Neuroprotective Effect and Potential of Cellular Prion Protein and Its Cleavage Products for Treatment of Neurodegenerative Disorders: Part I. A Literature Review

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Abstract

Introduction: The cellular prion protein (PrP^C) is well known for its pathogenic roles in prion diseases, several other neurodegenerative diseases (such as Alzheimer's disease), and multiple types of cancer, but the beneficial aspects of PrP^C and its cleavage products received much less attention.

Areas covered: Here the authors will systematically review the literatures on the negative as well as protective aspects of PrP^C and its derivatives (especially PrP N-terminal N1 peptide and shed PrP). The authors will dissect the current findings on N1 and shed PrP, including evidence for their neuroprotective effects, the categories of PrP^C cleavage, and numerous cleavage enzymes involved. The authors will also discuss the protective effects and therapeutic potentials of PrP^C-rich exosomes. The cited articles were obtained from extensive PubMed searches of recent literature, including peer-reviewed original articles and review articles.

Expert Opinion: PrP and its N-terminal fragments have strong neuroprotective activities that should be explored for therapeutics and prophylactics development against prion disease, Alzheimer's disease and a few other neurodegenerative diseases. The strategies to develop PrP-based therapeutics and prophylactics for these neurodegenerative diseases will be discussed in a companion article (Part II).

Keywords

Alpha-cleavage; ADAM; Aβ and other toxic oligomers; Alzheimer's disease; neurodegenerative diseases; N1 peptide; neuroprotection; prion protein; shedding; therapeutics

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1. Overview of cellular PrP

The cellular prion protein (PrP^C) is known for its essential roles in prion diseases (PrD), a group of fatal and transmissible neurodegenerative diseases affecting humans and several mammal species[1–3]. Common PrD includes various forms of Creutzfeldt-Jakob disease (CJD) in humans, bovine spongiform encephalopathy in cattle, scrapie in goats and sheep, and chronic wasting disease in cervids (deer, elk, moose).

PrP^C has been implicated in a large number of biological or pathological processes. Its diverse biological roles have been recently reviewed[4–5], including neuronal survival[6–8], stress protection[9–14], inhibition of neuronal excitation and copper homeostasis[15–19], peripheral myelin maintenance[20], cellular proliferation and differentiation[8, 21–24], immune function[25–26], iron uptake[27], and circadian rhythm[28–30]; but some of the implied functions are questioned[4]. Pathologically, PrP^C is a critical player in several neurodegenerative protein-misfolding diseases. It is essential for both prion replication and prion pathogenesis in PrD[31]. It has also been reported to serve as the receptor for cytotoxic amyloid-β (Aβ) oligomers in Alzheimer's disease (AD), toxic soluble aggregates of Tau in AD and a few other common neurodegenerative diseases involving Tau[32–33] and α-synuclein (αSyn) oligomers that are critical in Parkinson's disease (PD) and other synucleinopathies[34–35]. It is worth noting that a recent article reports no binding of PrP^C to αSyn oligomers and the absence of PrP^C had no effect on the toxicity of αSyn oligomers[36], raising questions on the role of PrP^C in αSyn toxicity. Knocking-out or knocking-down the PrP gene expression showed only limited negative effects in mice[37–40] or cattle[41]. Goats naturally devoid of PrP also appear largely healthy[42]. Some modest defects such as progressive demyelinating neuropathy of the peripheral nervous system[20,43], impaired hippocampus-dependent spatial-learning and long-term potentiation[44], and sensitivity to oxidative stress[45] were later detected in PrP-null goat and/or mice. These factors make PrP^C a highly attractive target for development of prevention and therapeutics against PrD, AD, PD, and several other neurodegenerative diseases.

PrP^C is a universally expressed glycoprotein that attaches to the outer layer of the cell membrane via a glycosylphosphatidylinositol (GPI) anchor, with highest expressions in the nervous system, muscles, and lymphoid tissues[46–51] and expression in developing embryos[52]. The mature mouse PrP^C is ~210 amino acids long, consisting of a flexible unstructured N-terminal domain (residues 23–120), a highly structured globular C-terminal domain (residues 121–231) with three α-helices, two β-sheets, a disulfide bond, two Asn-linked glycans, and a GPI anchor[53–55] (Figure 1). PrP^C undergoes various cleavages under physiological and pathological conditions, some of which are beneficial and protective. During *de novo* prion formation and seeded prion replication, PrP^C undergoes a not well understood process of conformational changes into the misfolded aggregated prion or scrapie form (PrP^{Sc})[56–57]. One study suggests that intracellular prion conversion occurs primarily in the multivesicular bodies[58] that derive exosomes. PrP^{Sc} in turn leads to neural damages and eventually clinical symptoms and death in a progressive process that requires cell surface PrP^C and involves the cytotoxic oligomeric PrP^{Sc} [59], but the detailed mechanisms are still unclear[3]. Recent studies show that prion infectivity and prion toxicity

can be separated[60] and high-density oligomeric PrPs found in rapidly progressive AD patient brains are neurotoxic and may contribute to pathogenesis[61].

Here we review the various protective forms of PrP^C (including its cleavage and derivative products) as well as the enzymes involved in the cleavages, with an emphasis on the N-terminal fragment derived from the PrP^C α-cleavage, the shed full-length PrP derived from PrP^C shedding, and PrP^C-containing exosomes.

2. Beneficial PrP forms and protective PrP processing

There are three main types of PrP^C processing: α-cleavage, β-cleavage, and shedding (Figure 1B). The α-cleavage occurs at the 110/111 or 111/112 peptide bond of the hydrophobic central region of PrP, resulting in the membrane-attached C-terminal C1 fragment[62] and the N-terminal N1 peptide (88–89 amino acid residues) released to the extracellular space. The β-cleavage cuts towards the C-terminal end of the octapeptide repeat region, creating the membrane-attached C2 fragment and releasing the N-terminal N2 fragment[62–64]. Shedding of PrP is achieved through cleavages near the C-terminus of PrP^C (residues 228–231) or within the GPI anchor, releasing anchorless full length PrP^C from the cell surface[65–68].

As will be discussed in detail below, α-cleavage and shedding of PrP^C and the resulting PrP peptides or anchorless PrP protein are beneficial (Figure 2). The β-cleavage of PrP^C also seems to be cytoprotective. The β-cleavage of PrP^C was reported to involve reactive oxygen species (ROS), and it is thought to be an early and critical event in protection against oxidative stress, since cells expressing PrP mutants incapable of β-cleavage had increased sensitivity to oxidative stress when challenged with H_2O_2 and Cu^{2+} [69]. The C-terminal β-cleavage product (C2) does not appear to have significant protective effects, but one recent report shows that both N1 and N2 reduce reactive oxygen species and lead to decreased growth and differentiation of murine neural stem cells[70]. Recombinant ADAM8 has also been reported to cut within the octapeptide repeat region of recombinant PrP in vitro and this cleavage is influenced by Cu^{2+} and Zn^{2+} [71], but its biological relevance remains to be seen. Wik *et al.*[72] suggested that suppression of β-cleavage may make more PrP^C available for the protective α-cleavage and shedding processes.

2.1 α-Cleavage of PrP and the cleavage products

The α-cleavage appears to be the most beneficial PrP^C processing. It cleaves at the central hydrophobic region of PrP^C to produce the N-terminal N1 fragment and the C-terminal C1 fragment (Figure 1B), leading to reduction of the amount of cell surface PrP^C that is not only the substrate for prion replication but also a key mediator of toxicity in prion diseases, AD, and other neurodegenerative diseases. Of the two fragments produced, N1 is neuroprotective[73–74] (see details in section 2.4) and murine C1 inhibits prion replication in a dominant-negative fashion mice[75–76]. A recent report shows that a highly conserved seven residue deletion (Δ190–196) of the bovine C1 fragment leads to spontaneous prions from the mutant C1 in the RK13 rabbit kidney cells[77], but such spontaneous mutant C1 prions failed to infect cells expressing wild type PrP[77], further confirming the resistance of wild type C1 to prion conversion. Nevertheless, the prion convertibility of C1 from

humans and other species has not been investigated. C1 also has a negative side: it has been reported to upregulate the transcription and activity of p53 and potentiate caspase 3 activation induced by staurosporin (a proapoptotic molecule) in HEK293 cells[78]. Consistent with these observations, we found that p53 is involved in the primary myopathy in a mouse model induced to overexpress wild type PrP and accumulate high levels of C1 in the skeletal muscles[79–80]. Fortunately, the negative activities of C1 may be counteracted by N1 through down-regulating p53 expression[73]. Moreover, transgenic mice overexpressing PrP(23–111) that corresponds to GPI-anchored C1 did not appear to exhibit neurological deficits or histological lesions[75], consistent with the notion that C1 may be pro-apoptotic only under apoptotic stimuli and is nontoxic under normal conditions[81]. The α-cleavage of PrP^C also has additional biological functions: Bremer *et al.* reported that the α-cleavage of PrP^C is essential for myelin maintenance in peripheral nerves after examining four independent PrP-knockout mouse strains[20].

The α-cleavage of PrP was first discovered as the cleavage of the hydrophobic region at the 110/111 or 111/112 peptide bond, resulting in a GPI-anchored C1 fragment[62]. A later study using TSM1 neurons and HEK293 cells overexpressing wild-type (wt) PrP^C and 3F4-tagged murine PrP^C revealed that cellular PrP can be cleaved to release a soluble 11–12-kDa N-terminal peptide termed N1[82]. This α-cleavage occurs within the hydrophobic central domain of PrP (residues 105–120) since its deletion abolishes α-cleavage and partial deletion led to reduction in α-cleavage proportional to the size of the deletions[83]. However, the cleavage site itself is unexpectedly tolerant to sequence variations as long as overall hydrophobicity is maintained[83]. The sequence variation tolerance of the α-cleavage site may be an evolutionarily advantageous feature, as the α-cleavage of PrP^C confers protection to neurons and other cell types.

Two studies suggest that the α-cleavage occurs late in the secretory pathway and several enzymes may be involved, most notably ADAM9, ADAM10, and ADAM17[84–85]. Inhibition, overexpression, or knock-out of ADAM10 or ADAM17 in cultured cells revealed that ADAM10 contributes to constitutive N1 production while ADAM17 (also named TACE) mainly participates in regulated N1 formation[85]. A later report found that reducing endogenous ADAM9 expression using antisense cDNA lowered N1 secretion and co-expression of ADAM10 and ADAM9 led to enhanced α-cleavage in human PrP-expressing fibroblasts[84]. However, transient transfection of ADAM9 into primary fibroblasts derived from ADAM10 KO mice failed to increase N1 production, suggesting that ADAM9 likely indirectly regulates the α-cleavage by modulating ADAM10 activity through shedding of the ADAM10 ectodomain. Forced PrP dimerization has also been reported to enhance the α-cleavage of mouse PrP^C [86], but it is still unclear whether physiological PrP dimers confer the same effect.

Some reports cast doubts on the role of ADAM9, ADAM10 or ADAM17 in the α-cleavage of PrP^C. Taylor *et al.*[68] reported that the overexpression of ADAM9, ADAM10 or ADAM17, or the siRNA knockdown of ADAM9 and ADAM10 in murine PrP^C expressing HEK cells did not alter the amount of C1 fragment relative to full-length PrP^C, indicating a lack of influence of these three ADAMs on the α-cleavage. This was corroborated by a later study from Wik and colleagues[72] showing that the addition of σ-phenanthroline (a general

ADAM inhibitor) and TAPI-1 (an ADAM17 inhibitor) to bovine PrP^C-expressing baby hamster kidney-21 (BHK) cells did not reduce α-cleavage by measuring the kinetics of the generation of the C1 fragment. It is worth noting these two studies both quantified C1 to measure α-cleavage activity. In addition, Altmeppen et al.[87] showed that the α-cleavage of PrP^C as measured by both C1 and N1 levels was not changed in primary neurons derived from neuron-specific ADAM10-KO mice and concluded that ADAM10 is not involved in the α-cleavage of PrP^C. On the other hand, McDonald et al.[71] showed that recombinant ADAM10 and ADAM17 can both cleave recombinant murine PrP in vitro at the 119/120 peptide bond (termed $α_3$-cleavage), which is 9–10 residues downstream of the normal α-cleavage site, but such $α_3$-cleavage has not been confirmed in cells or animals.

We have reported that ADAM8 is the primary α-cleavage enzyme for PrP^C in muscles based on in vitro experiments using recombinant PrP and recombinant ADAM8 as well as data from cultured muscle cell line (C2C12) and muscle tissues of PrP transgenic mice and ADAM8 knockout mice[88]. We found that ADAM8 protein level positively correlates with C1 production in the skeletal muscles of mice and in the C2C12 myoblast cell line, and that recombinant ADAM8 directly cleaved recombinant PrP to generate the C1 fragment in vitro. ADAM8 also contributes to the α-cleavage of PrP^C in the brain, although not as the primary α-secretase (Liang and Kong, unpublished data). McDonald et al.[71] studied PrP α-cleavage with recombinant mouse PrP and recombinant ADAM8, ADAM10 and ADAM17. They found that, in addition to cleaving at the previously reported α-cleavage site (109/110) (termed $α_1$-cleavage), ADAM8 can also cut within the octapeptide repeats and at 116/117 (termed $α_2$-cleavage), and the cleavage site preference is influenced by Cu^{2+} and Zn^{2+}. The novel ADAM8 cleavage sites have not been confirmed by studies in cells or animals.

The mechanisms underlying the contradicting results concerning the roles of ADAM9, ADAM10 and ADAM17 in α-cleavage of PrP^C are unclear. Cissé and colleagues[84] and Vincent and colleagues[85] (2001) both concluded that ADAM10 and ADAM17 are implicated in the α-cleavage, based on their data using the SAF-32 antibody (recognizing PrP amino acids [aa] 79–92) to detect N1. In contrast, Taylor and colleagues[68] used the 6H4 antibody (recognizing PrP aa144–152) to detect C1 and reached the opposite conclusion, i.e., ADAM9, ADAM10, and ADAM17 are not involved. On the other hand, Altmeppen and colleagues[87] used the POM2 antibody (recognizing PrP octarepeat region) to detect N1 and used the POM1 antibody (recognizing PrP C-terminal aa121–230) to detect C1 and concluded that ADAM10 is not involved in α-cleavage. N1 is easily degraded and difficult to quantify whereas C1 is much more stable and easier to measure accurately. If the unusual α-cleavages by ADAM8, ADAM10 and ADAM17 reported by McDonald et al.[71] can be confirmed in cell and animal studies, it will establish the roles of ADAM10 and ADAM17 in the α-cleavage of PrP^C and help reconcile the conflicting findings in the other cell and animal studies utilizing various monoclonal anti-PrP antibodies. Concurrent measurements of both N1 and C1 with appropriate anti-PrP antibodies, or revisiting the N1 measurements under stringent conditions, may also help resolve the controversy.

Alternatively, it is conceivable that, when one α-cleavage enzyme (such as ADAM10) is knocked down, the very limited impact on overall α-cleavage could be due to compensation from another α-cleavage enzyme(s) that cleaves at the same site or a slightly different

location in the hydrophobic region. In addition, enhancing the activity of one α-cleavage enzyme may fail to augment overall α-cleavage because another α-cleavage enzyme(s) may be downregulated concurrently.

In addition, it is entirely possible, even expected, that the enzyme(s) responsible for the α-cleavage of PrP^C may vary with the cell types and their tissue environment. Further investigations that measure both N1 and C1 in different cell types in vitro and in vivo will be necessary to fully identify all the enzymes involved in the crucial α-cleavage of PrP^C in each cell type.

2.2 Shedding of PrP and extracellular full-length PrP

Shedding of PrP^C from the cell surface increases extracellular PrP levels with a concurrent decrease of cell surface PrP^C, both leading to protection against the toxicity of misfolded protein oligomers in AD and other diseases. PrP^C shedding is the process in which cell surface PrP^C is cleaved near the GPI anchor by a secretase-like protease enzyme termed "sheddase" or at the GPI anchor by phospholipases, releasing an anchorless full-length PrP^C molecule into the extracellular space[87]. Mass spectrometric analysis shows that this shedding cleavage takes place just three amino acids away from the GPI anchor, between residues 228/229[87]. Several studies suggest that ADAM9 and ADAM10 are involved in PrP shedding[68,71,87,89].

Using recombinant mouse PrP and recombinant ADAM8, ADAM10 and ADAM17, McDonald et al.[71] demonstrated that ADAM10 can cleave PrP near its C-terminal end in test tubes, but ADAM8 and ADAM17 showed no shedding activities. Through overexpression and siRNA knockdown experiments in HEK cells, Taylor and colleagues[68] found that ADAM9 and ADAM10, but not ADAM17, are implicated in PrP shedding. Overexpression of ADAM10 alone led to a significant increase in the amount of shed PrP in the conditioned cell culture medium, and co-overexpression of ADAM9 and ADAM10 led to even more shed PrP, while overexpression of ADAM17 had no significant effect on PrP shedding. Moreover, siRNA knockdown of endogenous ADAM enzymes found that the knockdown of either ADAM9 or ADAM10 reduced the amount of shed PrP in the conditioned medium. In contrast, when ADAM10 is knocked down, ADAM9 overexpression did not affect shedding of PrP, indicating that the effect of ADAM9 on PrP shedding is achieved through modulating the activity of ADAM10. In addition, Altmeppen and colleagues[87] found that there was a 77% decrease of shed PrP^C in the culture medium of primary neurons derived from neuron-specific ADAM10 KO mice when compared to the littermate controls. Expression of ADAM10 from a plasmid in the ADAM10-KO neurons restored PrP^C shedding. Together these findings confirm that ADAM10 is the primary sheddase for PrP^C and ADAM9 affects PrP^C shedding through modulating ADAM10.

2.3 Full length PrP

2.3.1 Protective effects of full length PrP—Full length PrP^C has been reported to possess protective effects in vitro and in vivo. Cell surface PrP^C enhances neuronal survival through activation of the cAMP-dependent PKA pathway[6–8]. Cell surface PrP^C serves as a trophic receptor that activates cAMP/protein kinase A (PKA) to protect against anisomycin-

induced cell death in cell cultures of retinal explants[6] and staurosporine-induced cell death in primary hippocampal slices[8]. The protective effect of PrP[C] is also corroborated by Chen and colleagues[7], who observed increased apoptosis of primary cerebellar neurons from PrP-null mice compared to primary cerebellar neurons from wt mice. PrP[C] was also reported to be necessary for recombinant stress-inducible protein 1 (STI1)-induced neuritogenesis through activating the MAPK pathway in primary hippocampal slices[8].

Full length PrP[C] has also been observed to be protective under cellular stress. PrP[C] levels were found to be elevated in neuronal soma in the gray matter in ischemic or hypoxic human or mouse brains and the absence of PrP[C] led to bigger infarct in ischemic mouse brains[10]. An independent study confirmed this report and found that these protective effects required the N-terminal octarepeat region of PrP[C] [11]. In addition, PrP[C] has been reported to enhance the cell viability through Fyn kinase activation in immortalized hippocampal neuronal cells exposed to hydrogen peroxide[12] and in neuronal cells (SH-SY5Y and N2a) treated with kainite (an excitotoxin)[13].

Axonal PrP[C] that can undergo α-cleavage appears to play an important role in peripheral myelin maintenance, since axon morphometry analysis demonstrated that all four independent PrP[C]-deficient mouse strains experience chronic demyelinating polyneuropathy[20]. In addition, α-cleavage of PrP[C] was found to be elevated in ScN2a neuroblastoma cells treated with soluble αSyn aggregates, which may have contributed to the observed enhanced clearance of the toxic αSyn aggregates[90].

PrP[C] monomers can also form dimers[13,91] under physiological conditions, through interactions of the hydrophobic central domain[86,92]. PrP dimerization seems to be beneficial through promoting PrP[C] α-cleavage[86], mediating stress protective effects[13], and inhibiting prion replication in a dominant-negative manner[93].

Transgenic expression of a dimeric PrP-immunoglobulin Fcγ fusion protein was also shown to delay PrP[Sc] accumulation and disease onset in wildtype mice intracerebrally or intraperitoneally inoculated with prions[94].

2.3.2 The negative roles of full-length PrP[C]—Given the protective effects of PrP[C], it is tempting to adopt overexpression of full-length PrP[C] as a therapeutic approach. However, this strategy carries multiple risks, since PrP[C] plays a negative role in several neurodegenerative diseases (such as PrD and AD) as well as in several types of cancer and other diseases.

PrP[C] has been reported to be a critical toxicity mediator for Aβ aggregates in AD. PrP[C] binds to synthetic or AD brain-derived Aβ and shows the highest affinity for its oligomeric form over monomers and fibrils[95–97]. The crucial sites for PrP[C]-Aβ interaction in solutions in vitro have been determined as the N-terminal residues 23–27 and the ~92–110 region of PrP[C] [98]. To mediate Aβ toxicity, PrP[C] binds to metabotropic glutamate receptor 5 (mGluR5) on the cell surface to form co-receptors for Aβ oligomers[99] and mediate Aβ toxicity[100]. The PrP[C]-mGluR5 co-receptor triggers toxic signaling pathways upon binding with Aβ oligomers, activating the Fyn kinase (a member of the Src family kinases)[99,101], which in

turn causes activation of NMDAR through phosphorylation of its NR2B subunit, leading to calcium influx and cell death[101].

Moreover, Aβ-induced lactate dehydrogenase (LDH) release and dendritic spine loss also require the PrP^C-Aβ oligomer-mGluR5 complex in mouse hippocampal neuronal cultures[99, 101]. In addition, PrP^C is implicated in Aβ-induced impairment of long-term potentiation (LTP)[35,95], but this is disputed by other reports that found no significant effect of PrP^C levels on Aβ-induced LTP inhibition in PrP null mice[102] or cells ablated or overexpressing PrP[103]. The reasons for this discrepancy are unclear, but could be due to differences in the models (cell types, mouse strains) and reagents (Aβ preparations) used.

PrP^C also seems to mediate αSyn oligomer toxicity through the same pathway as that for Aβ oligomers[104]. Cell surface PrP^C was reported to preferentially bind to soluble aggregates or shorter fibrils of αSyn over their monomeric and large fibrillary forms[35,90] and αSyn oligomer binding is dependent on similar PrP regions (aa23–31 and aa92–110)[35] implicated in Aβ oligomer binding. PrP^C has also been implicated in the internalization of recombinant αSyn fibrils by cultured N2a cells and mouse primary hippocampal neurons. The rate of αSyn uptake was significantly lower in PrP-null cells[90,105,106] and in PrP-null mice[90], and there was less αSyn aggregation, astroglial activation and loss of dopaminergic neurons in the brains of PrP-null mice after intracerebral inoculation with recombinant αSyn fibrils[90]. In addition, PrP-null mice did not exhibit αSyn-induced LTP inhibition and treatment with an anti-PrP antibody reversed the αSyn-induced LTP defect in hippocampal slices from a transgenic mouse model of PD[104]. These observations further validate the role of cell surface PrP^C as a critical mediator of αSyn toxicity.

Cell surface PrP^C seems to play a similar role in Tau aggregate-induced toxicity that requires the same N-terminal PrP regions (aa23–31 and aa92–110) used by Aβ and αSyn oligomers[35,107–108]. Recombinant PrP is reported to show a higher affinity for soluble recombinant Tau aggregates than for Tau monomers or end-stage fibrils in vitro[35]. Again, PrP^C seems to mediate the internalization and toxicity of Tau aggregates, but the mechanisms are less well understood. The uptake of recombinant Tau K18 amyloids was reported to be greatly reduced in N2a cells ablated for PrP^C [108], and soluble Tau aggregates-induced neurotoxicity is abolished when PrP gene is ablated, knocked-down, or when neurons are pre-treated with anti-PrP blocking antibodies in mouse primary neurons[35]. PrP^C is also involved in toxicity induced by soluble Tau aggregates in vivo[109]. Intracerebroventricular administration of anti-PrP antibody 6D11 (epitope aa95–105) prior to intrahippocampal injection with recombinant Tau aggregates or human AD brain-derived Tau abrogated the inhibition of LTP in male rats[109].

Elevated PrP^C levels are associated with several cancers[110–114]. For instance, Pan and colleagues[110] reported that high levels of PrP^C found in gastric cancer tissues enhanced invasion and metastasis, probably via the PrP^C N-terminal region-mediated activation of MMP11 through the MEK/ERK pathway. Furthermore, the increased PrP^C expression in cancer cells seems to be directly correlated with shorter survival for pancreatic cancer patients[112].

Elevated PrP[C] levels will also increase the susceptibility and sensitivity to prions since PrP[C] is the essential substrate for prion replication and necessary for prion pathogenesis. Moreover, too much PrP[C] may worsen AD, PD and other neurodegenerative diseases because PrP[C] is a key receptor for the toxic Aβ, Tau, and αSyn oligomers[34–35,95, 97,100–101,107–109,115–116].

In addition, overexpression of wild type full-length PrP alone can cause diseases. Transgenic mice overexpressing wt PrP from hamster or mice exhibited spontaneous degeneration of muscles and peripheral and central nervous systems[117]. Similarly, transgenic mice overexpressing wt mouse PrP[C] ten-fold developed a progressive neurological illness with tremor, paralysis of the hind limbs, and abnormal posture, accompanied by accumulation of non-transmissible but neurotoxic PrP aggregates, and significant granule neuron degeneration and synaptic terminal enlargement[118]. We also found that muscle-specific overexpression of wild type PrP[C] led to a primary myopathy that is associated with muscular accumulation of the C1 fragment[80] and involves the p53 pro-apoptotic pathway[81]. Moreover, TSM1 neuronal cells and HEK293 kidney cells overexpressing full-length PrP[C] were found to be more prone to apoptosis after treatment with proapoptotic agents[119].

In summary, overexpression of full-length PrP[C] has many serious caveats, which makes it a very risky therapeutic strategy.

2.4 N1 peptide

2.4.1 Protective effects of N1 peptide—The extracellular N1 peptide, one of the α-cleavage products of PrP[C], has been shown to be broadly protective to cells from toxic molecules, including reactive oxygen species and multiple toxic protein oligomers implicated in AD, PD and other diseases, and might ultimately enhance neuronal viability.

In 2009, Guillot-Sestier and colleagues[73] first demonstrated that recombinant N1 fragment, but not the N2 fragment, shows cytoprotective function. They found that the N1 peptide show antiapoptotic effect through lowering caspase 3 activation in a dose-dependent fashion in mouse primary cortical neurons treated with staurosporin, and it protects against apoptosis through lowering p53 activity, as seen by a 46% reduction in mRNA transcription in HEK293 cells and 91% reduction in rat retinal cells under pressure-induced ischemia.

Recombinant N1 also protects primary cultured neurons against toxicity and cell death triggered by Aβ oligomers in conditioned medium from HEK293 cells overexpressing mutant APPs associated with familial AD or extracts from AD brain tissues[120]. A later report found that recombinant N1 binds to Aβ oligomers with high affinity and inhibits fibrillization of Aβ monomers and the association between recombinant N1 and Aβ oligomers is dependent on two positively charged regions (aa23–31 and aa95–105) of N1 and the intervening region (aa32–94)[121].

The mechanism of N1 neutralization of Aβ toxicity has been studied. Béland and colleagues[122] reported that coincubation of recombinant N1 and Aβ oligomers secreted by CHO-7PA2 cells causes a conformational change in Aβ oligomers that turns Aβ oligomers into insoluble, amorphous Aβ aggregates that cannot assemble with other

Aβ, thereby removing Aβ oligomers from the normal fibrillization pathway. N1 also co-immunoprecipitated with Aβ in the guanidine-extractable fraction from postmortem AD brain tissues, suggesting that N1 may coaggregate with Aβ in vivo as well[122]. By measuring the ratio of deglycosylated full length PrP^C and C1 in postmortem AD patient brains, Béland and colleagues[122] also observed an increase in α-cleavage of PrP^C, supporting the idea that the PrP^C α-cleavage is an endogenous neuroprotective mechanism against AD. Recombinant full-length PrP and N1 may also inhibit Aβ oligomerization and neutralize the toxicity of pre-existing Aβ oligomers, possibly by blocking Aβ oligomers from binding with cell surface PrP^C in hippocampal slices and neurons from wt and PrP-null mice[123]. Preincubation of these recombinant PrP forms with preformed Aβ oligomers before treating rat hippocampal slices was shown to decrease LDH release and restore LTP, further suggesting a protective effect of recombinant full-length PrP and N1 against Aβ[123]. Furthermore, preincubation of recombinant N1 with Aβ oligomers reduced the loss of synaptic markers in primary neurons and rescued Aβ oligomer–induced behavior deficits in C57/BL6 mice after intracerebroventricular injection[123].

In a very recent study, recombinant N1 induced secretion of the Cxcl10 cytokine and enhanced the metabolism and morphology of microglia that required direct cell-to-cell contact in a neuron-microglia co-culture system, suggesting that N1 may also improve neuronal cell viability[124].

2.4.2   Potential caveats of N1 peptide—There are seemingly significant yet misinformed concerns on the safety of using N1 as a therapeutic agent. There are a few reports suggesting that the N-terminal region of PrP is toxic. Sonati *et al.*[125] reported that the anti-prion antibodies induce toxicity in mice and in cerebellar organotypic cultured slices where the N-terminal flexible tail of cell-surface PrP^C plays an important role. The same group subsequently showed that transgenic mice expressing an internally truncated PrP protein (141–225), in which the N-terminal region (aa 1–140) and the GPI-anchor signal peptide (aa 232–254) are retained, developed a progressive, inexorably lethal neurodegeneration morphologically and biochemically similar to that triggered by antibodies against the PrP globular domain[126]. In this case, the toxic PrP is a GPI-anchored cell surface protein that contains the flexible N-terminal tail (aa23–128) and part of the 1st α-helix of PrP^C. In addition, when the PrP N-terminal tail (aa23–109) is expressed as a GPI-anchored GFP fusion protein (aa1-109-GFP-GPI anchor), it is toxic by inducing ionic currents[127]. Moreover, antibodies targeting epitopes in the C-terminal domain of PrP^C induce ionic currents and cause degeneration of dendrites on murine hippocampal neurons that are dependent on the presence of the N-terminal tail of PrP^C, and there is intramolecular docking between N- and C-terminal domains of PrP^C [127]. These data support an intramolecular auto-inhibitory mechanism for PrP toxicity, but all the evidences for the toxicity of PrP N-terminal fragments are based on GPI-anchored PrP forms located on the cell surface. This cell surface PrP location allows for ionic currents or other toxic signaling to damage the host cells. There is no evidence suggesting that PrP N1 and similar N-terminal fragments are toxic when they exist as free molecules in the extracellular space, such as after α-cleavage of PrP^C, expressed as a secreted peptide, or administered as a recombinant peptide.

The significance of the reported p53 suppression by N1[73] needs to be investigated. It is interesting to note that p53 binds directly to the promoter region of the PrP gene to activate its transcription[128]. We have reported the association of enhanced muscular p53 activity in a primary myopathy associated with dramatic overexpression of PrP and elevated PrP α-cleavage in the muscles of an inducible mouse model[79–80].

3.   PrP shedding, shed PrP, and extracellular full-length PrP

The nearly full-length shed anchorless PrP is believed to be protective as well[129], but no study has directly investigated the effect of shed PrP. There are several reports implying that the process of PrP shedding itself is beneficial since it reduces cell surface PrP^C levels[87,130], thereby decreasing levels of the essential substrate for prion replication and a critical receptor for several toxic protein aggregates as described above.

3.1   Beneficial effects of shed PrP

Several studies using recombinant full-length PrP have demonstrated its protective effect, and the potential benefits of shed PrP have been reviewed in depth[129]. Recombinant full-length PrP has been reported to induce neuritogenesis. Incubation of full-length recombinant hamster or mouse PrP with embryonic rat hippocampal neurons was reported to increase the development of synapses and length of neuronal networks[131]. Likewise, in a hippocampal slice culture system, soluble full-length recombinant mouse PrP induced a concentration-dependent neurite outgrowth and rapid growth cone turning towards the source of PrP, both requiring the presence of cell surface GPI-anchored PrP^C [132]. Recombinant full-length PrP also inhibits both fibrillization of Aβ1–42 monomers and Aβ aggregate toxicity in cultured neurons[133] and neutralizes Aβ oligomer toxicity in cultured slices of AD mouse brains[121].

Recombinant full-length PrP has also been reported to bind to toxic Tau oligomers and aSyn oligomers[35]. Although experimental evidence is still lacking, it is very likely recombinant full-length PrP will also neutralize the toxicity of Tau oligomers and αSyn oligomers. It is worth noting that recombinant full-length PrP is not an ideal substitute for shed PrP^C since recombinant PrP generated from bacteria does not have the O-linked glycans that shed PrP^C possesses.

Enhanced shedding of cell surface PrP^C has been implicated in protection against prions. Heiseke *et al.*[130] reported that, in cultured murine N2a neuronal cells, overexpression of a sorting nexin (SNX33) increased shedding of PrP^C, reduced the cell surface PrP^C level, and lowered PrP^{Sc} formation by ~60% in persistently scrapie infected cells and inhibited scrapie infection in naïve cells. Transgenic overexpression of ADAM10, the primary PrP^C sheddase, led to reduced full-length PrP^C levels and prolonged survival after prion infection in mice[134], and neuron-specific knockout of ADAM10 led to elevated PrP^C levels, increased PrP^{Sc} accumulation and reduced incubation time following prion infection[135]. However, there is one report arguing that enhanced shedding of PrP^C may not reduce prion replication in cultured scrapie-infected cells[68]. In persistently scrapie-infected ScN2a mouse neuroblastoma cells, suppressing ADAM10 with an inhibitor did not change the amount of protease-resistant PrP^{Sc}. Moreover, overexpression of ADAM10 from a plasmid failed to alter the percentage of protease-resistant PrP^{Sc}-positive cells, and the cell surface PrP^C

levels were not found to be altered in these cells[68]. Further investigation is needed to clarify this issue.

The mechanism of PrP^{Sc} accumulation inhibition by PrP^C shedding is not well understood, but cell surface PrP levels, which can be reduced by enhanced PrP shedding, are known to be inversely correlated with incubation times after prion infection in mouse models[135–137]. Reduction of cell surface PrP^C levels through PrP shedding in peripheral tissues may also slow neuroinvasion. PrP^C expression in the follicular dendritic cells of the spleen has been shown to be critical for neuroinvasion after peripheral prion inoculations[138–139]. In addition, transgenic mice expressing only GPI-anchorless PrP in an endogenous PrP-null background exhibited slow and infrequent CNS neuroinvasion after peripheral prion inoculations[140], which is consistent with a potential protective effect of increased PrP shedding.

3.2 Potential caveats of shed PrP

Shed PrP exists in extracellular space, which should mimic transgenically expressed anchorless PrP or administered recombinant full-length PrP to confer protection against toxic oligomeric aggregates of amyloidogenic proteins by sequestering these toxic molecules in the extracellular space. However, enhancing PrP^C shedding may also lead to negative biological activities in several ways.

First, shed PrP may serve as a good substrate for prion replication. Mice co-expressing anchorless PrP^C and GPI-anchored PrP^C have been reported to show accelerated prion disease[141] after prion inoculation. Extensive prion spread[142–143] and very high levels of infectious prions[144–145] were also found in transgenic mice expressing anchorless PrP after prion inoculation. Second, ADAM10 is the sheddase for several important cell surface proteins[146], so enhancing ADAM10 activity may have negative consequences for cancer[147–150], Fragile X Syndrome[151], and Huntington's Disease[152]. Third, treatment with recombinant full-length PrP, and by extension, enhancement of PrP^C shedding, may risk inducing inflammation in the CNS. Full-length recombinant human PrP induced the release of inflammatory mediators IL-6 and CCL2 in primary human astrocytes[153] and of CCL2, CXCL-12, and IL-8 in both uninfected and HIV-infected human primary monocytes[154]. CCL2 and TNF-α were shown to enhance the activity of ADAM10 (the PrP^C sheddase) in human astrocytes infected with HIV[154], and soluble PrP^C levels were elevated in the CSF of HIV-infected people with cognitive impairment[153]. These data suggest a positive feedback loop whereby extracellular PrP induces the release of cytokines, which augments ADAM10 activity that leads to elevated extracellular PrP levels through enhanced shedding of PrP^C.

4. Exosomal PrP

4.1 Protective effects of exosomal PrP

PrP^C on exosomes has also been shown to be protective against Aβ toxicity[155–158]. Exosomal PrP isolated from N2a cells was found to bind preferentially to smaller synthetic Aβ$_{42}$ aggregates in a PrP^C-dependent manner in vitro[158]. Moreover, exosomal surface PrP^C derived from hippocampal cells and injected intracerebroventricularly into the brain of rats was reported to bind to Aβ-derived diffusible ligands (ADDLs) prepared from synthetic

$A\beta_{1-42}$ in vivo[157]. The mechanism through which exosomal PrP^C binds to $A\beta$ has not been reported, but it likely depends on the same PrP regions (aa23–31 and aa95–105) that are crucial for recombinant N1 binding to $A\beta$[121]. Exosomal PrP^C has also been reported to protect against $A\beta$-related toxicity. First, preincubation of synthetic $A\beta_{42}$ with PrP-containing exosomes accelerated $A\beta_{42}$ aggregation into its fibrillar form, reduced synthetic $A\beta$ uptake, and abolished $A\beta_{42}$-induced apoptosis[158]. It is possible that, like extracellular recombinant PrP and N1, exosomal PrP^C may also inhibit the formation of toxic oligomeric $A\beta$ and promote the formation of fibrillary $A\beta$. Second, intracerebroventricular injection of PrP^C-containing exosomes rescued the LTP inhibition induced by ADDLs or AD brain extracts in the rat brains[157]. Third, exosomes isolated from murine N2a cells or mouse primary cortical neurons expressing PrP^C enhanced the clearance of endogenous $A\beta$ aggregates after intrahippocampal injection in an APP transgenic model, likely through exosome binding with the aggregates that upregulate internalization and degradation of the aggregates by microglia[155–156]. Continuous intraventricular administration of N2a cell-derived exosomes also ameliorated synaptic dysfunction in an APP transgenic mouse model, as seen by higher synaptophysin immunoreactivities compared to vehicle-treated mice[155].

Since full-length recombinant and neuronal PrPs have also been shown to bind to Tau and αSyn oligomers[35], exosomal PrPs can be expected to confer protection similarly by sequestering the toxic Tau/αSyn oligomers in the extracellular space, thereby hindering the toxic signaling through preventing Tau/αSyn oligomers from binding with cell surface PrP^C in the CNS of patients suffering from various tauopathies and synucleinopathies.

4.2   Potential caveats of exosomal PrP

Exosomes may be a powerful vehicle to harness and deliver the protective powers of PrP^C, but using exosomal PrP^C for therapeutic purposes may carry the same risks as using shed PrP or recombinant full-length PrP, such as facilitating the replication and spread of prions. Exosomes have been shown to associate with PrP^{Sc} in the culture media of prion infected cells[159]. Exosomes derived from prion-infected neuronal cell lines were reported to initiate prion propagation in uninfected non-neuronal cells and induce PrD when injected into mice[160], and blood-derived exosomes from prion-infected mice were infectious when injected into the Tga20 mice that overexpress mouse PrP^C [161]. Although there is no direct study yet, exosomal PrP^C might also induce CNS inflammation in a positive feedback loop involving ADAM10, as has been shown for recombinant full-length PrP. More work needs to be done to examine other biological activities that exosomal PrP^C may possess. In addition, exosomes contain many other molecules on its surface and its internal space, and some of them may induce toxic signaling or alter the recipient cell functions after being internalized[162–165].

5.   Conclusion

In summary, extracellular forms of PrP, including shed full-length PrP, exosomal full-length PrP, the N1 peptide released by PrP^C α-cleavage from cell surface, or externally administered recombinant full-length PrP or N1 peptide, have shown protective effect against certain stresses and several toxic molecules that are critical

in various neurodegenerative diseases, including PrD, AD, PD, and other tauopathies (such as progressive supranuclear palsy and frontotemporal lobar degeneration) and synucleinopathies (such as dementia with Lewy bodies, multiple system atrophy, and Lewy body variant of AD). These observations suggest that extracellular forms of PrP have great prophylactic and therapeutic potentials (Figure 2). We hypothesize that elevating the extracellular levels of one or more of these protective PrP forms is a promising broad-spectrum strategy against these devastating neurodegenerative diseases. A companion article (Part II) explores and evaluates this strategy for prophylactic and therapeutic purposes.

6. Expert opinion

The cellular PrP has been confirmed or implicated in many biological and pathological processes. On the one hand, it is suggested to play critical biological roles such as in development, myelin maintenance and cellular protection. On the other hand, it promotes cancer, is essential for prion replication and pathogenesis, and seems to be a mediator for toxicity through serving as a key receptor for several toxic oligomers of misfolded proteins (Aβ, Tau, and αSyn) in common neurodegenerative diseases, such as AD and other tauopathies as well as PD and other synucleinopathies. Cellular PrP is also subject to several types of regulated cleavage processing, including α-cleavage, β-cleavage and shedding, all appear to be protective at varying degrees. Unfortunately, PrP is still generally perceived as a "bad" protein by most and the positive side of PrP is often ignored.

Here we highlight the positive aspects and beneficial forms of PrP through reviewing the extensive literature on posttranslational PrP cleavages and the PrP cleavage products as well as the biological and pathological effects of cellular PrP and its cleavage products when localized on the cell surface or in the extracellular space. It is clear that most of the pathological effects of PrP are associated with cell surface full-length PrP and several PrP forms are protective when presented in the extracellular space. These protective PrP forms include the N1 peptide (the N-terminal PrP fragment derived from α-cleavage of cell surface PrP), the shed PrP (derived from shedding of cell surface PrP, primarily by ADAM10 in the brain), and exosomal PrP, all of which exist in the extracellular space. These extracellular beneficial PrP forms protect against the toxicity of misfolded proteins through sequestering the toxic oligomeric forms in the extracellular space and prevent the initiation of toxic signaling cascades that ultimately lead to cytotoxicity and neurodegeneration. In the case of N1, it can also directly reduce the toxic Aβ oligomers by inhibiting its formation and promoting its further aggregation into large fibrils that are much less toxic. The potential caveats of these beneficial PrP forms are also discussed, which mostly involve the risks of activating inflammation and facilitating the replication and spread of prions (and other misfolded protein aggregates in the case of exosomes). The N1 peptide and possibly other PrP N-terminal peptides do not have these drawbacks, making it a prime candidate for broad spectrum PrP-based therapeutics and prophylactics against PrD, AD, PD and other related neurodegenerative diseases.

The α-cleavage of PrP appears to be the most beneficial PrP processing since the two products (N1 and C1) are both protective and the negative effect of C1 is counteracted by N1, and the cleavage process itself reduces the level of cell surface PrP that is a substrate

for prion replication and a critical mediator of toxicity in PrD, AD and several other neurodegenerative diseases. Therefore, enhancing PrP α-cleavage is a promising strategy to develop treatments against these diseases. Given the essential roles of cell surface PrP in prion replication and pathogenesis, the RNA interference strategy targeting cellular PrP mRNAs has been tried with great success in treating prion diseases in mouse and cell models[166–170]. Since cellular PrP also mediates the toxicity of Aβ, Tau, and αSyn oligomers, it is reasonable to expect similar success through knocking down cellular PrP expression. Taking it one step further, simultaneous elevation of a PrP N-terminal peptide and knocking-down of the cellular PrP should be an even better strategy to develop safe and effective treatments and prevention for these neurodegenerative diseases. The various PrP-based strategies to develop broad spectrum treatments and prevention of PrD, AD, PD and a few other neurodegenerative diseases are covered in depth in the companion paper (Part II).

Acknowledgements

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Article highlights

- The full length cellular prion protein (PrP^C) has diverse biological roles such as neuronal survival, stress protection, neuronal excitation, peripheral myelin maintenance, and cellular proliferation and differentiation, yet PrP-null animals are largely normal.

- PrP^C is essential for prion diseases and serves as a key common receptor for a few toxic protein oligomers in Alzheimer's disease (AD) and a few other common neurodegenerative diseases involving Tau or α-synuclein (αSyn).

- PrP^C can undergo various cleavages under physiological and pathological conditions, some of which have been shown to be protective, most notably α-cleavage and shedding.

- Extracellular forms of PrP such as shed full-length PrP, exosomal full-length PrP, the N1 peptide released by PrP^C α-cleavage from cell surface (PrP-N), or recombinant PrP forms have been shown to be protective against toxic stressors.

- The N1 peptide derived from α-cleavage of cell surface PrP and recombinant N1 can protect against the toxicity of Aβ oligomers by inhibiting Aβ oligomer formation, hindering toxic signaling, and promoting oligomer aggregation into larger fibrils that are less toxic.

- Knocking down PrP^C expression while elevating the extracellular level of the PrP N-terminal peptide should have excellent prophylactic and therapeutic potential against several neurodegenerative diseases including PrD and AD, which will be explored in depth in the companion paper (Dexter and Kong, 2021).
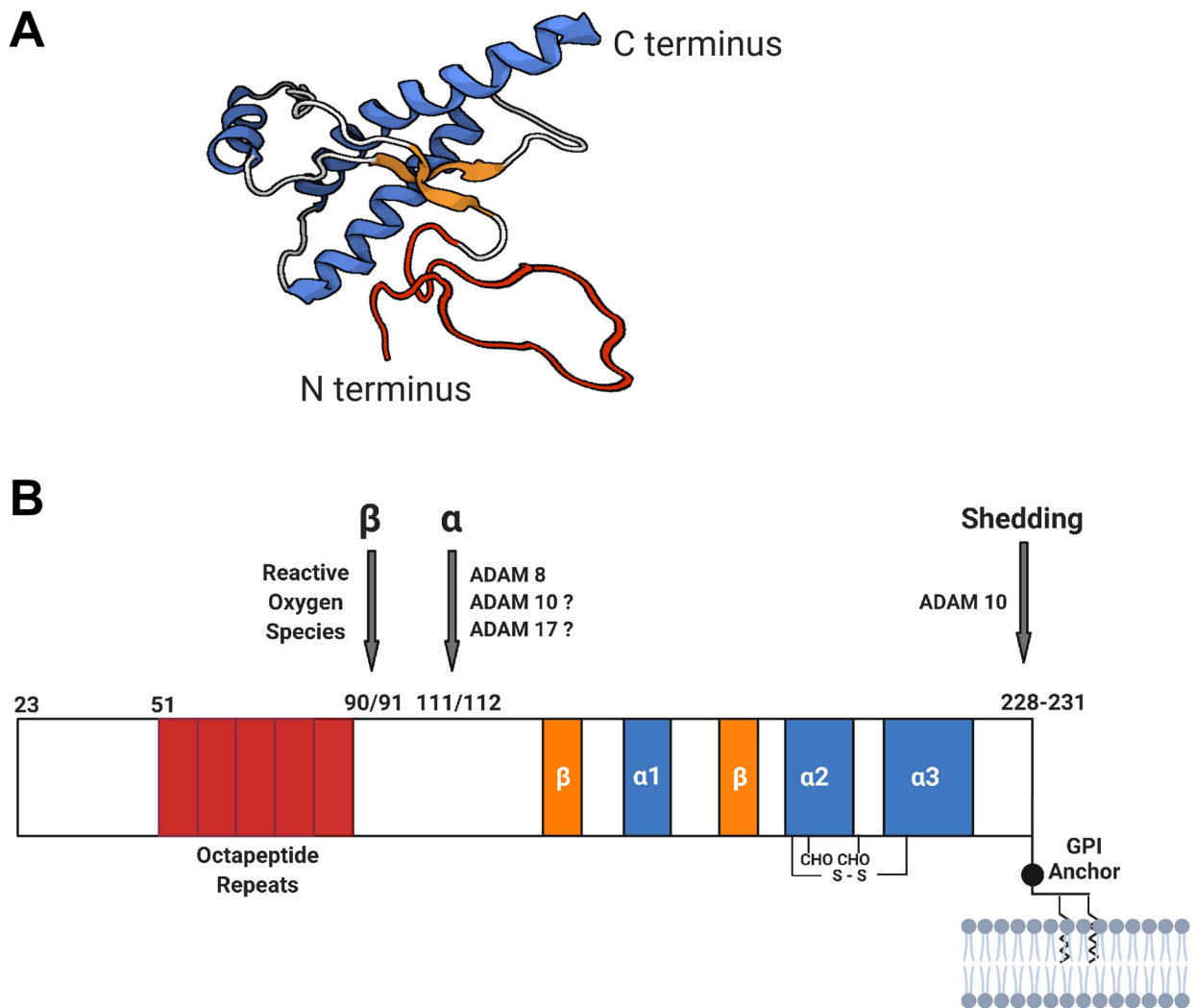
A

B

Figure 1. Diagrams of the structure and cleavages of cellular PrP.

A. 3D model of human PrP structure[55]. The segments of different secondary structures are color coded: α-helices (blue), β-sheets (orange), loops (gray), and unstructured (red). **B.** Schematic diagram of PrP cleavage sites (modified from Figure 1 in ref 81). The mature PrP^C is ~210 amino acids long, consisting of a flexible unstructured N-terminal domain (residues 23–120) and a highly structured globular C-terminal domain (residues 121–231) composed of three α-helices, two β-sheets, a disulfide bond, two Asn-linked glycans, and a C-terminal glycosylphosphatidylinositol (GPI) anchor. PrP can undergo cleavages at different positions: within the hydrophobic region (residues 109–120) (α-cleavage), within or at the end of the octapeptide repeats region (residues 51–91) (β-cleavage), and at or near the GPI anchor [within residues 228–231 or possibly within the GPI anchor (not depicted)] (shedding). The suspected or confirmed enzymes or molecules involved in these cleavages are marked. The β-cleavage of PrP^C produces the N2 and C2 fragments that both appear to be neutral.
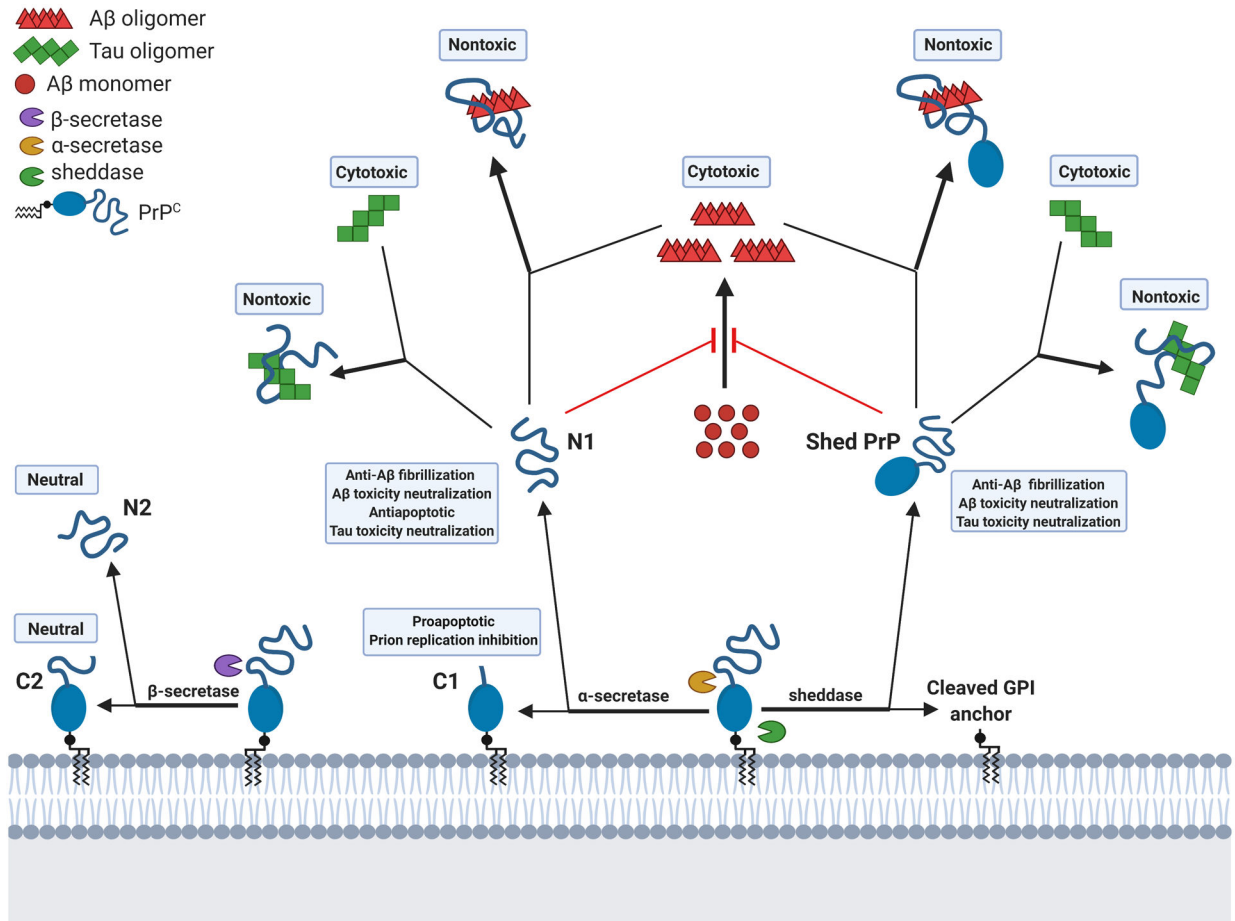
Figure 2. Protective activities of PrP and its N-terminal fragment released from cell surface by α-cleavage or shedding.

The N1 peptide, the N-terminal fragment of PrP released to the extracellular space by α-cleavage of PrP^C, can prevent Aβ fibrillization, neutralize Aβ and Tau toxicity through binding to the neurotoxic Aβ or Tau oligomers, and prevent stress-induced apoptosis. Shed PrP is shown (or expected to show) similar protective activities. Both N1 and shed PrP can associate with other toxic oligomeric misfolded proteins such as αSyn in Parkinson's disease (not depicted). C1, the C-terminal fragment from α-cleavage of PrP^C, is reported to inhibit prion replication and seems to be proapoptotic, although transgenic mice overexpressing C1 appear normal.