Published in final edited form as:

Curr Opin Struct Biol. 2009 February ; 19(1): 14–22. doi:10.1016/j.sbi.2008.12.007.

Prion Protein Misfolding and Disease

Roger A. Moore, Lara M. Taubner, and Suzette A. Priola

Rocky Mountain Laboratories, Laboratory of Persistent Viral Diseases, NIAID, NIH, 903 S. 4th St., Hamilton, Montana 59840, Fax: (406)-363-9286

Abstract

Transmissible spongiform encephalopathies (TSEs or prion diseases) are a rare group of invariably fatal neurodegenerative disorders that affect humans and other mammals. TSEs are protein misfolding diseases that involve the accumulation of an abnormally aggregated form of the normal host prion protein. They are unique among protein misfolding disorders in that they are transmissible and have different strains of infectious agent that are associated with unique phenotypes in vivo. A wealth of biological and biophysical evidence now suggests that the molecular basis for prion diseases may be encoded by protein conformation. The purpose of this review is to provide an overview of the existing structural information for prion protein within the context of what is known about the biology of prion disease.

Introduction

The transmissible spongiform encephalopathies (TSEs) are a group of rare neurodegenerative diseases that include scrapie in sheep, chronic wasting disease (CWD) in deer and elk, and bovine spongiform encephalopathy (BSE) in cattle. There are three categories of TSE disease in humans: 1) sporadic, the most common form of which is Creutzfeldt Jakob disease (CJD), 2) heritable, which is associated with mutations within the prion protein gene and, 3) acquired, which is a result of ingestion or inoculation of TSE contaminated materials. Both kuru, a TSE once endemic in the Fore tribe of New Guinea and variant CJD, which has been linked to BSE and can be transmitted via blood, are examples of acquired forms of TSE. There are neither preclinical diagnostic tests nor effective therapeutics for TSEs. As a result, following the onset of clinical signs, these diseases are inevitably fatal.

The TSEs fall within the spectrum of diseases of protein misfolding because the primary pathogenic event associated with disease onset is the conformational transformation of the host prion protein (PrP). PrP is a ubiquitous mammalian glycoprotein which, after cleavage of both a signal peptide and glycosylphosphatidylinositol (GPI) membrane anchor sequence, is approximately 208 amino acids (amino acid residues 23–231). It is processed through the secretory pathway where it is glycosylated at two N-linked glycosylation sites and eventually attached to the cell surface via the GPI anchor. During TSE pathogenesis, the soluble, protease sensitive form of PrP (PrP^{sen}) is refolded and converted into an insoluble protease-resistant form, PrP^{res}. PrP^{res} has the same primary sequence as PrP^{sen} but a different secondary structure. Thus, where PrP^{sen} is higher in α -helical content, PrP^{res} is higher in β -sheet content (see below) [1].

Corresponding authors: Moore, Roger A. (E-mail: rmoore@niaid.nih.gov phone: 406-363-9391) and Priola, Suzette A. (E-mail: spriola@niaid.nih.gov phone: 406-363-9319).

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

Conversion of PrP-sen to PrP-res most likely occurs on the cell surface and/or along the endocytic pathway via a mechanism of seeded polymerization, a process which can be broadly divided into two steps. In the rate limiting step, ordered aggregates or “seeds” of PrP-res bind PrP-sen. In the second step, the newly bound PrP-sen is conformationally altered, via a poorly understood process, into more PrP-res. PrP-res can accumulate either intra- or extracellularly, mainly within tissues of the central nervous system and lymphoreticular system. Formation of PrP-res is associated with the neuronal loss which leads to the characteristic spongiform appearance of a TSE-infected brain and is considered diagnostic for TSE diseases [1].

TSEs, also known as prion diseases after the prion protein, are unique among protein misfolding diseases in that they are transmissible. In fact, prion diseases have all of the characteristics of more conventional viral or bacterial agents in that they can replicate, have different strains of agent which are associated with different prion disease phenotypes in vivo, and demonstrate very strong species specificities. PrP-res is tightly associated with infectivity and recent in vitro data support the hypothesis that PrP-res itself is a primary component of the TSE infectious agent [2•,3•]. The challenge within the prion disease field is thus to understand how all the different aspects of prion pathogenesis can be explained within the context of the underlying structures of both PrP-sen and PrP-res.

Structural biology of PrP-sen

High resolution NMR structures were first obtained from C-terminal mouse PrP-sen (residues 121–231) expressed in *Escherichia coli* [4•]. As shown in Figure 1A, normal PrP contains three alpha helices separated by regions of loop/turn with two small regions of beta strand located upstream of the first and second alpha helices. Subsequent NMR-based models demonstrated that the C-terminal globular domain of PrP-sen was folded similarly across multiple mammalian species [5–9] and that the N-terminal portion of PrP from 23–120 was largely disordered [10]. The crystallization of recombinant PrP-sen has also led to high resolution structures which, other than being dimeric in one instance, were very similar to the structure derived by NMR [11;12]. Importantly, the NMR structure of PrP-sen purified from the brains of cattle was shown to be virtually identical in overall fold to that of *E. coli* derived PrP-sen, further validating the results using *E. coli* derived PrP [13•].

The primary structure of PrP-sen and PrP-res formation

Early in vivo experiments took advantage of the species barriers to TSE infection between mice and hamsters and showed that the middle third of PrP-sen (residues ~108–189) determined whether or not a mouse was susceptible to hamster scrapie [14]. In vitro scrapie-infected tissue culture models demonstrated that this central region controlled the species-specific formation of PrP-res and thus provided the basis for a molecular mechanism of TSE species barriers in vivo [15•,16;17]. If there was sufficient homology from residues 108–189 between exogenous PrP-res from one species and endogenous PrP-sen of another species, PrP-res would convert PrP-sen to more PrP-res and the species barrier would be crossed. If there was insufficient homology between the exogenous PrP-res and the endogenous PrP-sen, PrP-res would not efficiently convert PrP-sen and the species barrier would be maintained [14–17].

Thus, data from both in vivo and in vitro studies indicated that either the primary or secondary structural components within the middle third of PrP-sen were important for conversion. NMR structures of PrP-sen showed that these critical residues (~108–189) contain most of the folded domain including both β -strands, the first two α -helices, and the loop/turn regions connecting them. This important region notably does not contain the third α -helix (Box 1). Further mapping of the amino acids important in species-specific PrP-res formation showed that it was residues within the loop structures that were the most important (Figure 1B–D). Homology at PrP-sen

amino acid residues 138 in mouse [16•], 155 in hamster [17], 171 in sheep [18;19], and 129 in human [20] were essential for the efficient formation of PrP-res in vitro. In vivo, these same residues strongly influence species barriers to TSE infection [21–23].

The fact that the most important residues in PrP-res formation are not the same among species strongly suggests that it is not amino acid homology but rather slight variations within the loop/turn structures of PrP itself that are important in the conversion of PrP-sen to PrP-res [24]. Recent NMR data demonstrating that there are subtle but significant differences in the way that the solvent exposed loop regions of PrP-sen are ordered among species support this interpretation [25;26]. It is also consistent with the original suggestion of Riek et al. that, during PrP-res formation, the two small regions of β -strand upstream of the loop regions act as a nucleation site for the conformational conversion of the loop/turn and α -helical structures in PrP-sen to β -sheet in PrP-res [4••]. Interestingly, the loop between the second β -strand and α -helix 2 displays structural variation among species, with a significantly higher degree of order in the β 2- α 2 loop sequences from TSE susceptible species such as elk, sheep and bank voles [26]. Specifically, asparagine at position 170, carried by both bank voles and cervids, has been implicated in higher susceptibilities to TSE disease [27]. Thus, it seems that these highly exposed surface regions defined by loops between the small β -strands and the α -helices may be involved in intermolecular contacts between PrP-sen and PrP-res.

Role of PrP-sen secondary structures in PrP-res formation

Using the NMR structures as a guide, the major secondary structural elements of PrP-sen have been either mutated or deleted entirely and their effect on in vivo disease [28;29] and in vitro PrP-res formation [30] have been studied. Experiments using transgenic mice over-expressing PrP-sen deleted within the unstructured N-terminus showed that this region strongly influenced the susceptibility to and the incubation times of prion disease [29]. In vitro studies using similar N-terminal deletions have shown that residues between 108 and 124 are important in PrP-res formation [31]. The N-terminus contains a sequence of eight amino acids repeated 5 times (the octapeptide repeat region, residues 52–90) which contain histidine and are known to bind copper and zinc [32]. Recent studies have shown that while the number of octapeptide repeats does not influence the conformation of abnormal PrP, it does effect the kinetics of PrP-res formation by influencing both PrP-sen aggregation and the rate at which PrP-sen binds to PrP-res [33]. Thus, during PrP-res formation the N-terminus likely influences not only the initial PrP-sen/PrP-res binding step (via the octapeptide repeats) but also the actual conversion of PrP-sen to PrP-res (via residues 108–124).

Understanding how each α -helix and β -strand of PrP-sen contributes to PrP-res formation is much more problematic given that, unlike mutations within the disordered N-terminus, mutation of these well-defined secondary structures can lead to aberrantly processed PrP-sen [30]. As a result, it is often difficult to distinguish the role of these structures in PrP-res formation from their potentially critical role in appropriate PrP-sen folding and processing. Furthermore, the effect of these mutations is unpredictable. For example, in studies using scrapie-infected tissue culture cells, deletion of the second β -strand led to aberrantly processed PrP-sen. However, the additional deletion of the first α -helix appeared to restore normal PrP-sen processing [30]. Neither of these constructs was able to form PrP-res in vitro. By contrast, transgenic mice expressing a similar PrP-sen mutant (i.e. without the first α -helix and second β -strand) but with an additional N-terminal deletion of amino acid residues 23–88, were fully susceptible to scrapie infection and accumulated PrP-res [28]. Clearly, caution should be used when using PrP deletion mutants to determine the influence of PrP-sen secondary structures on PrP-res formation.

PrP-res structure and disease

Most researchers appear to now accept the protein-only theory of prion diseases which states that PrP-res is the infectious agent of TSE disease. Distinct prion strains are perhaps the most difficult aspect of TSEs to explain within the context of the protein-only hypothesis. Strains are defined biologically by disease incubation time, clinical signs and pathology and biochemically by the size, glycosylation and protease resistance of PrP-res. Notably, if PrP-res is the sole component of the infectious agent, then one has to consider how prion disease phenotypes are determined. The most likely explanation is that prion strain phenotypes are encoded with exquisite precision by the tertiary and quaternary structure of PrP-res. Various studies have suggested that TSE strain discrimination is indeed conformation-based. For example, the analysis of PrP-res with structure-specific antibodies [34] or luminescence reactions in which conjugated polymers interact with PrP-res in a structure-specific manner [35] support this idea. Thus, it is critical to resolve the structural components of PrP-res in order to understand how different prion strains work in vivo.

While a great deal is known about PrP-sen structure and how specific mutations in PrP-sen can influence PrP-res formation, the structure of PrP-res is unknown since it has not yet been purified sufficiently for high-resolution structural studies. Attempts to purify PrP-res from the brains of infected animals invariably yield heterogeneous mixtures and aggregation states not amenable to characterization by NMR spectroscopy or X-ray crystallography. This lack of high resolution data has made it necessary to focus more intently upon lower resolution techniques for structure determination, notably the ultrastructure and the secondary structure of PrP-res. For example, electron microscopy has been used extensively to characterize PrP-res aggregates. Such samples can display a wide spectrum of morphologies and aggregation characteristics, from highly ordered fibrillar deposits to apparently amorphous aggregates [36•]. It is not uncommon to observe electron microscopy images of PrP-res deposits that show fibrils and amorphous aggregates on the same grid square. Studies using circular dichroism and FTIR have shown that PrP-res can contain up to ~50% β -sheet structure but still retain a substantial amount of its original α -helical character [37,38•,39]. Importantly, FTIR has also been used to distinguish PrP-res from different strains by defining structural differences in β -sheet conformation [40•,41,42].

PrP-res and amyloid fibrils share common features

The ability of prions to amplify themselves in vivo bears a striking mechanistic resemblance to the way that amyloid fibrils grow through seeded polymerization from a reservoir of precursor protein. About forty amyloid diseases have now been identified, and they are typically defined by the presence of amyloid fibrils [43•]. By contrast, PrP-res is only sometimes observed in the form of amyloid, with some strains forming more amyloid during the disease process than others. However, the similarities between amyloid and prion diseases are nevertheless intriguing enough to make detailed comparisons worthwhile.

Amyloid fibrils, whether formed in vivo or in vitro, all display a characteristic cross- β folding pattern in which β -strands align to form sheets that are perpendicular to the fibril axis while hydrogen bonding between strands is parallel to the fibril axis [44–46]. An impressive array of biophysical methods has been developed to probe amyloid structure, and now unbiased atomic models of the cross- β spine from short peptides are available [47•,48]. Perhaps the highly specific molecular contacts among side chain residues in the tightly interdigitated dry interface of the β -strands in those short amyloid peptides constitute the same type of interface in the β -sheets of PrP-res, making the strain-specific conformations that define TSEs. However, such highly detailed structural models for larger proteins such as PrP are not yet available.

PrP, like most amyloid-disease related proteins, can be induced to form amyloid-like fibrils in vitro under the right conditions. Consequently, researchers have created amyloid-like fibrils from various PrP sequences in order to elucidate certain aspects of TSE disease and PrP-res structure. One particularly illustrative model has been the recombinant peptide PrP23–144, which is associated with a form of cerebral amyloid angiopathy [49], and forms amyloid-like fibrils in vitro under non-denaturing conditions from an intrinsically disordered structure [50]. Further studies showed that PrP23–144, like other amyloidogenic peptides, has a high degree of conformational plasticity and can be induced to form a wide range of fibril morphologies [51]. Using PrP23–144, Surewicz and his co-workers showed that the critical amino acids 138 and 139 determined a species-specific seeding preference [52]. For example, Syrian hamster PrP23–144 fibrils did not effectively seed human PrP23–144 and vice-versa due to an amino acid mismatch in these residues. This finding supported earlier results demonstrating the importance of residue 138 for the species-specific formation of PrP-res in mouse-scrapie infected tissue culture cells [16•]. These results helped to illustrate that PrP conversion or the lack thereof could be thought of in terms of structural compatibility between isoforms of PrP-sen and PrP-res without the need for any non-PrP co-factor.

Full-length recombinant PrP (residues ~23–231, numbering dependent upon the species) can also be induced to form amyloid-like fibrils (Figure 2A), herein termed r-PrP-res [53–57]. The ability to produce fibrils by seeded polymerization at neutral pH from a completely folded tertiary structure using essentially the complete PrP coding sequence represents an important step towards building a useful model for PrP misfolding in TSE disease [53]. In contrast to PrP23–144 and various other partial PrP sequences, full-length r-PrP does not readily form amyloid-like fibrils unless denaturants are used to partially unravel the tertiary structure prior to fibrillization. This requirement for non-physiological buffer conditions has made it difficult use r-PrP-res fibrils to examine phenomena which require a high degree of seeding specificity, such as would be required if modeling the species barrier or protein-based strain differentiation [58].

Additionally, fibrillization of r-PrP does not necessarily lead to an infectious PrP-res conformation. Numerous attempts to instigate TSE disease with amyloid-like fibrils have failed [e.g. 59]. Although there has been a report of recombinant mouse PrP89–231 amyloid-like fibrils triggering prion disease in transgenic mice over-expressing the same sequence, these results have yet to be repeated [60•]. One possible explanation for the paucity of infectious r-PrP-res samples is that only a small fraction of a very large number of possible r-PrP conformations may represent the correct tertiary and quaternary architecture necessary for the formation of infectious PrP-res.

It has been reported that the protease resistant β -sheet core of full-length recombinant amyloid-like human r-PrP-res fibrils is derived from residues ~169–220 [61]. This 8 kDa segment (similar to that shown in Figure 2B) is composed of β -strands oriented in a parallel, in-register alignment with tight alignment between the side chains [62•]. By contrast, immunoblots of PK-treated bona fide PrP-res suggest that the primary protease-resistant core is ~17 kDa, which is more than twice the molecular weight of the r-PrP-res β -sheet core [63]. It is not clear why r-PrP-res fibrils would not reflect those key characteristics of bona fide PrP-res, except that making r-PrP-res in vitro commonly involves the use of denaturants such as guanidine hydrochloride [53] or detergents like SDS [64•,65] to prompt fibrillization, and thus does not closely enough match the oligomerization environment found in vivo. This may help to explain why r-PrP-res differs structurally from PrP-res formed in vivo (Figure 2C).

However, there is still hope that future demonstrations of prion infectivity may be achieved by screening r-PrP-res formulations for those that contain just the right cross- β fold structure required to complex with PrP-sen in vivo. In fact, it has been demonstrated that r-PrP-sen can

be seeded by minute quantities of brain-derived PrP-res, resulting in a small proportion of the original r-PrP-sen molecules reorganizing into r-PrP-res with a PK resistant β -sheet that extends to ~16 kDa [64;66]. These studies showed that r-PrP-res can indeed acquire alternative conformations, although it remains to be determined whether or not these PrP-res seeded conformations, or some variation thereof, have acquired infectious properties.

Existing models of PrP-res structure

Since high-resolution structural details of PrP-res aggregates do not exist, the two most widely cited structural models for PrP-res were both done *in silico* [67•,68•]. The β -helix model, proposed by Fred Cohen and coworkers [68], is based upon a threading analysis methodology developed earlier [69]. The work was done largely in coordination with electron microscopy experiments [70•] using hamster PrP-res, whereby refined electron density maps from the microscopic observation of ordered two-dimensional arrays were used as constraints [68•]. The β -helix model proposes that the major conformation changes in the conversion process occur within residues 90–112 and extend beyond residue 138 (hamster numbering). The proposed β -sheet core region in this model would form left-handed β -helices within residues 89–175 coordinated in PrP trimers that would then stack upon each other perpendicular to the fibril axis. The disulfide linkage and glycosylation would be retained outside of the stackable β -helix core and the C-terminal residues including α -helices 2 and 3 would not be affected.

The spiral protofibril model, proposed by DeMarco and Daggett, is fundamentally different from the β -helical model. The original spiral model was derived from molecular dynamics simulations using the mutant D147N composed of hamster PrP residues 109–219 [67•]. At the heart of the spiral model are four β -strands, termed E1–E4, spanning residues within the 116–164 region. The first new strand proposed (E1; 116–119) is N-terminal to the first β strand originally present in PrP-sen. The second two extended structures (E2, E3) represent an extension of the original two β strands in PrP-sen. The spiral model is shown in Box 1 with key residues involved in extended β -sheet like structure shown as arrows. Importantly, none of the extended sheet structures would significantly affect the original α -helices, thus allowing them to retain their native conformation. A critical comparison between the spiral and β -helix model has also been published [71].

Finally, as discussed above, another structure has recently emerged, based upon experimental data obtained from site-directed spin labeling and EPR spectroscopy on r-PrP-res amyloid-like fibrils [62•]. This structural model is markedly different from the other two in that the β -sheet core extends from approximately 169–220 and none of the original α -helical character from PrP-sen is retained. By contrast, experimental evidence for PrP-res implicates N-terminal residues ~90–120 in the β -sheet core [72] and retention of α -helical character in the C-terminal residues [38].

Conclusion

High-resolution structures of PrP-sen are well established and have provided useful information regarding the influence of various regions in the PrP-sen to PrP-res conversion process. In particular, a key loop region composed of residues ~165–171 displays structural variations between species and is thought to influence species-specific intermolecular contacts, perhaps acting as a nucleation site in the conversion process. However, there are no corresponding structures of PrP-res due to problems with insolubility and heterogeneity of purified samples. Analysis of the secondary structure of PrP-res has revealed a high percentage of acquired β -sheet structure and it has been shown that the conformation of this β -sheet can vary between PrP-res samples from different TSE strains. PrP-res is sometimes but not always found in the form of amyloid fibrils, further illustrating the complex nature of potential PrP

conformations. It is thus critical to more thoroughly investigate PrP-res structure in order to understand what conformations may be associated with TSE infectivity.

Acknowledgements

The authors would like to thank Anita Mora for help with the graphics.

Reference List

1. Priola SA, Vorberg I. Molecular aspects of disease pathogenesis in the transmissible spongiform encephalopathies. *Mol Biotechnol* 2006;33:71–88. [PubMed: 16691009]

- 2••. Castilla J, Saa P, Hetz C, Soto C. In vitro generation of infectious scrapie prions. *Cell* 2005;121:195–206. [PubMed: 15851027]The first demonstration that infectious prion particles could be generated in vitro by mixing uninfected and prion-infected brain homogenates in serial rounds of dilution and sonication. This method is now widely termed protein misfolding cyclic amplification, or PMCA.
- 3••. Deleault NR, Harris BT, Rees JR, Supattapone S. Formation of native prions from minimal components *in vitro*. *Proc Natl Acad Sci USA* 2007;104:9741–9746. [PubMed: 17535913] Demonstrated that prions could be formed without infectious PrP-res as a seed from a minimal set of components including native PrP-sen, lipid molecules, and a synthetic polyanion.
- 4••. Riek R, Hornemann S, Wider G, Billeter M, Glockshuber R, Wuthrich K. NMR structure of the mouse prion protein domain PrP(121–231). *Nature* 1996;382:180–182. [PubMed: 8700211]First NMR solution structure of the globular fold of PrP-sen.
5. Zahn R, Liu A, Luhrs T, Riek R, von Schroetter C, Garcia FL, Billeter M, Calzolari L, Wider G, Wuthrich K. NMR solution structure of human prion protein. *Proc Natl Acad Sci USA* 2000;97:145–150. [PubMed: 10618385]
6. Garcia FL, Zahn R, Riek R, Wuthrich K. NMR structure of the bovine prion protein. *Proc Natl Acad Sci USA* 2000;97:8334–8339. [PubMed: 10899999]
7. Wuthrich K, Riek R. Three - dimensional structures of prion proteins. *Adv Prot Chem* 2001;57:55–82.
8. Lysek DA, Schorn C, Nivon LG, Esteve-Moya V, Christen B, Calzolari L, von SC, Fiorito F, Herrmann T, Guntert P, Wuthrich K. Prion protein NMR structures of cats, dogs, pigs, and sheep. *Proc Natl Acad Sci USA* 2005;102:640–645. [PubMed: 15647367]
9. Calzolari L, Lysek DA, Perez DR, Guntert P, Wuthrich K. Prion protein NMR structures of chickens, turtles, and frogs. *Proc Natl Acad Sci USA* 2005;102:651–655. [PubMed: 15647366]
10. Riek R, Hornemann S, Wider G, Glockshuber R, Wuthrich K. NMR characterization of the full-length recombinant murine prion protein, *mPrP*(23–231). *FEBS Lett* 1997;413:282–288. [PubMed: 9280298]
11. Haire LF, Whyte SM, Vasisht N, Gill AC, Verma C, Dodson EJ, Dodson GG, Bayley PM. The crystal structure of the globular domain of sheep prion protein. *J Mol Biol* 2004;336:1175–1183. [PubMed: 15037077]
12. Knaus KJ, Morillas M, Swietnicki W, Malone M, Surewicz WK, Yee VC. Crystal structure of the human prion protein reveals a mechanism for oligomerization. *Nat Struct Biol* 2001;8:770–774. [PubMed: 11524679]
- 13•. Hornemann S, Schorn C, Wuthrich K. NMR structure of the bovine prion protein isolated from healthy calf brains. *EMBO Rep* 2004;5:1159–1164. [PubMed: 15568016]This work showed that the three-dimensional structure of natural mammalian PrP and genetically engineered recombinant PrP are essentially the same, thus providing validation to the majority of structural studies which must rely on recombinant PrP.
14. Scott M, Groth D, Foster D, Torchia M, Yang SL, DeArmond SJ, Prusiner SB. Propagation of prions with artificial properties in transgenic mice expressing chimeric PrP genes. *Cell* 1993;73:979–988. [PubMed: 8098995]
- 15•. Scott MR, Kohler R, Foster D, Prusiner SB. Chimeric prion protein expression in cultured cells and transgenic mice. *Protein Sci* 1992;1:986–997. [PubMed: 1338978]First definitive mapping study showing that it was the central region of the PrP primary amino acid sequence that influenced the species-specific conversion of PrP-sen to PrP-res.
- 16•. Priola SA, Chesebro B. A single hamster amino acid blocks conversion to protease-resistant PrP in scrapie-infected mouse neuroblastoma cells. *J Virol* 1995;69:7754–7758. [PubMed: 7494285]First demonstration that sequence homology at a single amino acid residue could determine whether or not conversion of PrP-sen to PrP-res was possible.
17. Priola SA, Chabry J, Chan K. Efficient conversion of normal prion protein (PrP) by abnormal hamster PrP is determined by homology at amino acid residue 155. *J Virol* 2001;75:4673–4680. [PubMed: 11312338]
18. Bossers A, Belt PBGM, Raymond GJ, Caughey B, de Vries R, Smits MA. Scrapie susceptibility-linked polymorphisms modulate the *in vitro* conversion of sheep prion protein to protease-resistant forms. *Proc Natl Acad Sci USA* 1997;94:4931–4936. [PubMed: 9144167]

19. Bossers A, de Vries R, Smits MA. Susceptibility of sheep for scrapie as assessed by in vitro conversion of nine naturally occurring variants of PrP. *J Virol* 2000;74:1407–1414. [PubMed: 10627551]
20. Raymond GJ, Hope J, Kocisko DA, Priola SA, Raymond LD, Bossers A, Ironside J, Will RG, Chen SG, Petersen RB, Gambetti P, Rubenstein R, Smits MA, Lansbury PT Jr, Caughey B. Molecular assessment of the transmissibilities of BSE and scrapie to humans. *Nature* 1997;388:285–288. [PubMed: 9230438]
21. Goldmann W, Hunter N, Smith G, Foster J, Hope J. PrP genotype and agent effects in scrapie: change in allelic interaction with different isolates of agent in sheep, a natural host of scrapie. *J Gen Virol* 1994;75:989–995. [PubMed: 7909834]
22. Palmer MS, Dryden AJ, Hughes JT, Collinge J. Homozygous prion protein genotype predisposes to sporadic Creutzfeldt-Jakob disease. *Nature* 1991;352:340–342. [PubMed: 1677164]
23. Hunter N, Foster JD, Goldmann W, Stear MJ, Hope J, Bostock C. Natural scrapie in a closed flock of Cheviot sheep occurs only in specific PrP genotypes. *Arch Virol* 1996;141:809–824. [PubMed: 8678828]
24. Vorberg I, Groschup MH, Pfaff E, Priola SA. Multiple amino acid residues within the rabbit prion protein inhibit formation of its abnormal isoform. *J Virol* 2003;77:2003–2009. [PubMed: 12525634]
25. Gossert AD, Bonjour S, Lysek DA, Fiorito F, Wuthrich K. Prion protein NMR structures of elk and of mouse/elk hybrids. *Proc Natl Acad Sci USA* 2005;102:646–650. [PubMed: 15647363]
26. Christen B, Perez DR, Hornemann S, Wuthrich K. NMR Structure of the Bank Vole Prion Protein at 20 degrees C Contains a Structured Loop of Residues 165–171. *J Mol Biol.* 2008
27. Sigurdson CJ, Aguzzi A. Chronic wasting disease. *Biochim Biophys Acta* 2007;1772:610–618. [PubMed: 17223321]
28. Supattapone S, Bosque P, Muramoto T, Wille H, Aagaard C, Peretz D, Nguyen HO, Heinrich C, Torchia M, Safar J, Cohen FE, DeArmond SJ, Prusiner SB, Scott M. Prion protein of 106 residues creates an artificial transmission barrier for prion replication in transgenic mice. *Cell* 1999;96:869–878. [PubMed: 10102274]
29. Fischer M, Rulicke T, Raeber A, Sailer A, Moser M, Oesch B, Brandner S, Aguzzi A, Weissmann C. Prion protein (PrP) with amino-proximal deletions restoring susceptibility of PrP knockout mice to scrapie. *EMBO J* 1996;15:1255–1264. [PubMed: 8635458]
30. Vorberg I, Chan K, Priola SA. Deletion of beta-strand and alpha-helix secondary structure in normal prion protein inhibits formation of its protease-resistant isoform. *J Virol* 2001;75:10024–10032. [PubMed: 11581371]
31. Lawson VA, Priola SA, Wehrly K, Chesebro B. N-terminal truncation of prion protein affects both formation and conformation of abnormal protease-resistant prion protein generated in vitro. *J Biol Chem* 2001;276:35265–35271. [PubMed: 11466311]
32. Walter ED, Stevens DJ, Visconte MP, Millhauser GL. The prion protein is a combined zinc and copper binding protein: Zn²⁺ alters the distribution of Cu²⁺ coordination modes. *J Am Chem Soc* 2007;129:15440–15441. [PubMed: 18034490]
33. Moore RA, Herzog C, Errett J, Kocisko DA, Arnold KM, Hayes SF, Priola SA. Octapeptide repeat insertions increase the rate of protease-resistant prion protein formation. *Protein Sci* 2006;15:609–619. [PubMed: 16452616]
34. Safar J, Wille H, Itri V, Groth D, Serban H, Torchia M, Cohen FE, Prusiner SB. Eight prion strains have PrP(Sc) molecules with different conformations [see comments]. *Nat Med* 1998;4:1157–1165. [PubMed: 9771749]
35. Sigurdson CJ, Nilsson KP, Hornemann S, Manco G, Polymenidou M, Schwarz P, Leclerc M, Hammarstrom P, Wuthrich K, Aguzzi A. Prion strain discrimination using luminescent conjugated polymers. *Nat Methods* 2007;4:1023–1030. [PubMed: 18026110]
- 36••. Merz PA, Somerville RA, Wisniewski HM, Iqbal K. Abnormal fibrils from scrapie-infected brain. *Acta Neuropathol* 1981;54:63–74. [PubMed: 7195134] First transmission electron microscopy images of scrapie associated fibrils. The images were obtained before it was known that PrP-res was a major constituent of the fibrils.
37. Han S, Hill AF. Analysis of PrP conformation using circular dichroism. *Methods Mol Biol* 2008;459:145–159. [PubMed: 18576154]

- 38•. Caughey BW, Dong A, Bhat KS, Ernst D, Hayes SF, Caughey WS. Secondary structure analysis of the scrapie-associated protein PrP 27–30 in water by infrared spectroscopy. *Biochemistry* 1991;30:7672–7680. [PubMed: 1678278] This study demonstrated that FTIR could be applied to the analysis of PrP-res aggregates.
39. Pan K-M, Baldwin M, Nguyen J, Gasset M, Serban A, Groth D, Mehlhorn I, Huang Z, Fletterick RJ, Cohen FE, Prusiner SB. Conversion of alpha-helices into beta-sheets features in the formation of the scrapie prion protein. *Proc Natl Acad Sci USA* 1993;90:10962–10966. [PubMed: 7902575]
- 40••. Caughey B, Raymond GJ, Bessen RA. Strain-dependent differences in beta-sheet conformations of abnormal prion protein. *J Biol Chem* 1998;273:32230–32235. [PubMed: 9822701] Demonstrated that FTIR could be used to differentiate α -sheet conformation of PrP-res strains providing support for the hypothesis that prion strains are conformation-based.
41. Spassov S, Beekes M, Naumann D. Structural differences between TSEs strains investigated by FT-IR spectroscopy. *Biochim Biophys Acta* 2006;1760:1138–1149. [PubMed: 16730908]
42. Thomzig A, Spassov S, Friedrich M, Naumann D, Beekes M. Discriminating scrapie and bovine spongiform encephalopathy isolates by infrared spectroscopy of pathological prion protein. *J Biol Chem* 2004;279:33847–33854. [PubMed: 15155741]
- 43•. Chiti F, Dobson CM. Protein misfolding, functional amyloid, and human disease. *Annu Rev Biochem* 2006;75:333–366. [PubMed: 16756495] Extensive review article for further reading on the role of amyloid in human disease.
44. Nelson R, Eisenberg D. Structural models of amyloid-like fibrils. *Adv Protein Chem* 2006;73:235–282. [PubMed: 17190616]
45. Tycko R. Characterization of amyloid structures at the molecular level by solid state nuclear magnetic resonance spectroscopy. *Methods Enzymol* 2006;413:103–122. [PubMed: 17046393]
46. Nelson R, Eisenberg D. Recent atomic models of amyloid fibril structure. *Curr Opin Struct Biol* 2006;16:260–265. [PubMed: 16563741]
- 47••. Sawaya MR, Sambashivan S, Nelson R, Ivanova MI, Sievers SA, Apostol MI, Thompson MJ, Balbirnie M, Wiltzius JJ, McFarlane HT, Madsen AO, Riekel C, Eisenberg D. Atomic structures of amyloid cross-beta spines reveal varied steric zippers. *Nature* 2007;447:453–457. [PubMed: 17468747] The first fully objective atomic model of the interface between β -strands of amyloid-like fibrils.
48. Wiltzius JJ, Sievers SA, Sawaya MR, Cascio D, Popov D, Riekel C, Eisenberg D. Atomic structure of the cross-beta spine of islet amyloid polypeptide (amylin). *Protein Sci* 2008;17:1467–1474. [PubMed: 18556473]
49. Ghetti B, Piccardo P, Spillantini MG, Ichimiya Y, Porro M, Perini F, Kitamoto T, Tateishi J, Seiler C, Frangione B, Bugiani O, Giaccone G, Prelli F, Goedert M, Dlouhy SR, Tagliavini F. Vascular variant of prion protein cerebral amyloidosis with tau-positive neurofibrillary tangles: the phenotype of the stop codon 145 mutation in PRNP. *Proc Natl Acad Sci USA* 1996;93:744–748.
50. Kundu B, Maiti NR, Jones EM, Surewicz KA, Vanik DL, Surewicz WK. Nucleation-dependent conformational conversion of the Y145Stop variant of human prion protein: structural clues for prion propagation. *Proc Natl Acad Sci USA* 2003;100:12069–12074. [PubMed: 14519851]
51. Moore RA, Hayes SF, Fischer ER, Priola SA. Amyloid formation via supramolecular peptide assemblies. *Biochemistry* 2007;46:7079–7087. [PubMed: 17521170]
52. Surewicz WK, Jones EM, Apetri AC. The emerging principles of mammalian prion propagation and transmissibility barriers: Insight from studies in vitro. *Acc Chem Res* 2006;39:654–662. [PubMed: 16981682]
53. Breydo L, Makarava N, Baskakov IV. Methods for conversion of prion protein into amyloid fibrils. *Methods Mol Biol* 2008;459:105–115. [PubMed: 18576151]
54. Luhrs T, Zahn R, Wuthrich K. Amyloid formation by recombinant full-length prion proteins in phospholipid bicelle solutions. *J Mol Biol* 2006;357:833–841. [PubMed: 16466741]
55. Leffers KW, Wille H, Stohr J, Junger E, Prusiner SB, Riesner D. Assembly of natural and recombinant prion protein into fibrils. *Biol Chem* 2005;386:569–580. [PubMed: 16006244]
56. Tattum MH, Cohen-Krausz S, Thumanu K, Wharton CW, Khalili-Shirazi A, Jackson GS, Orlova EV, Collinge J, Clarke AR, Saibil HR. Elongated oligomers assemble into mammalian PrP amyloid fibrils. *J Mol Biol* 2006;357:975–985. [PubMed: 16473369]

57. Lee S, Eisenberg D. Seeded conversion of recombinant prion protein to a disulfide-bonded oligomer by a reduction-oxidation process. *Nat Struct Biol* 2003;10:725–730. [PubMed: 12897768]
58. Makarava N, Lee CI, Ostapchenko VG, Baskakov IV. Highly promiscuous nature of prion polymerization. *J Biol Chem* 2007;282:36704–36713. [PubMed: 17940285]
59. Hill AF, Antoniou M, Collinge J. Protease-resistant prion protein produced in vitro lacks detectable infectivity. *J Gen Virol* 1999;80:11–14. [PubMed: 9934677]
60. Legname G, Baskakov IV, Nguyen HO, Riesner D, Cohen FE, DeArmond SJ, Prusiner SB. Synthetic mammalian prions. *Science* 2004;305:673–676. [PubMed: 15286374] Reported that amyloid-like fibrils of recombinant mouse PrP^{89–230} generated in vitro could initiate prion disease in transgenic mice overexpressing the same PrP sequence.
61. Lu X, Wintrodde PL, Surewicz WK. Beta-sheet core of human prion protein amyloid fibrils as determined by hydrogen/deuterium exchange. *Proc Natl Acad Sci USA* 2007;104:1510–1515. [PubMed: 17242357]
62. Cobb NJ, Sonnichsen FD, McHaourab H, Surewicz WK. Molecular architecture of human prion protein amyloid: a parallel, in-register beta-structure. *Proc Natl Acad Sci USA* 2007;104:18946–18951. [PubMed: 18025469] Model of r-PrP-res based upon site-directed spin labeling with EPR spectroscopy. Demonstrated that the core of full-length amyloid-like PrP fibrils maps to C-terminal residues 160–220, forming single-molecule layers with parallel, in-register alignment of beta-strands.
63. Kocisko DA, Lansbury PT Jr, Caughey B. Partial unfolding and refolding of scrapie-associated prion protein: evidence for a critical 16-kDa C-terminal domain. *Biochemistry* 1996;35:13434–13442. [PubMed: 8873612]
64. Atarashi R, Moore RA, Sim VL, Hughson AG, Dorward DW, Onwubiko HA, Priola SA, Caughey B. Ultrasensitive detection of scrapie prion protein using seeded conversion of recombinant prion protein. *Nat Methods* 2007;4:645–650. [PubMed: 17643109] Demonstrated that PrP-sen could be used to detect minute amounts of PrP-res and that the resulting r-PrP-res could acquire a conformation more closely resembling that of the PrP-res seed.
65. Stohr J, Weinmann N, Wille H, Kaimann T, Nagel-Steger L, Birkmann E, Panza G, Prusiner SB, Eigen M, Riesner D. Mechanisms of prion protein assembly into amyloid. *Proc Natl Acad Sci USA* 2008;105:2409–2414. [PubMed: 18268326]
66. Atarashi R, Wilham JM, Christensen L, Hughson AG, Moore RA, Johnson LM, Onwubiko HA, Priola SA, Caughey B. Simplified ultrasensitive prion detection by recombinant PrP conversion with shaking. *Nat Methods* 2008;5:211–212. [PubMed: 18309304]
67. Demarco ML, Daggett V. From conversion to aggregation: protofibril formation of the prion protein. *Proc Natl Acad Sci USA* 2004;101:2293–2298. [PubMed: 14983003] Model of hamster PrP-res based upon molecular dynamics simulations.
68. Govaerts C, Wille H, Prusiner SB, Cohen FE. Evidence for assembly of prions with left-handed beta-helices into trimers. *Proc Natl Acad Sci USA* 2004;101:8342–8347. [PubMed: 15155909] Model of PrP-res based upon threading.
69. Bowie JU, Luthy R, Eisenberg D. A method to identify protein sequences that fold into a known three-dimensional structure. *Science* 1991;253:164–170. [PubMed: 1853201]
70. Wille H, Michelitsch MD, Guenebaut V, Supattapone S, Serban A, Cohen FE, Agard DA, Prusiner SB. Structural studies of the scrapie prion protein by electron crystallography. *Proc Natl Acad Sci USA* 2002;99:3563–3568. [PubMed: 11891310] Construction of low-resolution models of PrP-res structure based upon electron microscopy experiments.
71. Demarco ML, Silveira J, Caughey B, Daggett V. Structural Properties of Prion Protein Protofibrils and Fibrils: An Experimental Assessment of Atomic Models. *Biochemistry* 2006;45:15573–15582. [PubMed: 17176078]
72. Peretz D, Williamson RA, Matsunaga Y, Serban H, Pinilla C, Bastidas RB, Rozenshteyn R, James TL, Houghten RA, Cohen FE, Prusiner SB, Burton DR. A conformational transition at the N terminus of the prion protein features in formation of the scrapie isoform. *J Mol Biol* 1997;273:614–622. [PubMed: 9356250]

Figure 1. Critical loop regions of PrP-sen

Key PrP-sen residues in surface-exposed critical loop regions are illustrated in red. Experimental results from different research groups suggest that these key residues may represent contact sites between PrP-sen and PrP-res. A) Ribbon diagram of hamster PrP-sen (pbd 1b10) with residues Met139, Asn155 and Asn170 in red. B) Closer view of residue 139 (residue 138 in mouse PrP), found to be critical for conversion of PrP-sen to PrP-res. C) Expanded view of residue 155, shown in red. Sequence homology between PrP-sen and hamster PrP-res at amino acid residue 155 influenced the conversion efficiency of a protease-resistant product induced by hamster PrP-res [17]. D) Residue 170 is found in the $\beta 2$ - $\alpha 2$ loop and is important in susceptibility to TSE disease. Figure 1 was created with PyMOL. DeLano, Warren L. The PyMOL Molecular Graphics System (2008) DeLano Scientific, Palo Alto, CA, USA <http://www.pymol.org>.

Figure 2. r-HaPrP-res has a different secondary structure than 263K HaPrP-res

Amyloid-like r-PrP-res fibrils made in our laboratory can be PK digested resulting in removal of the N-terminus and generation of a C-terminal protease resistant core. Panel A) shows a representative example of amyloid-like fibrils of hamster r-PrP-sen (residues 23–231) grown at 37 °C with 200rpm in denaturing buffer. B) SDS-PAGE and staining of the PK-treated fibrils with Coomassie blue shows two bands of approximately 8 and 10 kDa, neither of which react with monoclonal antibody 3F4, which is specific for residues 109–112, suggesting that the cleavage site is C-terminal to this epitope. However the 10 kDa band does react with antibody R18, which is specific for residues 142–155, helping to confirm that a 10kDa fragment is present. C) ATR-FTIR spectra of PK-treated r-PrP-res23–231 (from samples shown in panel

B) or PrP90–231 show essentially the same β -sheet conformation in the 1610–1640 cm^{-1} region of the spectrum. However, a comparison of these spectra to that of PK-treated PrP-res from hamster TSE strain 263K shows a markedly different banding pattern in this region. A representative immunoblot of PK and non-PK treated 263K PrP-res developed with the monoclonal antibody 3F4 is shown on the far right of panel B.