Chapter 5
Insoluble Cellular Prion Protein

Wen-Quan Zou

Abstract The detergent-soluble cellular prion protein (PrP^{C}) and its detergent-insoluble infectious isoform (PrP^{Sc}) are two major conformers of the prion protein. Soluble PrP^{C} has been the only isoform detected in the normal mammalian brain. In 2006, however, we identified an insoluble PrP^{C} conformer (termed $iPrP^{C}$) in uninfected human and animal brains. This article highlights the physiochemical properties of $iPrP^{C}$, a conformer distinct from PrP^{C} or PrP^{Sc}, and discusses its formation and probable pathophysiology.

Keywords Prion protein • Prion disease • Insoluble prion protein • Alzheimer disease • Variably protease-sensitive prionopathy • Dementia • Memory

5.1 Introduction

The cellular prion protein (PrP^{C}) is a universally expressed membrane protein present predominantly in the central nervous system (CNS). Deposition in the CNS of its pathologic isoform (PrP^{Sc}), derived from PrP^{C} via a conformational transition, is a molecular hallmark of prion diseases, a group of fatal transmissible neurodegenerative disorders in humans and animals. Although the physiologic functions of PrP^{C} are unclear, it has nevertheless been proposed that PrP^{C} has beneficial and deleterious effects on cognition (Collinge et al. 1994; Laurén et al. 2009; Linden et al. 2008; Westaway et al. 2011). Moreover, it has been well demonstrated that the coexistence of PrP^{C} and PrP^{Sc} is the prerequisite for the emergence of prion diseases (PrDs).

W.-Q. Zou, M.D., Ph.D. (✉)
Department of Pathology and Department of Neurology, National Prion Disease Pathology Surveillance Center, Case Western Reserve University,
2085 Adelbert Rd, Cleveland, OH 44106, USA
e-mail: wenquan.zou@case.edu

W.-Q. Zou and P. Gambetti (eds.), *Prions and Diseases: Volume 1, Physiology and Pathophysiology*, DOI 10.1007/978-1-4614-5305-5_5,

The two PrP conformers mainly studied so far are believed to be implicated in these diseases. PrP^{C} and PrP^{Sc} share the same primary sequence but have distinct secondary structures (Meyer et al. 1986; Caughey et al. 1991; Pan et al. 1993). PrP^{C} is monomeric, rich in α-helical structure, sensitive to proteinase K (PK) digestion, soluble in non-denaturing detergents, non-precipitable by anti-DNA antibodies or DNA-binding proteins, noninfectious, and present in both uninfected and scrapie-infected brains. PrP^{Sc}, on the other hand, is oligomeric or aggregate, rich in β-sheet structure, partially resistant to PK digestion, insoluble in detergents, precipitable by anti-DNA antibodies or DNA-binding proteins, infectious, and present only in infected brains. Soluble PrP^{C} has been the only conformer detected in the uninfected mammalian brain. In contrast, insoluble PrP^{Sc} exhibits chameleon-like conformations, which may underlie the distinct prion strains and phenotypes of PrDs identified in animals and humans (Bessen and Marsh 1992; Parchi et al. 1996; Caughey et al. 1998; Safar et al. 1998; Zou and Gambetti 2007; Collinge and Clarke 2007). Recent identification of insoluble cellular PrP ($iPrP^{C}$) in the uninfected human and animal brain raises two possibilities: that the PrP^{C} species in the brain may also exhibit chameleon-like conformations that are implicated in the beneficial or deleterious effects of PrP^{C}, and that these species may play a role in the pathogenesis of PrDs and other neurodegenerative disorders (Yuan et al. 2006; Zou 2010; Zou et al. 2011b).

5.2 Prion Protein Is Characterized by the Presence of an Intrinsically Chameleon-Like Conformation

Studies using recombinant PrP (rPrP) in vitro indicated that PrP possesses a highly variable conformation. In aqueous solutions, rPrP could be folded into pH-dependent α-helical conformations, a thermodynamically more stable β-sheet, and various stable or transient intermediates (Zhang et al. 1997). A stopped-flow kinetic study demonstrated that PrP folded by a three-state mechanism involving a monomeric intermediate (Apetri and Surewicz 2002). It was found that the population of this partially structured PrP intermediate increased in the presence of relatively low concentrations of urea and was more stable at acidic pH 4.8, compared to neutral pH 7.0. Moreover, this approach revealed that PrP mutations, linked with naturally occurring familial prion diseases, showed a pronounced stabilization of the folding intermediate (Apetri et al. 2004). This characteristic strongly suggested that these intermediates play a crucial role in PrP conversion and serve as direct precursors of the pathologic PrP^{Sc} isoform. The existence of a PrP folding intermediate was also indicated by hydrogen exchange experiments (Nicholson et al. 2002), and by studies using high pressure NMR and fluorescence spectroscopy (Kuwata et al. 2002; Martins et al. 2003). In addition to a β-oligomer and an amyloid fibril (Baskakov et al. 2001; Morillas et al. 2001; Lu and Chang 2002; Sokolowski et al. 2003; Baskakov et al. 2004), two additional polymeric transient intermediates were also identified during fibrillogenesis of rPrP in vitro (Baskakov et al. 2002).

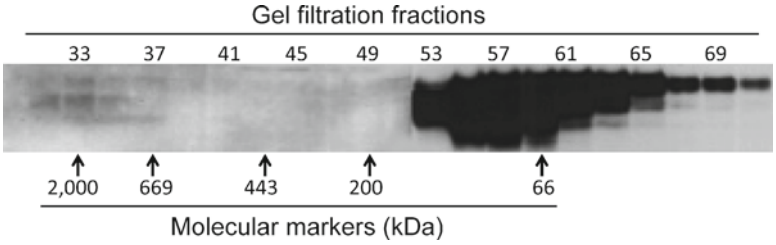

Fig. 5.1 Gel filtration of PrP from uninfected human brains. Gel filtration fractions of uninfected brain homogenates were subjected to SDS-PAGE and Western blotting with 3F4. Molecular mass (kDa) of various PrP species recovered in different fractions is indicated by an *arrow* and molecular mass markers used include dextran blue (2,000 kDa), thyroglobulin (669 kDa), apoferritin (443 kDa), β-amylase (200 kDa), and albumin (66 kDa). PrP was detected not only in fractions with molecular mass less than 66 kDa after fraction 59 but also in fractions with molecular mass greater than 66 kDa before fraction 59 including fraction 33 containing large PrP aggregates (2,000 kDa)

PrP^C in vivo is anchored to the cell membrane. Several experiments have indicated that the PrP conformation is affected by its local conditions. For example, the interaction of the anchorless recombinant PrP with lipids in a membrane-like environment resulted in a conformational transition (Wang et al. 2007; Re et al. 2008). Increasing the local concentration of membrane-anchored PrP^C seems to induce a conformational transition accompanied by oligomerization of PrP^C (Elfrink et al. 2008). Therefore, the tendency of PrP to form multiple nonnative β-sheet-rich isoforms in vitro, as demonstrated in biophysical studies on rPrP, may represent a unique intrinsic feature of this protein.

5.3 Insoluble Cellular Prion Protein Is Present in Normal Mammalian Brains

If the tendency of PrP to form multiple conformations in vitro represents a unique intrinsic feature of this protein, it is conceivable that other PrP conformers would be present in the normal brain in addition to the well-characterized PrP^C. To test for this possibility, we examined uninfected human and animal brains using a combination of biophysical and biochemical approaches to determine whether there are additional PrP conformers (Yuan et al. 2006). We identified a novel conformer which forms insoluble cellular PrP aggregates and protease-resistant PrP species in uninfected human brains (Yuan et al. 2006). Using gel filtration, we revealed that PrP in uninfected human brains is present not only in monomers but also in oligomers and large aggregates (Fig. 5.1). The new PrP conformer, which we termed insoluble cellular PrP ($iPrP^C$), accounts for approximately 5–25% of total PrP including full-length and N terminally truncated forms, and a portion of $iPrP^C$ is resistant to PK digestion even at 50 μg/ml (Yuan et al. 2006). Notably, the PK-resistant $iPrP^C$ has an immunoreactive behavior different from that of classic PrP^{Sc} detected in prion-infected brains; its affinity is much lower for 3F4 while higher for 1E4, compared to the

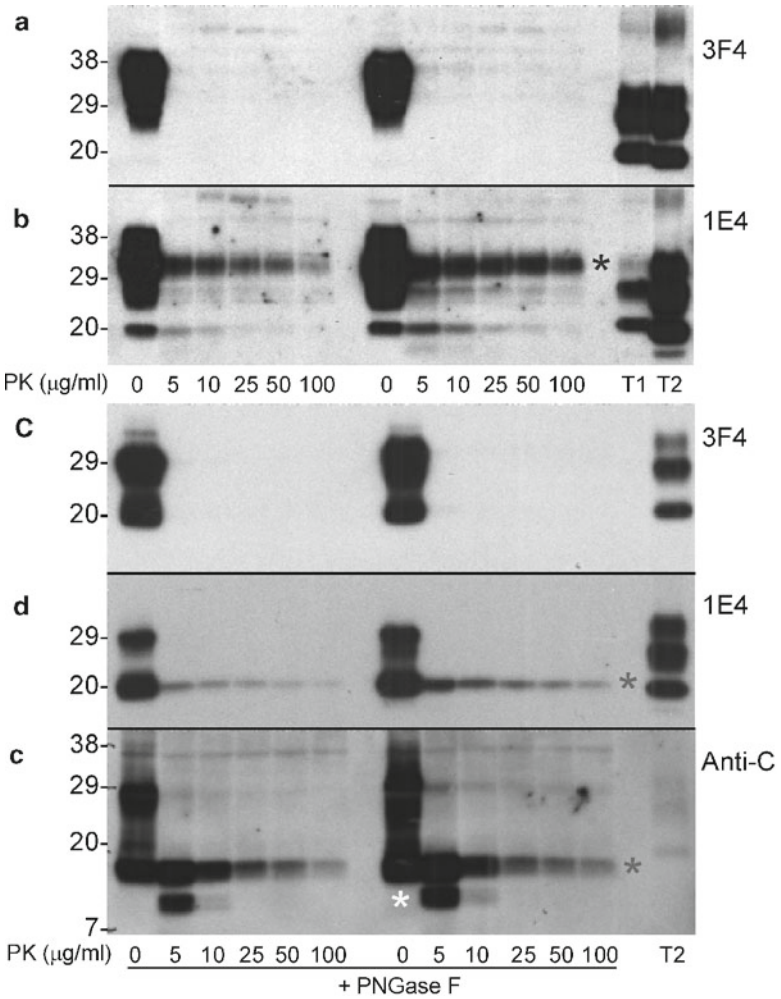

Fig. 5.2 PK-resistance of PrP in uninfected human brains. Brain homogenates from two uninfected human brains received at autopsy were treated with PK at 0, 5, 10, 25, 50, 100 μg/ml (*upper two panels A* and *B*) or PK plus PNGase F (*lower three panels C*, *D*, and *E*). The samples were subjected to SDS-PAGE and Western blotting with 3F4, 1E4, and Anti-C antibodies. No PK-resistant PrP was detectable with 3F4 antibody. In contrast, PK-resistant PrP was detected with 1E4 and Anti-C up to 100 μg/ml. With PK alone, three PrP bands migrating at 30-29 kDa, 27-26 kDa, and 21-20 kDa were detected, in which the *upper* band (~30-29 kDa, *dark asterisk*) was predominant while the intensity of the *middle* band was *lowest*, which is apparently different from those of PrP^{Sc} type 1 (T1) and type 2 (T2). After PNGase F treatment, only one band was detected with 1E4 and Anti-C migrating at ~20 kDa and ~18 kDa, respectively (PrP^{*20} and PrP^{*18}, *gray asterisk*). Interestingly, a band migrating at ~12–13 kDa was also detected with Anti-C at low PK concentration (5–10 μg/ml, *white asterisk*)

affinity of those antibodies for classic PrP^{Sc} (Yuan et al. 2006, 2008; Zou et al. 2010a, 2011a) (Fig. 5.2). The epitopes of the two antibodies 3F4 and 1E4 are adjacent and the C terminus of the 1E4 epitope between PrP97-105 is connected to the N terminus of the 3F4 epitope between PrP 106-112 (Yuan et al. 2008; Zou et al.

2010a). Antibody 3F4 is the most widely used antibody in the detection of human PrP^{C} and PrP^{Sc}, including PrP^{Sc} types 1 and 2 seen in sCJD and inherited CJD, and the internal PrP^{Sc} fragment PrP7-8 seen in GSS. In addition, the new conformer reveals high affinity for the gene 5 protein (g5p, a single-stranded DNA-binding protein) and sodium phosphotungstate (NaPTA), both of which also specifically bind to PrP^{Sc} but not to soluble PrP^{C} (Zou et al. 2004; Yuan et al. 2006; Safar et al. 1998; Wadsworth et al. 2001). To rule out the possibility that PrP aggregates detected in the uninfected human brain result from postmortem autolysis of autopsy tissues or from other neurodegenerative disorders, we also examined frozen uninfected human biopsy brain tissues or normal animal brain tissues from hamsters and cows. We discovered that the insoluble PrP^{C} was also detectable in these tissues, a finding which confirmed that $iPrP^{C}$ is a de novo generated PrP conformer (Yuan et al. 2006). Using gel filtration, we recently further demonstrated that not only soluble PrP^{C} monomers but also soluble PrP^{C} oligomers are present in the uninfected human brain (Xiao et al. 2012).

The presence of PrP conformers besides the typical PrP^{C} in uninfected brains was also implied in observations reported by other groups. Consistent with our findings, small amounts of PrP (less than 5% of total PrP^{C}) were also reported to be precipitated by NaPTA from uninfected human brains (Wadsworth et al. 2001). Moreover, by a differential SDS solubility assay, PrP^{C} species with either lower or higher solubility were differentiated in brain homogenates of noninfected humans, sheep, and cattle (Kuczius et al. 2009, 2011). Notably, a purified hamster brain PrP^{C} displayed an unexpectedly high β-sheet component under native conditions; this finding provided the first evidence that the full-length native PrP^{C} isolated from animal brains exhibited intrinsic conformational plasticity (Pergami et al. 1999). Moreover, mammalian brain PrP^{C} from six species was observed to be initially degraded to an intermediate fragment prior to complete proteolysis, suggesting an intrinsic partial PK-resistance (Buschmann et al. 1998). Interestingly, PrP aggregates have also been reported in pancreatic beta-cells of uninfected rats in response to hyperglycemia (Strom et al. 2007). In sum, the cumulative evidence shows that insoluble PrP^{C} is present in tissues and organs from uninfected animals and humans.

5.4 Spontaneous Formation of the Insoluble Cellular Prion Protein Has Been Modeled with Cultured Cells and May Result from PrP Cytosolic Accumulation

Lehmann and Harris (1996) modeled spontaneous formation of PrP^{Sc}-like insoluble PrP in cultured Chinese hamster ovary (CHO) cells expressing wild-type or mutant mouse PrP. Significant amounts of mutant PrP with point mutation at residue 199 (E199K) (~60%) or six octapeptide repeat insertion mutation between residues 51 and 90 (~90%) linked to inherited human prion disease were detergent; notably approximately 15% wild-type PrP^{C} was also detergent insoluble (Lehmann and Harris 1996). While approximately 5% mutant PrP was resistant to the digestion by PK at 3.3 μg/ml for 20 min, wild-type PrP was completely degraded. Because the

two mutant PrP molecules but not wild-type PrP were tightly associated with the plasma membrane, it was hypothesized that acquisition of PrP^{Sc}-like properties results from an alternation in membrane topology or affinity (Lehmann and Harris 1996). Using the same models, the same group further identified a three-step endocytic pathway by which mutant PrP forms a PrP^{Sc}-like conformer: initially hydrophobic, then detergent insoluble, and finally partially PK resistant (Daude et al. 1997). Using human neuroblastoma cells, Singh et al. also revealed that PrP with Q217R mutation linked to GSS formed a PrP^{Sc}-like form (Singh et al. 1997).

In addition to above PrP mutations, the two N-linked glycosylation sites located at residue 181, Asn–Ile–Thr residues 181–183, and at residue 197, Asn–Phe–Thr residues 197–199 (Puckett et al. 1991) are believed to play a crucial role in the stabilization of prion protein conformation. The naturally occurring mutations at residue 183, Thr to Ala (PrP^{T183A}), or at residue 198, Phe to Ser (PrP^{F198S}), falling in the two consensus sites, are linked to two distinct familial prion diseases (Nitrini et al. 1997; Tagliavini et al. 1991). Elimination of either site, or of both by mutagenesis of hamster PrP in CV1 cells, induced intracellular accumulation of mutant proteins (Rogers et al. 1990). Lehmann and Harris observed that mouse PrP mutated at T182 alone, or at both T182 and T198 in CHO cells, failed to reach the cell surface but the PrP with T198 mutation did. Moreover, all three mutant PrP's acquired PrP^{Sc}-like physicochemical properties reminiscent of PrP^{Sc}; PrP^{Wt} did so only when synthesized in the presence of N-linked glycosylation inhibitor tunicamycin (Lehmann and Harris 1997). Using M17 cells expressing human PrP^{N181G} or PrP^{T183A}, Capellari et al. observed that PrP^{N181G}, but not PrP^{T183A}, reached the cell surface even though both mutations eliminated glycosylation at the first site (Capellari et al. 2000). This observation indicates that the Thr to Ala mutation itself, rather than the elimination of the first glycosylation site, altered the physical properties of the mutant protein (Capellari et al. 2000). Although the F198S mutation falls within the second glycosylation site, Asn–Phe–Thr residues 197–199, PrP^{F198S} slightly increased the efficiency of glycosylation at the first glycosylation site (N181), and greatly increased the efficiency of glycosylation at the second site (N197) in cultured cells (Zaidi et al. 2005).

To further investigate the formation of $iPrP^{C}$ and the effect of mutations on the formation of $iPrP^{C}$, we examined $iPrP^{C}$ in cultured M17 cells expressing human wild-type (PrP^{Wt}) and mutant PrP (Yuan et al. 2008; Zou et al. 2011a). We confirmed that the de novo generated iPrP was detectable not only in cells expressing mutant PrP (PrP^{T183A} or PrP^{F198S}) linked to naturally occurring genetic Creutzfeldt–Jakob disease and Gerstmann–Sträussler–Scheinker disease, respectively, but also in cells expressing wild-type PrP. Compared to cells expressing wild-type PrP, significantly increased amounts of iPrP forming PrP aggregates and PK-resistant PrP were found in cells expressing mutant PrP. Most of PrP^{T183A} was composed of oligomers and large aggregates; virtually no monomeric form was present. In PrP^{F198S}, however, monomeric species were still dominant despite an increase in the amounts of aggregates. The enhanced tendency of PrP^{T183A} to form aggregates may result from the intracellular accumulation of the mutant protein. The F198S mutation did not

significantly diminish the ability of PrP^{F198S} to reach the cell surface (Zaidi et al. 2005), although the mutation may change the structure around the V14 epitope previously found to be localized between human PrP185-196 (Zou et al. 2011a; Moudjou et al. 2004; Rezaei et al. 2005). Therefore, the majority of the $iPrP^{C}$ associated with the T183A mutation may result from PrP intracellular accumulation, raising the possibility that $iPrP^{C}$ is derived predominantly from intracellular PrP species. Immunofluorescence microscopy of tagged PrP also indicated that PrP^{T183A} accumulates within the cell, whereas PrP^{F198S} was distributed both inside the cell and on the cell surface, consistent with previous observations (Zou et al. 2011a; Capellari et al. 2000; Zaidi et al. 2005).

In uninfected cultured cells, we also confirmed that the PK-resistant PrP exhibited higher affinity for 1E4 than for 3F4 that was initially observed in tissue samples (Zou et al. 2011a; Yuan et al. 2006, 2008). In Western blotting with cell lysates, virtually no PrP was detected by 1E4, and PrP was detectable only after PK treatment. However, PrP was stainable by 1E4 in fixed cultured cells treated with or without PK although the PrP signal was weaker in treated than in untreated cells (Zou et al. 2011a). It is worth noting that an antibody against human PrP95-110 (termed 8G8), that actually extends merely two more amino acids toward the N and C terminuses of the 1E4 epitope, respectively, stained PrP-expressing cells with a brilliant cytoplamic fluorescence (Krasemann et al. 1999). However, the number of positive cells was smaller than that of cells strained with antibodies against other PrP regions. Moreover, despite sharing a similar amino acid sequence within the corresponding region, only cattle, but not mouse and hamster PrP, was observed to react with 8G8 (Krasemann et al. 1999). In contrast to 3F4, 1E4 indeed seems to detect intracellular PrP in cultured cells (Zou et al. 2011a). Therefore, like 8G8, 1E4 may recognize a PrP species with a unique conformation in its epitope region.

In the absence of scrapie infection, aggregation of the cellular wild-type prion protein in cultured cells was first observed only when proteasome inhibitors were used (Yedidia et al. 2001). It was later reported that PrP^{Wt} accumulated in the cytoplasm of cultured cells under other conditions as well, such as in a reducing environment, or when expressing PrP without both N and C terminal signal peptides (Ma and Lindquist 2001, 2002; Drisaldi et al. 2003; Grenier et al. 2006). Cytosolic PrP forms aggregates that are insoluble in non-ionic detergents and partially resistant to PK (Ma and Lindquist 2001). Accumulated cytosolic PrP aggregates induced by ER stress and inhibition of proteasomal activity were recently observed to travel through the secretory pathway and reach the plasma membrane (Nunziante et al. 2011). Cytosolic PrP was observed not only in cultured cells but also in subpopulations of neurons in the hippocampus, neocortex, and thalamus in uninfected wild-type mice (Mironov et al. 2003). In addition, soluble PrP^{C} was observed to switch to insoluble PrP^{C} by treatment with acidic buffers in vitro (Zou and Cashman 2002).

The above observations with cell models may suggest that the formation of $iPrP^{C}$ or the aggregation of PrP^{C} is associated not only with mutations of the protein but also with altered cellular conditions that cause abnormal traffic and distribution of PrP in cells including reductive/oxidative stress.

5.5 Physiology and Pathophysiology of Insoluble PrP^C

5.5.1 Long-Term Memory Storage

The $iPrP^C$ with a conformation potentially different from soluble PrP^C may have a physiologic function. It has been hypothesized that prion-like conformational changes are indispensable for the maintenance of structural synaptic changes required for long-term memory (Si et al. 2003, 2010; Papassotiropoulos et al. 2005; Shorter and Lindquist 2005). Conceivably, the conversion of soluble PrP^C monomers into insoluble PrP oligomers or aggregates could be associated with long-term memory storage in the normal human brain (Zou et al. 2011c). The $iPrP^C$ molecule is able to bind to g5p, the single-stranded DNA-binding protein (Yuan et al. 2006, 2008). The possible binding of $iPrP^C$ to mRNA in vivo cannot be ruled out. Based on the observation that 24 h after a word-list learning task, carriers of either the polymorphism methionine/methionine (M/M) at residue 129 (129MM) or M/valine (V) (129 MV) genotype recalled 17% more information than did 129VV carriers (Papassotiropoulos et al. 2005). The PrP gene is believed to be genetically associated with human long-term memory performance. Therefore, the polymorphism at the residue 129 of PrP may participate in mediating human memory, in which the 129 M allele may have a beneficial effect on long-term memory. Interestingly, the impact of a putative PrP conformation rather than pathologic PrP^{Sc} on long-term memory in healthy humans was proposed to be related to physiologically occurring conformational changes (Tompa and Friedrich 1998; Papassotiropoulos et al. 2005).

5.5.2 Prion Disease

The in vivo pathway by which PrP^C forms PrP^{Sc} remains poorly understood. Two non-exclusive conversion models were proposed: *refolding* (Griffith 1967; Prusiner 1991) and *seeding* (Jarrett and Lansbury 1993). In the former, the exogenous PrP^{Sc} binds to the PrP^C species that has been partially unfolded and the PrP^{Sc}-bound PrP^C molecule undergoes a refolding process during which the nascent PrP^{Sc} is derived from this PrP^C species via a conformational transition. The latter proposes that a small amount of abnormal PrP^{Sc} or PrP^{Sc}-like form (PrP*) is present in the normal brain and is in reversible equilibrium with PrP^C. When several monomeric PrP* molecules form a highly ordered nucleus, PrP^C is converted to PrP^{Sc} polymers. Obviously, two key elements are required by the seeding model. One is the presence in the uninfected brain of a small amount of endogenous PrP^{Sc} or PrP* and the second is the formation of PrP^{Sc}-derived oligomers. The seeding model, with the two elements, has been recapitulated in vitro using PrP from various fungal and mammalian sources (Ross et al. 2005; Castilla et al. 2005; Tanaka et al. 2005).

Because $iPrP^C$ possesses PrP^{Sc}-like physicochemical properties, it is possible that $iPrP^C$ represents endogenous PrP^{Sc}, an intermediate form (PrP*) between PrP^C and

PrP^{Sc}, or silent prion (Hall and Edskes 2004; Weissmann 2004; Yuan et al. 2006; Zou et al. 2011a). Based on the observation that the brains of bigenic mice are capable of clearing prions, it has been proposed that the normal brain contains low levels of PrP^{Sc} (Safar et al. 2005). Under normal circumstances, despite the presence of a small amount of PrP^{Sc}, the brain may maintain equilibrium between the formation and clearance of PrP^{Sc}. The amount of PrP^{Sc} may be too small to induce a neurodegenerative disorder, which presumably, remain in a silent state. However, prion diseases may be triggered when the levels of the silent prions are significantly increased due to infection, PrP mutation, or unknown causes. Using protein misfolding cyclic amplification (PMCA), Barria and coworkers generated a new infectious prion without adding exogenous PrP^{Sc} seeds (Barria et al. 2009). This study may raise two possibilities (1) PMCA replicated an intermediate PrP^{Sc} which was present in the brain homogenate; or (2) the silent prion was activated by the sonication–incubation cycles during PMCA.

As mentioned above, $iPrP^{C}$ possesses a unique immunoreactive behavior showing poor affinity for 3F4 and higher affinity for 1E4, different from other types of human PrP^{Sc} identified so far (Yuan et al. 2006, 2008; Zou et al. 2011a). The two antibodies have adjacent epitopes on PrP (Yuan et al. 2008; Zou et al. 2010b). Thus, the possibility cannot be ruled out that iPrP is a distinct PrP species with an altered conformation and that $iPrP^{C}$ may be a conformer which, when it increases, induces an atypical form of prion disease. Some previous observations with experimental animals may favor this hypothesis. A novel neurologic syndrome was reported in Tg mice overexpressing wild-type PrP and these mice exhibited degeneration of skeletal muscle, peripheral nerves, and the central nervous system (Westaway et al. 1994). The increased amounts of wild-type PrP^{C} might form aggregates that induce degeneration in those mice. Chiesa et al. indeed observed that homozygous Tg mice overexpressing wild-type PrP at approximately tenfold but not hemizygous mice overexpressing wild-type PrP at approximately fivefold developed a spontaneous neurodegenerative disorder manifesting tremor and paresis (Chiesa et al. 2008). Nevertheless, abnormal PrP deposits and enlarged synaptic terminals with a dramatic proliferation of membranous structures were found in both types of mice. It was also observed that the overexpressed PrP assembled into insoluble aggregates with mild PK resistance but acquired no infectivity (Chiesa et al. 2008). Misfolding and neurotoxicity of wild-type PrP in transgenic flies were observed to be sequence dependent: Hamster PrP formed large amounts of PrP aggregates with spongiform degeneration, whereas rabbit PrP formed only small amounts of PrP aggregates without spongiform degeneration (Fernandez-Funez et al. 2010). Moreover, the same study also found that although small amounts of PrP aggregates were similarly detected in young flies (day 1) expressing hamster PrP, spongiform degeneration was not evident. Therefore, the small amounts of PrP aggregates were unable to induce spongiform degeneration. Interestingly, spongiform degeneration was observed to occur in older flies (day 30) only when the concentrations of PrP aggregates increased.

The same unique immunoreactivity behavior with 1E4 has also been observed in a new PrP^{Sc} species we recently identified from variably protease-sensitive prionopathy (VPSPr), a novel human prion disease (Gambetti et al. 2008; Zou et al. 2010b). VPSPr exhibits an abnormal PrP species with peculiar glycosylation and enzymatic

proteolysis (Zou et al. 2010b, 2011c). The 1E4-detected pathogenetic PK-resistant PrP^{Sc} with a ladder-like electrophoretic profile is the molecular hallmark of VPSPr. PrP^{Sc} from VPSPr exhibits not only the peculiar immunoreactivity behavior but also three PK-resistant core fragments, which is similar to $iPrP^{C}$ (Zou et al. 2010b, 2011c). These similarities may suggest that they share a common molecular metabolic pathway. Similar to sCJD, VPSPr affects patients regardless of their PrP genotypes defined by 129 MV polymorphism; however, the allelic prevalence is distinct in the two diseases (Zou et al. 2010b; Gambetti et al. 2011a). Notably, the amounts of PK-resistant PrP^{Sc} in VPSPr seem to be dependent on the polymorphism, a characteristic which has not been observed in sCJD. Preliminary data revealed no clinical phenotype during the normal life span of the transgenic mice expressing human PrP-129V at sixfold inoculated with brain homogenates from cases of VPSPr-129VV (Gambetti et al. 2011a), suggesting that the infectivity of PrP^{Sc} from VPSPr may be much lower than that of PrP^{Sc} from sCJD. Only 30% of the mice exhibited peculiar PrP plaques with a distinctive topography and minimal or no spongiform degeneration, compared to the typical neuropathological changes found in 100% mice inoculated with the classical sCJD control. Some of these mice inoculated with VPSPr also had the PK-resistant PrP^{Sc} whose profile exhibited the ladder-like electrophoresis detected by 1E4. Therefore, it is possible that VPSPr characterized by the deposition in the brain of $iPrP^{C}$-like PrP^{Sc} represents a prion disease, distinct from classical prion diseases and bearing more resemblance to other neurodegenerative diseases such as Alzheimer disease and tauopathies (Gambetti et al. 2011b). Because of the similarities between $iPrP^{C}$ and PrP^{Sc} from VPSPr, the possibility that VPSPr results from an increase in the amount of $iPrP^{C}$ cannot be excluded.

5.5.3 Alzheimer Disease

The insoluble PrP^{C} has been recently demonstrated to be the main species that interacts with Aβ in AD (Zou et al. 2011b). Moreover, using a peptide membrane array involving 13-mer human PrP peptides, and two Aβ peptides (Aβ42 and Aβ40), we identified 17 Aβ binding regions distributed on N terminal, internal, and C terminal PrP domains. Two distinct types of Aβ-binding sites were differentiated: one specifically binds to Aβ42 and the other binds to both Aβ42 and Aβ40. Notably, Aβ42-specific binding sites are localized predominantly in the octapeptide repeat region, whereas sites that bind both Aβ40 and Aβ42 are mainly in the extreme N terminal and C terminal domains of PrP (Zou et al. 2011b). Our study is consistent with other observations. PrP deposits often histologically accompany Aβ-positive plaques in AD brains (Esiri et al. 2000; Ferrer et al. 2001; Kovacs et al. 2002). In addition, Freir et al. also observed that interaction between PrP and toxic Aβ assemblies can be therapeutically targeted at multiple sites (Freir et al. 2011), indicating that their binding sites are not limited only to the internal domain. Remarkably, Kudo et al. showed more recently that not only anti-PrP antibodies but also PrP^{C} peptides identified in our previous study (Zou et al. 2011b) rescued Aβ oligomer-induced neurotoxiciy (Kudo et al. 2012).

Although the exact biological relevance of the interaction between iPrP^{C} and Aβ remains unclear, aggregation of one protein was observed to facilitate aggregation of the other (Morales et al. 2010). Moreover, synergistic interactions between other amyloidogenic proteins associated with neurodegeneration have also been reported to promote each other's fibrillization, amyloid deposition, and formation of filamentous inclusions in transgenic mice (Schwarze-Eicker et al. 2005). An increase in the efficiency of Aβ42 aggregation in vitro was dependent on PrP^{Sc} dosage (Morales et al. 2010). Moreover, insoluble PrP^{Sc} aggregates also seemed to facilitate Aβ42 aggregation in vivo; AD mice developed a strikingly higher load of cerebral amyloid plaques that appeared much faster in prion infected than in uninfected mice (Morales et al. 2010). Our finding that Aβ42 binds to iPrP may suggest that iPrP (the PrP^{Sc}-like forms in uninfected human brains) facilitates the fibrillization of Aβ42 in AD. Similarly, the possibility should be considered that a significant increase in the total number of Aβ plaques observed in bigenic mice overexpressing PrP (Schwarze-Eicker et al. 2005) might result from an increase in the formation of iPrP. Since the less toxic insoluble Aβ42 aggregates constitute the end products of highly toxic soluble Aβ42 oligomers, it is conceivable that formation of the large aggregates facilitated by iPrP^{C} may reduce the amount of Aβ42 oligomers. The decrease in the levels of toxic Aβ42 oligomers would then attenuate the cognitive impairment induced by Aβ42 oligomers in AD. If this is the case, iPrP^{C} may play a protective role in AD. Given that iPrP^{C} interacts with insoluble Aβ42, whereas soluble PrP^{C} binds soluble Aβ42 in vivo (Zou et al. 2011b), it is possible that distinct PrP conformers binding to different Aβ42 species thereby function either as receptors for soluble Aβ42 oligomers or as modulators of insoluble Aβ42 deposition. It would be interesting to test this hypothesis by intracerebrally injecting anti-PrP antibodies against either soluble or insoluble PrP species in AD animal models. This experiment would establish that the multiple conformers of PrP^{C} are coupled with its beneficial and deleterious effects.

5.6 Conclusions

Like PrP^{Sc} whose chameleon-like conformations are believed to link to transmissible and non-transmissible prion diseases with highly heterogeneous phenotypes (Zou 2007; Zou and Gambetti 2007), the chameleon-like conformations of PrP^{C} may be linked to its beneficial and deleterious effects (Zou et al. 2011c). Demonstration of the presence of insoluble PrP in normal mammalian brains and its potential association with AD and atypical prion disease may open a new avenue in the exploration of prion formation and in the physiology and pathophysiology of prion protein.

Acknowledgements The author wants to thank Dr. Xiangzhu Xiao and Jue Yuan for the technical support in Western blotting analysis for the two figures. This work was supported by the National Institutes of Health R01NS062787, the University Center on Aging and Health with the support of the McGregor Foundation and the President's Discretionary Fund (Case Western Reserve University), the Alliance BioSecure, and the CJD Foundation.

References

Apetri AC, Surewicz WK (2002) Kinetic intermediate in the folding of human prion protein. J Biol Chem 277:44589–44592

Apetri AC, Surewicz K, Surewicz WK (2004) The effect of disease-associated mutations on the folding pathway of human prion protein. J Biol Chem 279:18008–18014

Barria MA, Mukherjee A, Gonzalez-Romero D, Morales R, Soto C (2009) De novo generation of infectious prions in vitro produces a new disease phenotype. PLoS Pathog 5:e1000421

Baskakov IV, Legname G, Prusiner SB, Cohen FE (2001) Folding of prion protein to its native alpha-helical conformation is under kinetic control. J Biol Chem 276:19687–19690

Baskakov IV, Legname G, Baldwin MA, Prusiner SB, Cohen FE (2002) Pathway complexity of prion protein assembly into amyloid. J Biol Chem 277:21140–21148

Baskakov IV, Legname G, Gryczynski Z, Prusiner SB (2004) The peculiar nature of unfolding of the human prion protein. Protein Sci 13:586–595

Bessen RA, Marsh RF (1992) Identification of two biologically distinct strains of transmissible mink encephalopathy in hamsters. J Gen Virol 73:329–334

Buschmann A, Kuczius T, Bodemer W, Groschup MH (1998) Cellular prion proteins of mammalian species display an intrinsic partial proteinase K resistance. Biochem Biophys Res Commun 253:693–702

Capellari S, Zaidi SI, Long AC, Kwon EE, Petersen RB (2000) The Thr183Ala mutation, not the loss of the first glycosylation site, alters the physical properties of the prion protein. J Alzheimers Dis 2:27–35

Castilla J, Saá P, Hetz C, Soto C (2005) In vitro generation of infectious scrapie prions. Cell 121:195–206

Caughey BW, Dong A, Bhat KS, Ernst D, Hayes SF, Caughey WS (1991) Secondary structure analysis of the scrapie-associated protein PrP 27-30 in water by infrared spectroscopy. Biochemistry 30:7672–7680

Caughey B, Raymond GJ, Bessen RA (1998) Strain-dependent differences in beta-sheet conformations of abnormal prion protein. J Biol Chem 273:32230–32235

Chiesa R, Piccardo P, Biasini E, Ghetti B, Harris DA (2008) Aggregated, wild-type prion protein causes neurological dysfunction and synaptic abnormalities. J Neurosci 28:13258–13267

Collinge J, Clarke AR (2007) A general model of prion strains and their pathogenicity. Science 318:930–936

Collinge J, Whittington MA, Sidle KC, Smith CJ, Palmer MS, Clarke AR et al (1994) Prion protein is necessary for normal synaptic function. Nature 370:295–297

Daude N, Lehmann S, Harris DA (1997) Identification of intermediate steps in the conversion of a mutant prion protein to a scrapie-like form in cultured cells. J Biol Chem 272(17): 11604–11612, Erratum in J Biol Chem 2000;275:1520

Drisaldi B, Stewart RS, Adles C, Stewart LR, Quaglio E, Biasini E, Fioriti L, Chiesa R, Harris DA (2003) Mutant PrP is delayed in its exit from the endoplasmic reticulum, but neither wild-type nor mutant PrP undergoes retrotranslocation prior to proteasomal degradation. J Biol Chem 278:21732–21743

Elfrink K, Ollesch J, Stöhr J, Willbold D, Riesner D, Gerwert K (2008) Structural changes of membrane-anchored native PrP(C). Proc Natl Acad Sci USA 105:10815–10819

Esiri MM, Carter J, Ironside JW (2000) Prion protein immunoreactivity in brain samples from an unselected autopsy population: findings in 200 consecutive cases. Neuropathol Appl Neurobiol 26:273–284

Fernandez-Funez P, Zhang Y, Casas-Tinto S, Xiao X, Zou WQ, Rincon-Limas DE (2010) Sequence-dependent prion protein misfolding and neurotoxicity. J Biol Chem 285:36897–36908

Ferrer I, Blanco R, Carmona M, Puig B, Ribera R, Rey MJ et al (2001) Prion protein expression in senile plaques in Alzheimer's disease. Acta Neuropathol 101:49–56

Freir DB, Nicoll AJ, Klyubin I, Panico S, Mc Donald JM, Risse E, Asante EA, Farrow MA, Sessions RB, Saibil HR, Clarke AR, Rowan MJ, Walsh DM, Collinge J (2011) Interaction

between prion protein and toxic amyloid β assemblies can be therapeutically targeted at multiple sites. Nat Commun 2:336–340
Gambetti P, Dong Z, Yuan J, Xiao X, Zheng M, Alshekhlee A et al (2008) A novel human disease with abnormal prion protein sensitive to protease. Ann Neurol 63:697–708
Gambetti P, Puoti G, Zou WQ (2011a) Variably protease-sensitive prionopathy: a novel disease of the prion protein. J Mol Neurosci 45:422–424
Gambetti P, Zou WQ, Torres JM, Soto C, Notari S, Espinosa JC et al (2011b) Variably protease-sensitive prionopathy: transmissibility and PMCA studies. Prion 5:14
Grenier C, Bissonnette C, Volkov L, Roucou X (2006) Molecular morphology and toxicity of cytoplasmic prion protein aggregates in neuronal and non-neuronal cells. J Neurochem 97:1456–1466
Griffith JS (1967) Self-replication and scrapie. Nature 215:1043–1044
Hall D, Edskes H (2004) Silent prions lying in wait: a two-hit model of prion/amyloid formation and infection. J Mol Biol 336:775–786
Jarrett JT, Lansbury PT Jr (1993) Seeding "one-dimensional crystallization" of amyloid: a pathogenic mechanism in Alzheimer's disease and scrapie? Cell 73:1055–1058
Kovacs GG, Zerbi P, Voigtländer T, Strohschneider M, Trabattoni G, Hainfellner JA et al (2002) The prion protein in human neurodegenerative disorders. Neurosci Lett 329:269–272
Krasemann S, Jürgens T, Bodemer W (1999) Generation of monoclonal antibodies against prion proteins with an unconventional nucleic acid-based immunization strategy. J Biotechnol 73:119–129
Kuczius T, Karch H, Groschup MH (2009) Differential solubility of prions is associated in manifold phenotypes. Mol Cell Neurosci 42:226–233
Kuczius T, Wohlers J, Karch H, Groschup MH (2011) Subtyping of human cellular prion proteins and their differential solubility. Exp Neurol 227:188–194
Kudo W, Lee HP, Zou WQ, Wang X, Perry G, Zhu X, Smith MA, Petersen RB, Lee HG (2012) Cellular prion protein is essential oligomeric amyloid-β induced neuronal cell death. Hum Mol Genet 21(5):1138–1144
Kuwata K, Li H, Yamada H, Legname G, Prusiner SB, Akasaka K et al (2002) Locally disordered conformer of the hamster prion protein: a crucial intermediate to PrPSc? Biochemistry 41:12277–12283
Laurén J, Gimbel DA, Nygaard HB, Gilbert JW, Strittmatter SM (2009) Cellular prion protein mediates impairment of synaptic plasticity by amyloid-beta oligomers. Nature 457:1128–1132
Lehmann S, Harris DA (1996) Two mutant prion proteins expressed in cultured cells acquire biochemical properties reminiscent of the scrapie isoform. Proc Natl Acad Sci USA 93:5610–5614
Lehmann S, Harris DA (1997) Blockade of glycosylation promotes acquisition of scrapie-like properties by the prion protein in cultured cells. J Biol Chem 272:21479–21487
Linden R, Martins VR, Prado MA, Cammarota M, Izquierdo I, Brentani RR (2008) Physiology of the prion protein. Physiol Rev 88:673–728
Lu BY, Chang JY (2002) Isolation and characterization of a polymerized prion protein. Biochem J 364:81–87
Ma J, Lindquist S (2001) Wild-type PrP and a mutant associated with prion disease are subject to retrograde transport and proteasome degradation. Proc Natl Acad Sci USA 98:14955–14960
Ma J, Lindquist S (2002) Conversion of PrP to a self-perpetuating PrPSc-like conformation in the cytosol. Science 298:1785–1788
Martins SM, Chapeaurouge A, Ferreira ST (2003) Folding intermediates of the prion protein stabilized by hydrostatic pressure and low temperature. J Biol Chem 278:50449–50455
Meyer RK, McKinley MP, Bowman KA, Braunfeld MB, Barry RA, Prusiner SB (1986) Separation and properties of cellular and scrapie prion proteins. Proc Natl Acad Sci USA 83:2310–2314
Mironov A Jr, Latawiec D, Wille H, Bouzamondo-Bernstein E, Legname G, Williamson RA, Burton D, DeArmond SJ, Prusiner SB, Peters PJ (2003) Cytosolic prion protein in neurons. J Neurosci 23:7183–7193

Morales R, Estrada LD, Diaz-Espinoza R, Morales-Scheihing D, Jara MC, Castilla J et al (2010) Molecular cross talk between misfolded proteins in animal models of Alzheimer's and Prion diseases. J Neurosci 30:4528–4535

Morillas M, Vanik DL, Surewicz WK (2001) On the mechanism of alpha-helix to beta-sheet transition in the recombinant prion protein. Biochemistry 40:6982–6987

Moudjou M, Treguer E, Rezaei H, Sabuncu E, Neuendorf E, Groschup MH, Grosclaude J, Laude H (2004) Glycan-controlled epitopes of prion protein include a major determinant of susceptibility to sheep scrapie. J Virol 78:11449

Nicholson EM, Mo H, Prusiner SB, Cohen FE, Marqusee S (2002) Differences between the prion protein and its homolog Doppel: a partially structured state with implications for scrapie formation. J Mol Biol 316:807–815

Nitrini R, Rosemberg S, Passos-Bueno MR, da Silva LS, Iughetti P, Papadopoulos M, Carrilho PM, Caramelli P, Albrecht S, Zatz M, LeBlanc A (1997) Familial spongiform encephalopathy associated with a novel prion protein gene mutation. Ann Neurol 42:138–146

Nunziante M, Ackermann K, Dietrich K, Wolf H, Gädtke L, Gilch S, Vorberg I, Groschup M, Schätzl HM (2011) Proteasomal dysfunction and endoplasmic reticulum stress enhance trafficking of prion protein aggregates through the secretory pathway and increase accumulation of pathologic prion protein. J Biol Chem 286:33942–33953

Pan KM, Baldwin M, Nguyen J, Gasset M, Serban A, Groth D et al (1993) Conversion of alpha-helices into beta-sheets features in the formation of the scrapie prion proteins. Proc Natl Acad Sci USA 90:10962–10966

Papassotiropoulos A, Wollmer MA, Aguzzi A, Hock C, Nitsch RM, de Quervain DJ (2005) The prion gene is associated with human long-term memory. Hum Mol Genet 14:2241–2246

Parchi P, Castellani R, Capellari S, Ghetti B, Young K, Chen SG et al (1996) Molecular basis of phenotypic variability in sporadic Creutzfeldt–Jakob disease. Ann Neurol 39:767–778

Pergami P, Poloni TE, Corato M, Camisa B, Ceroni M (1999) Prions and prion diseases. Funct Neurol 14:241–252

Prusiner SB (1991) Molecular biology of prion diseases. Science 252:1515–1522

Puckett C, Concannon P, Casey C, Hood L (1991) Genomic structure of the human prion protein gene. Am J Hum Genet 49:320–329

Re F, Sesana S, Barbiroli A, Bonomi F, Cazzaniga E, Lonati E, Bulbarelli A, Masserini M (2008) Prion protein structure is affected by pH-dependent interaction with membranes: a study in a model system. FEBS Lett 582:215–220

Rezaei H, Eghiaian F, Perez J, Doublet B, Choiset Y, Haertle T, Grosclaude J (2005) Sequential generation of two structurally distinct ovine prion protein soluble oligomers displaying different biochemical reactivities. J Mol Biol 347:665–679

Rogers M, Taraboulos A, Scott M, Groth D, Prusiner SB (1990) Intracellular accumulation of the cellular prion protein after mutagenesis of its Asn-linked glycosylation sites. Glycobiology 1:101–109

Ross ED, Minton A, Wickner RB (2005) Prion domains: sequences, structures and interactions. Nat Cell Biol 7:1039–1044

Safar J, Wille H, Itri V, Groth D, Serban H, Torchia M et al (1998) Eight prion strains have PrP(Sc) molecules with different conformations. Nat Med 4:1157–1165

Safar JG, DeArmond SJ, Kociuba K, Deering C, Didorenko S, Bouzamondo-Bernstein E et al (2005) Prion clearance in bigenic mice. J Gen Virol 86:2913–2923

Schwarze-Eicker K, Keyvani K, Görtz N, Westaway D, Sachser N, Paulus W (2005) Prion protein (PrPc) promotes beta-amyloid plaque formation. Neurobiol Aging 26:1177–1182

Shorter J, Lindquist S (2005) Prions as adaptive conduits of memory and inheritance. Nat Rev Genet 6:435–450

Si K, Lindquist S, Kandel ER (2003) A neuronal isoform of the aplysia CPEB has prion-like properties. Cell 115:879–891

Si K, Choi YB, White-Grindley E, Majumdar A, Kandel ER (2010) Aplysia CPEB can form prion-like multimers in sensory neurons that contribute to long-term facilitation. Cell 140:421–435

Singh N, Zanusso G, Chen SG, Fujioka H, Richardson S, Gambetti P, Petersen RB (1997) Prion protein aggregation reverted by low temperature in transfected cells carrying a prion protein gene mutation. J Biol Chem 272:28461–28470

Sokolowski F, Modler AJ, Masuch R, Zirwer D, Baier M, Lutsch G et al (2003) Formation of critical oligomers is a key event during conformational transition of recombinant syrian hamster prion protein. J Biol Chem 278:40481–40492

Strom A, Wang GS, Reimer R, Finegood DT, Scott FW (2007) Pronounced cytosolic aggregation of cellular prion protein in pancreatic beta-cells in response to hyperglycemia. Lab Invest 87:139–149

Tagliavini F, Prelli F, Ghiso J, Bugiani O, Serban D, Prusiner SB, Farlow MR, Ghetti B, Frangione B (1991) Amyloid protein of Gerstmann-Sträussler-Scheinker disease (Indiana kindred) is an 11 kd fragment of prion protein with an N-terminal glycine at codon 58. EMBO J 10:513–519

Tanaka M, Chien P, Yonekura K, Weissman JS (2005) Mechanism of cross-species prion transmission: an infectious conformation compatible with two highly divergent yeast prion proteins. Cell 121:49–62

Tompa P, Friedrich P (1998) Prion proteins as memory molecules: an hypothesis. Neuroscience 86:1037–1043

Wadsworth JD, Joiner S, Hill AF, Campbell TA, Desbruslais M, Luthert PJ, Collinge J (2001) Tissue distribution of protease resistant prion protein in variant Creutzfeldt–Jakob disease using a highly sensitive immunoblotting assay. Lancet 358:171–180

Wang F, Yang F, Hu Y, Wang X, Wang X, Jin C, Ma J (2007) Lipid interaction converts prion protein to a PrPSc-like proteinase K-resistant conformation under physiological conditions. Biochemistry 46:7045–7053

Weissmann C (2004) The state of the prion. Nat Rev Microbiol 2:861–871

Westaway D, DeArmond SJ, Cayetano-Canlas J, Groth D, Foster D, Yang SL et al (1994) Degeneration of skeletal muscle, peripheral nerves, and the central nervous system in transgenic mice overexpressing wild-type prion proteins. Cell 76:117–129

Westaway D, Alier K, Vergote D, MacTavish D, Mercer R, Fu W et al (2011) Prion proteins and the Alzheimer disease Aβ amyloid cascade. Prion 5:1–2

Xiao X, Yuan J, Zou WQ (2012) Isolation of soluble and insoluble PrP oligomers in the normal human brain. J Vis Exp 68:e3788, DOI: 10.3791/3788

Yedidia Y, Horonchik L, Tzaban S, Yanai A, Taraboulos A (2001) Proteasomes and ubiquitin are involved in the turnover of the wild-type prion protein. EMBO J 20:5383–5391

Yuan J, Xiao X, McGeehan J, Dong Z, Cali I, Fujioka H et al (2006) Insoluble aggregates and protease-resistant conformers of prion protein in uninfected human brains. J Biol Chem 281:34848–34858

Yuan J, Dong Z, Guo JP, McGeehan J, Xiao X, Wang J et al (2008) Accessibility of a critical prion protein region involved in strain recognition and its implications for the early detection of prions. Cell Mol Life Sci 65:631–643

Zaidi SI, Richardson SL, Capellari S, Song L, Smith MA, Ghetti B, Sy MS, Gambetti P, Petersen RB (2005) Characterization of the F198S prion protein mutation: enhanced glycosylation and defective refolding. J Alzheimers Dis 7:159–171, discussion 173–180

Zhang H, Stockel J, Mehlhorn I, Groth D, Baldwin MA, Prusiner SB et al (1997) Physical studies of conformational plasticity in a recombinant prion protein. Biochemistry 36:3543–3553

Zou WQ (2007) Transmissible spongiform encephalopathy and beyond (E-letter). Science. http://www.sciencemag.org/content/308/5727/1420.long/reply#sci_el_10316. Accessed 20 Sep 2007

Zou WQ (2010) Chameleon-like prion protein and human cognition. Curr Top Biochem Res 12:1–8

Zou WQ, Cashman NR (2002) Acidic pH and detergents enhance in vitro conversion of human brain PrPC to a PrPSc-like form. J Biol Chem 277:43942–43947

Zou WQ, Gambetti P (2007) Prion: the chameleon protein. Cell Mol Life Sci 64:3266–3270

Zou WQ, Zheng J, Gray DM, Gambetti P, Chen SG (2004) Antibody to DNA detects scrapie but not normal prion protein. Proc Natl Acad Sci USA 101:1380–1385
Zou WQ, Langeveld J, Xiao X, Chen S, McGeer PL, Yuan J et al (2010a) PrP conformational transitions alter species preference of a PrP-specific antibody. J Biol Chem 285:13874–13884
Zou WQ, Puoti G, Xiao X, Yuan J, Qing L, Cali I et al (2010b) Variably protease-sensitive prionopathy: a new sporadic disease of the prion protein. Ann Neurol 68:162–172
Zou RS, Fujioka H, Guo JP, Xiao X, Shimoji M, Kong C, Chen C, Tasnadi M, Voma C, Yuan J, Moudjou M, Laude H, Petersen RB, Zou WQ (2011a) Characterization of spontaneously generated prion-like conformers in cultured cells. Aging 3:968–984
Zou WQ, Xiao X, Yuan J, Puoti G, Fujioka H, Wang X et al (2011b) Amyloid-{beta}42 interacts mainly with insoluble prion protein in the Alzheimer brain. J Biol Chem 286:15095–15105
Zou WQ, Zhou X, Yuan J, Xiao X (2011c) Insoluble cellular prion protein and its association with prion and Alzheimer diseases. Prion 5:172–178