

Commentary & View

Antagonistic roles of the N-terminal domain of prion protein to doppel

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Prion protein (PrP)-like molecule, doppel (Dpl), is neurotoxic in mice, causing Purkinje cell degeneration. In contrast, PrP antagonizes Dpl in trans, rescuing mice from Purkinje cell death. We have previously shown that PrP with deletion of the N-terminal residues 23–88 failed to neutralize Dpl in mice, indicating that the N-terminal region, particularly that including residues 23–88, may have trans-protective activity against Dpl. Interestingly, PrP with deletion elongated to residues 121 or 134 in the N-terminal region was shown to be similarly neurotoxic to Dpl, indicating that the PrP C-terminal region may have toxicity which is normally prevented by the N-terminal domain in cis. We recently investigated further roles for the N-terminal region of PrP in antagonistic interactions with Dpl by producing three different types of transgenic mice. These mice expressed PrP with deletion of residues 25–50 or 51–90, or a fusion protein of the N-terminal region of PrP with Dpl. Here, we discuss a possible model for the antagonistic interaction between PrP and Dpl.

The normal prion protein, termed PrP^C, is a membrane glycoprotein tethered to the outer cell surface via a glycosylphosphatidylinositol (GPI) anchor moiety.^{1,2} It is ubiquitously expressed in neuronal and non-neuronal tissues, with highest expression in the central nervous system, particularly in neurons.³ The physiological function of PrP^C remains elusive. We and others have shown that PrP^C functionally antagonizes doppel (Dpl), a PrP-like GPI-anchored protein with ~23% identity in amino acid composition to PrP, protecting Dpl-induced neurotoxicity in mice.^{4–7} Dpl is encoded on *Prnd* located downstream of the PrP gene (*Prnp*) and expressed in the testis, heart, kidney and spleen of wild-type mice but not in the brain where PrP^C is actively expressed.^{4,5,8} However, when ectopically expressed in brains, particularly in cerebellar Purkinje cells, Dpl exerts a neurotoxic activity, causing ataxia and Purkinje cell degeneration in NgsK, Rcm0 and Zrch II lines of mice devoid of PrP^C (*Prnp*^{0/0}).^{4,9,10} In these mice, Dpl was abnormally controlled by the upstream *Prnp* promoter.^{4,5} This is due to targeted deletion of part of *Prnp* including a splicing acceptor of exon 3.¹¹ Pre-mRNA starting

from the residual exon1/2 of *Prnp* was abnormally elongated until the end of *Prnd* and then intergenically spliced between the residual *Prnp* exons 1/2 and the *Prnd* coding exons.^{4,5} As a result, Dpl was ectopically expressed under the control of the *Prnp* promoter in the brain, particularly in neurons including Purkinje cells.^{4,5} In contrast, in other *Prnp*^{0/0} lines, such as Zrch I and Npu, the splicing acceptor was intact, resulting in normal Purkinje cells without ectopic expression of Dpl in the brain.⁴

The molecular mechanism of the antagonistic interaction between PrP^C and Dpl remains unknown. We recently showed that the N-terminal half of PrP^C includes elements that might mediate cis or trans protection against Dpl in mice, ameliorating Purkinje cell degeneration.¹² We also showed that the octapeptide repeat (OR) region in the N-terminal domain is dispensable for PrP^C to neutralize Dpl neurotoxicity in mice.¹² Here, possible molecular mechanisms for the antagonism between PrP^C and Dpl will be discussed.

Lack of a cis-Protective Element Renders PrP and Dpl Neurotoxic

PrP^C largely comprises of two domains, the N-terminal and C-terminal domains (Fig. 1A). The N-terminal domain is highly flexible, lacking identifiable secondary structures.¹³ This domain includes the OR region, which is unique to all PrP molecules.² In contrast, the C-terminal domain forms a globular structure with three α -strands and two short β -strands.¹³ Interestingly, PrP with the N-terminal residues 32–121 or 32–134 deleted, termed PrP Δ 32–121 and PrP Δ 32–134 (Constructs 1 and 2 in Fig. 1B), respectively, was shown to be neurotoxic.¹⁴ This caused ataxia and cerebellar neurodegeneration, including granule or Purkinje cell death in Zrch I *Prnp*^{0/0} mice.^{14,15} These results suggest that PrP^C is potentially neurotoxic via the C-terminal domain but under normal conditions the neurotoxicity of the C-terminal domain may be masked by the N-terminal domain.

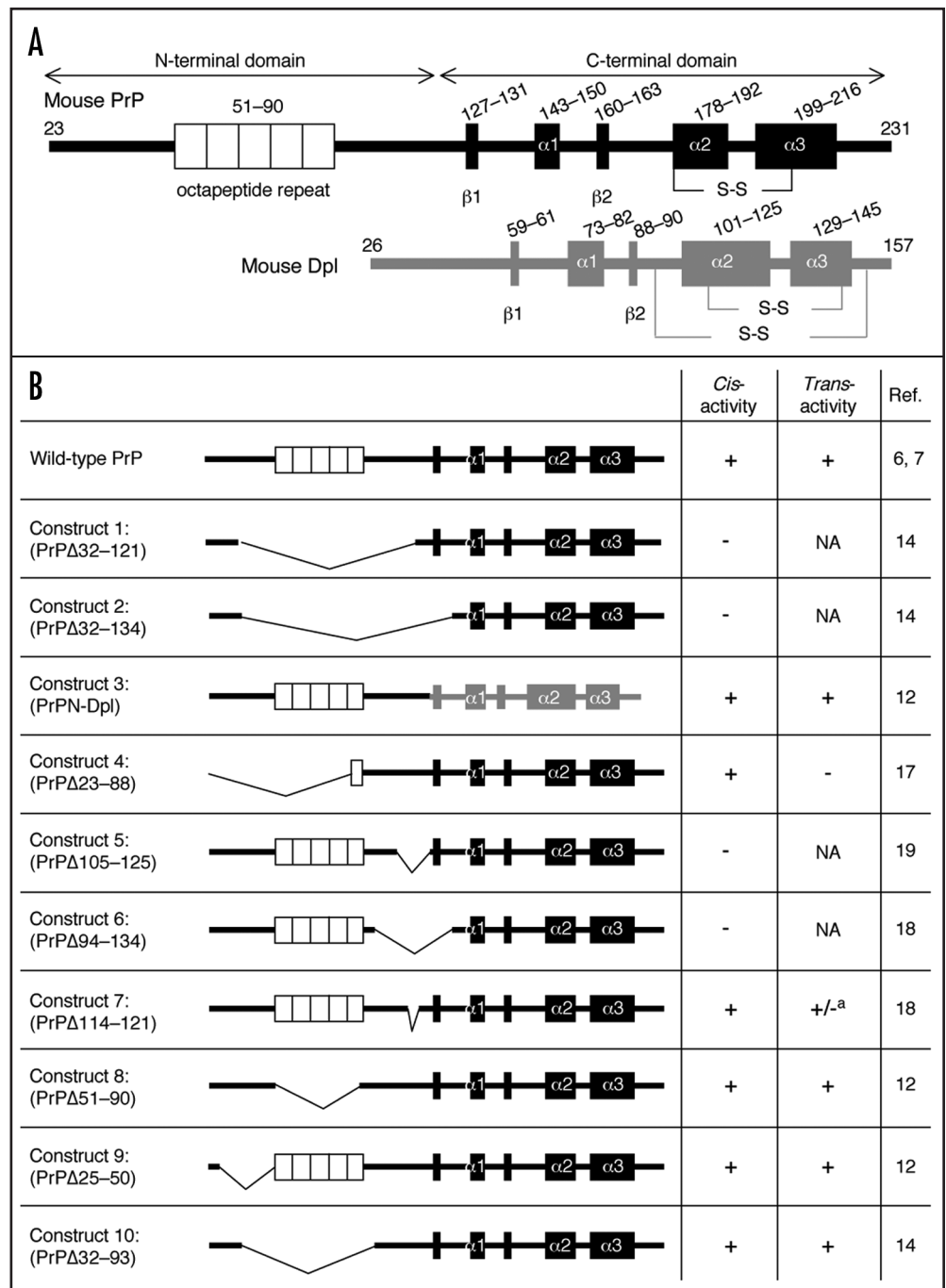
Dpl is a homologue of the C-terminal globular domain of PrP^C (Fig. 1A).¹⁶ However, Dpl lacks the amino acid sequences corresponding to the N-terminal half of PrP^C (Fig. 1A). It is therefore conceivable that the neurotoxicity of Dpl might be due to lack of the corresponding N-terminal part of PrP^C. Consistent with this, we recently showed that PrP^N-Dpl (Construct 3 in Fig. 1B), a fusion protein of the N-terminal residues 1–124 of PrP^C and the residues 58–179 of Dpl, was itself non-toxic in mice.¹² It induced neither ataxia nor Purkinje cell degeneration in Zrch I *Prnp*^{0/0} mice, even when transgenically expressed in the brain under the control of the *Prnp* promoter.¹² Constructs 1 and 2 cover most of

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Figure 1. (A) Schemes of wild-type mouse PrP and Dpl. Mouse PrP is first translated as a precursor protein consisting of 254 amino acids. The N-terminal 22 and C-terminal 23 hydrophobic amino acids are removed as a signal peptide and a GPI-anchor signal sequence, respectively. The N-terminal half of PrP^C is highly flexible and lacks identifiable secondary structure. The octapeptide repeat (OR) region, comprising five copies of a P(H/Q)GGG(G)WGQ octapeptide sequence, is located in the N-terminal domain. The OR region is thought to mediate anti-oxidative activity by binding to Cu²⁺ via histidine residues. However, the exact function of this region remains to be elucidated. The C-terminal half of PrP^C forms a globular structure with three α -helices (α 1-3) and two short anti-parallel β -strands (β 1, β 2). The second and third helices are linked by a disulfide bond (-S-S-). The precursor protein of Dpl consists of 179 amino acids. The N-terminal 25 and C-terminal 22 hydrophobic residues may be removed as signal peptide and GPI-anchor signals, respectively. Dpl is a structural homologue of the C-terminal globular domain of PrP^C, sharing ~23% identical amino acids and is composed of three α -helices (α 1-3) and two short anti-parallel β -strands (β 1, β 2). Two disulfide bonds (-S-S-) are formed. However, Dpl lacks the corresponding N-terminal part of PrP^C. (B) Structural schemes of PrPs with deletion of various regions and PrPN-Dpl, the fusion protein composed of the N-terminal region of PrP with Dpl, with their cis- and trans-protective activity against Dpl or toxic PrPs are shown. α : Construct 7 is itself non-toxic. However, it has different affects on neurotoxic Constructs 2 and 6: It enhances the toxicity of Construct 6 but diminishes that of Construct 2. NA: data are not available.



the Dpl-homologous C-terminal part of PrP. These observations strongly suggest that Dpl might undergo the same or very similar molecular processes as toxic PrP molecules do to perform their neurotoxicity in mice.

In contrast to the Constructs 1 and 2, PrPΔ23-88 (Construct 4 in Fig. 1B) is non-toxic in mice.¹⁷ This indicates that the central region including residues 89-121, which are deleted in the toxic Constructs 1 and 2 but intact in the non-toxic Construct 4, may include an element(s) that mediates the cis-protection against the neurotoxic C-terminal domain. Indeed, PrP with deletion of the central residues 105-125 or 94-134 (Constructs 5 and 6 in Fig. 1B) was shown to be neurotoxic, causing cerebellar degeneration or demyelination in mice.^{18,19} However, no neurotoxicity was detected for PrP with

deletion of only eight amino acids (residues 114-121) in the central region (Construct 7 in Fig. 1B).¹⁸ These results suggest that the cis-protective activity of the central region might be regulated in a highly integrated way, which might be impaired by deletion of a large part of the region rather than any specific amino acids or small areas.

Trans-Protection by PrP Against Dpl

Trans-protective activity of various PrP constructs against Dpl or the toxic truncated PrPs is summarized in Figure 1B. Wild-type PrP^C has the potential to abrogate Dpl neurotoxicity in trans. The ataxia and Purkinje cell degeneration, which were induced by transgenic expression of Dpl in the brain, could be attenuated in mice carrying the wild-type but not the knockout genetic background

for *Prnp*.^{6,7} We previously showed that Construct 4, which lacks the N-terminal residues 23–88, completely lost the ability to rescue an ataxic Ngsk line of *Prnp*^{0/0} mice from Dpl-induced Purkinje cell degeneration.¹⁷ We also recently showed that Construct 3 in which the PrP N terminal region (residues 23–124) was fused to Dpl (residues 58–179) mitigated the neurotoxicity of transgenically expressed wild-type Dpl in mice, prolonging the times to the onset of ataxia and Purkinje cell degeneration.¹² These results indicate that the N-terminal domain, particularly that encompassing residues 23–88, might include an element(s) that mediates the antagonistic function of PrP^C against Dpl in trans. However, the trans-protective element might require cis-protective activity to function, because the neurotoxic Constructs 5 and 6 include the trans-elements but not the cis-element.^{18,19}

Residues 23–88 cover the entire pre-OR and almost the entire OR except for two amino acids (residues 89 and 90). We recently investigated the role of the OR and the pre-OR in the trans-neuroprotection of PrP^C against Dpl by producing transgenic mice expressing Constructs 8 or 9.¹² They expressed PrP with deletion of the entire OR (residues 51–90) or most of the pre-OR (residues 25–50) except for residues 23 and 24.¹² Complete rescue from ataxia and Purkinje cell degeneration was detected in mice co-expressing the OR-lacking Construct 8 and Dpl in the absence of wild-type PrP^C,¹² clearly indicating that the OR is dispensable for PrP^C protection against Dpl-neurotoxicity in trans. The pre-OR-lacking Construct 9 also blocked Dpl-neurotoxicity in mice in a manner dependent on its expression level, prolonging the onset of ataxia and Purkinje cell death.¹² Shmerling et al. reported that the cerebellar granule cell death induced by the neurotoxic Construct 2 in Zrch I *Prnp*^{0/0} mice could be abrogated by Construct 10.¹⁴ Construct 10 lacks the entire OR and part of the pre-OR. These findings indicate that the OR and part of the pre-OR are also unnecessary for PrP^C to antagonize the neurotoxicity of truncated PrPs in trans.

Two amino acids (residues 23 and 24) of the pre-OR are commonly intact in the trans-protective molecules, including wild-type PrP^C and Constructs 3 as well as 8–10, but not in the non-protective Construct 4. It is therefore possible that these two residues are important for the trans-neuroprotection of PrP^C against Dpl or the toxic truncated PrPs. Interestingly, the two amino acids are followed by residues starting from 51 in Construct 9, generating a new N-terminal sequence (KKPQG GTWG), which is very similar to the N-terminal 9 residues (KKRPKPGGW) of wild-type PrP^C. Six out of nine of these amino acids are identical. It is thus possible that this newly generated N-terminal sequence might mimic the function of wild-type N-terminal 9 residues. This N-terminal sequence also remains intact in the other protective Constructs 3, 8 and 10. This therefore suggests that rather than the two amino acids, the 9 N-terminal residues may be relevant to the trans-neuroprotection of PrP^C against Dpl. It might be alternatively possible that the trans-neuroprotection of PrP^C against Dpl may be impaired only by a large deletion of the N-terminal domain, such as deletion of the residues 23–88, but not by small deletions such as deletion of part of pre-OR and/or OR.

Possible Mechanism of Antagonistic Interaction between PrP and Dpl

The exact mechanism by which PrP^C antagonizes Dpl, preventing Purkinje cell degeneration, remains elusive. Accumulating evidence indicates that PrP^C might function as a neuroprotective molecule by exerting anti-apoptotic activities. Indeed, we and others showed that *Prnp*^{0/0} mice were highly sensitive to ischemic or traumatic brain damage, developing more severe apoptotic neuronal cell death than in wild-type mice.^{20–23} Moreover, it was reported that hippocampal neuronal cell lines established from *Prnp*^{0/0} mice easily succumbed to apoptosis after serum withdrawal, and that expression of either PrP^C or the anti-apoptotic molecule Bcl-2 rescued cell lines from the apoptosis.²⁴ PrP^C also prevented Bax-induced apoptosis in human primary neurons.²⁵ Interestingly, PrP lacking OR failed to rescue the cells from the apoptosis, which was induced by serum withdrawal or Bax, and Zrch I *Prnp*^{0/0} mice from ischemic brain damage.^{25–27} This indicates that the OR is essential for the neuroprotective activity of PrP^C. However, we demonstrated that the OR is dispensable for PrP^C to antagonize Dpl in mice.¹² Shmerling et al. also showed that the OR is unnecessary for PrP^C to antagonize the neurotoxicity of truncated PrPs.¹⁴ These indicate that the neuroprotective activity of PrP^C, especially mediated via the OR, may not be required for the antagonistic function of PrP^C against Dpl.

Some models postulate that PrP^C interacts with an as yet unidentified transmembrane molecule that transmits a neuroprotective or cell survival signal.^{14,18} Dpl and the toxic truncated PrPs could bind to the molecule, but generate no signal due to lack of the N-terminal domain, resulting in neuronal cell death. According to these models, PrP molecules that fail to generate the signal should be toxic, like Dpl or the toxic truncated PrPs. However, inconsistent with this, we previously showed that Construct 4 lacking residues 23–88, failed to elicit the antagonistic signal against Dpl but was itself non-toxic to neurons.¹⁷

Wong et al. reported that Dpl-expressing Rcm0 *Prnp*^{0/0} mice produced oxidative stress of radical oxygen species or nitric oxide in their brains much more than non-expressing Npu *Prnp*^{0/0} mice.²⁸ This therefore suggested that Dpl may actively produce the neurotoxic signal, causing neuronal cell degeneration. Dpl is a GPI-anchored membrane glycoprotein, thus requiring interaction with a transmembrane molecule to transmit the signal (Fig. 2A). The toxic PrP molecules may interact with the molecule via the Dpl-homologous C-terminal domain in the same way as Dpl, eliciting a neurotoxic signal (Fig. 2A). However, the central region may interfere with the interaction, thereby preventing the neurotoxicity of the C-terminal of PrP in cis (Fig. 2B). Rambold et al. reported that PrP with the domain spanning central residues 113–133 deleted, termed PrPΔHD, failed to form a homo-dimer, being toxic by inducing apoptosis in human neuroblastoma SH-SY5Y cells.²⁹ It is thus possible that the central residues may be involved in dimerization of PrP, thereby preventing the C-terminal domain of PrP from interaction with the transmembrane molecule (Fig. 2B). Alternatively, the central residues may form intra-molecular interaction with the C-terminal region of PrP, thereby inhibiting the binding between it and the transmembrane molecule (Fig. 2B). The N-terminal region which mediates the trans-protective activity, may also bind to the transmembrane molecule only when the cis-element is intact, but

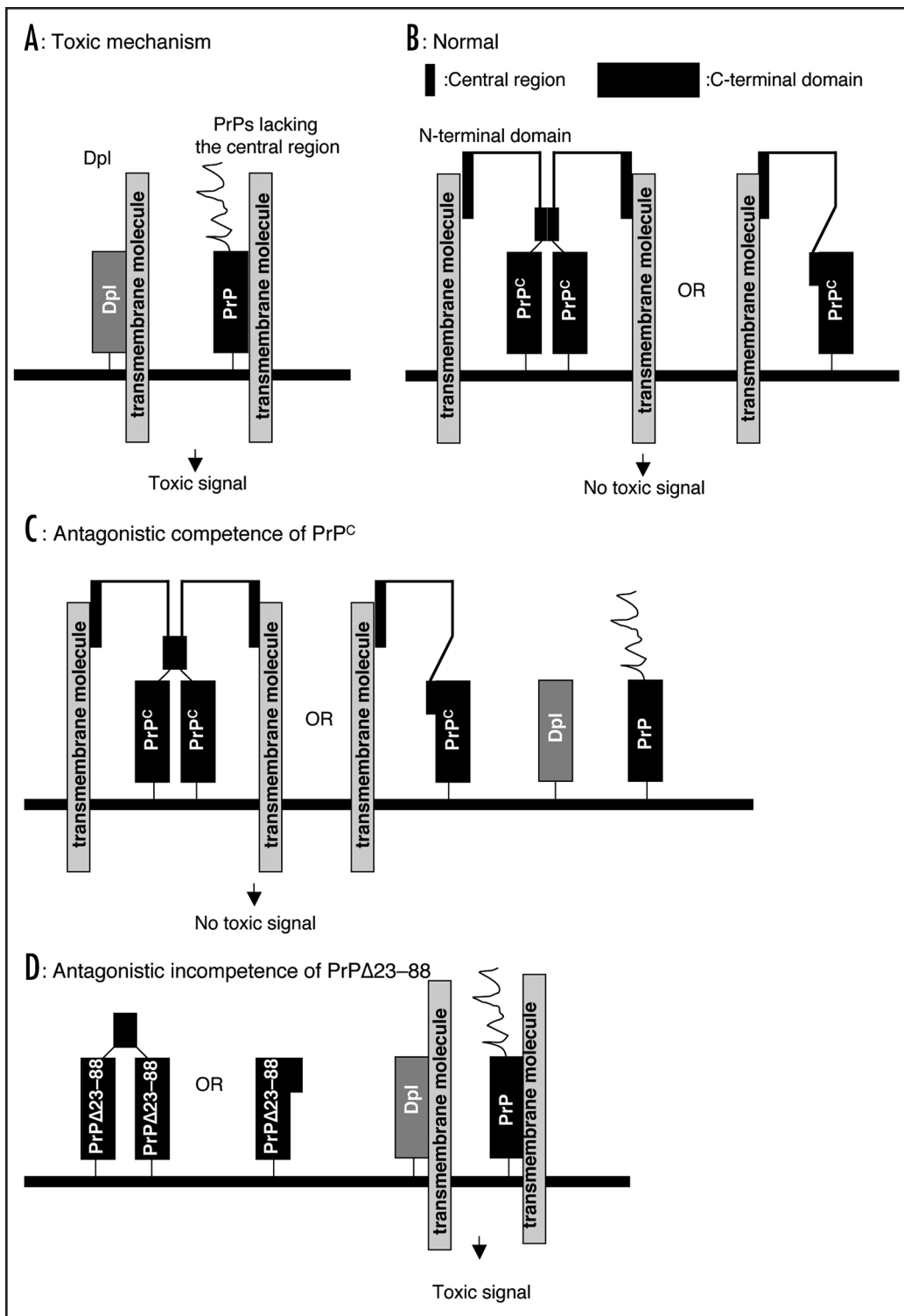


Figure 2. A possible mechanism for the antagonistic interaction of PrP^C and Dpl or the toxic PrPs. (A) Dpl binds to a putative transmembrane molecule, producing a toxic signal. Toxic PrPs with deletion of the central region, such as Constructs 1, 2, 5 and 6, bind to the transmembrane molecule via the Dpl-homologous C-terminal area in the same way as Dpl, eliciting a similar toxic signal. (B) Under normal conditions, wild-type PrP^C binds to the transmembrane molecule via the N-terminal region but not its C-terminal region because it forms either a homo-dimer linked via the central region or a monomer with the central region interacting with part of the C-terminal domain. The N-terminal region acquires binding affinity to the molecule only when the central region is intact. However, this type of interaction produces no toxic signal. (C) PrPs with part of the N-terminal region and with the central region both intact, such as trans-protective PrPs, have a higher affinity for the transmembrane molecule than Dpl or the toxic PrPs, resulting in trans-protection against Dpl and the toxic PrPs. (D) Construct 4 (PrPΔ23-88) still has potential to form a homo-dimer due to the residual central region or a monomer with the residual central region masking part of the C-terminal region, similarly to wild-type PrP^C. Therefore, Construct 4 cannot form a complex with the transmembrane molecule via the C-terminal region, generating no toxic signal. In addition, by lacking part of the N-terminal domain, Construct 4 has no affinity for the transmembrane molecule, losing trans-protective activity against Dpl.

or the Dpl-homologous C-terminal region of PrP and the putative transmembrane molecule (Fig. 2A). The cis- and trans-neuroprotective activity may be mediated by disturbing the interaction in cis or trans via the central or N-terminal regions, respectively (Fig. 2B–D). The neurotoxic PrP peptide, PrP106-126, and neurotoxic monoclonal anti-PrP antibodies, IgG D13 and P, which recognize an epitope

(residues 95–105) very adjacent to the central region, may impair the cis-activity of the central region and promote the neurotoxic binding between the C-terminal region of PrP^C and the transmembrane molecule, inducing neuronal cell death.^{30,31} However, this model can be verified only when the putative transmembrane molecule is identified.

Implication for Prion Diseases

Many lines of evidence indicate that conformational conversion of PrP^C into the abnormally folded amyloidogenic isoform, PrP^{Sc}, plays a pivotal role in the pathogenesis of transmissible spongiform encephalopathies or prion diseases, including Creutzfeldt-Jakob disease in humans and bovine spongiform encephalopathy in cattle.³² However, the molecular mechanism by which neurons undergo degenerative death remains unknown. PrP^{Sc} differs from PrP^C in tertiary structure.³³ PrP^C is rich in α -helix content while PrP^{Sc} have a markedly increased content of β -sheet.³³ Thus, due to the structural changes, the central region of PrP^{Sc} may lose its cis-activity and PrP^{Sc} therefore might interact with the putative transmembrane molecule, causing neuronal degeneration. Alternatively, association between PrP^C and PrP^{Sc} during the structural conversion might impair the *cis*-activity of the associating PrP^C, subsequently inducing neuronal cell death. N-terminally truncated forms of protease-resistant PrP have been reported to accumulate in the brains of patients affected with prion diseases and in persistently infected cultured cells.^{34,35} It may be also conceivable that these N-terminally truncated PrP fragments possess a neurotoxic potential equivalent to that of Dpl and Constructs 1 and 2 due to deletion of the *cis*-element. Thus, elucidation of a molecular mechanism of the antagonistic interaction between Dpl and PrP^C could be useful for understanding of the molecular pathogenesis of prion diseases.

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