

Prion Protein and Metal Interaction: Physiological and Pathological Implications

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Abstract

Metal induced free radicals are important mediators of neurotoxicity in several neurodegenerative conditions such as Alzheimer's disease, Parkinson's disease, and Huntington's disease. Similar evidence is now emerging for prion diseases, a group of neurodegenerative disorders of humans and animals. The main pathogenic agent in all prion disorders is PrP^{Sc} (PrP^{Sc}), a β -sheet rich isoform of a normal cell surface glycoprotein known as the prion protein (PrP^C). Deposits of PrP^{Sc} in the brain parenchyma are believed to induce neurotoxicity through poorly understood mechanisms. Recent reports suggest that imbalance of brain metal homeostasis is a significant cause of PrP^{Sc}-associated neurotoxicity, though the underlying mechanisms are difficult to explain based on existing information. Proposed hypotheses include a functional role for PrP^C in metal metabolism, and loss of this function due to aggregation to the disease associated PrP^{Sc} form as the cause of brain metal imbalance. Other views suggest gain of toxic function by PrP^{Sc} due to sequestration of PrP^C-associated metals within the aggregates, resulting in the generation of redox-active PrP^{Sc} complexes. The physiological implications of some PrP^C-metal interactions are known, while others are still unclear. The pathological implications of PrP^C-metal interaction include metal-induced oxidative damage, and in some instances conversion of PrP^C to a PrP^{Sc}-like form. Despite its significance, only limited information is available on PrP-metal interaction and its implications on prion disease pathogenesis. In this review, we summarize the

physiological significance and pathological implications of PrP-metal interaction on prion disease pathogenesis.

Introduction

The current challenge facing prion research is the lack of an effective therapeutic strategy for prion disorders, a group of invariably fatal neurodegenerative conditions of humans and animals. Research in this area has been hampered due to incomplete understanding of the underlying mechanisms of neurotoxicity in these devastating diseases. The most favored hypothesis supported by numerous studies suggests that neurotoxicity in all prion disorders is mediated by PrP-scrapie (PrP^{Sc}), a β -sheet rich conformation of a cell surface glycoprotein known as the prion protein (PrP^C). The change in conformation of PrP^C to PrP^{Sc} confers certain biochemical and biophysical properties to PrP^{Sc}, which, unlike its counterpart PrP^C, becomes insoluble in non-ionic detergents and resists limited digestion by proteinase-K (PK). Deposits of PrP^{Sc} in the brain parenchyma are considered the principal cause of neurotoxicity, though the pathways involved in this process are poorly understood (Prusiner, 1998; Aguzzi and Polymenidou, 2004, Caughey and Baron, 2006). Intense research in this area has clarified several aspects of this process. It is now clear that PrP^{Sc} in the extracellular space does not induce toxicity in the absence of PrP^C expression on the neuronal plasma membrane, implicating PrP^C in mediating the toxic signal (Malluci et al., 2003; Chesebro et al., 2005). On the other hand, accumulation of PrP^{Sc} only on astrocytes induces neurotoxicity, suggesting indirect pathways of toxicity by PrP^{Sc} (Jeffrey et al., 2004). Suggested pathways include secretion of toxic chemokines and factors by microglia in response to PrP^{Sc}, physical damage to the membrane structure by PrP^{Sc} aggregates, interference with synaptic transmission, and toxicity through a labile by-product of the PrP^C to PrP^{Sc} conversion reaction (reviewed in Harris and True, 2006; Caughey and Baron, 2006). Evidence from cell model studies of familial prion disorders indicates abnormal processing and metabolism of mutant PrP as a possible cause of cytotoxicity, though the mechanism varies with specific mutations (Jin et al., 2000; Mishra et al., 2002; Gu et al., 2003). Other mechanisms such as activation of cell death pathways due to intracellular accumulation of PrP^{Sc} (Yadavalli et al., 2004; Kristiansen et al., 2005), cross-linking of neuronal PrP^C on the plasma membrane by PrP^{Sc} (Solfrosi et al., 2004), and toxicity due to C-transmembrane and cytosolic forms of PrP^C through unspecified pathways have also been suggested, but the details of underlying mechanisms remain unclear (Hegde et al., 1998; 1999; Ma et al., 2002; Ma and Lindquist, 2002; Heller et al., 2003; Wang et al., 2006).

An overlooked but equally important cause of neurotoxicity is the loss of normal function of PrP^C due to conversion to the PrP^{Sc} form. Information on this subject is limited since the normal function of PrP^C remains ambiguous despite its ubiquitous presence and conservation through

Abbreviations

Fe²⁺: Ferrous iron
 Fe³⁺: Ferric iron
 AD: Alzheimer's disease
 CJD: Creutzfeldt-Jakob disease
 HFE: hemochromatosis protein
 HD: Huntington's disease
 LIP: labile iron pool
 N2a: mouse neuroblastoma cells
 PD: Parkinson's disease
 PK: proteinase-K
 PrP^{-/-}: mice lacking PrP expression
 PrP^C: prion protein
 PrP^{Sc}: PrP-scrapie
 ScN2a: scrapie infected mouse neuroblastoma cells
 SOD: Cu/Zn superoxide dismutase
 Tf: transferrin, TfR: transferrin receptor

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evolution. Transgenic mice lacking PrP^C expression (PrP^{-/-}) do not develop a specific phenotype unless a prion-like protein Doppel is up regulated, making it difficult to ascertain the functional role of PrP^C (Bueller et al., 1992). Other experimental models suggest several unrelated functions, including a role in signal transduction, cell adhesion, copper uptake and transport, as an anti-oxidant and anti-apoptotic protein, and others (Bounhar et al., 2001; Roucou et al., 2004; 2005; Roucou and LeBlanc, 2005; Harris and True, 2006). The diverse functions attributed to PrP^C probably reflect its involvement in an essential biochemical pathway that affects several cellular processes. Loss of this activity by the change in conformation of PrP^C to PrP^{Sc} is likely to induce toxicity due to loss of an essential function, together with gain of certain toxic properties by PrP^{Sc}.

One such pathway may involve the functional role of PrP^C in copper and iron metabolism. Since these metals are redox-active, abnormal metabolism of either of these metals is likely to induce neurotoxicity due to the generation of free radicals. Such a mechanism has been observed in neurodegenerative conditions associated with protein aggregation such as Alzheimer's disease (AD) and Parkinson's disease (PD) (Moos and Morgan, 2004; Gerlach et al., 1994; Hayashi et al., 2006; Gaasch et al., 2007; Smith et al., 1997; 2007; Barnham and Bush, 2008). Recent reports indicate the presence of a similar phenomenon in prion disorders (Petersen et al., 2005; Singh et al., 2009a). Markers of oxidative stress and imbalance of metal homeostasis have been reported in prion disease affected brains, lending support to this assumption (Kim et al., 2000;

Rossi et al 2004; Petersen et al., 2005; Pamplona et al., 2008; Singh et al., 2009a). In this review, we summarize evidence related to the interaction of PrP^C with various metals, followed by the physiological and pathological implications of PrP-metal interaction. In particular, the role of PrP^C in copper and iron metabolism is reviewed since these metals are essential for several metabolic processes, and are also toxic if mismanaged due to their redox-active nature.

PrP-metal interactions

Several studies have reported the interaction of PrP^C with metals using *in vitro* and *in vivo* models. The *in vitro* studies have been more revealing due to the simplicity and accuracy of the readout compared to cell and animal models where metal metabolism is complex and the interaction of individual proteins with metals is often missed due to low affinity or their transient nature. Observations from *in vitro* studies using re-folded, full-length recombinant PrP or its fragments have lead to important findings regarding PrP-metal interaction and its significance to prion disease pathogenesis. It is now clear that recombinant PrP binds several divalent cations, including copper, iron, zinc, manganese and nickel (Pan et al., 1992; Hornshaw et al., 1995a; 1995b; Brown et al., 2000; Jackson et al., 2001; Jones et al., 2004; 2005; Basu et al., 2007). The highly conserved octa-peptide repeat region of PrP is the principal metal binding site, and its affinity for metals is highest for copper, followed by nickel, zinc, and manganese (Jackson et al. 2001). A diagrammatic representation of the interaction of PrP^C with copper and

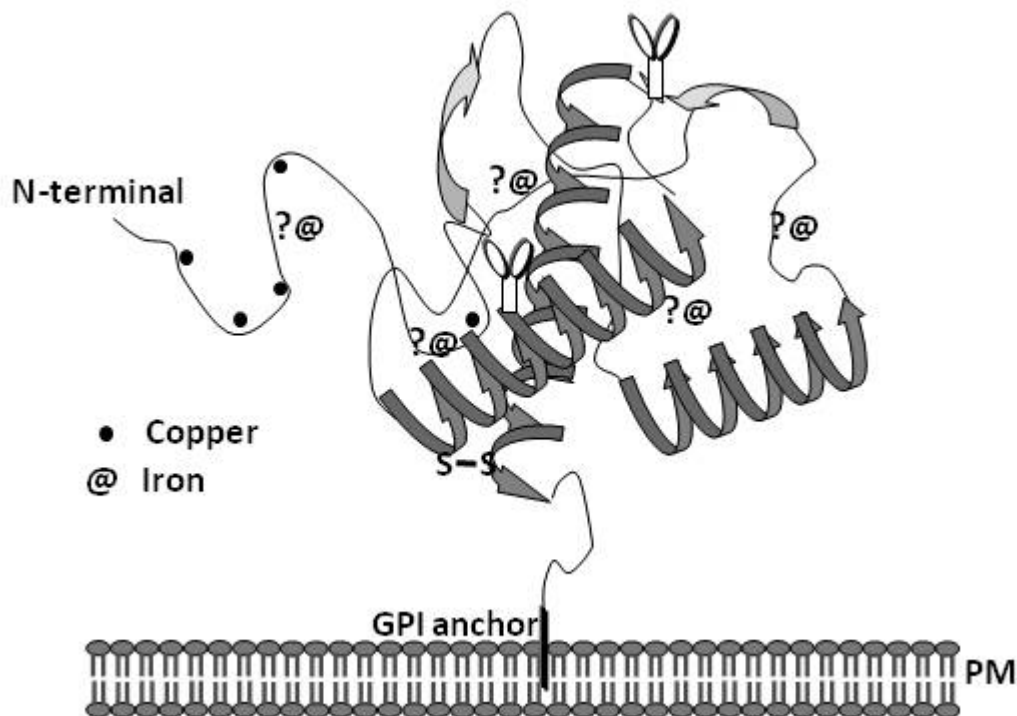


Figure 1. A model of PrP^C demonstrating the known copper binding sites in the octa-peptide region and histidine residues 96 and 111. The association of PrP^C with iron is based on unpublished data from recombinant full length PrP^C and its fragments.

iron is shown in Figure 1. The interaction of PrP^C with metals is important to understand because of the physiological and pathological implications of this association. For example, PrP^C is believed to mediate the uptake of copper and iron, suggesting a role in cellular copper and iron metabolism (Brown and Harris, 2003; Singh et al., 2009b,c). On the other hand, interaction of recombinant and cell associated PrP^C with certain metals induces a change in its conformation to the disease associated PrP^{Sc} form, suggesting their role in prion disease pathogenesis (Deleaut et al., 2007; Basu et al., 2007). The conversion of PrP^C to the PrP^{Sc} form increases its affinity for nickel while decreasing the binding of zinc and manganese, indicating specific interaction of different metals with the normal and the disease associated PrP^{Sc} form (Jackson et al., 2001).

The association of PrP^C with copper is better characterized than its interaction with other metals. Four copper binding sites have been identified within the octapeptide repeat region of PrP^C with additional sites on histidine residues 96 and 111 (Burns et al., 2003). Purified PrP^C from mouse and human brains also binds copper, substantiating the *in vitro* observations (Brown et al., 2001; Wong et al., 2001b). The affinity of PrP^C for copper is higher compared to zinc, and even a large excess of zinc cannot displace copper from the octapeptide repeat region of full-length PrP^C under *in vitro* conditions (Jackson et al., 2001; Walter et al., 2007). However, physiologically relevant levels of zinc in cell models alter the distribution of PrP^C bound copper with relative ease, indicating the influence of other catalytic factors in determining the metal ion occupancy of PrP^C (Watt and Hooper, 2003). These observations suggest that the interaction of PrP^C with zinc may be more significant given the relative abundance of this metal in the brain (Qin et al., 2002; Watt and Hooper, 2003; Walter et al., 2007; Kenward et al., 2007). PrP^C also binds manganese, probably in the C-terminal region between residues 91-230 or overlapping with the copper binding site at His-96 (Treiber et al., 2007; Brazier et al., 2008). Although these observations are useful, the data need to be interpreted with caution since *in vitro* reactions do not always represent the complex milieu of PrP^C in cells or in the brain.

The interaction of PrP^C with some of these metals alters its structure such that it simulates PrP^{Sc} in certain biochemical properties including detergent insolubility and resistance to limited digestion by proteinase-K. Other metals induce this change in PrP^C by their absence such as in the presence of a specific chelator, suggesting that modification of metal ion occupancy of PrP^C is equally significant in bringing about this change (Deleaut et al., 2007). Thus, addition of manganese promotes a change in the conformation of recombinant PrP^C to PrP^{Sc}, while zinc is believed to inhibit fibril formation and promote inter-molecular reactions (Brown et al., 2000; Giese et al., 2004; Tsenkova et al., 2004; Bocharova et al., 2005; Kim et al., 2005; Abdelraheim et al., 2006; Treiber et al., 2006; Kenward et al., 2007). Addition of zinc and copper to the toxic peptide of PrP, a model often used for studying prion-mediated toxicity, increases its aggregation and toxicity to cells (Jobling et al., 1999; 2001). A similar change in the conformation of PrP^C to a PrP^{Sc}-like form is noted when PrP-expressing yeast cells are grown in medium supplemented with copper or manganese (Treiber et al., 2006). This reaction is reversed by adding specific chelators such as bathocuproinedisulfonic acid and clioquinol to

the medium, reinforcing the idea that only specific metals induce this reaction. Despite this information and additional data emphasizing the significance of PrP^C and PrP^{Sc}-metal interaction, neither the physiological nor the pathological significance of this interaction is clearly understood.

Physiological significance of PrP-metal interaction

Observations from neuroblastoma cells suggest that PrP^C binds extracellular copper ions and delivers to endocytic compartments, functioning as a copper uptake protein (Pauly and Harris, 1998; Brown and Harris, 2003). The octapeptide repeats of PrP^C are essential for this process since deletion of this region inhibits copper uptake (Perera and Hooper, 2001). The octapeptide repeat region is also believed to function as a reductase, reducing Cu (II) ions before transport across the endosomal membrane to the cytosol (Miura et al., 2005). Although these observations suggest that PrP^C may be a major copper delivery protein, surprisingly, there is minimal difference in the copper content of brains from wild type and transgenic mice lacking PrP expression (PrP^{-/-}) (Giese et al., 2005). However, contradictory studies claim a significant difference in copper levels between wild type, PrP^{-/-}, and scrapie infected mouse brains, suggesting a prominent role for PrP^C in maintaining copper homeostasis in the brain (Brown et al., 1997, 1998; Wong et al., 2001c; Thackray et al., 2002). Likewise, conflicting results regarding brain copper levels have been reported in PrP^C over-expressing mice (Herms et al., 1999; Kretzschmar et al., 2000; Waggoner et al., 2000; Stuermer and Plattner, 2005), leaving the matter unsettled.

The interaction of PrP^C with iron is a relatively recent finding, and the physiological significance of this association is becoming increasingly clear from studies indicating a functional role for PrP^C in cellular iron uptake and transport (Basu et al., 2007; Singh et al., 2009b; 2009c). Unlike most other divalent cations, iron is an essential component of enzymes and proteins and is required for optimal neuronal growth and function (Beard and Connor, 2003). On the other hand, iron is also considered a toxin due to its ability to exist in two oxidation states (ferric Fe³⁺ and ferrous Fe²⁺) (Thompson et al., 2001; Kaplan, 2002). Due to this reason, iron transport into and out of the cells is tightly regulated. It is surprising that PrP^C influences the cellular iron pool within this tightly regulated mechanism of iron uptake, transport, and utilization (Singh et al., 2009b; 2009c). These observations have significant bearing on the pathogenesis of prion disorders since aggregation of PrP^C is likely to disturb cellular iron homeostasis, resulting in neurotoxicity.

PrP^C has been demonstrated to influence iron metabolism in cells expressing normal and mutant PrP forms and in PrP^{-/-} transgenic mouse models (Singh et al., 2009b, 2009c). Over-expression of PrP^C in cultured neuroblastoma cells increases the cellular labile iron pool (LIP) and iron saturation of ferritin, suggesting a role for PrP^C in iron uptake. It is interesting to note that cells expressing pathogenic and non-pathogenic mutations of PrP alter the cellular LIP and iron saturation of ferritin differentially. This difference in the cellular iron content is maintained even when cells are cultured in the presence of excess extracellular iron, indicating a dominant role in iron uptake (Singh et al., 2009b). Furthermore, stimulation of endocytosis by a PrP-specific antibody increases intracellular iron stores, suggesting that PrP^C mediates uptake of iron from the extra-

cellular milieu. Unlike ceruloplasmin, PrP^C does not mediate the efflux of excess iron from cells, confirming its role as an iron uptake protein (Jeong and David, 2003; Singh et al., 2009b). Presently it is unclear whether PrP^C mediates iron uptake using a novel pathway or by interacting with the conventional pathway of iron uptake and transport. It has been hypothesized that PrP^C may influence iron uptake by interacting with the transferrin/transferrin receptor pathway as described for HFE (Waheed et al., 2002), or function as a ferric reductase to facilitate the transport of ferric iron from endosomes to cytosolic ferritin (Singh et al., 2009b).

Similar observations suggesting a positive effect of PrP^C on systemic iron levels are noted when wild type mice are compared with PrP^{-/-} mouse models. Deletion of PrP^C in PrP^{-/-} mice induces iron deficiency in the latter by decreasing the efficiency of iron transport from the intestinal lumen to the blood stream, and uptake of iron from the blood by parenchymal cells and cells of the hematopoietic lineage. Re-expression of PrP^C corrects the iron deficiency in these mice, confirming the functional role for PrP in iron uptake (Singh et al., 2009c). Considering that PrP^{-/-} mice are only mildly iron deficient, it is likely that PrP^C modulates

the function of other iron uptake proteins or is involved in a pathway that compensates for its absence.

An important question that remains unanswered is the binding site and the affinity of PrP^C for iron. *In vitro* experiments using recombinant PrP^C and its fragments indicate that the octapeptide repeat region of PrP^C is not essential for iron binding, and the iron and copper binding regions of PrP^C do not overlap (unpublished observations). Limited data using different denaturing conditions suggest that the interaction of PrP^C with iron depends on the conformation of PrP^C rather than a specific amino acid sequence. These studies are limited to recombinant PrP^C since it is difficult to identify iron associated cellular PrP^C, suggesting that either the interaction of PrP^C with iron is transient, or the affinity of PrP^C for iron is not high enough for easy identification. A diagrammatic representation of possible mechanisms of iron uptake by PrP^C is depicted in Figure 2. It is likely that after binding iron, PrP^C moves from lipid rich domains in the plasma membrane to the vicinity of TfR, where it might influence the binding of Tf to the TfR, internalization of PrP^C/TfR/Tf complex, or endocytosis of Tf/PrP^C complex. It is also possible that PrP^C influences iron

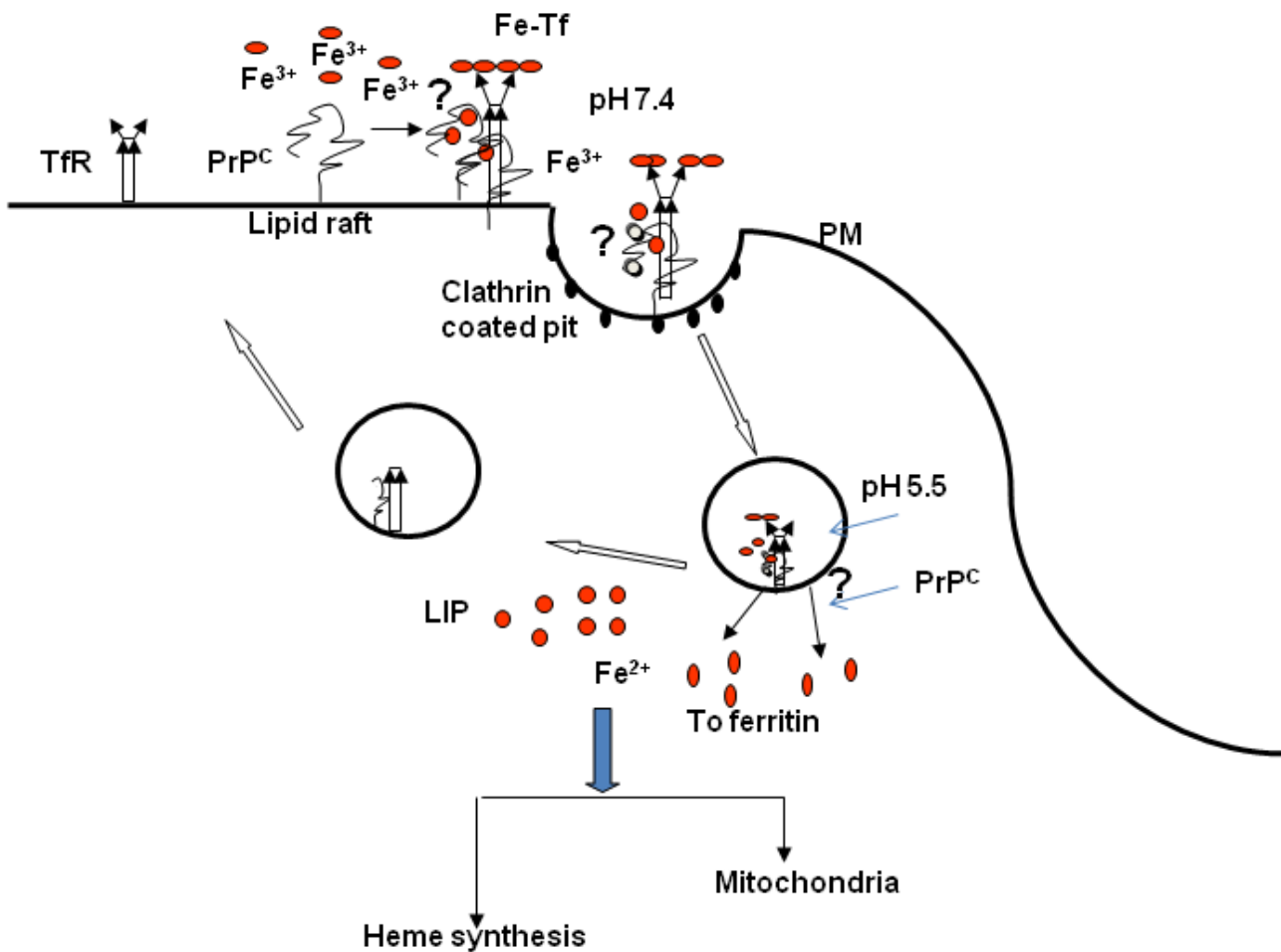


Figure 2. A representation of possible mechanisms by which PrP^C is likely to mediate cellular iron uptake. At the plasma membrane, PrP^C may bind iron directly for transport to the cytosol through endocytosis. It is also likely that PrP^C influences iron uptake indirectly by modulating the endocytosis association or endocytosis of the Tf/TfR complex. Alternately, PrP^C may facilitate the transport of iron from the endosomal compartment to the cytosol by functioning as a ferric-reductase. In the cytosol, iron is used for metabolic processes, or stored in ferritin in a relatively inert form.

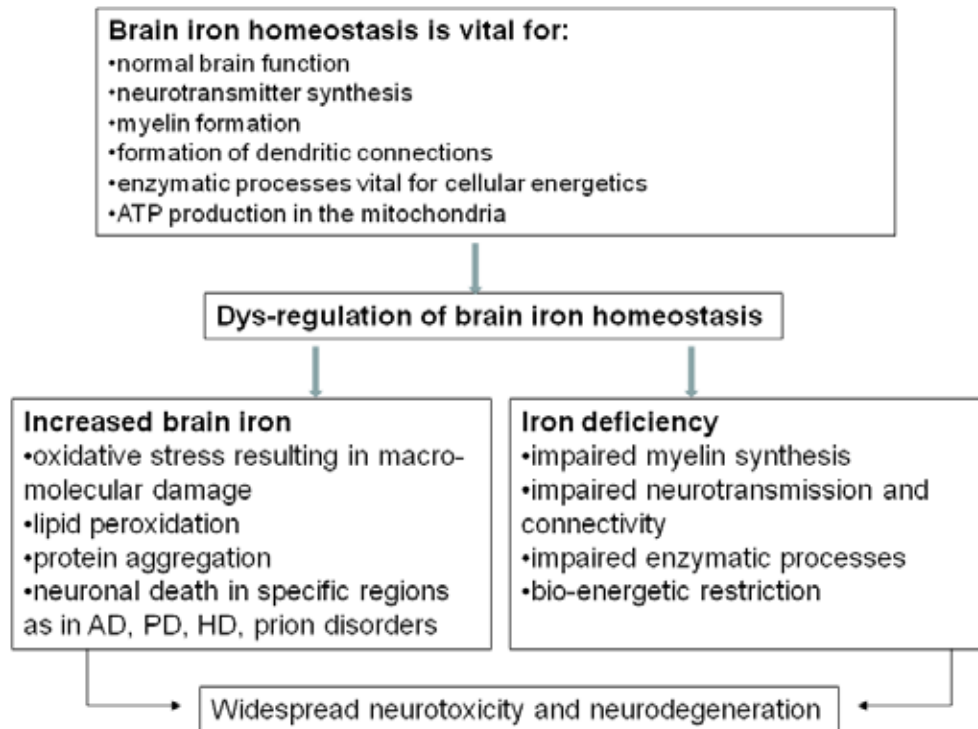


Figure 3. A model demonstrating the vital role of iron in brain function such as neurotransmitter synthesis, myelin formation, maintenance of dendritic connections, and other metabolic processes. Dys-regulation of brain iron homeostasis can induce neurotoxicity both by iron excess, and by iron deficiency. The former is a consequence of the redox-active nature of iron leading to oxidative stress, and the latter due to impairment of essential metabolic processes.

transport from the endosomes to the cytosol by functioning as a ferric-reductase as demonstrated for copper (Miura et al., 2005).

Pathological implications of PrP-metal interaction

Copper and iron have significant pathological implications due to their redox-active nature. For example, both copper and iron induce the conversion of PrP^C to the PrP^{Sc} form (Kim et al., 2005). Addition of copper increases the protease resistance and infectivity of denatured PrP^{Sc} *in vitro*, while copper chelation *in vivo* delays the onset of disease in prion infected mice (Pauly and Harris, 1998; Quaglio et al., 2001; Sigurdsson et al., 2003; Kuczius et al., 2004). Paradoxically, copper treatment inhibits PrP^{Sc} formation in cells infected with mouse prions and delays the onset of disease in scrapie infected hamsters, demonstrating a protective role, perhaps by augmenting the function of PrP^C as a Cu/Zn superoxide dismutase (SOD) (Vassallo and Herms, 2003; Hijazi et al., 2003; Kiachopoulos et al., 2004; Orem et al., 2006). These conflicting observations can perhaps be explained from *in vitro* studies where copper inhibits the amplification of PrP^{Sc} from purified brain-derived PrP^C and recombinant PrP^C by stabilizing its α -helical structure, while it enhances the β -sheet structure of preformed PrP fibrils, thereby increasing their PrP^{Sc} content (Liu et al., 2007). Copper could therefore delay or augment disease progression based on the time when it is introduced to the animal.

An important consideration in evaluating the pathogenic consequences of PrP^C-metal interaction is the cellular compartment in which metal bound PrP^C is exposed to

free radicals. It has been observed that exposure of PrP^C expressing cells to a source of redox-active metal such as ferrous chloride induces the generation of PrP^{Sc}-like aggregates on the plasma membrane that accumulate within lysosomes in association with ferritin (Basu et al., 2007). These aggregates are also redox-active and initiate the generation of additional PrP-ferritin aggregates, propagating the PrP^{Sc}-like conformation within cells (Basu et al., 2007). Likewise, exposure of PrP^C expressing cells to hemin, an iron containing compound, also results in the aggregation and degradation of PrP^C (Lee et al., 2007), implicating redox-iron in the generation and propagation of PrP^{Sc}. Similar aggregation of α -synuclein is noted in response to iron and copper (Paik et al., 2000; Ostrerova-Golts et al., 2000; Golts et al., 2002; Takahashi et al., 2007), suggesting that protein aggregation by redox-active metals is not specific to PrP^C. However, the generation of additional PrP^{Sc} aggregates by redox-active PrP^{Sc} appears to be unique to PrP, explaining in part the propagation of PrP^{Sc} once initiated. Surprisingly, chelation of iron from diseased brain homogenates decreases the total amount of PK-resistant PrP^{Sc}, suggesting that iron is also involved in the stability of PrP^{Sc} (Basu et al., 2007). This observation is of immense prophylactic value since iron chelation can be used as a means to decrease the infectivity of prion contaminated material.

Observations from mouse models of prion disease demonstrate an increase in the levels of iron regulatory proteins 1 and 2 and iron storage protein ferritin in the hippocampus and cerebral cortex of diseased brains,

indicating the presence of brain iron imbalance (Kim et al., 2007). A similar imbalance of cellular iron homeostasis is observed in scrapie infected mouse neuroblastoma cells (ScN2a) that also show increased susceptibility to iron induced oxidative stress (Fernaesus et al., 2005; Fernaeus and Land, 2005). Imbalance of iron homeostasis is also observed in prion disease affected human, mouse, and hamster brains that demonstrate a phenotype of iron deficiency in the presence of increased total and redox-active Fe²⁺ iron (Singh et al., 2009a). The iron deficiency in scrapie infected hamster brains is noted ~6 weeks after inoculation of the infectious material and correlates with PrP^{Sc} levels, perhaps due to sequestration of iron in PrP^{Sc}-ferritin complexes (Singh et al., 2009a). The redox-active nature of PrP^{Sc}-ferritin aggregates is likely to induce the aggregation of additional PrP^C, creating an ongoing state of iron imbalance and associated neurotoxicity in the diseased brain (Mishra et al., 2004; Basu et al., 2007). The associated iron deficiency caused by PrP^{Sc}-ferritin aggregates is equally harmful especially since iron is required for several vital metabolic processes (Figure 3).

In addition to copper and iron, alteration in the homeostasis of manganese has also been reported in prion disease affected human and animal brains. In scrapie infected mice, levels of manganese are elevated in the peripheral blood, while in diseased cattle and sheep increased levels are noted in both the blood and brain tissue (Wong et al., 2001a, 2001b; Thackray et al., 2002; Hesketh et al. 2007; 2008). Surprisingly, altered levels of manganese have also been reported in scrapie resistant sheep that do not develop clinical disease, making it unlikely that the change is due to the disease process *per se*. However, limited studies make it difficult to explain the generality or the underlying cause of this abnormality. Other diseases that show elevated levels of manganese include hemochromatosis and diseases of the blood-brain barrier, information that may help in understanding the basis of manganese imbalance in prion disorders. Other reports indicate a decrease in the levels of copper and zinc in the brains of scrapie infected mice, although blood levels of zinc increase during disease progression as noted for manganese (Wong et al. 2001a, 2001b; Thackray et al., 2002). In human brains affected with sCJD, PrP^C-associated copper is replaced by zinc, resulting in the loss of SOD-like activity of PrP^C, a possible cause of increased neurotoxicity due to oxidative stress. Further studies are necessary to understand this phenomenon fully.

Summary and Perspective

Despite overwhelming evidence implicating PrP^{Sc} in the pathogenesis of prion disorders, the mechanistic details underlying the neurotoxicity associated with these disorders remain unclear. The association of PrP^C with redox-active metals such as copper and iron provides new insight into the role of metal-induced oxidative stress in these disorders. Observations from cell and mouse models suggest that PrP^C is involved in copper and iron uptake. Although PrP^C is not a major iron or copper modulating protein, compromised levels of these metals due to aggregation of PrP^C to the PrP^{Sc} form are likely to affect cell health due to their redox-active nature. At the same time, PrP^{Sc} assumes a redox-active nature due to sequestration of iron in PrP^{Sc}-ferritin aggregates, providing a logical explanation

for imbalance of iron homeostasis in prion disease affected brains. This phenotype is likely to induce oxidative stress and neuronal damage, and could contribute significantly to prion disease associated neurotoxicity. Although the role of redox-active metals in prion disease pathogenesis is still at its formative stage, sufficient information exists to prompt future investigations on this subject that may lead to the development of anti-oxidants and metal chelators as useful therapeutic agents.

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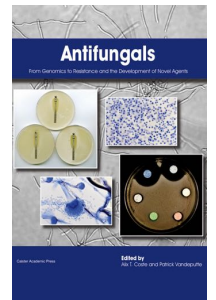
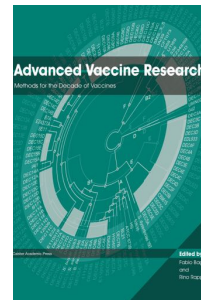
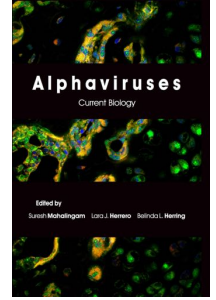
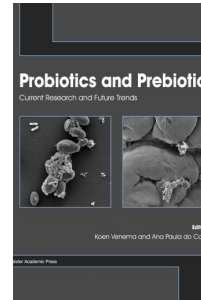
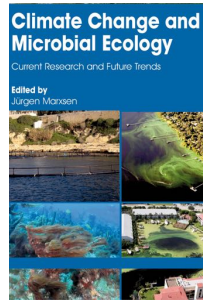
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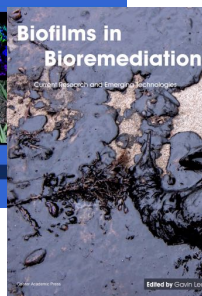
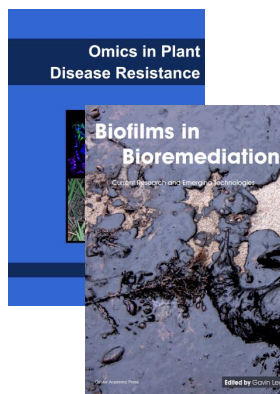
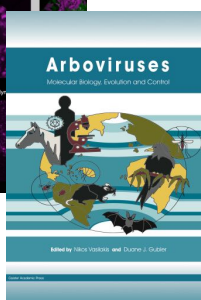
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