The Role of GPI-anchored PrP^C in Mediating the Neurotoxic Effect of Scrapie Prions in Neurons

Helois E. Radford and Giovanna R. Mallucci*

In vivo models of Neurotoxicity Group, MRC Toxicology Unit, Hodgkin Building, University of Leicester, Lancaster Road, Leicester, LE1 9EH, UK

Abstract

There are two central phenomena in prion disease: prion replication and prion neurotoxicity. Underlying them both is the conversion of a host-encoded ubiquitously expressed protein, prion protein (PrP^C), into a partially-protease resistant isoform, PrP^{Sc}, which accumulates in the brain. PrP^{Sc} is associated with both pathology and infectivity (Prusiner, 1991). In the absence of PrP^C, PrP^{Sc} cannot be generated and PrP-null mice do not propagate infectivity or develop pathology on infection with scrapie (Bueler et al., 1993; Manson et al., 1994; Sailer et al., 1994). However, while PrP^C expression is fundamental to both prion infectivity and neurodegeneration, the uncoupling of these processes is a growing concept in the field. This dissociation is evident in subclinical states of prion infection (Hill et al., 2000), where PrP^{Sc} levels are high in the absence of disease, and in transgenic mice experiments, where, despite extra-neuronal PrP^{Sc} accumulation, even in very high amounts, there is no neurotoxicity (Chesebro et al., 2005; Mallucci et al., 2003). Both these models have further implications. Thus depleting PrP^C from neurons (but not glia) of prion-infected mice prevents clinical disease (Mallucci et al., 2003), and detaching it from the surface of cells by removing its anchor does the same (Chesebro et al., 2005). The uncoupling toxicity and infectivity has implications for the nature of the neurotoxic species; these mouse models suggest that the site for the generation of this species is intra-neuronal. This review considers the role of neuronal surface-expressed PrP^C in mediating toxicity in prion infection, and the dissociation of this from the deposition of PrP^{Sc}.

Introduction: concepts of neurotoxicity in prion disease

Prion diseases, or transmissible spongiform encephalopathies, are fatal neurodegenerative conditions of humans and animals, which are transmissible within or between mammalian species. They are associated with the accumulation in the brain of an abnormal, partially protease-resistant, isoform of host-encoded prion protein (PrP). The normal cellular isoform, PrP^C, is widely expressed but found at the highest level in the CNS as a glycosylated, glycosylphosphatidylinositol (GPI) anchored cell-surface protein (Caughey et al., 1989; Stahl et al., 1987).

The disease-related isoform (PrP^{Sc}) is derived from PrP^C by a post-translational process that involves conformational change and aggregation. PrP^C is rich in α-helical structure while PrP^{Sc}, which is found as insoluble aggregates, appears to be predominantly composed of ß-sheet structure. Many studies support the 'protein-only' hypothesis (Griffith, 1967; Prusiner, 1982) namely that an abnormal PrP isoform is the principal, and possibly the sole, constituent of the transmissible agent or prion. PrP^{Sc} is thought to act as a conformational template, recruiting PrP^C to form further PrP^{Sc}. Prion propagation may involve recruitment of an alternately folded form of PrP^C, into PrP^{Sc} aggregates, the process being driven thermodynamically by intermolecular interactions (Jackson et al., 1999).

However, the cause of neuronal death in prion disease remains unclear, in particular, the role played by PrP^{Sc}. The idea that neurodegeneration follows from direct toxicity of PrP^{Sc} and/or prions (Prusiner, 1991) has been increasingly challenged. PrP^{Sc} does not seem to have direct toxic effects on neurons *in vivo,* despite *in vitro* evidence for both full length PrP^{Sc} (Muller et al., 1993) and shorter fragments (Forloni et al., 1993) being toxic to primary cultured neurons. There are several inherited prion diseases in which PrP^{Sc} is not detected in significant amounts despite fatal clinical disease (Collinge et al., 1995; Medori et al., 1992; Tateishi et al., 1992), and often there is poor correlation between PrP^{Sc} deposits and clinical signs or neuronal loss (Collinge et al., 1995; Hsiao et al., 1990; Medori et al., 1992). Most tested potential therapeutic agents that target the accumulation of PrP^{Sc} have shown disappointing therapeutic benefit in prion infected animals. At best, they prolong incubation periods, even if given before, or at the time of, peripheral infection, but they do not prevent neurodegeneration and death (see (Mallucci and Collinge, 2005; Trevitt and Collinge, 2006) for reviews). What is clear is that host PrP^C is absolutely necessary for prion-induced neurotoxicity as well as for PrP^{Sc} production. This is seen in the resistance of PrP-null mice to prion neurotoxicity (Brandner et al., 1996; Bueler et al., 1993; Manson et al., 1994; Sailer et al., 1994), and in the rescue of neurons from degeneration when PrP^C is 'removed' from them, preventing ongoing neuronal prion replication (Mallucci et al., 2003; Mallucci et al., 2007; White et al., 2008) (see below). But even where PrP^C is present to support prion replication and mediate the neurotoxicity of PrP^{Sc}, PrP^{Sc} accumulation and toxicity can be uncoupled. This is seen in subclinical forms of prion infection in which animals can harbour high levels of infectivity, and PrP^{Sc}, without clinical disease (Hill and Collinge, 2003; Hill et al., 2000; Race et al., 2001), or where prion replication is extra-neuronal (Chesebro et al., 2005; Mallucci et al., 2003). In mice where neuronal PrP^C is deleted during the course of infection, but glial replication of PrP^{Sc} continues (Mallucci et al., 2003), and in mice in which the GPI-anchor is removed, detaching PrP from the cell surface but allowing replication to occur away from the cell bodies and processes (Chesebro et al., 2005), neurotoxicity does not occur despite extensive PrP^{Sc} accumulation.

One hypothesis is that if PrP^{Sc} itself is not directly neurotoxic, it is the depletion of PrP^C during prion replication that damages cells. The normal function of

*grm7@le.ac.uk

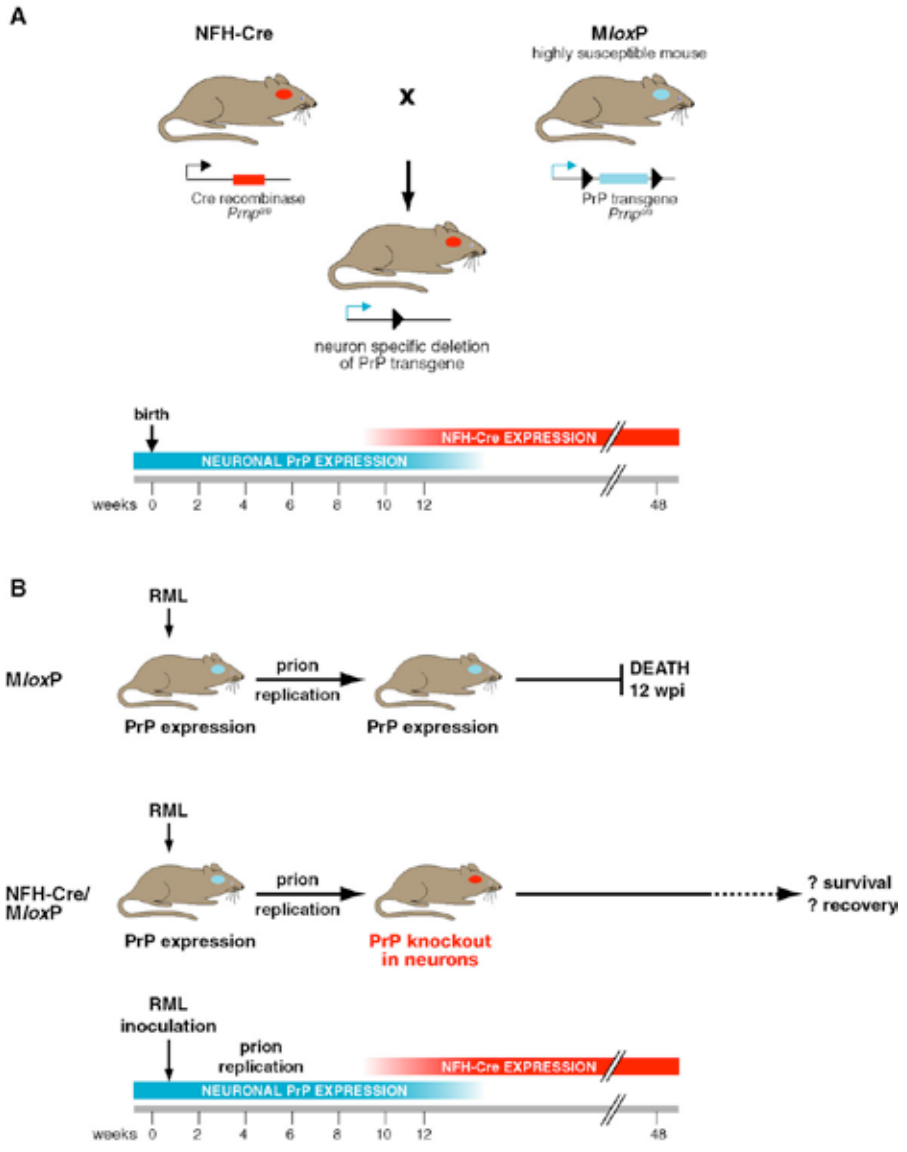

Figure 1. Adult onset PrP^C knockout in neurons by Cre-mediated recombination in mice; a model for studying neurotoxicity in prion disease **A.** NFH-Cre mice express Cre recombinase in neurons at ~ 9 weeks of age which deletes PrP transgenes from M*lox*P mice. **B.** The model is used to study the effects of neuronal PrP^C knockout during RML prion infection. Both NFH-Cre/M*lox*P and M*lox*P control mice replicate prions for 8 weeks, after which double transgenic animals lose neuronal PrP expression.

PrP^C is incompletely understood, and there is evidence that it may be neuroprotective (see review by Martins et al, this issue) and that aberrant trafficking or topology can be neurotoxic (Hegde et al., 1998; Hegde and Rane, 2003; Ma and Lindquist, 2002) (reviewed by Miesbauer et al; this issue). Yet the lack of neurodegeneration in either acquired (Mallucci et al., 2002), or embryonic (Bueler et al., 1993; Manson et al., 1994; Sailer et al., 1994), PrP knockout mice and in mice lacking correctly anchored (and hence functional) PrP (Chesebro et al., 2005), argues against this.

The uncoupling of PrP^{Sc} and neurotoxicity indicates that it is not the aggregated, protease resistant core of the molecule that is toxic to cells. But this begs the question of what the toxic species is? The central role of PrP^C conversion in the process suggests the generation of a toxic intermediate species, which may never aggregate, or whose toxicity is in any case independent of eventual aggregation. The possibility that this species may not only be transient and soluble, but needs to be generated within neurons is discussed below along with alternative explanations.

Prion neurotoxicity: a critical role for neuronal expression and surface anchoring of PrP^C?

The demonstration of recovery of early degenerative prion pathology and behavioral and neurophysiological deficits in mice in which neuronal PrP^C was depleted during infection, gives potentially important insights into possible mechanisms of prion neurotoxicity. The evidence comes from use of a mouse model in which PrP^C is depleted from neurons in the

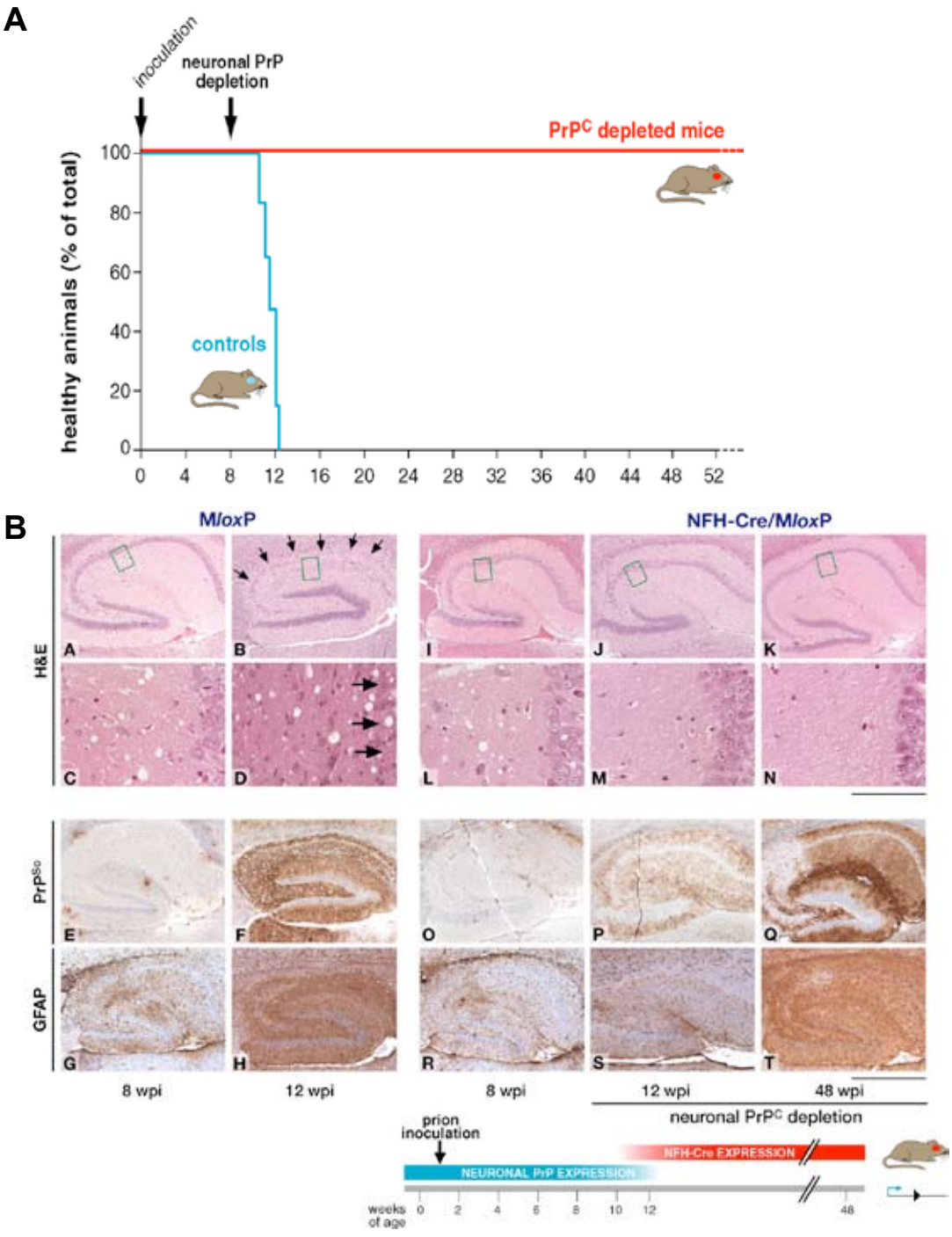

Figure 2. Scrapie-infected NFH-Cre/tg37 mice survive long term after Cre-mediated PrP^{C} depletion and early pathology reverses despite ongoing extra-neuronal PrP^{Sc} accumulation. **A.** Kaplan-Meier survival plot of RML scrapie prion-inoculated mice. All control M*lox*P mice (blue curve) succumbed to scrapie within 12 wpi (*n*=6). No animals with Cre-mediated PrP^{C} depletion at 8wpi (red line) succumbed to scrapie or show any clinical signs of disease by 52 wpi (*n*=6). The timing of inoculation and the onset of Cre-mediated PrP^{C} depletion 8 weeks into the course of infection, are indicated (arrows). **B.** Fixed sections of brains from scrapie-infected M*lox*P and NFH-Cre/M*lox*P mice, stained with haematoxylin and eosin (H&E), and immunostained for GFAP and PrP^{Sc} detection. Neuronal PrP depletion by Cre-mediated recombination in NFH-Cre/M*lox*P mice from 8 wpi is indicated. There is severe neuronal loss of hippocampal regions CA1-3 (arrows) (B,D) and indeed shrinkage of the entire hippocampus (B) in clinically sick M*lox*P mice, but no neuronal loss in prion-infected mice with Cre-mediated PrP^{C} depletion (I-K, L-N) up to 48 wpi. Early spongiform change is seen at 8 wpi in all mice (C,L), which recovers in scrapie-infected animals after Cre-mediated recombination (M,N). PrP^{Sc} accumulation (P,Q) and gliosis (S,T) continues in NFH-Cre/M*lox*P mice after Cre-mediated PrP^{C} depletion (R-T). Scale bar represents 320μm in all panels, except for panels C,D and L,M,N where it represents 80μm.

adult animal, after the nervous system is fully developed (Mallucci et al., 2002). Infecting these mice before knockout of PrP occurred allowed the effects of depleting neuronal PrP^{C} during the course of prion disease to be studied. In this system, PrP is expressed from 'floxed' transgenes (M*lox*P) and is deleted by expression of the enzyme Cre recombinase in neurons (from the NFH-Cre transgenes), see Figure 1. In contrast to PrP-null mice where deletion is embryonic, and global, the knockout of PrP here is acquired (at 9 weeks of age). Further, the knockout is neuron-specific, as the NFH promoter is expressed only in neurons. Mice were infected with prions at one week of age, 8 weeks before the onset of transgene mediated PrP depletion, allowing prion infection to develop over this time. By the time PrP depletion occurred, prion neuropathological change was established, with early spongiform change, astrocytosis and PrP^{Sc} deposition. Control M*lox*P animals without transgene meditated depletion at this stage of disease progressed to full prion neurodegeneration and death within 4 weeks. But animals in which PrP^{C} was knocked out at this time point survived, asymptomatic, long term (Figure 2A) and showed reversal of early spongiform change (Figure 2B, panels L,M,N). The animals were effectively clinically cured (Mallucci et al., 2003). This occurred despite the continued accumulation of extra-neuronal PrP^{Sc} over time to levels observed in terminally sick wild-type animals (Figure 2B, panel Q). This extra-neuronal conversion of PrP^{C} to PrP^{Sc} was not toxic to neurons, however. By 48 weeks post-infection, the animals had accumulated levels of PrP^{Sc} (and prion titres) as high as those seen in end-stage clinical prion disease in control mice, but no symptoms or neuronal loss were observed, suggesting that it is the occurrence of prion conversion within neurons that is pathogenic. Here, neurotoxicity and PrP^{Sc} accumulation are unequivocally uncoupled, and the data validated approaches in prion disease therapeutics targeting PrP^{C}; which was further confirmed using RNAi against PrP in prion infection (White et al., 2008). Further analysis of this model led to questions about the cell types involved in prion neurotoxicity, the nature of the toxic species and the timing of treatment in prion disease.

So does prion replication have to be intra-neuronal to be toxic? Certainly, this model supports this concept. Different cell types appear to be important in prion disease infectivity and pathogenesis. Both neurons and astrocytes propagate prions, and astrocytes may be the earliest sites of PrP^{Sc} accumulation (Diedrich et al., 1991). But astrocytic replication is not essential for prion neurotoxicity as expressing PrP in neurons alone in transgenic mice is sufficient to render the animals susceptible to prion disease (Race et al., 1995). Similarly, the data discussed above, where neuronal PrP^{C} depletion is protective against neurotoxicity, supports the argument that prion replication must be intra-neuronal to be toxic. Yet a mouse model in which PrP^{C} expression was directed towards astrocytes using the GFAP promoter to drive hamster PrP (HaPrP) expression also restored susceptibility to hamster scrapie prions in these mice (Raeber et al., 1997). These mice showed prion pathological changes (spongiform changes and astrogliosis), but no neuronal loss. Electron microscopy showed that neurons were primarily damaged and astrocytes appeared to not show any degenerative changes, although the $HaPrP^{Sc}$ mainly colocalised to astrocytes (Jeffrey et al., 2004). How does this reconcile with resistance of mice with acquired neuronal knockout of PrP to astrocyte PrP^{Sc} mediated neurotoxicity? The two models differ significantly and are not directly comparable for a number of reasons, including having been generated on different genetic backgrounds, expressing different species of PrP under different promoters and infection with different scrapie strains. GFAP-PrP mice over-express hamster PrP^{C} in astrocytes due to exponential induction of the GFAP promoter during infection, while in M*lox*P mice there is low-level astrocytic expression of mouse PrP^{C} expression under its own promoter. Further, 263K scrapie used to infect the GFAP-PrP mice produces very little neuronal loss, in contrast to the severe neurodegeneration induced by RML infection of M*lox*P mice. Further, it is increasingly clear that GFAP promoter activity is not entirely restricted to astrocytes. It is widely expressed in developing cerebellar interneurons, contributing up to 30% of the final population of these cells (Silbereis et al., 2009) and is also widely expressed throughout life in neural stem cells in the adult forebrain in the subventricular zone (Morshead et al., 2003). Recent evidence from genetic fate mapping of postnatal GFAP positive cells reveals that they seed the postnatal brain with neural progenitors that in turn give rise to their mature neuronal progeny throughout the CNS, including the cerebral cortex (Ganat et al., 2006). Thus it is likely that the GFAP-PrP mice are indeed expressing PrP in a sufficient number of neurons, to restore susceptibility of these mice to prion neurotoxicity.

While this mouse model makes a strong case for prion neurotoxicity hinging on the intra-neuronal conversion of PrP^{C} to PrP^{Sc}, it is also possible that in fact this need for neuronal replication actually reflects a kinetic effect rather than cell specificity. Thus the effect of gene dosage, and hence levels of expression, of PrP are known to correlate inversely with prion incubation times, despite extensive build-up of PrP^{Sc} and prion titre (Bueler et al., 1994; Weissmann et al., 1994). After neuronal PrP knockout, total PrP levels are reduced (Mallucci et al., 2003; Mallucci et al., 2002; Mallucci et al., 2007) and neurons no longer propagate prions; glial prion replication taking over. Beyond the reversal of pathology and increased survival in this model, what is striking is that by 48 wpi (weeks post infection) levels of PrP^{Sc} and titres of prion infectivity are as high as in the terminally ill wild type controls. The key difference, apart from the localization of the PrP^{Sc} to astrocytes, is the rate at which this accumulation occurs. The levels of infectivity and prion replication in control mice rise from basal to end stage in 4 weeks, while in animals with neuronal PrP knockout, the same increase takes 40 weeks. It is therefore also possible that at a lower rate of production, neurons can 'clear' the toxic species, wherever this is produced.

Collinge and colleagues have proposed a model to explain a 'clearance theory' for PrP toxic species, which they term PrP^{L} (for PrP 'lethal'). During the conversion of PrP^{C} to PrP^{Sc}, the putative neurotoxic intermediate molecule, PrP^{L} might be formed (Hill and Collinge, 2003; Collinge and Clarke, 2007). Such an intermediate form of PrP may be different from the infectious form of PrP aggregates, and be rapidly cleared or sequestered into large PrP^{Sc} aggregates. According to this model, the rate of neurodegeneration would depend on the level of steady state 'PrP^{L}', which could explain the uncoupling of prion titre and neurotoxicity seen in experimental models. Preventing the conversion of PrP^{C} into PrP^{Sc} should also prevent the generation of 'PrP^{L}',

whereas targeting PrP^{Sc}, the end product of the conversion process, might increase the level of 'PrP^{L}'. This model is consistent with the data from neuronally PrP-deleted mice, where the knockdown would impact on steady state level of a neurotoxic intermediate.

Further analysis of the Cre-mediated PrP knockout animals certainly supports the generation of a toxic species that is transient and separate to PrP^{Sc}. There are lessons to be learnt specifically from the course of reversal of the early pathology in these mice. Analysis showed that early pathology correlated with early functional deficits that recovered rapidly when PrP was deleted, consistent with transient toxicity. Moreover, the earliest 'toxic' effects were functional, not degenerative, implying a disturbance of neuronal function upstream of neurodegeneration. Thus early spongiform change was associated with cognitive and behavioral deficits and impaired neurophysiological function that recovered post knockout (Mallucci et al., 2007). Coincident with earliest spongiform change, 8 wpi, mice lost the capacity for novel object recognition, a test of non-spatial memory, and showed significantly reduced species- specific burrowing behavior, both of which reflect hippocampal dysfunction. Neurophysiological examination revealed concurrent reduced synaptic responses in CA1 hippocampal neurons. Remarkably, within a week, soon after neuronal PrP depletion both cognitive/behavioral and synaptic deficits recovered and this recovery was sustained up to 30 wpi, in parallel with recovery of spongiform change and sustained neuroprotection. Further, the deficits occur before extensive PrP^{Sc} deposits accumulate and recover rapidly after PrP^C depletion, again supporting the concept that they are caused by a transient neurotoxic species, distinct from aggregated PrP^{Sc} (Mallucci et al., 2007). The behavioral changes can similarly be prevented by lentivirally-mediated knockdown of PrP^C using RNA interference (White et al., 2008).

Thus this model uncouples prion neurotoxicity and PrP^{Sc} and prion titre, strongly supports the generation of a transient toxic intermediate, and overall supports the concept that this generation needs to be intra-neuronal to manifest its neurotoxicity. More recent evidence also implicates the necessity of intra-neuronal, or at least neuronal surface prion replication for neurotoxicity. PrP^C (and PrP^{Sc}) is attached to the cell surface by a glycosyphosphatidylinositol (GPI) moiety added to its C-terminus during processing in the Golgi, which anchors it to the outer leaflet of the plasma membrane (Caughey et al., 1989; Stahl et al., 1987). PrP^C is localised to cholesterol-rich lipid rafts within the plasma membrane, in part mediated by the affinity of the GPI anchor for saturated lipid species (Kaneko et al., 1997; Madore et al., 1999; Taraboulos et al., 1995; Vey et al., 1996), although some studies have shown PrP^C association with rafts in an independent manner, determined by PrP^C's N-terminus (Baron and Caughey, 2003; Campana et al., 2007; Sanghera and Pinheiro, 2002; Walmsley et al., 2003). The function of GPI anchors is poorly understood, and GPI anchored proteins may serve a variety of functions (receptors, adhesion, enzymes, complement regulation, signal transduction). The enrichment of GPI anchors, along with glycosphingolipids and cholesterol, in rafts locates them within these relatively rigid platforms on which, through the interaction of surface and cytoplasmic proteins, several signal transduction pathways are triggered and vesicular trafficking is organized (Fontaine et al., 2003; Mukasa et al., 1995; Oxley and Bacic, 1999). Recent evidence also suggests that GPI-linkage is required for many proteins in order to assume their fully functional conformation (Macrae et al., 2005). GPI-anchorage might also be important for apical targeting of proteins in polarised cells such as those of the intestinal epithelium, which conceivably could apply also to neurons.

The role of the GPI anchor in PrP^C function is not clear, but is it necessary for the toxicity and replication of prions? In cell-free experiments and *in vitro* anchorless PrP^C can be converted to PrP^{Sc} (Caughey and Raymond, 1991; Kocisko et al., 1994; Lawson et al., 2001; Rogers et al., 1993). However, others report that removing the anchor from PrP^C by treating with phosphatidylinositol-specific phospholipase C (PI-PLC) before exposure to scrapie prevents both infection and propagation in N2a cells (Enari et al., 2001). PI-PLC removes the anchor from PrP^C but not from PrP^{Sc}, but cathepsin-D does cleave the GPI moiety from PrP^{Sc}. Cathespin-D treated RML brain homogenate can infect both N2a cells and wild type mice (Lewis et al., 2006), suggesting that the GPI anchor on PrP^{Sc} is not needed for infection.

Chesebro and co-workers generated mice expressing anchorless PrP^C to address the role of the anchor in prion replication and neurotoxicity *in vivo*. These animals produced anchorless soluble monomeric PrP^C (GPI^- PrP). In neurons cultured from these mice, GPI^- PrP was not detectable on the plasma membrane, instead, around 90% was secreted into the medium and the rest detected in the ER and Golgi (Campana et al., 2007; Chesebro et al., 2005). Thus GPI^- PrP is secreted rather than retained intracellulary, indeed intracellular levels of GPI^- PrP were similar to those in control mice (Chesebro et al., 2005). These animals were essentially healthy and viable, but most significantly, were resistant to prion disease. Animals expressing GPI^- PrP did not develop clinical prion disease symptoms when infected with various mouse prion strains (RML, ME7, 22L) up to 400-600 days post infection (dpi), compared to control wild type mice that developed clinical symptoms within 140-160 days, depending on the strain used (Chesebro et al., 2005). Strikingly, however, the animals had widespread amyloid plaques of PrP^{Sc} throughout the brain, particularly near blood vessels. Plaques were detected from around 70 dpi and were progressively more widely distributed after 213 dpi. Some animals had up to 40% more PrP^{Sc} than clinically sick infected non-transgenic controls. GPI^- PrP expression thus supports prion replication and PrP^{Sc} deposition, but not the development of neurotoxicity or clinical disease, reminiscent of neuronal PrP knockout mice (Figure 3). Again there is clear uncoupling of PrP^{Sc} accumulation and neurotoxicity, and the lack of surface anchoring of PrP^C in this model further supports a role for prion neuronal conversion, mediated by GPI-anchored PrP^C, as central to neurotoxicity. That it is PrP^C, and not PrP^{Sc}, that must be anchored is confirmed by the fact that blood or brain homogenates from RML infected GPI-negative mice were infectious to wild type mice (Chesebro et al., 2005; Trifilo et al., 2006), but not to other GPI^- PrP mice (Chesebro et al., 2005). The findings are consistent also with observations on the infectivity of cathepsin-D treated PrP^{Sc} (Lewis et al., 2006). Further, replacing the GPI anchor of PrP^C with its transmembrane domain, removes it from rafts and inhibits its conversion to PrP^{Sc} *in vitro* (Kaneko et al., 1997).

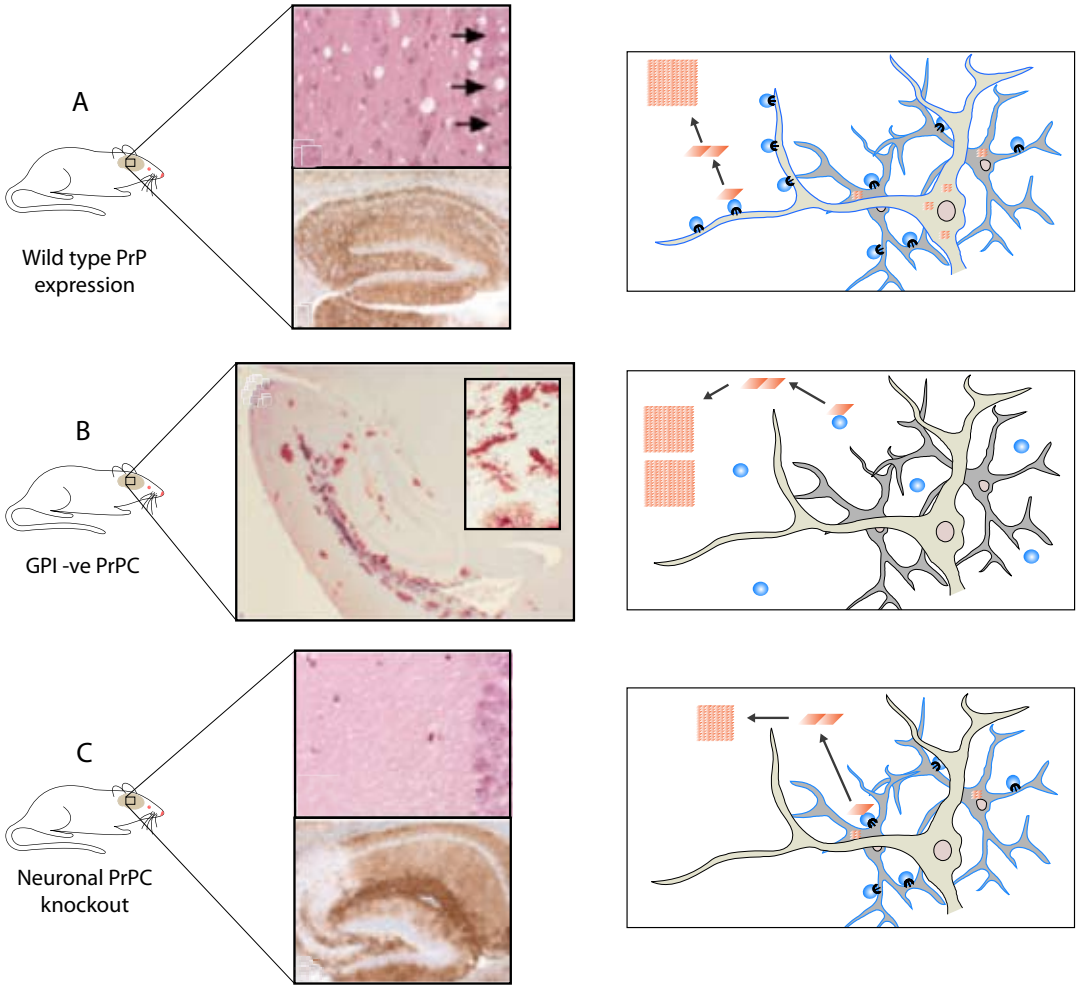

Figure 3. Uncoupling of PrP^{Sc} depostion and neurotoxicity in transgenic mice models. **A.** Mice expressing GPI-anchored PrP^{C} (blue circles = PrP^{C}; GPI anchor in black) physiologically on neurons (pale gray) and astrocytes (darker gray), replicate prions and deposit PrP^{Sc}, which aggregates (orange squares), causing typical prion neurotoxicity with neuronal loss and spongiosis (upper histology image) and PrP^{Sc} depostion (lower immunohistochemistry image). **B.** Mice expressing anchorless PrP^{C} (blue circles; no anchor) do not have PrP^{C} on the neuronal or astrocytic surface but secrete it, where it is converted into PrP^{Sc} and aggregates into amyloid plaques (histology panel). **C.** Mice with neuronal PrP knockout but expressing GPI-anchored PrP^{C} on astrocytes only generate PrP^{Sc} but are resistant to neurotoxicity (top histology panel). All mice generate PrP^{Sc} (lower immunohistochemistry panel) but the mice survive despite its accumulation, without neurotoxicity.

This work supports the hypothesis that the surface conversion of PrP^{C} to PrP^{Sc} requires PrP^{C}, but not PrP^{Sc}, to be GPI anchored. As neurons are the target of prion neurotoxicity it would appear that this process must occur on, or within, neurons. The data have been interpreted as reflecting prion neurotoxicity involving some perturbation of the normal function of PrP^{C}, possibly mediated through its anchor, in the presence of prion replication (Aguzzi, 2005). However, other interpretations are possible. The GPI^{-} PrP mouse model raises some questions. While they undoubtedly secrete soluble PrP, which is not located on the cell membrane and is involved in prion conversion extra-neuronally, these mice have rather low levels of mutant PrP expression. The authors describe levels of mRNA at about 50% wild type, but levels of actual protein appear lower still (see Chesebro et al; 2005, Figure S2). Gene dosage of PrP is a clear determinant of prion incubation time (Bueler et al., 1994; Manson et al., 1994; Weissmann et al., 1994) and even hemizygosity for wild type PrP leads to at least doubling of incubation time despite accumulating levels of PrP^{Sc} and prion titre. The reported survival of the GPI^{-} PrP mice is within this range, so is the survival unrelated to the anchoring but dependent on total levels of expression? It is likely that both the levels of PrP expression and the lack of anchor are significant. There is growing evidence in prion infected cell lines, in which protein trafficking has been selectively impaired, that the endosomal recycling compartment is the likely site of prion conversion (Marijanovic et al., 2009). This compartment is also known to be important in the pathway for internalization of GPI anchored proteins (Mayor et al., 1998), again supporting the need for a GPI anchor on PrP^{C} as being central to prion conversion, as anchorless PrP^{Sc} is infectious (Chesebro et al., 2005; Lewis et al., 2006).

Concluding remarks

The data discussed here unequivocally uncouple neurotoxicity and prion (and PrP^{Sc}) replication in prion disease, a concept described as early as 1994, when the effects of PrP gene dosage on incubation times were first described (Bueler et al., 1994; Weissmann et al., 1994). They also increasingly provide support for intra-neuronal generation of a toxic intermediate, where GPI-anchored PrP^C likely plays a crucial role. There are still many unanswered questions, not least what the neurotoxic species is and how, exactly, it causes neuronal dysfunction, and eventually death. The role of pharmacokinetic factors in clearing, depositing and aggregating PrP^{Sc} and its isoforms further complicates the interpretation of mechanisms of neurotoxicity in these disorders, but clearly is essential to an understanding of the real picture. Targeting the substrate of conversion, PrP^C, remains a therapeutically sound strategy, and new approaches may eventually provide clarity on the details of the toxic culprit and how it acts.

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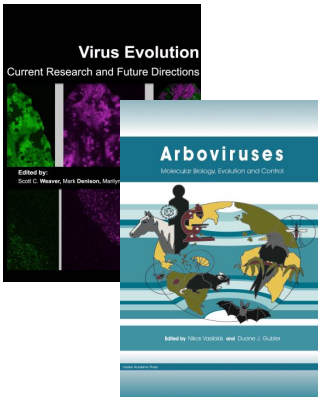

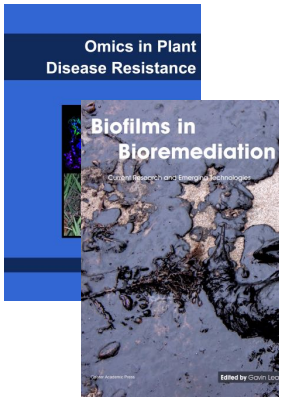

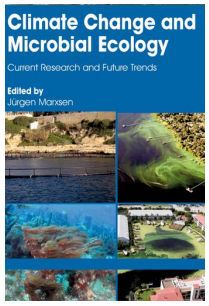

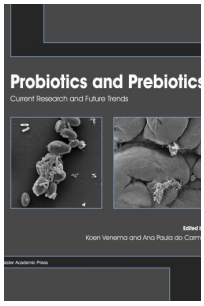

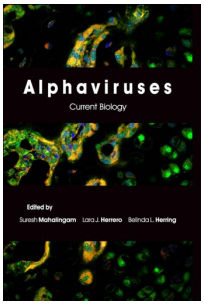

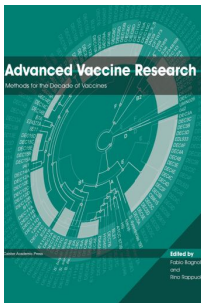

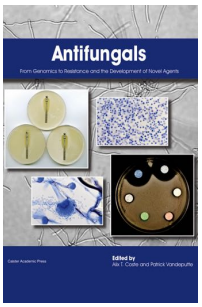

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