REVIEW

Molecular biology of prion protein and its first homologous protein

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Abstract : Conformational conversion of the normal cellular isoform of prion protein, PrP^{C}, a glycoprotein anchored to the cell membrane by a glycosylphosphatidylinositol moiety, into the abnormally folded, amyloidogenic prion protein, PrP^{Sc}, plays a pivotal role in the pathogenesis of prion diseases. It has been suggested that PrP^{C} might be functionally disturbed by constitutive conversion to PrP^{Sc} due to either the resulting depletion of PrP^{C} or the dominant negative effects of PrP^{Sc} on PrP^{C} or both. Consistent with this, we and others showed that mice devoid of PrP^{C} (PrP-/-) spontaneously developed abnormal phenotypes very similar to the neurological abnormalities of prion diseases, supporting the concept that functional loss of PrP^{C} might at least be partly involved in the pathogenesis of the diseases. However, no neuronal cell death could be detected in PrP-/- mice, indicating that the functional loss of PrP^{C} alone might not be enough to induce neuronal cell death, one of major pathological hallmarks of prion diseases. Interestingly, it was recently shown that the first identified PrP-like protein, termed PrPLP/Doppel (Dpl), is neurotoxic in the absence of PrP^{C}, causing Purkinje cell degeneration in the cerebellum of mice. Although it is not understood if PrP^{Sc} could have a neurotoxic potential similar to PrPLP/Dpl, it is very interesting to speculate that accumulation of PrP^{Sc} and the functional disturbance of PrP^{C}, both of which are caused by constitutive conversion, might be required for the neurodegeneration in prion diseases. J. Med. Invest. 54 : 211-223, August, 2007

Keywords **:** *prion, prion protein, prion protein-like protein, knockout mice, neurodegeneration*

INTRODUCTION

The normal cellular isoform of prion protein, designated PrP^{C}, is a membrane glycoprotein abundantly expressed in the central nervous system (CNS), particularly in neurons (1). Its structural counterpart, the abnormally folded, amyloidogenic isoform, termed PrP^{Sc}, is specifically present in the tissues affected by prion diseases (1). Prion diseases are a group of fatal neurodegenerative disorders including Creutzfeldt-Jakob disease in humans and scrapie and bovine spongiform encephalopathy in animals (2). The causative agents of the diseases, the so-called prions, are very different from conventional pathogens, such as bacteria and viruses (3). Prions lack a nucleic acid genome (3). According to the widely accepted protein-only hypothesis, prions are assumed to consist of PrP^{Sc} alone (4). However, the exact nature of prions still remains controversial. Here, I will discuss the nature of prions and the roles of PrP in the pathogenesis of prion diseases.

I will also discuss the normal functions of PrP^{C} and its antagonistic function to the first identified PrP-like protein, termed PrPLP/doppel (Dpl) (5).

Received for publication June 15, 2007 ; accepted July 16, 2007.

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PrPLP/Dpl is neurotoxic when ectopically expressed in neurons, causing Purkinje cell degeneration in mice, and PrP^{C} antagonizes the neurotoxicity, rescuing the mice from neurodegeneration (6, 7). However, unlike PrP^{C}, PrPLP/Dpl seems to have no potential to convert to a PrP^{Sc}-like infectious isoform (6, 8).

NORMAL AND ABNORMAL ISOFORMS OF PRP

Normal isoform of PrP

The gene for PrP, designated *Prnp*, is located on chromosomes 2 and 20 in human and mouse, respectively (9). Human and hamster *Prnp* consists of two exons (9). On the other hand, mouse, sheep, and rat *Prnp* contain three exons with exon 3 analogous to exon 2 of human and hamster *Prnp*. The protein coding sequence is present in the last single exon in all mammals. *Prnp* is constitutively expressed in various tissues, with highest expression in brain, particularly in neurons, and, to a lesser extent, in others including spleen, kidney, lung, and heart (10).

Mouse *Prnp* encodes the precursor protein consisting of 254 amino acids (Fig. 1). The 22 N-terminal hydrophobic amino acids are removed as a signal peptide when the nascent precursor protein enters the endoplasmic reticulum (ER) (Fig. 1). The 23 C-terminal hydrophobic amino acids are also cleaved as a glycosylphosphatidylinositol (GPI) -anchor signal sequence in the ER and, at the same time, a GPI anchor moiety is attached to the C-terminus of the serine residue at position 231 (Fig. 1) (1). Then, the protein is transported to the Golgi apparatus, where two asparagine residues at positions 181 and 197 undergo N-glycosylation (Fig. 1), and finally to the cell surface along the secretory pathway. As a result, mature PrP^{C}, consisting of the amino acid residues 23-231, is expressed as a membrane glycoprotein anchored to the cell surface via a GPI moiety.

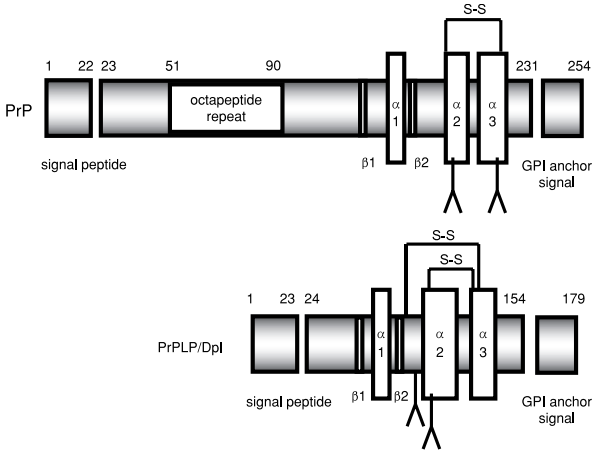

Fig. 1. Schematic structures of PrP and PrPLP/Dpl. α and β indicate α-helix and β-strand, respectively. S-S and Y indicate a disulfide bond and N-glycosylation, respectively. Arabic figures represent amino acid positions.

Abnormal isoform of PrP

The abnormal isoform, PrP^{Sc}, is specifically produced within prion infected cells, particularly in neurons. We and others showed that PrP^{Sc} failed to be produced in mice devoid of PrP^{C} (PrP-/-) (11-14), indicating that PrP^{C} is essential for the generation of PrP^{Sc}. Moreover, it was reported that PrP^{Sc}-like PrP could be generated from PrP^{C} in certain conditions *in vitro* (15). Therefore, it is thought that PrP^{Sc} is produced from PrP^{C}. However, it remains unknown whether or not PrP^{Sc} is produced from either mature PrP^{C} or immature unfolded PrP, or both.

PrP^{Sc} is identical to PrP^{C} in amino acid sequence. However, structural analysis of PrP^{C} and PrP^{Sc} using circular dichroism revealed marked differences in the protein structures of both proteins (Table 1). PrP^{C} has a lower content of β-sheet strands (3%) but a higher α-helix content (42%) (16). In contrast, PrP^{Sc} has a higher β-sheet content (43%) (16). It is therefore postulated that the transition of α-helices into β-sheet strands within PrP might be a key step in the generation of PrP^{Sc}.

Biochemical and structural properties of PrPs

PrP^{C} and PrP^{Sc} possess markedly different biochemical properties from each other, particularly in detergent solubility and resistance to proteinase digestion, probably due to the structural differences. PrP^{C} is highly soluble and easily digested by proteinase K whereas PrP^{Sc} readily aggregates to form

Table 1 Different biochemical and structural properties of PrP isoforms

PrP isoforms	secondary structure content		detergent solubility	proteinase K digestion
	α-helix	β-sheet		
Normal isoform (PrP^{C})	42%	3%	soluble	sensitive
Abnormal isoform (PrP^{Sc})	30%	43%	insoluble	relatively resistant

amyloid fibers and is relatively resistant to the digestion (Table 1) (1). These different biochemical properties are useful to distinguish PrP^{Sc} from PrP^{C}.

PrP^{Sc} remains structurally unresolved due to its predisposition to form aggregates. In contrast, the protein structure of PrP^{C} was resolved by nuclear magnetic resonance (NMR) analysis. According to NMR analysis, the N-terminal domain of PrP^{C} is highly flexible and lacks identifiable secondary structure while the C-terminal domain forms a globular structure with three α-helices and two short antiparallel β-strands (Fig. 1) (17). The second and third helices are linked by a disulfide bond (Fig. 1) (17).

The N-terminal domain includes a PrP-specific region, the so-called octapeptide repeat (OR) region, in which 8 amino acids are repeated 5 times in tandem (Fig. 1). This region is considered to bind Cu^{2+} via histidine residues and mediate anti-oxidative activities by activating Cu^{2+}-dependent antioxidant enzymes such as superoxide dismutase via transfer of bound Cu^{2+} to the enzymes (18, 19). However, the exact function of this region in anti-oxidative activities remains to be elucidated.

THE FIRST PRP-LIKE PROTEIN (PRPLP/DPL)

PrPLP/Dpl and PrP

We and others isolated a novel gene, termed *Prnd*, encoding the first PrP-like protein, PrPLP/Dpl, about 16-kb downstream of the PrP gene, *Prnp* (5, 7). PrPLP/Dpl is also a GPI-anchored membrane glycoprotein (5). Like PrP^{C}, PrPLP/Dpl is first translated into the precursor protein consisting of 179 amino acids and then undergoes several modifications, including cleavage of the 23 N-terminal and 25 C-terminal hydrophobic residues as a signal peptide and a GPI-anchor signal, respectively, N-glycosylation at two sites, and formation of two disulfide bonds (Fig. 1) (20).

PrPLP/Dpl and PrP share ~23% identical amino acids (5, 7). However, PrPLP/Dpl lacks a region corresponding to the N-terminal part of PrP^{C} (5, 7). The protein structural analysis clearly showed that PrPLP/Dpl is a structural homologue of the C-terminal globular domain of PrP^{C}, composed of three α-helices and two short antiparallel β-strands (Fig. 1) (21).

Normal functions of PrPLP/Dpl

PrPLP/Dpl mRNA is expressed in various tissues of adult wild-type mice, including the testis, heart, spleen and skeletal muscle (22). To investigate the physiological functions of PrPLP/Dpl in mice, Behrens, *et al.* produced mice devoid of PrPLP/Dpl, designated $Prnd^{neo/neo}$ mice (23). Interestingly, male mutant mice were sterile whereas female mutant mice were fertile. The testes in these mutant mice were macroscopically normal. However, the number of spermatozoa and motility of mutant sperm were significantly decreased. Moreover, the mutant sperm exhibited abnormal morphologies and impaired acrosome function. Consistently, it has been reported that PrPLP/Dpl is expressed in spermatids in mice and spermatozoa and Sertoli cells in humans (23, 24). These results indicate that PrPLP/Dpl is involved in spermatogenesis.

In contrast to PrP^{C}, PrPLP/Dpl was undetectable in the brains of adult wild-type mice (22). However, in neonatal mice, we found substantial expression of PrPLP/Dpl mRNA in their brains, preferentially in blood vessel endothelial cells. PrPLP/Dpl mRNA was already expressed 1 day after birth, peaked by around 1 week, and then decreased to an undetectable level by at least 8 weeks (22). Therefore, such developmental regulation of PrPLP/Dpl expression in brain blood vessels suggests that PrPLP/Dpl may be involved in the development of brain blood vessels and/or blood-brain barrier. However, no pathological abnormalities were detected in the tissues of $Prnd^{neo/neo}$ mice, including the brain, heart, spleen and skeletal muscle (23).

PRION RESEARCH IN MICE DEVOID OF PRP

Protein-only hypothesis and mice devoid of PrP

The protein-only hypothesis postulates that a prion is constituted of PrP^{Sc} alone (25). According to the hypothesis, a prion or PrP^{Sc} interacts with PrP^{C} expressed on the cell surface and induces changes in the conformation of the interacting PrP^{C} into that of PrP^{Sc}, resulting in generation of a new PrP^{Sc} molecule, or propagation of a prion (Fig. 2). However, the hypothesis is controversial. If the hypothesis is true, prions cannot propagate when PrP^{C} is absent. In contrast, if prions can propagate without PrP^{C}, the hypothesis is clearly negated. In other words, if PrP-/- mice can support prion propagation, the hypothesis is wrong, and vise versa.

To investigate validity of the protein-only hypothesis, we generated a line of PrP-/- mice, referred to

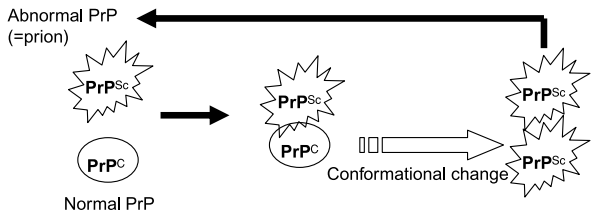

Fig. 2. A prion replication model according to the protein-only hypothesis. A prion is constituted of the abnormal isofrom of PrP, PrP^{Sc}, interacts with the normal isoform of PrP, PrP^{C}, and induces the conformational changes of the interacting PrP^{C} to produce a new molecule of PrP^{Sc} or a prion. The newly produced PrP^{Sc} or prion also converts another PrP^{C} into PrP^{Sc} or prion in the same way.

as Ngsk PrP-/- mice, and intracerebrally inoculated them with a mouse-adapted Fukuoka-1 prion (14). Wild-type (PrP+/+) mice developed disease-specific symptoms at 138±12 days and died 143±14 days after inoculation (Table 2). Microscopic examinations of the brains of these diseased mice showed profound vacuolation and gliosis, both of which are hallmarks of the pathological changes in prion diseases. In addition, PrP^{Sc} was markedly accumulated and prions were propagated in their brains. In contrast, no Ngsk PrP-/- mice showed such specific symptoms. They were all alive at least by 460 days after inoculation (Table 2). No disease-specific pathologies were observed in the brains of Ngsk PrP-/- mice sacrificed 400 days after inoculation. Moreover, neither accumulation of PrP^{Sc} nor prion propagation could be detected in their brains. Other investigators also showed similar results using other lines of PrP-/- mice (11-13). These results indicate that PrP^{C} is essentially required for prion propagation, and that prion propagation is linked to accumulation of PrP^{Sc}, strongly supporting the protein-only hypothesis.

Prolonged incubation times and less accumulation of PrP^{Sc} in mice heterozygous for PrP

We also inoculated Ngsk PrP+/- mice with the Fukuoka-1 prion (14). Compared with PrP+/+ mice, Ngsk PrP+/- mice developed the disease with considerably retarded incubation times of 259±27 days and died 269±27 days after inoculation (Table 2). The clinical symptoms and pathological changes in diseased Ngsk PrP+/- mice were indistinguishable from those of diseased PrP+/+ mice. However, very strangely, amounts of PrP^{Sc} accumulated in the brains of terminal PrP+/- mice were only half of those in terminal PrP+/+ mice (14). These results indicate that the expression levels of PrP^{C} prior to infection affect the timing of onset of disease and the accumulation levels of PrP^{Sc} but not the final severity and pathology of disease, and that PrP^{Sc} levels are not correlated with disease progression.

NORMAL FUNCTIONS OF PRP IN NEURONS AND GLIA

Higher brain functions and PrP

Since PrP^{C} is abundantly expressed in pyramidal neurons of the hippocampus, in which learning and memory processes are integrated, it has been suggested that PrP^{C} might be involved in learning and memory processes. Büeler, *et al.* produced a line of PrP-/- mice, Zrch I PrP-/-, and subjected them to behavioral tasks, such as a swimming navigation test and a Y-maze discrimination test (26). However, no different performance could be detected in these tests between the mutant mice and control PrP+/+ mice (26). On the other hand, Nishida, *et al.* reported poor performance in Ngsk PrP-/- mice using other behavioral tests, including a water-finding test and a conditioned passive-avoidance test (27). Thus, it may be possible that PrP^{C} is involved in certain types of learning and memory.

Collinge, *et al.* showed that long-term potentiation (LTP), a form of synaptic plasticity that is thought to be important for memory formation, was impaired in the hippocampal CA1 neurons of Zrch I PrP-/- mice using electrophysiological studies (28). Similar results were reported in another line of PrP-/- mice, Npu PrP-/- mice (29). Therefore, these results seem to support that PrP^{C} is involved in the processes of learning and memory. However, other investigators reported no such electrophysiological abnormalities in the hippocampus of Zrch I PrP-/- mice (30).

It was also shown that PrP^{C} is involved in the regulation of circadian rhythm (31). In both Zrch I PrP-/- and Npu PrP-/- mice, much more fragmented sleep was observed than in PrP+/+ mice (31). Moreover,

Table 2 Ngsk PrP-/- mice were resistant to prion disease.

Mouse genotype	Incubation time (mean±SD days)	Survival time (mean±SD days)
Wild-type	138±12	143±14
Ngsk PrP+/-	259±27	269±27
Ngsk PrP-/-	<460	<460

the mutant mice exhibited a much longer activity period of 23.9 h under constant darkness, compared to 23.3 h in PrP+/+ mice (31).

Axonal myelination and PrP

We found many vacuoles in the spinal cord and peripheral nervous system of Ngsk PrP-/- and Zrch I PrP-/- mice (32). Most of the vacuoles were surrounded by an enlarged myelin sheath, but in some cases splits within a myelin sheath formed vacuoles (32). In addition, large myelinated fibers were reduced in number and remaining axons were thinly myelinated (32). Subsequently, we could rescue Ngsk PrP-/- mice from the demyelination by transgenically expressing mouse PrP^C (32). These results indicate that PrP^C is involved in the organization of the myelin sheath.

PrP^C is expressed on the surface of oligodendrocytes and Schwann cells (33, 34), both of which form myelin sheaths in the CNS and the peripheral nervous system, respectively. It is therefore conceivable that PrP^C functions as an adhesion molecule within a myelin sheath and/or between a myelin sheath and an axon to form a tightly compacted myelin sheath. This might be consistent with the result that some vacuoles were formed due to splits within myelin sheaths. It is alternatively possible that PrP^C could be a trophic factor for these glial cells.

PRP AND PRPLP/DPL IN NEURODEGENERATION

Purkinje cell degeneration among different lines of mice devoid of PrP

No neuropathological abnormalities were reported in Zrch I PrP-/- and Npu PrP-/- mice (26, 35). However, very strangely, we noticed that Ngsk PrP-/- mice showed ataxic gait around 70 weeks after birth (36). In these ataxic mice, cerebellar Purkinje cells were dramatically decreased in number due to their degeneration and the molecular layer also became very thin, probably due to the loss of the dendritic trees of Purkinje cells (Fig. 3) (36). In contrast, no Purkinje cell degeneration could be detected in younger Ngsk PrP-/- mice and old PrP+/+ and Ngsk PrP+/- mice (36). We also confirmed that the ataxia and Purkinje cell degeneration in Ngsk PrP-/- mice could be successfully rescued by introduction of the transgene encoding PrP^C (Fig. 3) (32). Similar cerebellar degeneration was subsequently reported in other lines of PrP-/- mice, such as Rcm0 PrP-/-

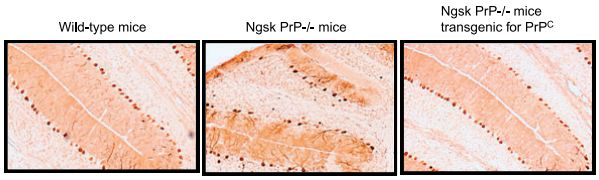

Fig. 3. Purkinje cell degeneration in Ngsk PrP-/- mice and Purkinje cells rescued in Ngsk PrP-/- mice transgenic for PrP^C. Purkinje cells are immunohistochemically stained using anti-calbindin antibodies and can be observed as brownish dots.

and Zrch II PrP-/- mice (5, 37). Taken together, these results indicated that the functional loss of PrP^C is responsible for Purkinje cell degeneration, although it remained unknown why the neurodegeneration was discrepant among different lines of PrP-/- mice.

In Zrch I PrP-/- mice, a part of the PrP open reading frame (ORF) was replaced with the neomycin phosphotransferase (neo) gene (Fig. 4) (26). In Npu PrP-/- mice, the neo gene was simply inserted into a unique site in the PrP-coding sequence (Fig. 4) (35). In contrast, in the ataxic lines of Ngsk PrP-/-, Rcm0 PrP-/-, and Zrch II PrP-/- mice, the entire ORF was completely deleted (Fig. 4) (5, 36, 37). It was therefore conceivable that in non-ataxic lines of Zrch I PrP-/- and Npu PrP-/- mice, some aspects of the normal function of PrP^C might remain intact because of incomplete disruption of the PrP allele. Consistently, it was reported that a fused mRNA consisting of the neo and the residual *Prnp* sequences was produced in their brains (26). Alternatively, it might be possible that the loss of PrP^C alone may not be enough to induce Purkinje cell degeneration, and that other factor(s), which are specifically associated with the ataxic lines of PrP-/- mice, together with the loss of PrP^C, are involved in the neurodegeneration.

Ectopic expression of PrPLP/Dpl associated with Purkinje cell degeneration

We found that, in the brains of Ngsk PrP-/- mice but not in Zrch I PrP-/- and PrP+/+ mice, the PrPLP/Dpl-coding exons were ectopically expressed as chimeric mRNAs with the residual non-coding *Prnp* exons 1 and 2 due to an abnormal intergenic splicing taking place between *Prnp* and *Prnd* (7). In Ngsk PrP-/- mice, due to lack of the 3' part of intron 2 including a splice acceptor, the pre-mRNA transcribed from the residual *Prnp* promoter could not efficiently undergo cleavage/polyadenylation at the end of *Prnp* (Fig. 5B). The unsuccessfully cleaved pre-mRNA was then elongated until the last

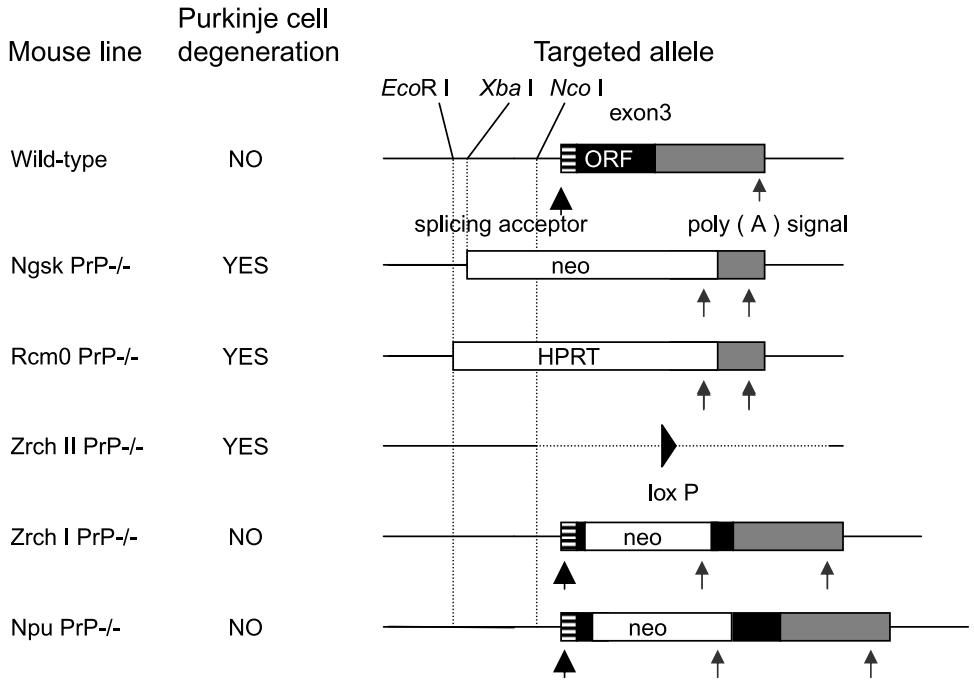

Fig. 4. Targeted PrP alleles among different lines of PrP-/- mice. In Ngsk PrP-/- mice, a 2.1-kb genomic DNA segment including 0.9-kb of intron 2, 10-bp of 5' untranslated region (UTR) of exon 3, the entire PrP ORF, and 0.45-kb of 3' UTR is replaced by the neo gene under the control of the mouse phosphoglycerate kinase (PGK) promoter. Rcm0 PrP-/- mice were generated by a similar targeting strategy utilized in Ngsk PrP-/- mice. The hypoxanthine phosphoribosyltransferase gene under control of the PGK promoter was used in Rcm0 PrP-/- mice as a selectable marker. In Zrch II PrP-/- mice, 0.27-kb of intron 2, the entire exon 3, and 0.6-kb of the 3' flanking DNA segment were targeted by a specific 34-bp *loxP* sequence. In these lines of PrP-/- mice, the entire PrP ORF is completely deleted. In contrast, Zrch I PrP-/- mice were generated by replacement of PrP codons 4-187 among a total of 254 codons with the neo gene under the control of the herpes simplex virus thymidine kinase promoter. Npu PrP-/- mice contain the disrupted *Prnp* alleles, in which the neo gene under the control of the mouse metalothioneine promoter was simply inserted into a unique *Knp* I site in the PrP-coding sequence.

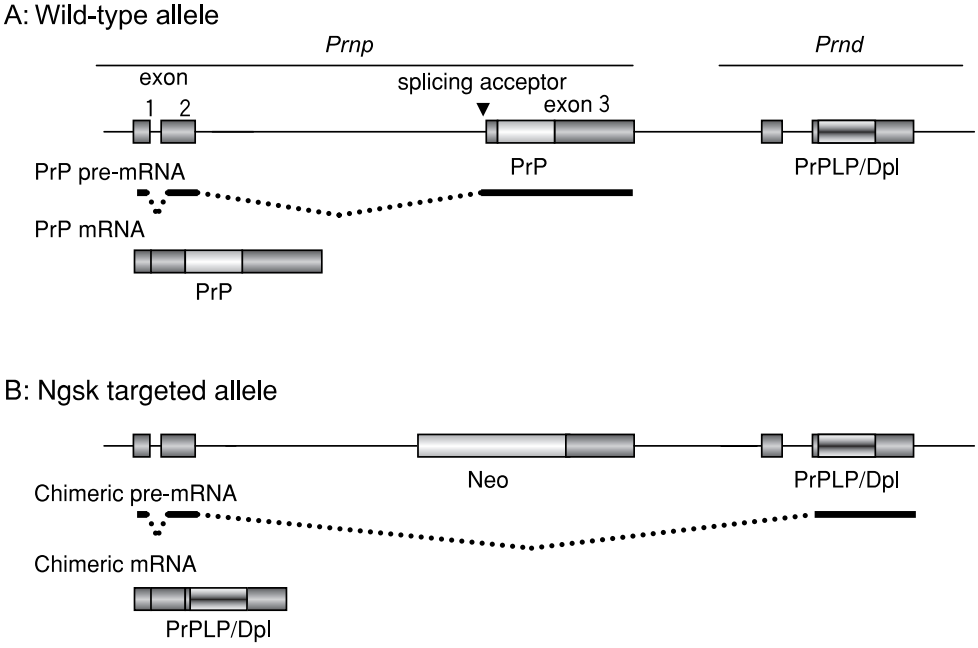

Fig. 5. Mechanism for the generation of PrPLP/Dpl-encoding chimeric mRNAs in Ngsk PrP-/- mice. In wild-type mice, the PrP pre-mRNA is normally cleaved and polyadenylated at the last exon of the *Prnp* (A). However, in Ngsk PrP-/- mice, due to lack of the 3' part of intron 2, the pre-mRNA transcribed from the *Prnp* promoter could not efficiently undergo cleavage/polyadenylation at the last exon of *Prnp*, being further elongated until the last exon of *Prnd*, and subjected to intergenic splicing between the residual *Prnp* exons 1/2 and the PrPLP/Dpl-coding exon (B). As a result, PrPLP/Dpl became abnormally expressed under the control of the *Prnp* promoter, leading to the ectopic expression of PrPLP/Dpl in the brains of ataxic lines of PrP-/- mice.

exon of *Prnd* and subjected to intergenic splicing between the residual *Prnp* exon 2 and the PrPLP/Dpl-coding exon, producing chimeric mRNAs consisting of the *Prnp* exons 1 and 2 and the PrPLP/Dpl-coding exons (Fig. 5B). As a result, *Prnd* became abnormally regulated under the control of the *Prnp* promoter in Ngsk PrP-/- mice and PrPLP/Dpl was ectopically expressed in the brains, especially in neurons and glial cells where the promoter is very active (7). Similar ectopic expression of PrPLP/Dpl was subsequently reported in other ataxic lines of PrP-/- mice, Rcm0 PrP-/- and Zrch II PrP-/- mice (5, 37). Taken together, these results indicate that the ectopic expression of PrPLP/Dpl in the absence of PrP^C might be responsible for Purkinje cell degeneration in the ataxic lines of PrP-/- mice.

PrPLP/Dpl in the absence of PrP causes Purkinje cell degeneration

To investigate the possibility that ectopic expression of PrPLP/Dpl in the absence of PrP^C could cause the Purkinje cell degeneration, we generated transgenic mice, referred to as tg(N-PrPLP/Dpl) mice, in which PrPLP/Dpl was specifically expressed in nearly all neurons including Purkinje cells under the control of the neuron-specific enolase promoter, and subsequently crossed them with the non-ataxic line of Zrch I PrP-/- mice (38). Tg(N-PrPLP/Dpl)32 mice expressed PrPLP/Dpl in the cerebellum at a level about 1-2 times more than that of Ngsk PrP-/- mice, developing ataxia at 58±15 days on the Zrch I PrP-/- background (Table 3). Purkinje cells were markedly decreased in number due to the degeneration in these ataxic tg mice. In contrast, neither ataxia nor Purkinje cell degeneration could be detected in the tg mice carrying the PrP+/+ background (Table 3). Another tg(N-PrPLP/Dpl)25 mouse line, in which PrPLP/Dpl was expressed in the cerebellum at a level less than a quarter that of Ngsk PrP-/- mice, also developed ataxia and Purkinje cell degeneration at 359±52 days on the Zrch I PrP-/- background but not on the PrP+/+ background (Table 3). These results clearly showed that the ectopic expression of PrPLP/Dpl is neurotoxic in the absence of PrP^C, causing the Purkinje cell degeneration, and that the neurotoxicity of PrPLP/Dpl is antagonized by PrP^C.

We also produced another type of tg mice, tg(P-PrPLP/Dpl) mice (38). In these tg mice, the expression of PrPLP/Dpl was specifically targeted to Purkinje cells under the control of the Purkinje cell protein-2 promoter (38). Like tg(N-PrPLP/Dpl) mice, tg(P-PrPLP/Dpl) mice developed ataxia and Purkinje cell degeneration on the Zrch I PrP-/- background but not on the PrP+/+ background (38). Tg(P-PrPLP/Dpl)26 and 27 mice showed ataxia at 268±28 and 167±13 days after birth, respectively, on the Zrch I PrP-/- background (Table 3). These results clearly indicate that PrPLP/Dpl ectopically expressed on Purkinje cells is itself neurotoxic to the cells.

Stoichiometrical antagonism between PrP and PrPLP/Dpl in neurodegeneration

The times to the onset of ataxia in tg(N-PrPLP/Dpl) mice were inversely correlated with the expression levels of PrPLP/Dpl (38). Tg(N-PrPLP/Dpl)32 mice expressed more PrPLP/Dpl in the cerebellum and developed the ataxia earlier than tg(N-PrPLP/Dpl)25 mice (Table 3). In contrast, the levels of PrP^C were correlated with the times of the onset. Tg(N-PrPLP/Dpl)32 and 25 mice showed significantly retarded onset of the ataxia on the Zrch I PrP+/- background, compared with the Zrch I PrP -/- background (Table 3). Thus, these results indicate that PrPLP/Dpl and PrP^C stoichiometrically antagonize each other to induce Purkinje cell degeneration.

N-terminal domain of PrP antagonistic for PrPLP/Dpl

PrP^C possesses the OR region-containing N-terminal domain whereas PrPLP/Dpl lacks the corresponding domain. It is therefore conceivable that the N-terminal domain might be important for PrP^C to antagonize against the PrPLP/Dpl neurotoxcity. To investigate the possibility, we introduced PrP with a deletion of the N-terminal residues 23-88

Table 3 PrPLP/Dpl stoichiometrically antagonizes PrP^C to induce ataxia

Tg	lines	Onset of ataxia on different genetic backgrounds (mean±SD days)		
		Zrch I PrP-/-	Zrch I PrP+/-	Wild-type
Tg(N-PrPLP/Dpl)	25	359±52	495±86	<600
	32	58±15	259±48	<600
Tg(P-PrPLP/Dpl)	26	268±28	463±81	<600
	27	167±13	391±108	<600

into Ngsk PrP-/- mice (39). As expected, the deletion mutant PrP failed to rescue Ngsk PrP-/- mice from ataxia and Purkinje cell degeneration (39). Ngsk PrP-/- mice expressing the deletion mutant developed ataxia and Purkinje cell degeneration on a time course identical to that of non-transgenic Ngsk PrP-/- mice (39). These clearly indicate that the N-terminal residues 23-88 are important for PrP^C to antagonize the neurotoxicity of PrPLP/Dpl. The N-terminal residues 23-88 include most of the OR region. Thus, it is suggested that this OR region could be important for PrP^C to protect Purkinje cells from PrPLP/Dpl-induced degeneration, although it remains to be investigated which region in the deleted N-terminal domain could be essential for the neuroprotection of PrP^C against PrPLP/Dpl.

We also introduced PrP carrying a familial prion disease-associated mutation (E199K) into Ngsk PrP-/- mice (39). Interestingly, these mice developed no ataxia and Purkinje cell degeneration (39), showing that the mutant PrP was fully functional for antagonizing the PrPLP/Dpl-induced neurotoxicity, suggesting that other disease-associated mutant PrPs are also functionally competent.

PRP AND PRPLP/DPL IN ISCHEMIC NEURONAL CELL DEATH

To assess whether PrP^C and PrPLP/Dpl could be involved in other types of neuronal cell death, we subjected Zrch I PrP-/- and Ngsk PrP-/- mice to transient forebrain ischemia (40). Interestingly, male Zrch I PrP-/- mice were very susceptible to the ischemia compared to control PrP+/+ mice, developing marked apoptosis in the hippocampal CA1 region (40). McLennan, *et al.* also reported that permanent occlusion of the middle cerebral artery increased the infarction volume in male Npu PrP-/- mice without no ectopic expression of PrPLP/Dpl in neurons (41). However, no apoptotic cell death could be detected in the CA 1 of female Zrch I PrP-/- mice (40). Taken together, these results indicate that PrP^C is involved in neuroprotection against brain ischemia, and that the neuroprotective function of PrP^C is masked by female-specific neuroprotective factor(s).

We also showed that, in contrast to Zrch I PrP-/- mice, both male and female Ngsk PrP-/- mice exhibited severe ischemic damage to CA1 neurons (40). Since Ngsk PrP-/- mice ectopically express PrPLP/Dpl in neurons, it is therefore conceivable that PrPLP/Dpl might counteract the female-specific neuroprotective function, thereby increasing the susceptibility of PrP^C-deficient neurons to ischemic insults.

ROLES OF PRP AND PRPLP/DPL IN NEURODEGENERATION

Neurotoxic PrPs

PrPLP/Dpl is a homologue of the C-terminal part of PrP^C. Interestingly, it was shown that the N-terminally truncated PrPs, PrPΔ32-121 and PrPΔ32-134, induced ataxia and cerebellar degeneration characterized by marked granule cell death in Zrch I PrP-/- mice and the neurotoxicity of these truncated PrPs was antagonized by the expression of full-length PrP^C (42). No Purkinje cell degeneration was observed in these mice because the truncated PrPs were not expressed in Purkinje cells of these mice due to the limited activity of the promoter used (42). Consistently, it was demonstrated that ataxia and Purkinje cell loss could be induced in Zrch I PrP-/- mice when PrPΔ32-134 was targeted to Purkinje cells (43). PrPΔ32-121 and PrPΔ32-134 encompass the homologous C-terminal part of PrP^C to PrPLP/Dpl. It is therefore very likely that PrPLP/Dpl and the truncated PrPs might use the same or a very similar molecular mechanism to execute the neurotoxicity.

Cis- and trans-neuroprotective function of PrP against PrPLP/Dpl

In contrast to neurotoxic PrPΔ32-121 and PrPΔ32-134, it was shown that PrPΔ23-88 was not neurotoxic, causing no Purkinje cell degeneration in Zrch I PrP-/- mice (39), suggesting that the neurotoxicity of the C-terminal domain of PrP^C is blocked by a cis-element(s) present in the region between the residues 89 and 121. This region, overlapping with the central hydrophobic part, is reported to comprise part of the binding sites for the heat shock protein, stress-inducible protein 1, and the extracellular matrix constituent glycosaminoglycans (44, 45). It is therefore possible that interaction of PrP^C with these molecules might be involved in the cis-inhibition of the neurotoxicity of the C-terminal domain of PrP^C. In contrast, PrPLP/Dpl and the truncated PrPs are unable to interact with these molecules, therefore acting as neurotoxic proteins.

PrP^C also neutralizes the neurotoxicity of PrPLP/Dpl and the truncated PrPs in trans, rescuing from

the Purkinje cell degeneration. Interestingly, we showed that PrPΔ23-88 has no such trans-neuroprotective activity, unable to antagonize against PrPLP/Dpl (39). It is therefore suggested that N-terminal residues 23-88 are involved in the trans-neuroprotection of PrP^C against PrPLP/Dpl and probably the truncated PrPs as well.

Antagonistic signals of PrP and PrPLP/Dpl in neuronal cell death

Kuwahara, *et al.* previously reported that hippocampal neuronal cells from PrP-/- mice easily undergo apoptosis after withdrawal of serum, which can be prevented by either re-introduction of PrP^C or by expressing the anti-apoptotic protein, Bcl-2 (46). Bounhar, *et al.* also showed that PrP^C protected human primary neurons from the apoptosis induced by the pro-apoptotic protein Bax (47). It is therefore suggested that PrP^C might be an anti-apoptotic protein while PrPLP/Dpl might be pro-apoptotic.

It was shown that the primary cultured cerebellar neurons from non-ataxic Npu PrP-/- mice were more sensitive to oxidative stress than those from PrP+/+ mice (19), indicating that PrP^C might be involved in mitigation of oxidative stress. Interestingly, Wong, *et al.* showed that oxidative stress was much more elevated in the brains of ataxic Rcm0 PrP-/- mice than in those of non-ataxic Npu PrP-/- mice (48). It is therefore suggested that, in contrast to PrP^C, PrPLP/Dpl aggravates oxidative stress. Oxidative stress is associated with overproduction of radical oxygens, such as superoxide and nitric oxide (NO). Interestingly, it was shown that enzymatic activity of superoxide dismutase, a superoxide-detoxifying enzyme, was significantly decreased in PrP-/- mice (18). Moreover, it was reported that recombinant PrPLP/Dpl was toxic to the cultured cerebellar neurons of non-ataxic Zrch I PrP-/- mice and that this PrPLP/Dpl-neurotoxicity could be prevented by L-*N*-acetyl methyl ester, a pharmacological inhibitor of NO synthases (49). It is therefore possible that PrPLP/Dpl might aggravate oxidative stress by overproducing NO and superoxide, thereby causing Purkinje cell death, and that PrP^C could detoxify it, preventing PrPLP/Dpl-induced neurodegeneration.

It was previously shown that, in Zrch I PrP-/- mice, Ca^{2+}-activated K^+ currents in hippocampal pyramidal neurons were abnormal and intracellular Ca^{2+} contents in cerebellar granule cells were altered (50). Moreover, we showed that the T- and L-type Ca^{2+}-antagonist, flunarizine, significantly reduced the PrPLP/Dpl-aggravated ischemic neuronal apoptosis in Ngsk PrP-/- mice (40). Since excess of intracellular Ca^{2+} has been shown to be toxic to neurons (51), it is alternatively possible that PrP^C could reduce the intracellular Ca^{2+} load in neurons, thereby being neuroprotective, and that, in contrast, PrPLP/Dpl increases the intracellular Ca^{2+} load in neurons, thereby enhancing the susceptibility of neurons to apoptosis.

A proposed model of antagonistic interaction between PrP and PrPLP/Dpl

Weissmann and colleagues have proposed an interesting hypothesis, in which two conjectural molecules, protein π, which is another PrP-like protein, and a cognate ligand for PrP^C, are introduced (42, 52). In normal mice, PrP^C binds its cognate ligand to elicit a neuroprotective signal, thereby Purkinje cells are able to survive. But, in ataxic lines of PrP-/- mice, PrPLP/Dpl or the neurotoxic truncated PrPs compete with PrP^C for the ligand and blocks the signal, resulting in Purkinje cell degeneration. In non-ataxic lines of PrP-/- mice, instead of PrP^C, the protein π could elicit the same neuroprotective signal via binding to the ligand, thereby causing no Purkinje cell degeneration. However, this hypothesis can be verified only when the putative molecules are identified.

FINDINGS IN PRP-NULL MICE AND PATHOGENESIS OF PRION DISEASES

The molecular pathogenesis of prion diseases remains elusive. Since PrP-/- mice were resistant to these diseases (11-14), it is considered that the conformational conversion of PrP^C to PrP^{Sc} plays an essential role in the pathogenesis of the diseases. However, the exact nature of this role has not been fully understood. PrP^{Sc} is markedly accumulated in infected brains due to constitutive conversion. Forloni, *et al.* showed that an amyloidogenic PrP peptide (PrP106-126) was highly toxic to primary cultured neurons (53). It is therefore conceivable that PrP^{Sc} might itself be neurotoxic. In contrast, Yokoyama, *et al.* showed that the PrP^C-specific immunoreactivity was decreased in the brain regions where PrP^{Sc} had accumulated in experimentally infected mice (54), indicating that PrP^C is reduced in the infected neurons due to conversion. It is therefore alternatively possible that PrP^C might be functionally disturbed due to its marked reduction, thereby resulting in the nerodegeneration. Or, both the accumu-

lation of PrP^{Sc} and the functional loss of PrP^{C} might be required for the neuronal cell death.

PrP-/- mice without ectopic expression of PrPLP/Dpl exhibited neurological abnormalities, including impairment of LTP, alteration in sleep and circadian rhythm, as well as demyelination in the spinal cord and peripheral nervous system (28, 31, 32). LTP is a form of synaptic plasticity that is thought to underlie memory formation. Memory loss or dementia is a common symptom in prion diseases (55). Alteration in sleep and circadian rhythms is also a symptom characteristic for the inherited human prion disease, fetal familial insomnia (55). Moreover, demyelinating peripheral neuropathy has been reported in some cases of inherited prion disease (56, 57). Taken together, these results strongly support that the functional loss of PrP^{C} is involved in these pathogenic changes in the diseases. However, no neuronal cell degeneration could be detected in mice devoid of PrP^{C} alone, indicating that loss of PrP^{C} alone might not be enough to induce neuronal cell death or that other neurotoxic mechanisms might associate with neuronal cell death in prion diseases.

Interestingly, the ectopic expression of PrPLP/Dpl, PrPΔ32-121, or PrPΔ32-134 in the absence of PrP^{C} caused degeneration of Purkinje cells and granule cells (6, 38, 42). Purkinje cells and granule cells are markedly degenerative in human prion diseases (55). It is therefore conceivable that PrPLP/Dpl and the truncated PrPs might be involved in the pathogenesis of the diseases. However, PrPLP/Dpl itself is unlikely to be involved in the pathogenesis of prion diseases because PrPLP/Dpl could not be detected in the brains affected by experimental prion diseases (6, 8). Instead, PrP^{Sc} is markedly accumulated and its fragmented C-terminal products are often observed in the affected brains (58). It is therefore very interesting to speculate that PrP^{Sc} or its fragmented products possess a neurotoxic potential equivalent to that of PrPLP/Dpl, PrPΔ32-121, or PrPΔ32-134. Thus, elucidation of the molecular mechanism for the Purkinje cell degeneration in ataxic lines of PrP-/- mice might be useful for understanding of the molecular pathogenesis of prion diseases.

ACKNOWLEDGMENTS

I would like to thank Dr. S. Katamine, Dr. K. Shigematsu, Dr. N. Nishida, Dr. R. Atarashi, Dr. A. Li, Dr. N. Yamaguchi, Dr. Y. Sakurai-Yamashita, and Mr. N. Okimura. This study is partly supported from the Ministry of Health, Labour and Welfare, Japan.

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