Published in final edited form as:

Exp Neurol. 2009 June ; 217(2): 347–352. doi:10.1016/j.expneurol.2009.03.017.

Prion Protein on Astrocytes or in Extracellular Fluid Impedes Neurodegeneration Induced by Truncated Prion Protein

Brent Race¹, Kimberly Meade-White¹, Richard Race¹, Frank Baumann², Adriano Aguzzi², and Bruce Chesebro¹

¹Laboratory of Persistent Viral Diseases, Rocky Mountain Laboratories, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Hamilton, MT, 59840 USA ²Institute of Neuropathology, University Hospital of Zürich, Schmelzbergstrasse 12, Zürich, CH8091 Switzerland

Abstract

Prion protein (PrP) is a host-encoded membrane-anchored glycoprotein which is required for susceptibility to prion disease. PrP may also be important for normal brain functions such as hippocampal spatial memory. Previously transgenic mice expressing amino terminally truncated mouse PrP ($\Delta 32$ –134) spontaneously developed a fatal disease associated with degeneration of cerebellar granular neurons as well as vacuolar degeneration of deep cerebellar and brain stem white matter. This disease could be prevented by co-expression of wild-type (WT) mouse PrP on neurons or oligodendroglia. In the present experiments we studied $\Delta 32$ –134 PrP transgenic mice with WT PrP expression restricted to astroglia, an abundant CNS cell-type important for neuronal viability. Expression of WT PrP in astroglia was sufficient to rescue 50% of mice from disease and prolonged survival by 200 days in the other 50%. We also found that transgenic mice expressing full-length soluble anchorless PrP had increased survival by 100 days. Together these two results indicated that rescue from neurodegeneration induced by $\Delta 32$ –134 PrP might involve interactions between neurons expressing truncated PrP and nearby astrocytes expressing WT PrP or extracellular fluid containing soluble WT PrP.

Keywords

Astroglia; glycoposphatidylinositol anchor; granule cell neurons; neurodegeneration; prion; truncated prion protein

Introduction

Prion protein (PrP) is well known as an important factor that influences susceptibility to transmissible spongiform encephalopathies (TSE) or prion diseases. PrP is expressed in nearly all mammalian tissues though the highest levels are found in the brain (Sakudo et al. 2006; Bendheim et al. 1992; Race et al. 1995). Despite its widespread expression, the normal cellular function of PrP remains unclear. Roles in neuroprotection and maintenance of myelin integrity *in vivo* have been reported (Weise et al. 2004; Baumann et al. 2007), and anti-apoptotic and anti-oxidative protective functions have been demonstrated *in vitro* (Kuwahara et al. 1999;

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Sakudo et al. 2003). Knockout mice devoid of PrP develop and reproduce normally (Bueler et al. 1992; Manson et al. 1994) however, such mice do show defects in certain behavioral and neurophysiological tests (Criado et al. 2005; Colling et al. 1996; Curtis et al. 2003; Mallucci et al. 2002; Manson et al. 1995).

To study the role of specific PrP regions required for TSE susceptibility, transgenic mice expressing amino-proximal PrP deletion mutants were generated previously by Shmerling et al (Shmerling et al. 1998). Surprisingly, uninfected mice lacking PrP amino acid residues 32–134 (ΔF PrP) spontaneously developed a severe, progressive neurologic disease. These mice initially had coarse tremors and gait abnormalities beginning around 5 weeks of age which progressed to significant wasting, hind limb paresis and death by 3–4 months of age. The CNS pathology found in these mice was two fold: severe depletion of the granular cell layer of the cerebellum and vacuolation of white matter of the cerebellum, brain stem and upper spinal cord. Interestingly, disease could be prevented in mice co-expressing wild-type PrP (Shmerling et al. 1998).

Previous studies showed that WT PrP expression restricted either to neurons or oligodendroglia was sufficient to mediate rescue from this neurodegenerative disease (Radovanovic et al. 2005). In the present experiments we studied the effect of WT PrP expression restricted to astrocytes which are another glial cell type involved in maintenance of neuronal viability. We also studied the effect of expression of WT soluble anchorless PrP in this model in order to test whether PrP in the extracellular fluid (ECF) unattached to any particular cell type might also be able to promote enhanced survival. Both astroglial PrP and soluble anchorless PrP were able to impede the process of neurodegeneration induced by ΔF PrP, suggesting that the sites involved in rescue might be accessible to ECF and might not be specific for a particular brain cell type.

Materials and Methods

Observation of mice

All animals were housed at the Rocky Mountain Laboratories (RML) in an AAALAC-accredited facility according to approved NIH RML animal use protocols. Mice were observed daily for onset of neurologic signs. Initial age at onset, neurologic symptom severity and lifespan data were collected for each mouse. When mice became weak and had difficulty reaching food and water they were euthanized. Mice in experimental groups that did not progress to a terminal state were observed for 400–600 days.

Transgenic mice

Generation of anchorless PrP mice (tgAnchorless) was done by modifying the “half-genomic” mouse PrP plasmid pHGPrP (Fischer et al. 1996), as previously described (Chesebro et al. 2005). The tgAnchorless mice were then backcrossed to a C57BL/10 background for greater than eight generations. Genome scan data suggests >98.6 % homology to C57BL/10. Construction of the transgenic mice expressing hamster PrP in neurons under control of the neuron-specific enolase promoter (tgNSE) was performed by standard techniques using a construct containing the neuron-specific enolase (NSE) promoter plus a 1 kb cDNA containing the hamster PrP open reading frame (Race et al. 1995). Transgenic mice expressing hamster PrP in astrocytes under control of the GFAP promoter (tgGFAP) mice have been described previously (Raeber et al. 1997). Mice expressing hamster PrP under the control of the endogenous mouse PrP promoter (Tg7) were also described previously (Race et al. 2000).

ΔF PrP mice were on a mixed 129/Sv-C57BL/6 background. Mice of the ΔF PrP $^{+/-}$, WT $^{-/-}$ genotype became too weak prior to sexual maturity to successfully reproduce so ΔF PrP mice

were maintained by breeding to WT C57BL/10 PrP^{+/-} mice. Offspring were genotyped for the presence of the Δ F PrP truncation using primers pE2 and Mut217 as described previously (Shmerling et al. 1998). Detection of the WT PrP gene was done using primers 624 (5'-AACCGTTACCCACCTCAGGGT) and 1319 (5'-GCGCTCCATCATCTTCACA).

Generation and identification of experimental mice

Hemizygous Δ F PrP^{+/-}WT^{+/-} mice were crossed to homozygous tg7, tgNSE, and tgGFAP mice and hemizygous tgAnchorless mice. Offspring were genotyped for the presence of the Δ F PrP and WT genes as described above. Detection of the tgAnchorless gene was done using primers 624 (above) and 2037 (5'-CAGGCGCCTCGAGACGCGTCA). To distinguish WT PrP from tgAnchorless PrP, an additional pair of primers that recognized WT PrP, but not tgAnchorless PrP, was used 2038 (5'-TCCCACGATCAGGAAGATGAG) and 2057 (5'-CCAAGGAGGGGTACCCAT).

To study the role of tgAnchorless PrP ^{+/+} vs. ^{+/-} genotypes we generated a segregating population of Δ F PrP^{+/-} mice which had either ^{+/+} or ^{+/-} anchorless PrP. Δ F PrP^{+/-}, tgAnchorless^{+/-} mice were mated to Δ F PrP^{-/-}, tgAnchorless^{+/+} mice. The resulting offspring were genotyped for the presence of the Δ F PrP gene. Mice positive for Δ F PrP were then genotyped by quantitative PCR to determine the zygosity of the tgAnchorless gene using an ABI Prism 7900 HT Sequence detection system and SDS 2.2.2 software. Probes and primers were designed to recognize mouse *PRNP* sequence present in tgAnchorless mice but absent in Δ F PrP^{+/-} mice. Probe (moPrP_{lower}418T): (5'-CGGTCCTCCCAGTCGTTGCCAAA), forward primer (moPrP-396F): (5'-CGTGAGCAGGCCCATGATC), reverse primer (moPrP-465R): (5'-GCGGTACATGTTTTTCACGGTAGT). Multiple repeats of these tests were done to confirm these results.

Western blotting detection of PNGaseF digested PrP

Brain was placed in a DNase/RNase free 1.5 ml tube and homogenized in ice cold sterile 0.01M Tris-HCL, pH 7.4 with 0.005M MgCl₂, 10 μ M leupeptin, 1 μ M pepstatin A, 1 μ g/ml aprotinin to 20% w/v. Samples were sonicated 1 minute then centrifuged at 5000 rpm for 10 minutes. The supernatant was mixed 1:1 in 2X sample buffer and boiled for 3 minutes. Twenty μ l aliquots (2 μ g tissue equivalents) for PNGaseF treatment were removed at this point. Reagents and enzymes for PNGaseF treatment were purchased from New England BioLabs (Beverly, MA, USA). Reaction conditions were as recommended by the manufacturer except that denaturing was done in a total volume of 20 μ L sodium dodecyl sulfate polyacrylamide gel electrophoresis sample buffer. Each sample was digested by using 1,000 U PNGaseF and incubated overnight at 37°C. Samples were run on 16% SDS-PAGE gels and proteins were transferred to Immobilon PVDF membranes. PrP bands were detected using anti-PrP rabbit polyclonal antibodies R20 (residues 218–232) (Caughey et al. 1991), R30 (residues 89–103) (Caughey et al. 1991), R18 (residues 142–155) (Lawson et al. 2001), mouse monoclonal antibody SAF-32 (residues 59–89) (Feraudet et al. 2005), and human recombinant antibody D13 (residues 96–103) (InPro). Primary antibodies were diluted 1:5000 in TBS-T + 2.5% skim milk and incubated for 1 hour. Membranes were rinsed with TBS-T buffer and incubated with respective secondary antibodies (GE Healthcare / Sigma) at a 1:5000 dilution in TBS-T for 30 min. Bands were detected using ECL substrate as directed (GE Healthcare).

Immunohistochemistry

Whole brains were removed and placed in 3.7% phosphate-buffered formaldehyde for 3 to 5 days before dehydration and embedding in paraffin. Serial 4 μ m sections were cut using a standard Leica microtome, placed on positively charged glass slides, and dried overnight at 56°C. Brain sections were stained by standard hematoxylin and eosin (H&E) and examined for lesions. Each slide was examined and scored subjectively for the presence of spongiform

change and loss of granular cell neurons. Images were collected using an Olympus BX51 microscope and Microsuite™ FIVE software.

Results

Expression of WT PrP restricted to astrocytes induces prolonged survival or rescue from death

In our experiments starting at 33 days mice expressing ΔF PrP developed a wobbly gait and fine tremors (Figure 1A). Several weeks later these mice progressed to hind limb paralysis, weakness and wasting, and between 64 and 126 days these mice had to be euthanized (Figure 1B). Co-expression of wild-type (WT) mouse PrP has been shown to prevent this clinical phenotype (Shmerling et al. 1998). To study the effects of WT PrP expression in different CNS cell types we used previously described transgenic mice which expressed WT hamster PrP restricted to astrocytes, neurons or multiple cell types (Race et al. 1995; Raeber et al. 1997; Race et al. 2000). These mice were crossed with mice expressing ΔF PrP and progeny co-expressing both ΔF PrP and WT hamster PrP were identified by PCR. In our experiments hamster PrP was similar to mouse PrP in mediating rescue from this disease. Mice co-expressing WT hamster PrP restricted to neurons (tgNSE) or on multiple CNS cell types (tg7) showed 100% survival over 400 days (Figure 1A and 1B). Furthermore, expression of WT hamster PrP restricted to astrocytes (tgGFAP) also showed complete rescue of 50% of the mice for over 400 days and prolongation of survival by around 220 days in the other 50% of mice (Figure 1A and 1B). This result indicated that astroglia and neurons could both be important participants in the rescue process.

PrP expression levels in brain of transgenic mice

Because previous results showed that the variation in expression levels of PrP molecules could alter the timing of neurodegeneration (Baumann et al. 2007; Li et al. 2007), we tested the brain expression levels of all PrP molecules in the co-expressing transgenic mice used in the present experiments. Immunoblot analysis indicated that expression of ΔF PrP was not altered by the presence of any of the WT PrP alleles used (Figure 2C). Therefore, prevention or delay of neurodegeneration by WT PrP in these mice was not due to alteration of expression of truncated PrP. Brain levels of WT hamster PrP expressed in tg7 or tgNSE mice (Figure 2A&B, lanes 7 and 8) were both slightly higher than the level in mice expressing hemizygous WT mouse PrP (Figure 2A&B, lane 5). Brain level of WT hamster PrP in tgGFAP mice was about 4-fold lower than in tgNSE mice (Figure 2A, lane 9 vs 8). The significant level of rescue from disease seen in tgGFAP mice, in spite of the lower PrP expression, indicated that astrocyte restricted PrP had a strong rescue effect in this system.

Soluble anchorless PrP expression prolongs survival of mice expressing ΔF PrP

The finding that astrocyte-restricted, oligodendrocyte-restricted and neuron-restricted PrP could all prevent neurodegeneration induced by ΔF PrP indicated that the rescue effect was not limited to a particular cell type. It seemed possible that rescue might involve molecular interactions of WT PrP from glia or neurons with a putative receptor on the surface of neurons targeted for degeneration by ΔF PrP. Such a receptor might be accessible to molecules in the adjacent extracellular fluid. To test this possibility, we studied the effect of expression of soluble anchorless WT PrP in this system. Mice expressing ΔF PrP plus one allele of anchorless PrP (hemizygous) had a 75 day prolongation of survival compared to mice expressing ΔF PrP alone (Figure 3B). In addition, we also generated a population of ΔF PrP mice expressing two alleles (homozygous) of anchorless PrP. In this experiment homozygous tgAnchorless mice had a 100 day prolongation of survival compared to mice expressing only ΔF PrP (Figure 3B). It should be noted that in both of these experiments all the mice expressing anchorless PrP ultimately died, so this protection was significantly less than that induced by the presence of

WT anchored PrP (Figure 3B). These results supported the conclusion that expression of anchorless PrP was responsible for the observed prolongation of survival times.

By western blot analysis expression of anchorless PrP in the hemizygous mice was about 6-fold less than in WT mice (Figure 2A, lanes 5 and 6), which appeared to account for the reduced rescue effect observed. In the homozygous mice the expression was double that seen in hemizygous mice (data not shown). This correlated with a further increase in survival (Figure 3B). However, the 75–100 day prolongation of survival in both these groups was highly significant ($P < 0.001$).

Analysis of neurodegeneration by histopathology

To determine whether the delay or rescue of clinical disease was accompanied by a delay in the histopathological features, brains of mice were examined microscopically. Terminal ΔF PrP mice at 101 days had atrophy of the granular cell layer of the cerebellum as well as vacuolation of the cerebellar and brainstem white matter (Figure 4A–C). In contrast, mice co-expressing ΔF PrP and WT mouse PrP appeared normal (Figure 4D–F). At 120 dpi mice co-expressing ΔF PrP plus WT PrP restricted to astrocytes (tgGFAP) or neurons (tgNSE) also had no lesions (Figure 4G–I, and J–L,). Mice co-expressing ΔF PrP plus soluble WT PrP (tgAnchorless) had detectable lesions consisting of thinning of the cerebellar granular layer and mild vacuolation of the cerebellar and brain stem white matter (Figure 4M–O), but these changes were much less than in mice expressing only ΔF PrP. These histopathology results correlated well with the alteration of the survival curves shown for the co-expression of these genes.

Discussion

Brain expression of truncated PrP molecules such as ΔF PrP is known to cause cerebellar degeneration and fatal clinical disease (Shmerling et al. 1998). Co-expression of WT PrP can prevent or delay the disease. The mechanism of disease induction by ΔF PrP is currently unknown. Previous authors have speculated that ΔF PrP might activate a cellular receptor to induce neurotoxicity (Shmerling et al. 1998; Li et al. 2007). However, the cellular site of such an effect remains unknown. Rescue of ΔF PrP induced brain disease by WT PrP expressed on specific cell types should provide clues to the location of rescue events and the range of cell types involved. In the present experiments astrocyte-restricted co-expression of WT PrP rescued 50% of ΔF PrP expressing mice from disease and prolonged survival in the other 50%. The present data on astrocytes together with previously published results using PrP expression restricted to neurons or oligodendroglia (Radovanovic et al. 2005) indicate that there is not a strong cell type specificity for the rescue by WT PrP in this system.

Expression of soluble anchorless PrP also significantly delayed fatal disease induced by ΔF PrP. Thus GPI-anchoring of PrP was not required for prolongation of survival in this model. The results also suggested that a putative receptor for the toxic signal by truncated PrP would likely have access to the extracellular fluid where most of the anchorless PrP is located. These data were in agreement with previous results showing that a soluble anchorless truncated PrP (121–231) was neurotoxic in vitro and that a soluble full-length PrP could block this toxicity (Daniels et al. 2001).

PrP expression levels also appeared to influence the extent of the rescue observed in our experiments, and others have previously noted similar effects (Radovanovic et al. 2005; Li et al. 2007; Baumann et al. 2007). With tg mice it is not possible to control the tg expression levels in individual lines, and we were not able to obtain lines expressing each transgene at 100% of WT which would have been ideal for optimal comparisons. Nevertheless, the amount of rescue and increased survival which we observed with lower expression levels of WT PrP

in astrocyte restricted tgGFAP mice and tgAnchorless PrP mice was significant, and thus contributed to our understanding of the location of the rescue process in this model.

There are two distinct neuropathological lesions which have previously been associated with disease induced by ΔF PrP: (1) apoptotic degeneration of the cerebellar granule cell layer and (2) vacuolation, astrocytosis and demyelination of cerebellar and brain stem white matter. These appear to be independent processes which both might contribute to the disease. In the present experiments the transgenes tested did not differentially prevent either of these two types of pathology, so astrocytic PrP and soluble PrP appeared to affect both of these pathological processes. However, differential rescue of these phenotypes has been seen by others. For example, in one study WT mouse PrP expression in oligodendroglia prevented white matter damage and clinical disease, but had little effect on granule cell degeneration. This suggested that the white matter damage was responsible for the clinical disease (Radovanovic et al. 2005). A similar conclusion was reached in other studies where clinical disease was seen when granule cell degeneration was prevented, but white matter damage persisted (Li et al. 2007; Baumann et al. 2007).

An analogous disease also occurs with abnormal brain expression of the Doppel gene, which shares structural and sequence homologies with ΔF PrP, and this disease can also be inhibited by WT PrP (Nishida et al. 1999). Recently, *in vitro* degeneration of cerebellar granule cell neurons associated with truncated PrP or Doppel was partially blocked by Shadoo, a CNS GPI-anchored membrane glycoprotein with homology and domain organization similar to the N-terminal portion of PrP (Watts et al. 2007). In the future it will be important to determine whether Shadoo can act *in vivo* to block the clinical disease and neuropathology associated with expression of truncated PrP and Doppel.

The finding that hamster PrP was similar to mouse PrP in blocking disease induced by ΔF PrP indicates that there is cross-recognition of the PrP molecules from these two species. This cross-protection is reminiscent of the ability of hamster PrP expression in neurons and other cells to prevent neurobehavioral and neurophysiological abnormalities seen in PrP null mice (Criado et al. 2005). In contrast, the specificity of this process differs greatly from that of species-specific prion disease infection, where mouse and hamster PrP encode specificity for different infectious prion agents. (Priola 1999; Race and Chesebro, B. 1998; Kimberlin and Walker, C. A. 1978)

Acknowledgments

This research was supported by the Intramural Research Program of the NIH, NIAID. The authors thank Cynthia Favara and Lori Lubke for work with histopathology, Lynne Raymond and Nicolette Arndt for assistance with animal breeding and husbandry, Anita Mora and Gary Hettrick for graphics assistance, and Drs. Sonja Best, John Portis, Byron Caughey, Sue Priola and Karin Peterson for helpful discussions concerning the manuscript.

Reference List

1. Baumann F, Tolnay M, Brabeck C, Pahnke J, Klotz U, Niemann HH, Heikenwalder M, Rulicke T, Burkle A, Aguzzi A. Lethal recessive myelin toxicity of prion protein lacking its central domain. *EMBO J* 2007;26:538–547. [PubMed: 17245436]
2. Bendheim PE, Brown HR, Rudelli RD, Scala LJ, Goller NL, Wen GY, Kascsak RJ, Cashman NR, Bolton DC. Nearly ubiquitous tissue distribution of the scrapie agent precursor protein. *Neurology* 1992;42:149–156. [PubMed: 1346470]
3. Bueler H, Fischer M, Lang Y, Bluethmann H, Lipp HP, DeArmond SJ, Prusiner SB, Aguet M, Weissmann C. Normal development and behaviour of mice lacking the neuronal cell-surface PrP protein. *Nature* 1992;356:577–582. [PubMed: 1373228]

4. Caughey B, Raymond GJ, Ernst D, Race RE. N-terminal truncation of the scrapie-associated form of PrP by lysosomal protease(s): implications regarding the site of conversion of PrP to the protease-resistant state. *J. Virol* 1991;65:6597–6603. [PubMed: 1682507]
5. Chesebro B, Trifilo M, Race R, Meade-White K, Teng C, LaCasse R, Raymond L, Favara C, Baron G, Priola S, Caughey B, Masliah E, Oldstone M. Anchorless prion protein results in infectious amyloid disease without clinical scrapie. *Science* 2005;308:1435–1439. [PubMed: 15933194]
6. Colling SB, Collinge J, Jefferys JG. Hippocampal slices from prion protein null mice: disrupted Ca(2+)-activated K⁺ currents. *Neurosci. Lett* 1996;209:49–52. [PubMed: 8734907]
7. Criado JR, Sanchez-Alavez M, Conti B, Giacchino JL, Wills DN, Henriksen SJ, Race R, Manson JC, Chesebro B, Oldstone MB. Mice devoid of prion protein have cognitive deficits that are rescued by reconstitution of PrP in neurons. *Neurobiol. Dis* 2005;19:255–265. [PubMed: 15837581]
8. Curtis J, Errington M, Bliss T, Voss K, MacLeod N. Age-dependent loss of PTP and LTP in the hippocampus of PrP-null mice. *Neurobiol. Dis* 2003;13:55–62. [PubMed: 12758067]
9. Daniels M, Cereghetti GM, Brown DR. Toxicity of novel C-terminal prion protein fragments and peptides harbouring disease-related C-terminal mutations. *Eur. J. Biochem* 2001;268:6155–6164. [PubMed: 11733010]
10. Feraudet C, Morel N, Simon S, Volland H, Frobert Y, Creminon C, Vilette D, Lehmann S, Grassi J. Screening of 145 anti-PrP monoclonal antibodies for their capacity to inhibit PrP^{Sc} replication in infected cells. *J. Biol. Chem* 2005;280:11247–11258. [PubMed: 15618225]
11. Fischer M, Rulicke T, Raeber A, Sailer A, Moser M, Oesch B, Brandner S, Aguzzi A, Weissmann C. Prion protein (PrP) with amino-proximal deletions restoring susceptibility of PrP knockout mice to scrapie. *EMBO J* 1996;15:1255–1264. [PubMed: 8635458]
12. Kimberlin RH, Walker CA. Evidence that the transmission of one source of scrapie agent to hamsters involves separation of agent strains from a mixture. *J. Gen. Virol* 1978;39:487–496. [PubMed: 96212]
13. Kuwahara C, Takeuchi AM, Nishimura T, Haraguchi K, Kubosaki A, Matsumoto Y, Saeki K, Matsumoto Y, Yokoyama T, Itoharu S, Onodera T. Prions prevent neuronal cell-line death. *Nature* 1999;400:225–226. [PubMed: 10421360]
14. Lawson VA, Priola SA, Wehrly K, Chesebro B. N-terminal truncation of prion protein affects both formation and conformation of abnormal protease-resistant prion protein generated in vitro. *J. Biol. Chem* 2001;276:35265–35271. [PubMed: 11466311]
15. Li A, Piccardo P, Barmada SJ, Ghetti B, Harris DA. Prion protein with an octapeptide insertion has impaired neuroprotective activity in transgenic mice. *EMBO J* 2007;26:2777–2785. [PubMed: 17510630]
16. Mallucci GR, Ratte S, Asante EA, Linehan J, Gowland I, Jefferys JG, Collinge J. Post-natal knockout of prion protein alters hippocampal CA1 properties, but does not result in neurodegeneration. *EMBO J* 2002;21:202–210. [PubMed: 11823413]
17. Manson JC, Clarke AR, Hooper ML, Aitchison L, McConnell I, Hope J. 129/Ola mice carrying a null mutation in PrP that abolishes mRNA production are developmentally normal. *Mol. Neurobiol* 1994;8:121–127. [PubMed: 7999308]
18. Manson JC, Hope J, Clarke AR, Johnston A, Black C, MacLeod N. PrP gene dosage and long term potentiation. *Neurodegeneration* 1995;4:113–114. [PubMed: 7600180]
19. Nishida N, Tremblay P, Sugimoto T, Shigematsu K, Shirabe S, Petromilli C, Erpel SP, Nakaoke R, Atarashi R, Houtani T, Torchia M, Sakaguchi S, DeArmond SJ, Prusiner SB, Katamine S. A mouse prion protein transgene rescues mice deficient for the prion protein gene from purkinje cell degeneration and demyelination. *Lab Invest* 1999;79:689–697. [PubMed: 10378511]
20. Priola SA. Prion protein and species barriers in the transmissible spongiform encephalopathies. *Biomed. Pharmacother* 1999;53:27–33. [PubMed: 10221165]
21. Race R, Chesebro B. Scrapie infectivity found in resistant species. *Nature* 1998;392:770. [PubMed: 9572135]
22. Race R, Oldstone M, Chesebro B. Entry versus blockade of brain infection following oral or intraperitoneal scrapie administration: role of prion protein expression in peripheral nerves and spleen. *J. Virol* 2000;74:828–833. [PubMed: 10623745]

23. Race RE, Priola SA, Bessen RA, Ernst D, Dockter J, Rall GF, Mucke L, Chesebro B, Oldstone MB. Neuron-specific expression of a hamster prion protein minigene in transgenic mice induces susceptibility to hamster scrapie agent. *Neuron* 1995;15:1183–1191. [PubMed: 7576660]
24. Radovanovic I, Braun N, Giger OT, Mertz K, Miele G, Prinz M, Navarro B, Aguzzi A. Truncated prion protein and Doppel are myelinotoxic in the absence of oligodendrocytic PrP^C. *J.Neurosci* 2005;25:4879–4888. [PubMed: 15888663]
25. Raeber AJ, Race RE, Brandner S, Priola SA, Sailer A, Bessen RA, Mucke L, Manson J, Aguzzi A, Oldstone MB, Weissmann C, Chesebro B. Astrocyte-specific expression of hamster prion protein (PrP) renders PrP knockout mice susceptible to hamster scrapie. *EMBO J* 1997;16:6057–6065. [PubMed: 9321385]
26. Sakudo A, Lee DC, Saeki K, Matsumoto Y, Itohara S, Onodera T. Tumor necrosis factor attenuates prion protein-deficient neuronal cell death by increases in anti-apoptotic Bcl-2 family proteins. *Biochem.Biophys.Res.Commun* 2003;310:725–729. [PubMed: 14550262]
27. Sakudo A, Onodera T, Sukanuma Y, Kobayashi T, Saeki K, Ikuta K. Recent advances in clarifying prion protein functions using knockout mice and derived cell lines. *Mini.Rev.Med.Chem* 2006;6:589–601. [PubMed: 16719834]
28. Shmerling D, Hegyi I, Fischer M, Blattler T, Brandner S, Gotz J, Rulicke T, Flechsig E, Cozzio A, von MC, Hangartner C, Aguzzi A, Weissmann C. Expression of amino-terminally truncated PrP in the mouse leading to ataxia and specific cerebellar lesions. *Cell* 1998;93:203–214. [PubMed: 9568713]
29. Watts JC, Drisaldi B, Ng V, Yang J, Strome B, Horne P, Sy MS, Yoong L, Young R, Mastrangelo P, Bergeron C, Fraser PE, Carlson GA, Mount HT, Schmitt-Ulms G, Westaway D. The CNS glycoprotein Shadoo has PrP(C)-like protective properties and displays reduced levels in prion infections. *EMBO J* 2007;26:4038–4050. [PubMed: 17703189]
30. Weise J, Crome O, Sandau R, Schulz-Schaeffer W, Bahr M, Zerr I. Upregulation of cellular prion protein (PrP^C) after focal cerebral ischemia and influence of lesion severity. *Neurosci.Lett* 2004;372:146–150. [PubMed: 15531106]

Figure 1.

Clinical onset (A) and survival (B) curves for mice expressing ΔF PrP with or without co-expression of WT hamster PrP using various promoters. Mice expressing ΔF PrP plus WT hamster PrP in neurons (tgNSE) or many cell types (tg7) survived until the end of the observation period (400–600 days). Symbols and N values for each group are as follows: ΔF only ($\blacksquare$), N=29; ΔF & tgGFAP ($\blacklozenge$), N=28; ΔF & tgNSE ($\bullet$), N=21; ΔF & tg7 ($\square$), N=34; ΔF & WT ($\circ$), N=33.

Figure 2.

Immunoblots of brain PrP from double transgenic mice using monoclonal antibody D13 or anti-PrP C-terminal peptide antibody R20.

(A.) Monoclonal antibody D13 (96–106): 30 min exposure time; lower panel (B), 2 min exposure time. Lanes 1 and 2, Wild type (WT); Lanes 3 and 4, ΔF PrP; Lane 5, ΔF & WT; Lane 6, ΔF & tgAnchorless; Lane 7, ΔF & tg7; Lane 8, ΔF & tgNSE; Lane 9, ΔF & tgGFAP. Samples in lanes 2 and 4–9 were treated with PNGase F (+) as described in methods and were loaded with 0.35 mg wet weight brain tissue equivalents. Lanes 1 and 3 were loaded with 0.5 mg brain tissue equivalents. In lanes 2, 5, 7–9 the main PrP band was accompanied by a smaller PrP fragment lacking the C-terminal epitope seen by R20 (note absence of this fragment in

Figure 2C). The D13 antibody does not react with ΔF PrP since the epitope is missing with the deletion (lanes 3,4). Results show that expression of hemizygous tgAnchorless PrP (lane 6) was 6–8-fold less than hemizygous WT (lane 5). Level of tgGFAP-PrP (lane 9) was 4–5-fold less than WT. Similar results were observed using three other antibodies including R30 (89–103), R18 (142–155) and SAF-32 (59–89). (C) Anti-hamster PrP peptide (218–232) antibody, R20. This antibody was used to detect ΔF PrP which lacked the residues detected by D13. The same samples used in A&B were used in C. Lanes 4–9 show a similar level of ΔF PrP at 15 kDa (arrowhead) indicating that in double transgenic mice expression of ΔF PrP was not altered by co-expression of the full-length PrP constructs. Interestingly, R20 detected WT PrP (lane 5) versus anchorless PrP (lane 6) at similar levels. This result differed from that seen with all other antibodies and appeared to be due to partial blocking of the C-terminal R20 epitope in WT PrP by interactions between N-terminal PrP residues and the GPI moiety.

Figure 3.

Clinical onset (A) and survival (B) curves for mice expressing ΔF PrP with or without co-expression of WT or anchorless mouse PrP. Mice expressing ΔF PrP plus WT PrP survived past 600 days. Symbols and N values for each group are as follows: ΔF (■), N=29; ΔF & tgAnchorless+/- (▼), N=35; ΔF & tgAnchorless+/+ (▲), N=30; ΔF & WT (○), N=33. Mantel-Cox statistical analysis of the ΔF and ΔF & tgAnchorless+/- survival curves were significantly different ($P < 0.0001$) as were the ΔF & tgAnchorless+/- (▼) compared to the ΔF & tgAnchorless+/+ (▲) mice ($P < 0.0001$).

Figure 4.

Histopathology of cerebellar regions of five mice differing in PrP genotype. Results show white matter vacuoles and loss of cerebellar granule cell layer neurons in mice expressing ΔF PrP alone (panels A–C). Similar lesions were not observed in mice co-expressing ΔF PrP and WT mouse PrP (WT) (panels D–F), astrocytic PrP (tgGFAP) (panels G–I) or neuronal PrP (tgNSE) (panels J–L). Mice coexpressing ΔF PrP and anchorless PrP (tgAnchorless) also showed a decrease in cerebellar neurons and an increase in white matter vacuoles but the onset of these lesions was delayed by several weeks (panels M–O). Results are representative of 4–6 mice examined in each group. Column 1, overview of cerebellum, H&E stain with enlarged areas (column 2) boxed. Column 2, granule cell layer, H&E stain. Loss of cerebellar granule layer cells is seen in panels B and N (mild). Column 3, deep cerebellar white matter, H&E stain. Arrows show vacuoles in panels C and O. Scale bars shown in the bottom panel of each column are representative for all the panels in that column: column 1: 500 μm , column 2: 100 μm , column 3: 25 μm .