, herpes simplex virus type 1.

These elegant studies demonstrate that autophagy induction in response to virus infection is one major route by which PRNP exerts antiviral activity. However, it is unlikely to be the only one, and distinct targeted defense mechanisms may be invoked against different infectious agents (Fig. 2). Moreover, as we will see in the next section, HSV usurps PRNP.

### HSV-1 NOT MERELY BLOCKS, BUT EXPLOITS, PRNP

In apparent conflict with antimicrobial role of PRNP, it was earlier reported that wild-type HSV-1 is not inhibited by PRNP, but in fact needs PRNP for efficient proliferation. The discovery of the HSV-1-

encoded 'anti-PRNP' function discussed above now provides fresh insights into these earlier reports.

In a key paper, Thackray and Bujdoso [92] reported that *Prnp* knockout mice are in fact much less sensitive to wild-type HSV-1 (isolate SC16) than are *Prnp* wild-type mice, and the survival of mice following lethal challenge was remarkably increased by *Prnp* knockout. Conversely, overexpression of wild-type PRNP dramatically boosted virus proliferation. In a follow-up paper, the same group confirmed earlier findings and reported significantly increased acute virus titers in brainstem of wild-type versus knockout animals [93].

These instrumental data indicate that, in addition to blocking PRNP-dependent antiviral autophagy, wild-type HSV-1 depends upon, and usurps, PRNP to foster virus proliferation. This is not unprecedented. HIV-1 exploits the classical antimicrobial peptide LL-37 to promote its own replication [94], and viruses such as HIV-1 and HSV-1 exploit cell-surface immunoreceptors which normally stimulate immunity to facilitate virus infection of immune cells. The mechanism by which HSV-1 usurps PRNP remains unknown, but may involve membrane interactions and/or nucleic acid binding (below).

#### ANTIMICROBIAL PEPTIDES ALSO BIND TO NUCLEIC ACIDS

Because antimicrobial peptides centrally target membranes as part of their antimicrobial activity [60, 95], they often also bind to nucleic acids. In vertebrates, membranes are highly enriched in negatively charged molecules including phospholipids and sulfated glycosaminoglycans, and bacterial membranes containing teichoic acids and lipopolysaccharides are also negatively charged. Thus, many antimicrobial peptides also bind to nucleic acids.

LL-37 has been shown to bind tightly to extracellular DNA plasmids and oligonucleotides [96, 97]. It can also migrate to the nucleus and modulate gene transcription [98]. Similar findings have been reported for A $\beta$ . Structural analysis indicated that A $\beta$  exhibits the signature characteristics of a nucleic acid-binding protein [99]. Moreover, direct binding to DNA has been described [100–104], and A $\beta$  is reported to enter the nucleus to bind directly to DNA to modulate transcription, targeting a specific A $\beta$ -interacting domain in the promoter regions of the key *APP*, *BACE1*, and *APOE* gene promoters [105]. Nucleic acid binding by A $\beta$  may also contribute to its

antiviral effects, such as by interfering with reverse transcription activity. For example, Wang et al. [106] found that A $\beta$  oligomers, but not monomers, robustly inhibited the reverse transcription activity of vertebrate telomerase enzyme TERT, probably by binding to the substrate RNA/DNA hybrid [106], raising the prospect that A $\beta$  nucleic acid binding might also inhibit the proliferation of retroviruses.

#### *Nucleic acid binding by PRNP: Inhibition of translation and induced PrP<sup>c</sup> to PrP<sup>Sc</sup> conversion as a sequestration strategy*

PRNP protein contains two basic regions in the N-terminus of the molecule, and moreover this region of the molecule is an 'intrinsically disordered region' (IDR) (Fig. 1). IDRs can refold around a molecule such as RNA so as to grasp the ligand, and the presence of an IDR is a characteristic of several RNA-binding proteins [107].

It has been established for many years that PRNP binds to nucleic acids ([108, 109], reviewed in [110, 111]). The presence of two basic regions (Fig. 1) suggests that PRNP may contain two binding sites for nucleic acids. In addition to binding to A $\beta$ , PRNP also interacts with several other nucleic acid-binding proteins – RNA-binding proteins were among the most significant hits in a microarray screen for PRNP binding partners [112]. Nucleic acid binding could contribute to the antiviral repertoire of PRNP. We (J.L.D.) previously reported that PRNP binding to HIV-1 mRNA blocks translation of the viral message, and native PRNP inhibited HIV-1 replication [84]. This activity has been confirmed for human, mouse, and hamster PRNP [113], and is thus evolutionarily conserved.

Does PRNP bind to nucleic acids *in vivo*? The evidence suggests that it does. For example, antibodies and binding proteins against single- and double-stranded DNA efficiently retrieve PRNP from TSE brain (CJD, BSE, scrapie), but not from control brain ([114]; antibodies against RNA were not tested). Importantly, specific nucleic acids precipitate the conversion of PrP<sup>c</sup> to PrP<sup>Sc</sup>, in which the protein refolds to adopt a  $\beta$ -sheet configuration and subsequently aggregates. Different nucleic acids differ in their ability to catalyze this transition. Binding of DNA can stimulate the  $\beta$ -sheet conversion, but aggregation is inhibited [109]. In detailed studies, Zeiler et al. [115] and Adler et al. [116] reported that different RNAs have widely different affinities for PrP<sup>c</sup>, with the highest binding being displayed

by highly structured RNAs with multiple double-stranded regions. In addition, only highly structured RNAs perturbed the conformation of PRNP in such a way as to promote the conversion of PRNP (PrP<sup>C</sup>) to PrP<sup>Sc</sup> [116]. The exact structural features are not yet known, although it was speculated that PRNP might be a sensor of abnormal RNAs containing non-Watson–Crick base pairs in double-stranded RNA [115], or of other motifs such as adjacent hairpins or quadruplex structures [117], and further work in this direction is certainly warranted.

We surmise that disease-associated aggregation of PRNP is likely to contribute to host defense, as it is for other antimicrobial peptides such as A $\beta$  [5]. In the case of A $\beta$  the specific trigger is not yet known, but for PRNP binding of specific abnormal RNAs causes refolding of the molecule and generation of the aggregation-prone PrP<sup>Sc</sup> form. Although this remains to be formally demonstrated, one may legitimately speculate that abnormal RNAs will thus become entrapped in an insoluble aggregate where they can no longer participate in cellular metabolism. Plausibly, sequestration of abnormal RNAs could be a component of the antimicrobial repertoire of PRNP (Fig. 2).

## CONCLUDING REMARKS

We have reviewed the enigmatic antiviral and proviral roles of PRNP, as well as binding of PRNP to AD A $\beta$ , a protein that is itself increasingly implicated as an antimicrobial agent [1–5]. The antimicrobial activities of both PRNP and A $\beta$  lend support to the theory that amyloid peptides may generally have antimicrobial activity [7, 8].

Both PRNP and A $\beta$  are high-profile molecules associated with human disease, and have thus been subject to intense scrutiny. Possible association of PRNP with other components of the innate immune system in addition to A $\beta$  has been less well investigated. Indeed, subtle deficits in cellular immunity have been reported in *Prnp* knockout mice [118], and PRNP has been implicated in defense against other forms of immunological and pathophysiological stress [119]. It is notable that both PRNP and A $\beta$  interact with the immunomodulatory protein APOE [32, 120], and a functional interaction between PRNP and  $\alpha$ -synuclein, the protein deposited in Parkinson's disease brain, has also been reported [121], of importance because of recent evidence that  $\alpha$ -synuclein itself could be an antimicrobial peptide [122–124],

lending further weight to the suggestion that amyloid peptides may generally have antimicrobial activity [7, 8].

Potential interactions with other components of the innate immune system such as LL-37, APP- and PRNP-like molecules, and other actors remain to be addressed in detail. Indeed, an interaction between A $\beta$  and LL-37 has recently been uncovered [125], and  $\alpha$ -synuclein was first discovered as a molecule that, like PRNP, binds with high affinity to A $\beta$  ([126]; reviewed in [127]). This raises the question of whether these molecules associate in a complex that appears to contain PRNP, APP/A $\beta$  and paralogues, APOE, other antimicrobial peptides, and RNA-binding proteins, among others. Do these constitute a multiprotein complex that is sprung into action following infection, and, if so, how would this process be triggered?

The prominent antiviral activity of PRNP (Table 1), reinforced by the finding that evolution has led HSV-1 to develop an 'anti-PRNP' function, argues that PRNP plays a role in innate immunity; PRNP oligomerization in response to binding of abnormal nucleic acids is also consistent with an innate immune defense strategy. It is also an intriguing fact that chain-terminating mutations in human *PRNP* are not only associated with brain disease, but chronic diarrhea is also a prominent feature in these patients [57], a typical presentation of innate immune deficiency (e.g., *NOD2* mutations in irritable bowel syndrome). By contrast, cattle and goats lacking PRNP appear to be overtly healthy [128, 129], although the extent to which *PRNP*-like genes (*doppel*, *PRND*, and *shadoo*, *SPRN*) might substitute for PRNP function in ruminants is not known, and the PRNP-deficient Norwegian goat line displays elevated levels of interferon-responsive gene expression that could be compatible with subclinical infection [130]. Direct challenge experiments have not been done.

Although in many scenarios PRNP acts to block viral proliferation, PRNP appears to be usurped and exploited by HSV-1. This is not unprecedented; for example, the classical antimicrobial peptide LL-37 stimulates, rather than inhibits, the life cycle of HIV-1 [94]. Looking wider, an antimicrobial role for PRNP may have implications for the pathoetiology of both TSEs and AD. Time will also tell whether other agents, in addition to HSV-1, might usurp the host defense roles of PRNP and/or A $\beta$  to ensure their own proliferation; further research into the roles of PRNP and A $\beta$  in innate immunity to infec-



tious agents is clearly warranted. To close, we quote Brentani and colleagues a decade ago: Time is ripe for examining possible loss-of-immune-function components of prion diseases in the context of peripheral infection [70].

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## CONFLICT OF INTEREST

The authors have no conflict of interest to report.

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