CHAPTER

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Molecular Basis of Prion Diseases

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OUTLINE

INTRODUCTION

The prion diseases are a closely related group of neurodegenerative conditions that affect both humans and animals. They have previously been described as the subacute spongiform encephalopathies, slow virus diseases and transmissible dementias, and include scrapie in sheep, bovine spongiform encephalopathy (BSE) in cattle, and the human prion diseases Creutzfeldt-Jakob disease (CJD), Gerstmann-Sträussler-Scheinker disease (GSS), fatal familial insomnia (FFI) and kuru (Collinge, 2001; Collinge, 2005). Prion diseases are unique in biology in that they manifest as sporadic, genetic and infectious disorders. Although historically rare in humans, affecting about one person per million worldwide per annum, the appearance of a novel human prion disease, variant CJD (vCJD), and the experimental confirmation that this is caused by the same prion strain as that causing BSE in cattle, raised the possibility that a major epidemic of vCJD would occur within the United Kingdom and other countries as a result of dietary or other exposure to BSE prions (Collinge, 1999; Collinge, 2005; Wadsworth & Collinge, 2007). These concerns, together with those of iatrogenic transmission of preclinical vCJD via medical and surgical procedures—since prions are known to resist conventional sterilization methods—have led to intense efforts to understand the molecular basis of prion propagation and to develop rational therapeutics. The wider relevance of prion-like mechanisms in other neurodegenerative diseases is also now becoming clear (Collinge & Clarke, 2007) and prion biology provides a key paradigm for protein misfolding diseases and protein-based inheritance with far-reaching implications for disease mechanisms, aging and evolution.

PRION DISEASES ARE BIOLOGICALLY UNIQUE

Discovery of the prion protein

The demonstration of transmission of prion disease by inoculation of scrapie brain isolates into sheep, and of kuru, CJD and GSS brain isolates into primates, formed the basis for the concept of "transmissible dementias." However, the nature of the transmissible agent has been a subject of heated debate for many years. The understandable initial assumption that the agent must be some form of virus was challenged by the failure to directly demonstrate such a virus (or indeed any immunological response to it) and by evidence indicating that the transmissible agent showed remarkable resistance to treatment expected to inactivate nucleic acids. Such findings led to suggestions that the transmissible agent may be devoid of nucleic acid (Alper et al., 1967) and might be a protein (Griffith, 1967). Subsequently, the progressive enrichment of brain homogenates for prion infectivity resulted in the isolation of a protease-resistant sialoglycoprotein, designated the prion protein (PrP) by Prusiner and co-workers in 1982. This protein was the major constituent of infective fractions and was found to accumulate in affected brains and sometimes to form amyloid deposits. The term prion (from *pro*teinaceous *in*fectious particle) was proposed by Prusiner to distinguish the infectious pathogen from viruses or viroids. Prions were defined as "small proteinaceous infectious particles that resist inactivation by procedures which modify nucleic acids" (Prusiner, 1982).

Prion protein is encoded by the host

At the time of its isolation, PrP was assumed to be encoded by a gene within the putative slow virus thought to be responsible for these diseases; however, amino acid sequencing of part of PrP and the subsequent recovery of cognate cDNA clones using an isocoding mixture of oligonucleotides led to the realization that PrP was encoded by a single-copy chromosomal gene rather than by a putative nucleic acid in fractions enriched for scrapie infectivity (Weissmann & Fleschig, 2003).

Aberrant metabolism of the prion protein is the central feature of prion disease

It is now clear that the central and unifying hallmark of the prion diseases is the aberrant metabolism of PrP, which can exists in distinct conformational states with different physicochemical properties. The normal cellular form of the protein, referred to as PrP^{C}, is a highly conserved cell surface glycosylphosphatidylinositol (GPI)-anchored sialoglycoprotein that is sensitive to protease treatment and soluble in detergents. Disease-associated isoforms of prion protein, generally referred to as PrP scrapie or PrP^{Sc}, are found only in prion-infected tissue as aggregated material and are partially resistant to protease treatment and insoluble in detergents. Due to its physiochemical properties, the precise atomic structure of the infectious particle or prion is still undetermined, but considerable evidence argues that prions are composed largely, if not entirely, of an abnormal isoform of PrP. The essential role of host PrP for prion propagation and pathogenesis is demonstrated by the fact that knockout mice lacking the PrP gene ($Prnp^{o/o}$ mice) are entirely resistant to prion infection, and that reintroduction of the murine PrP transgene restores susceptibility to infection (Weissmann et al., 2003). For comprehensive reviews of prion disease biology, see Prusiner, 1998; Collinge, 2001; Collinge & Clarke, 2007; Aguzzi & Calella, 2009.

ANIMAL PRION DISEASES

Scrapie and BSE

Scrapie is the prototypic prion disease and has been recognized as an enzootic disease of sheep and goats for more than 250 years. The etiology of natural scrapie has been the subject of intense debate for many years, but it is now clear that it is an infectious disease for which susceptibility is genetically modulated by the host. Following its discovery in 1985, BSE reached epidemic proportions in cattle within the United Kingdom, with smaller numbers in many other countries. Epidemiologic studies point to contaminated offal used in the manufacture of meat and bone meal and fed to cattle as the source of prions responsible for BSE, although

the nature and source of the original prion contamination remains unknown (Collinge, 2001). The host range of BSE prions appears to be unusually wide, affecting many other animal species. Foodstuffs contaminated with BSE appear to have caused prion disease in several other animal species in the UK, including domestic cats, exotic ungulates and captive cats in zoos. In addition to these "natural" infections, BSE has been experimentally transmitted to a wide variety of species.

Other animal prion diseases

Outbreaks of transmissible mink encephalopathy and chronic wasting disease (CWD) in captive populations of mink, mule deer and elk in the United States have also been attributed to prion-infected foodstuffs, although the origin of prion infection is unclear. Horizontal transmission of CWD prions appears to be highly efficient and CWD-infected wild cervids have now been reported in 14 U.S. states, 2 Canadian provinces and South Korea (Sigurdson, 2008). The possibility of a zoonotic transmission of CWD prions via diet is of particular concern in North America, where hunting of cervids is a popular sport. To date, CWD has not been reported in Europe, although surveillance has been limited.

HUMAN PRION DISEASES

Human prion diseases encompass the three etiological types of prion disease: inherited, sporadic and acquired forms.

Human prion disease most commonly presents itself sporadically

Approximately 85% of cases of human prion disease occur sporadically as Creutzfeldt–Jakob disease at a rate of roughly 1–2 cases per million of the population per year across the world, with onset generally in late adult life, a lifetime risk of ~1 in 30–50,000 and an equal incidence in men and women. While spontaneous conversion of PrP^{C} to PrP^{Sc} as a rare stochastic event or somatic mutation of the PrP gene resulting in expression of a pathogenic PrP mutant are plausible explanations for sporadic CJD, other causes may be involved in at least some cases, such as environmental exposure to human prions or exposure to animal prions (Collinge, 2005). In this regard, the number of prion strains causing sheep scrapie has yet to be established and epidemiological data cannot exclude this as a cause of a proportion of cases. Homozygosity for a common coding polymorphism at codon 129 of *PRNP* encoding either methionine or valine predisposes to the development of sporadic CJD (Collinge, 2005). Additionally, a *PRNP* susceptibility haplotype at or near the *PRNP* locus has been identified as indicating further genetic susceptibility to sporadic CJD (Collinge, 2005).

Pathogenic mutations in the prion protein gene cause inherited prion disease

Approximately 15% of human prion diseases are associated with autosomal dominant pathogenic mutations in *PRNP* (Figure 50-1). The identification of one of the pathogenic *PRNP*

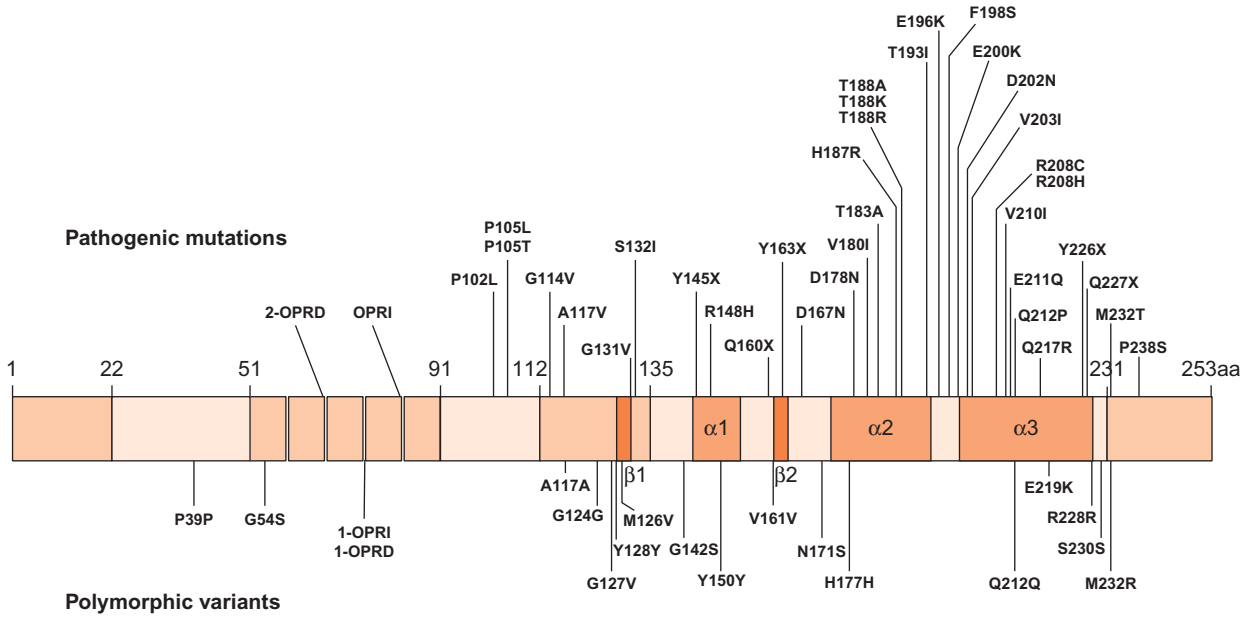

FIGURE 50-1 **Pathogenic mutations and polymorphisms in the human prion protein**. The pathogenic mutations associated with human prion disease are shown above the human PrP coding sequence. These consist of octapeptide repeat insertions (ORPI) within the octapeptide repeat region between codons 51 and 91, a 2-octapeptide repeat deletion (ORPD), and various point mutations causing missense or stop amino acid substitutions. Point mutations are designated by the wild-type amino acid preceding the codon number, followed by the mutant residue, using single-letter amino acid nomenclature. Polymorphic variants are shown below the PrP coding sequence.

mutations in a case with neurodegenerative disease allows diagnosis of an inherited prion disease and subclassification according to mutation and also permits presymptomatic genetic testing in affected families (Collinge, 2005). Over 30 pathogenic mutations have been described in two groups: (1) point mutations resulting in amino acid substitutions in PrP or production of a stop codon resulting in expression of a truncated PrP; and (2) insertions encoding additional integral copies of an octapeptide repeat present in a tandem array of five copies in the normal protein (Figure 50-1). How pathogenic mutations in *PRNP* cause prion disease has yet to be resolved, although, in most cases, the mutation is thought to lead to an increased tendency of PrP^{C} to form PrP^{Sc}. However, different mutations may have profoundly different structural consequences in the expressed protein, acting to destabilize the native PrP^{C} fold, to increase aggregation propensity, to alter cellular trafficking, or to stabilize alternative protein (PrP^{Sc}) structures. While a wealth of data from acquired or sporadic CJD indicates that residue 129 polymorphism critically dictates thermodynamic preferences for disease-related PrP isoforms (Collinge & Clarke, 2007), the full spectrum of effects that different pathogenic *PRNP* mutations have remains unclear. Experimentally manipulated mutations of the prion gene can lead to spontaneous neurodegeneration without the formation of detectable protease-resistant PrP. These findings raise the question of whether all inherited forms of human prion disease invoke disease through the same mechanism, and in this regard it is currently unknown whether all are transmissible by inoculation.

Acquired human prion diseases include kuru and variant CJD

Although the human prion diseases are transmissible diseases, acquired forms have, until recently, been confined to rare and unusual situations. The two most frequent causes of iatrogenic CJD occurring through medical procedures are implantation of dura mater grafts and treatment with human growth hormone derived from the pituitary glands of human cadavers. Less frequent occurrences of human prion disease have resulted from iatrogenic transmission of CJD during corneal transplantation, contaminated electroencephalographic (EEG) electrode implantation and surgical operations using contaminated instruments or apparatus. The most well-known incidents of acquired prion disease in humans resulting from a dietary origin have been cases of kuru, which was transmitted by cannibalism among the Fore linguistic group of the Eastern Highlands in Papua New Guinea (Collinge et al., 2006), and more recently the occurrence of vCJD, which is causally related to BSE in cattle, in the United Kingdom and other countries (Collinge, 2005). Incubation periods of acquired prion diseases in humans can exceed 50 years, and it remains to be seen how many cases of vCJD will eventually occur within the United Kingdom and elsewhere (Collinge, 1999; Collinge, 2001; Collinge et al., 2006).

Prion protein polymorphism contributes genetic susceptibility to prion disease

PRNP codon 129 polymorphism plays a key role in contributing genetic susceptibility to acquired human prion disease (Collinge, 2005). In iatrogenic CJD caused by exposure to contaminated pituitary hormones, *PRNP* codon 129 heterozygotes have a longer mean incubation period. All cases of neuropathologically confirmed vCJD have to date been methionine homozygotes, and homozygotes of either allele have an earlier age of onset for kuru (Collinge, 2005). Kuru imposed strong balancing selection on the Fore population, essentially eliminating *PRNP* 129 homozygotes. Elderly survivors of the kuru epidemic, who had multiple exposures at mortuary feasts, are, in marked contrast to younger unexposed Fore, predominantly *PRNP* 129 heterozygotes (Mead et al., 2003). Recently a novel protective PrP variant with a coding change at residue 127 has been found to colocalize with kuru exposure (Mead et al., 2009b). Variants at codons 127 and 129 of *PRNP* demonstrate the population genetic response to an epidemic of prion disease and represent a powerful episode of recent selection in humans (Mead et al., 2003; Mead et al., 2009b).

Human prion diseases are clinically heterogeneous

Human prion diseases are associated with a range of clinical presentations and are classified by both clinicopathological syndrome and etiology, with subclassification according to molecular criteria. All forms are invariably fatal following clinical onset; however, there is marked phenotypic variability depending upon etiology. Progressive dementia, cerebellar ataxia, pyramidal signs, chorea, myoclonus, extrapyramidal features, pseudobulbar signs, seizures and amyotrophic features are seen in variable combinations. The clinical presentation in classical CJD is a rapidly progressive dementia accompanied by myoclonus followed by decline to akinetic mutism and death, often within 3–4 months. Traditionally, inherited prion diseases have been classified by the presenting clinical syndromes, which fall into three main subdivisions: GSS, CJD and FFI. GSS classically presents as a chronic cerebellar ataxia with pyramidal features, with dementia occurring much later in a clinical course that is typically much longer than that seen in classical CJD. FFI is characterized by progressive, untreatable insomnia, dysautonomia, dementia and selective thalamic degeneration; FFI is most commonly associated with a missense mutation at codon 178 of *PRNP*, although sporadic FFI with no causative mutation in *PRNP* has been reported. The availability of direct gene markers for inherited prion diseases has enabled identification of highly atypical cases and has widened the known phenotypic range for these disorders. Some families show remarkable phenotypic variability that can encompass both CJD- and GSS- like cases as well as other cases that do not conform to CJD, GSS or FFI phenotypes. Because of its extensive phenotypic variability and ability to mimic other neurodegenerative conditions, notably Alzheimer's disease, prion disease and *PRNP* analysis should be considered in the investigation of all presenile ataxias and dementias, even in the absence of an apparent family history of neurodegenerative illness (Collinge, 2005). The clinical features of kuru consist of a progressive cerebellar ataxia accompanied by dementia in the later stages, and death, which usually occurs within 12 months. Iatrogenic prion disease arising from intracerebral or optic inoculation

usually manifests itself clinically as classical CJD, while CJD arising from a peripheral route of inoculation, such as pituitary growth hormone, commonly presents with a progressive ataxia. The early clinical presentation of vCJD resembles kuru more than classical CJD and consists of behavioral and psychiatric disturbances, peripheral sensory disturbance and cerebellar ataxia. For reviews on human prion disease see Collinge, 2005; Wadsworth et al., 2008.

PRION DISEASE PATHOLOGY AND PATHOGENESIS

Peripheral pathogenesis involves the lymphoreticular system

Although the pathological consequences of prion infection occur in the central nervous system and experimental transmission of these diseases is most efficiently accomplished by intracerebral inoculation, most natural infections do not occur by these means. Indeed, administration to sites other than the central nervous system is known to be associated with much longer incubation periods, which may extend over decades (Collinge, 2001; Collinge et al., 2006). Experimental evidence suggests that this latent period is associated with clinically silent prion replication in lymphoreticular tissue, whereas neuroinvasion takes place later. The *M* cells in the intestinal epithelium mediate prion entry from the gastrointestinal lumen into the body. Follicular dendritic cells (FDCs) may have a major role in prion replication and are the principal site of accumulation of disease-associated PrP^{Sc} within secondary lymphoid organs. B-cell–deficient mice are resistant to intraperitoneal inoculation with prions, probably because of their involvement with FDC maturation and maintenance. The interface between FDCs and sympathetic nerves represents a critical site for the transfer of lymphoid prions into the nervous system; however, the mechanism by which this is achieved remains unknown. Distinct forms of prion disease show differences in lymphoreticular involvement that may be related to the etiology of the disease or to divergent properties of distinct prion strains. For a review of prion disease peripheral pathogenesis, see Aguzzi & Calella, 2009.

Prion disease produces characteristic pathology in the central nervous system

The brains of patients or animals with prion disease frequently show no recognizable abnormalities on gross examination at necropsy; but microscopic examination of the central nervous system typically reveals characteristic histopathological changes, consisting of neuronal vacuolation and degeneration, which give the cerebral gray matter a microvacuolated or "spongiform" appearance, and a reactive proliferation of astroglial cells. Although spongiform degeneration is frequently detected, it is not an obligatory neuropathologic feature of prion disease; the presence of astrogliosis and microgliosis, although not specific to the prion diseases, is more constantly seen. The lack of a classical lymphocytic inflammatory response is also an important characteristic. Demonstration of abnormal PrP immunoreactivity or, more specifically, biochemical detection of PrP^{Sc} in brain material by immunoblotting techniques, is diagnostic of prion disease. Some forms of prion disease are characterized by deposition of amyloid plaques composed of insoluble aggregates of PrP (Brandner et al., 2008). Amyloid plaques are a notable feature of kuru and GSS but they are less frequently found in the brains of patients with sporadic CJD, who typically show a diffuse pattern of abnormal PrP deposition (Figure 50-2). The histopathological features of vCJD are relatively consistent when compared to sporadic CJD and distinguish vCJD from other human prion diseases. The most distinctive feature of vCJD is the presence of large numbers of PrP-positive amyloid plaques that differ in morphology from the plaques seen in kuru and GSS in that the surrounding tissue in vCJD takes on a microvacuolated appearance, giving the plaques a florid appearance (Figure 50-2). vCJD is distinct in its pathogenesis from other human prion diseases as PrP^{Sc} is readily detectable in lymphoreticular tissues in vCJD and not in classical CJD, kuru or inherited cases of human prion disease. Tonsil biopsy is used for antemortem diagnosis of vCJD, and to date has shown 100% sensitivity and specificity for diagnosis of vCJD, including at an early clinical stage. The demonstration of extensive lymphoreticular involvement in the peripheral pathogenesis of vCJD raises concerns that iatrogenic transmission of vCJD prions through medical procedures may be a major public health issue (Collinge, 2005; Wadsworth & Collinge, 2007).

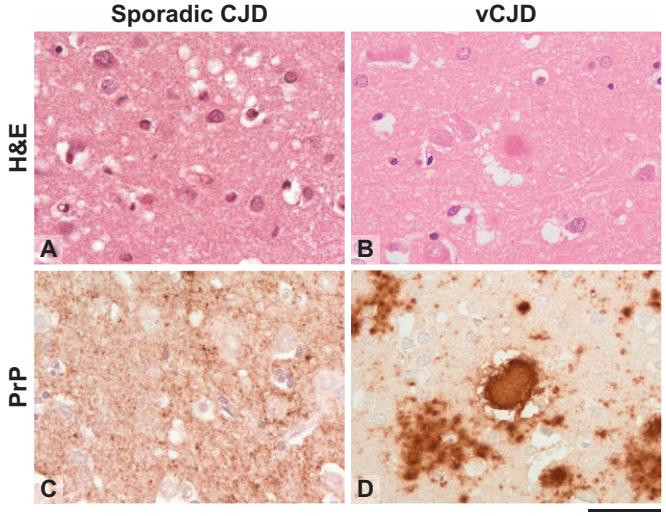

FIGURE 50-2 **Prion disease pathology**. Brain sections from sporadic CJD **(A, C)** and vCJD **(B, D)** show spongiform neurodegeneration following hematoxylin and eosin staining **(H & E)** and abnormal PrP immunoreactivity following immunohistochemistry using an anti-PrP monoclonal antibody (**PrP**). Abnormal PrP deposition in sporadic CJD most commonly presents as diffuse, synaptic staining, whereas vCJD is distinguished by the presence of florid PrP plaques consisting of a round amyloid core surrounded by a ring of spongiform vacuoles. Scale bar: 50μm. Figure courtesy of Prof. Sebastian Brandner.

THE PROTEIN-ONLY HYPOTHESIS OF PRION PROPAGATION

A wide body of data now supports the idea that infectious prions consist principally or entirely of an abnormal isoform of PrP (Prusiner, 1998; Collinge, 2001; Riesner, 2003; Collinge & Clarke, 2007). Disease-associated PrP, designated PrP^{Sc}, is derived from PrP^{C} by a post-translational mechanism and neither amino acid sequencing nor systematic study of known post-translational modifications have shown any consistent covalent differences between PrP^{C} and PrP^{Sc} (Prusiner, 1998; Collinge, 2001).

Prion propagation involves conversion of PrP^{C} to PrP^{Sc}

The protein-only hypothesis, in its current form, argues that prion propagation occurs through PrP^{Sc} acting to replicate itself with high fidelity by recruiting endogenous PrP^{C} and that this conversion involves only conformational change (Prusiner, 1998; Collinge, 2001; Collinge & Clarke, 2007). It is hypothesized that prions are self-propagating fibrillar or amyloid forms of PrP in which the ends of the propagating fibrils constitute the infectious entity and the exponential rise in prion titer during disease progression is a consequence of fiber fragmentation (Figure 50-3). However, the underlying molecular events during infection that lead to the conversion of PrP^{C} to PrP^{Sc} and how PrP^{Sc} accumulation leads to neurodegeneration remain poorly defined. The most coherent and general model thus far proposed is that the protein PrP fluctuates between a dominant native state, PrP^{C}, and a series of minor conformations, one or a set of which can self-associate in an ordered manner to produce a stable supramolecular structure, PrP^{Sc}, composed of misfolded PrP monomers. Once a stable "seed" structure is formed, PrP can then be recruited, leading to an explosive, autocatalytic formation of PrP^{Sc}. Such a mechanism could underlie prion propagation and account for the transmitted, sporadic and inherited etiologies of prion disease. Initiation of a pathogenic self-propagating conversion reaction, with accumulation of aggregated PrP, may be induced by exposure to a "seed" of aggregated PrP following prion inoculation, as a rare stochastic conformational change, or as an inevitable consequence of expression of a pathogenic PrP^{C} mutant that is predisposed to form misfolded PrP (Figure 50-3). Such a system would be extremely sensitive to three factors: (1) overall PrP^{C} concentration; (2) the equilibrium distribution between the native conformation and the self-associating conformation; and (3) complementarity between surfaces that come together in the aggregation step. All three of these predictions from this minimal model are manifested in the etiology of prion disease: an inversely proportional relationship between PrP^{C} expression and prion incubation period in transgenic mice; predisposition by relatively subtle mutations in the protein sequence; and a requirement for molecular homogeneity between PrP^{Sc} and PrP^{C} for efficient prion propagation (Prusiner, 1998; Collinge & Clarke, 2007). It is clear that a full understanding of prion propagation will require knowledge of both the structure of PrP^{C} and PrP^{Sc} and the mechanism of conversion between them.

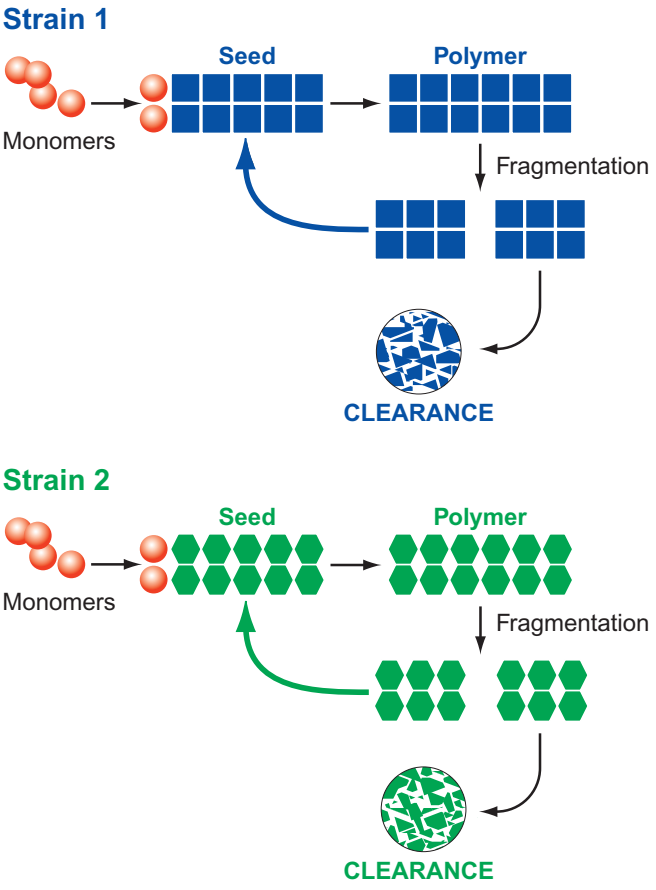

FIGURE 50-3 **The protein-only model of prion propagation and basis of prion strains.** Prion propagation proceeds by recruitment of PrP monomers onto a preexisting PrP polymer template followed by fission to generate more templates in an autocatalytic manner. This mechanism can account for the transmitted, sporadic and inherited etiologies of prion disease. Initiation of a pathogenic self-propagating conversion reaction may be induced by exposure to a "seed" of PrP^{Sc} following prion inoculation, or as a rare stochastic conformational change in wild-type PrP^{C}, or as an inevitable consequence of expression of a pathogenic PrP^{C} mutant that is predisposed to form misfolded PrP. Distinct PrP polymer types with different PrP conformations and assembly states can propagate themselves, accounting for different prion strains.

CHARACTERIZATION OF PRP^{C}

PrP^{C} has a predominantly alpha-helical conformation

The conformation of the cellular isoform of PrP was first established by NMR measurements made on the recombinant mouse protein. Since then, NMR measurements on recombinant hamster and human PrP show that they have essentially the same conformation (Wuthrich & Riek, 2001) and these data have been supported by crystallographic determination of structure (Antonyuk et al., 2009). Following cleavage of an N-terminal signal peptide and removal of a C-terminal peptide on addition of a GPI anchor, the mature PrP^{C} species

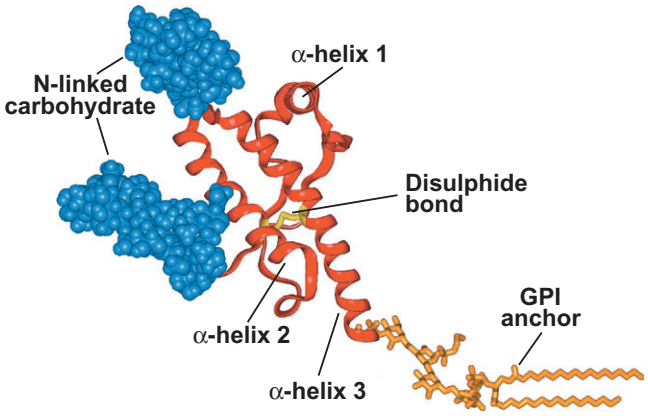

FIGURE 50-4 **Model structure of PrP^C.** The conformation of recombinant mouse PrP (residues 124-231) determined by NMR is shown in red ribbon. The single disulfide bridge linking α-helixes 2 and 3 is shown in yellow. N-linked carbohydrate groups are shown as space-filling structures in blue. The glycosylphosphatidylinositol (GPI) anchor that attaches PrP to the outer surface of the cell membrane is shown in gold.

consists of an N-terminal region of about 100 amino acids, which is unstructured in the isolated molecule in solution, and a C-terminal segment, also around 100 amino acids in length. The C-terminal domain is folded into a largely α-helical conformation (three α-helices and a short anti-parallel β-sheet) and stabilized by a single disulfide bond linking helices 2 and 3 (Figure 50-4). There are two asparagine-linked glycosylation sites (Figure 50-4). The N-terminal region contains a segment of five repeats of an eight-amino-acid sequence (the octapeptide-repeat region), expansion of which by insertional mutation leads to inherited prion disease. While unstructured in the isolated molecule, this region contains a tight binding site for a single Cu^{2+} ion with a second tight Cu^{2+} ion-binding site present upstream of the octapeptide-repeat region but before the structured C-domain. Both high-affinity Cu^{2+} binding sites can also coordinate Zn^{2+} ions with dissociation constants of physiological relevance, and it is thought that the N-terminal region of PrP may acquire coordinated structure *in vivo* through interaction with either Cu^{2+}or Zn^{2+} ions. The structured C-domain folds and unfolds reversibly in response to chaotropic denaturants, and it appears that there are no populated intermediates in the folding reaction and that the protein displays unusually rapid rates of folding and unfolding. These data suggest that PrP^{Sc} is unlikely to be formed from a kinetic folding intermediate, as has been hypothesized in the case of amyloid formation in other systems, and is more likely to be formed from the unfolded state of PrP (Collinge et al., 2007). Pathogenic mutations in *PRNP* seen in inherited prion diseases may produce disease by destabilizing PrP^C, which would predispose the molecule to aggregate.

Reverse genetics approaches to studying PrP^C

Reintroduction of PrP transgenes into knockout mice lacking the PrP gene ($Prnp^{o/o}$ mice) restores susceptibility to infection in a species-specific manner that allows reverse genetics approaches to studying structure–function relationships in PrP (for review see Weissmann & Fleschig, 2003). PrP in its entirety is unnecessary for prion propagation. Not only can the unstructured N-terminal 90 amino acids be deleted, but also the first α-helix, the second β-strand and part of helix 2. In transgenic animals, a 106-amino-acid fragment of the protein comprising PrPΔ23-88 and Δ141-176 conferred susceptibility to, and propagation of, prions (Weissmann & Fleschig, 2003). Notably, while expression of PrP N-terminal deletion mutants to residue 106 are tolerated and supportive of prion propagation, deletion beyond this leads to severe ataxia and neuronal loss in the granular cell layer of the cerebellum (Weissmann & Fleschig, 2003). Intriguingly, the Doppel protein (Dpl), which has a structure similar to N-terminally truncated PrP, causes a similar cerebellar effect when ectopically expressed in the brain. The severity of neurotoxicity correlates with the level of Dpl expression and can be rescued by PrP^C expression, indicating that PrP^C, Dpl and ΔPrP might compete for a common hypothetical receptor or ligand L_{PrP} that transduces neuroprotective signals when bound to PrP^C but not when bound to Dpl or ΔPrP (Weissmann et al., 2003). This model also proposes the existence of a PrP^C-like protein termed Π that is capable of compensating for the absence of PrP^C in $Prnp^{0/0}$ mice. It has been demonstrated that the protein Sho is a GPI-anchored neuronal glycoprotein present in the CNS from early postnatal life that can counteract the neurotoxic effects of either Dpl or ΔPrP and is therefore a candidate for Π (Watts et al., 2007).

The function of PrP^C remains unknown

PrP is highly conserved among mammals, is identified in marsupials, birds, amphibians and fish, and may therefore be present in all vertebrates. It is expressed during early embryogenesis and is found in most tissues in the adult with the highest levels of expression in the central nervous system, particularly in association with synaptic membranes. PrP is also widely expressed in cells of the immune system. Because it is a GPI-anchored cell surface glycoprotein, PrP may have a role in cell adhesion or signaling processes, but its precise cellular function has remained obscure. Newly synthesized PrP^C is transported to the cell surface from which it has been reported to cycle via either clathrin-coated vesicles or caveolae-dependent endocytic pathways. A range of PrP^C-interacting molecules has been identified; however, despite a growing body of evidence suggesting that PrP^C may play a role in cell survival through interaction with apoptotic pathways, the *in vivo* relevance of such interactions has yet to be convincingly demonstrated. A role for PrP in copper or zinc metabolism or transport seems possible and disturbance of this function by the conformational transition from normal to disease-related isoforms of PrP may be involved in prion-related neurotoxicity.

PrP knockout mice have subtle abnormalities

While PrP^C is absolutely required for prion propagation and neurotoxicity, knockout of PrP^C in embryonic models or in adult brain has no overt phenotypic effect that influences lifespan or fertility. These findings demonstrate that acute loss

of neuronal PrP in adulthood is tolerated, and that prion neurodegeneration is not due to loss of normal PrP function in neurons (Mallucci & Collinge, 2005; Aguzzi & Calella, 2009). However, *Prnp*$^{o/o}$ mice are not entirely normal, (for reviews see Collinge, 2001; Weissmann & Fleschig, 2003; Aguzzi & Calella, 2009). In addition to a proposed role for PrP^{C} in providing neuroprotective signals, abnormalities in synaptic physiology, circadian rhythms, cognition and olfactory physiology have been reported (Collinge, 2001; Aguzzi & Calella, 2009). Important functional correlates of abnormalities of synaptic transmission in *Prnp*$^{o/o}$ mice include cognitive deficits and impairment of olfactory physiology which can be rescued by transgenic neuronal expression of PrP (Aguzzi & Calella, 2009). It has been revealed that axonal PrP expression is required for peripheral myelin maintenance (Bremer et al., 2010) and this finding correlates strongly with earlier demonstrations of extensive demyelination in transgenic mice expressing mutated PrP with deletions in the central domain (Aguzzi & Calella, 2009). Importantly, despite current uncertainties regarding the conversion of PrP^{C} to PrP^{Sc} and possible mechanisms of neurotoxicity (Collinge & Clarke, 2007), the prevention of this conversion in neurons by conditional knock out of PrP^{C} has been shown to prevent disease progression and to reverse early degenerative changes (Mallucci et al., 2003). These data have firmly established PrP^{C} as the prime target for rational therapeutics in prion disease (Mallucci & Collinge, 2005; Nicoll & Collinge, 2009).

CHARACTERIZATION OF PrP^{SC}

PrP^{Sc} has a predominantly beta-sheet conformation

PrP^{Sc} is extracted from affected brains as highly aggregated detergent insoluble material that is not amenable to high-resolution structural techniques. However, Fourier transform infrared spectroscopic methods show that PrP^{Sc}, in sharp contrast to PrP^{C}, has a high β-sheet content. PrP^{Sc} is covalently indistinguishable from PrP^{C} but can be distinguished from PrP^{C} by its partial resistance to proteolysis and its marked insolubility in detergents; (for reviews see Prusiner, 1998; Riesner, 2003). Under conditions in which PrP^{C} exists as a detergent-soluble monomer and is completely degraded by the nonspecific protease proteinase K, PrP^{Sc} exists in an aggregated form with the C-terminal two thirds of the protein showing marked resistance to proteolytic degradation, leading to the generation of amino-terminally truncated fragments of di-, mono- and non-glycosylated PrP (Figure 50-5). For a particular PrP^{Sc} isoform, all three PrP^{Sc} glycoforms show equivalent accessibility to proteinase K and are cleaved at the same point in the N-terminus of the protein (Figure 50-5).

Prion structure remains unknown

The precise structure of the infectious agent in prion disease remains unclear. While there is no evidence for a specific prion-associated nucleic acid (Prusiner, 1998; Safar et al., 2005), purified prion rods do contain an inert polysaccharide

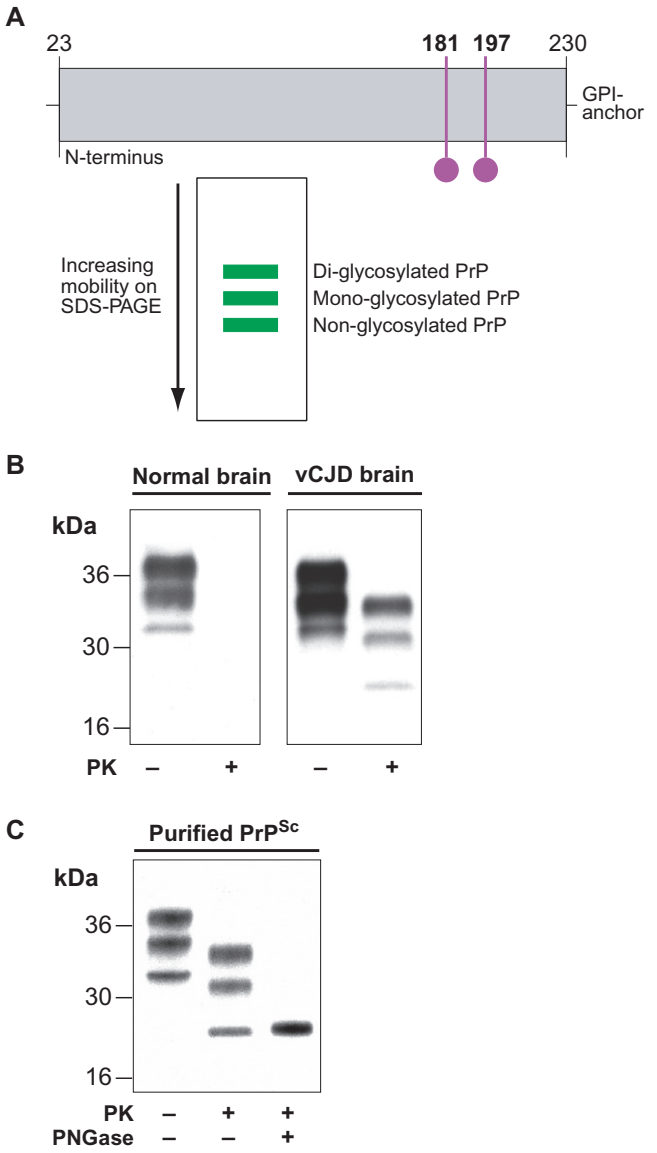

FIGURE 50-5 **Western blot analysis of PrP. (A)** Following cleavage of an N-terminal signal peptide and removal of a C-terminal peptide on addition of a GPI anchor, mature human PrP^{C} consists of a 208-residue polypeptide that contains two sites for N-linked glycosylation at asparagine residues 181 and 197. PrP^{C} is expressed as di-, mono-, and non-glycosylated forms, giving rise to three principal PrP bands after sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). **(B)** Western blot analysis of normal human brain and vCJD brain homogenates before and after treatment with proteinase K (PK). PrP^{C} in both normal and vCJD brain is completely degraded by proteinase K, whereas PrP^{Sc} present in vCJD brain shows resistance to proteolytic degradation leading to the generation of amino-terminally truncated fragments of di-, mono- and non-glycosylated PrP. **(C)** Western blot analysis of purified PrP^{Sc} from vCJD brain before and after treatment with proteinase K (PK) or after consecutive treatment with proteinase K and peptide-N-glycosidase F (PNGase). Proteinase K cleaves at the same point in the N-terminus of all three PrP^{Sc} glycoforms, as removal of N-linked carbohydrate with PNGase results in the generation of a single band corresponding to the nonglycosylated proteolytic fragment of PrP^{Sc}.

scaffold (Riesner, 2003). The current working hypothesis is that an abnormal isoform of PrP (PrP^{Sc}) is the infectious agent (Prusiner, 1998; Riesner, 2003; Collinge & Clarke, 2007; Aguzzi & Calella, 2009). To date however, the most highly enriched preparations of prions contain an excess of PrP per infectious particle (Safar et al., 2005; Silveira et al., 2005) and it remains unclear whether only a minority of PrP molecules are actually infectious or whether PrP aggregates of defined size are necessary for infectivity (Silveira et al., 2005). Understanding the ratio of PrP molecules to infectivity is highly convoluted, however, because of rapid clearance of prions from the brain after intracerebral challenge (Safar et al., 2005). While abnormal isoforms of PrP are undoubtedly the major constituents of mammalian prions, there is a growing consensus in the field that something may be missing (Deleault et al., 2007). In this regard, the precise identification of minor 'contaminants' that co-purify with PrP^{Sc} may still be of critical importance to understanding infectious prion composition and the determinants of strain or the ability of a prion to infect a host (pathogenicity).

In vitro generation of alternative PrP conformations and prion infectivity

Direct *in vitro* mixing experiments were the first attempts to produce PrP^{Sc} *in vitro* (Caughey, 2003). In such experiments, PrP^{Sc} was used in excess as a seed to convert PrP^{C} to a protease-resistant form, designated PrP^{Res}. While there are now many historical examples in the literature of conditions that generated PrP^{Res}, such reactions did not produce demonstrable *de novo* prion infectivity (Caughey, 2003). Likewise, numerous studies examining the activity of alternative conformational states of recombinant PrP enriched for beta-sheet secondary structure failed to show that they were sufficient to cause prion disease in an experimental host. However, the discovery of a protein-misfolding, cyclic amplification (PMCA) system established that substantial amplification of PrP^{Res} and prion infectivity can be achieved in the absence of living cells (Castilla et al., 2008). Despite the significance of this discovery, proof of the protein-only hypothesis still requires *in vitro* prion amplification to be achieved using entirely defined substrates rather than brain homogenate. Attempts to achieve this have shown that non-proteinaceous cofactors (including lipids and polyanionic compounds such as RNA) may be necessary to catalyze the generation of infectious prions from highly purified recombinant PrP produced in *E. coli* (Deleault et al., 2007). Although the generation of infectious prions from recombinant PrP alone appears possible (Colby et al., 2009; Kim et al., 2010), to date the low prion titer of such preparations has precluded meaningful structural analysis.

THE MOLECULAR BASIS OF PRION STRAIN DIVERSITY

A major problem for the "protein-only" hypothesis of prion propagation has been how to explain the existence of multiple isolates, or strains, of prions. Prion strains are distinguished by their biological properties: they produce distinct incubation periods and patterns of neuropathological targeting (so-called lesion profiles) in defined inbred mouse lines (Collinge & Clarke, 2007). As they can be serially propagated in inbred mice with the same *Prnp* genotype, they cannot be encoded by differences in PrP primary structure. Usually, distinct strains of conventional pathogen are explained by differences in their nucleic acid genome. However, in the absence of such a scrapie genome, alternative possibilities must be considered. Weissmann proposed a "unified hypothesis" where, although the protein alone was argued to be sufficient to account for infectivity, it was proposed that strain characteristics could be encoded by a small cellular nucleic acid, or "co-prion" (Weissmann, 1991). Although this hypothesis leads to the testable prediction that strain characteristics, unlike infectivity, would be sensitive to UV irradiation, no such test has been reported. At the other extreme, the protein-only hypothesis would have to explain how a single polypeptide chain could encode multiple disease phenotypes. Clearly, understanding how a protein-only infectious agent could encode such phenotypic information is of considerable biological interest.

Prion strain diversity appears to be encoded by PrP itself

Support for the contention that strain specificity may be encoded by PrP itself was provided by study of two distinct strains of transmissible mink encephalopathy prions which can be serially propagated in hamsters, designated *hyper* and *drowsy*. These strains can be distinguished by differing physiochemical properties of the accumulated PrP^{Sc} in the brains of affected hamsters. Following limited proteolysis, strain-specific migration patterns of PrP^{Sc} on Western blots are seen that relate to different N-terminal ends of PrP^{Sc} following protease treatment, implying differing conformations of PrP^{Sc} (Bessen et al., 1994). Subsequently, it was discovered that different human PrP^{Sc} isoforms propagate in the brain of patients with phenotypically distinct forms of CJD (Collinge et al., 1996; Parchi et al., 1996; Telling et al., 1996; Collinge, 2005; Wadsworth & Collinge, 2007). It is crucial that these biochemical properties of PrP^{Sc}, indicative of differences in PrP conformation and glycosylation, could be maintained after passage in transgenic mice expressing human PrP consistent with these biochemical differences encoding strain variation (Collinge et al., 1996). Further evidence for PrP conformation enciphering strain diversity was provided by similar studies with transgenic mice expressing a chimeric human mouse PrP (Telling et al., 1996). Collectively, these findings suggested that mammalian prion strain variation is encoded by a combination of PrP conformation and assembly state, and this hypothesis has now been strongly supported by many other studies involving different prion strain–host combinations (for review see Prusiner, 1998; Safar et al., 1998; Collinge & Clarke, 2007; Castilla et al., 2008; Aguzzi & Calella, 2009).

Distinct PrP^{Sc} types are seen in human prion disease

The different fragment sizes seen on Western blots after treatment with proteinase K suggests that there are several different human PrP^{Sc} conformations, referred to as molecular

strain types (Figure 50-6). These types can be further classified by the ratio of the three PrP bands seen after protease digestion, corresponding to amino-terminally truncated cleavage products generated from di-, mono- or non-glycosylated PrP^{Sc}. Four types of human PrP^{Sc} have now been commonly identified using molecular strain typing (Figure 50-6), although much greater heterogeneity seems likely (Collinge, 2005; Wadsworth & Collinge, 2007). Sporadic and iatrogenic CJD and kuru are associated with PrP^{Sc} types 1-3, while type 4 PrP^{Sc} is uniquely associated with vCJD and is characterized by a fragment size and glycoform ratio that is distinct from PrP^{Sc} types 1-3 but similar to PrP^{Sc} seen in BSE and BSE when transmitted to several other species (Collinge, 2001; Wadsworth & Collinge, 2007; Wadsworth et al., 2008). Polymorphism at *PRNP* residue 129 dictates the propagation of distinct PrP^{Sc} types in humans (Collinge & Clarke, 2007; Wadsworth & Clarke, 2007) and it has become clear that prion strain selection and the propagation of distinct PrP^{Sc} types may be crucially influenced by other genetic loci of the host genome (Collinge, 2001; Collinge & Clarke, 2007; Mead et al., 2009a). The identification of strain-specific PrP^{Sc} structural properties allows an etiology-based classification of human prion disease by typing of the infectious agent itself. Molecular strain typing of prion isolates currently facilitates molecular diagnosis of sporadic and variant CJD and provides the means for epidemiological studies investigating the etiologies of sporadic CJD and BSE-related human prion disease. Stratification of human prion disease by molecular criteria may enable recognition of a change in relative frequencies of particular disease subtypes in relation to either BSE exposure patterns or iatrogenic sources of vCJD prions.

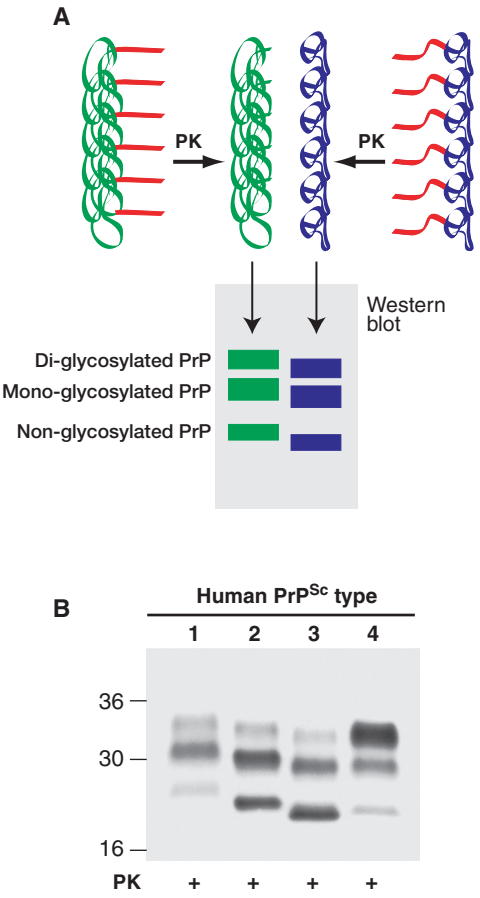

FIGURE 50-6 **Molecular analysis of PrP^{Sc} isoforms. (A)** The schematic demonstrates the principle of molecular strain typing of PrP^{Sc} isoforms by limited proteolytic digestion and Western blotting. Two distinct aggregates of PrP^{Sc} with differing conformations (shown in green or blue) present different accessibilities to proteinase K within the N-terminal region of the protein (shown in red). Disparity in the most C-terminal scissile bond accessible to proteinase K results in a different mobility of C-terminal PrP^{Sc} proteolytic fragments generated from of di-, mono- and non-glycosylated PrP^{Sc} that can be visualized by western blotting. **(B)** Western blot showing proteinase K digestion products from distinct human PrP^{Sc} conformers designated PrP^{Sc} types 1–4. PrP^{Sc} types 1-3 are seen in the brain of classical forms of CJD (either sporadic or iatrogenic CJD) and kuru, PrP^{Sc} type 4 is uniquely seen in vCJD brain and differs markedly in the proportions of di- and mono-glycosylated glycoforms.

Difficulties in defining human prion strains

The hypothesis that alternative conformations or assembly states of PrP provide the molecular substrate for a significant part of the clinicopathological heterogeneity seen in human prion diseases and that this relates to the existence of distinct human prion strains is supported by considerable experimental evidence (Prusiner, 1998; Collinge & Clarke, 2007; Castilla et al., 2008) and by the demonstration of protein-conformation–based inheritance mechanisms of yeast prions (Wickner et al., 2007). Despite these advances, the precise molecular basis of mammalian prion strain diversity is unknown. A major confounding issue in this regard has been in resolving whether relatively subtle conformational differences in PrP^{Sc} are biologically important and accurately reflect the propagation of distinct human prion strains. This is particularly true in sporadic CJD, where progress has been severely hampered by a lack of transgenic modeling data to firmly distinguish the identity of distinct prion strains and their defining molecular and neuropathological phenotypes (Wadsworth & Collinge, 2007). This fundamental problem coupled with the difficulties and variability of the biochemical methods used to distinguish PrP^{Sc} types has so far precluded an internationally accepted classification system for human prion strains (Collinge, 2001; Wadsworth & Collinge, 2007). In this regard, the increasingly recognized co-occurrence of different PrP^{Sc} types in the same brain and the recognition that protease-sensitive pathological isoforms of PrP may have a significant role in both animal and human prion disease have further confounded progress. All of these factors, together with the known ability of genetic background to influence prion strain selection (Collinge, 2001; Collinge & Clarke, 2007; Mead et al., 2009a) and knowledge that route of transmission in acquired human prion disease may dramatically influence clinical and neuropathological presentation (Wadsworth et al., 2008), have re-emphasized the requirement to remove host variability by identifying distinct human prion strains in appropriate transgenic models.

PRION TRANSMISSION BARRIERS

Prion transmission between species is limited by a barrier

A "species barrier" restricts transmission of prion disease between different mammalian species. Usually, on primary passage of prions from species A to species B, not all inoculated animals of species B develop disease, and those that do have much longer and more variable incubation periods than those that are seen with transmission of prions within the same species. On second passage of infectivity to further animals of species B, transmission parameters resemble those of within-species transmissions, with most if not all animals developing the disease with short and consistent incubation periods. Species barriers can therefore be quantified by measuring the fall in mean incubation period on primary and second passage, or, perhaps more rigorously, by a comparative titration study. The effect of a very substantial species barrier is that few, if any, animals succumb to clinical prion disease at all on primary passage, and then only at incubation periods approaching the natural lifespan of the species concerned (Collinge, 2001; Collinge & Clarke, 2007).

Both PrP sequence and prion strain type influence prion transmission barriers

Early studies of the molecular basis of the species barrier argued that it principally resided in differences in PrP primary structure between the species from which the inoculum was derived and the inoculated host. However, it has been clear for many years that prion strain type also has a crucial effect on species barriers. For example, the natural transmission of BSE to a wide variety of hosts, and the subsequent demonstration that transmission of BSE from these different species (all with varied PrP primary amino acid sequences) to mice results in the maintenance of a 'BSE-like' strain, provides a key example of where effects from a prion strain appear more important than PrP primary sequence homology to such barriers (Collinge & Clarke, 2007). Similarly, after transmission to transgenic mice expressing only human PrP, there is disparity in the behavior of sporadic or vCJD prions that have originated in humans expressing wild-type PrP of identical primary sequence. Whereas sporadic CJD prions have transmission characteristics consistent with the complete absence of a species barrier, vCJD prions have transmission properties that are completely distinct from other human prions but closely similar to those of cattle BSE and that are consistent with the presence of a barrier (Collinge, 2001; Collinge et al., 2007; Wadsworth et al., 2008). The term "species barrier" does not seem appropriate to describe such effects, and the general term "transmission barrier" now seems more preferable (Collinge & Clarke, 2007).

A conformational selection model of prion transmission barriers

A conformational selection model of prion transmission barriers has been proposed that encompasses contributions from both PrP sequence and strain-specific PrP^{Sc} structural properties (Collinge, 1999; Collinge, 2001; Collinge & Clarke, 2007). Both PrP amino acid sequence and prion strain type will affect the three-dimensional structure of glycosylated PrP, which will presumably, in turn, affect the efficiency of the protein-protein interactions thought to determine prion propagation. Mammalian PrP genes are highly conserved and presumably only a restricted number of different PrP^{Sc} conformations (that are highly stable and can therefore be serially propagated) will be permissible thermodynamically and will constitute the range of prion strains seen. PrP glycosylation may be important in stabilizing particular PrP^{Sc} conformations. While a significant number of different PrP^{Sc} conformations may be possible among the range of mammalian PrPs, only a subset of these would be allowable for a given single mammalian PrP sequence. Substantial overlap between the favored conformations for PrP^{Sc} derived from species A and species B might therefore result in relatively easy transmission of prion diseases between these two species, while two species with no preferred PrP^{Sc} conformations in common would have a large barrier to transmission (and indeed transmission would necessitate a change of strain type). According to such a model of a prion transmission barrier, BSE may represent a thermodynamically highly favored PrP^{Sc} conformation that is permissive for PrP expressed in a wide range of different species, accounting for the remarkable promiscuity of this strain in mammals (Collinge, 1999; Collinge, 2001; Collinge & Clarke, 2007). Homozygosity at polymorphic residue 129 of human PrP remains the key genetic susceptibility factor for sporadic and acquired prion disease, and in vCJD it represents the strongest known common genetic susceptibility polymorphism in any human disease (Collinge, 2005; Mead et al., 2009a). Heterozygosity at codon 129 is thought to confer resistance to prion disease by inhibiting homologous protein–protein interactions essential for efficient prion replication, while the presence of methionine or valine at residue 129 controls the propagation of distinct human prion strains via conformational selection (Collinge, 1999; Collinge, 2001; Collinge & Clarke, 2007). To date, the repertoire of pathogenic PrP isoforms that can be stably propagated by human PrP with 129 methionine or 129 valine remains unknown (Wadsworth & Collinge, 2007). Contributions of other components to transmission barriers are possible and may involve interacting cofactors which mediate the efficiency of prion propagation, although no such factors have yet been convincingly identified (Caughey, 2003).

Subclinical forms of prion disease pose a risk to public health

The assessment of species barriers has historically relied on the development of a clinical disease in inoculated animals; however, during infection with prion diseases, infectious titers in the brain rise progressively throughout prolonged, clinically silent incubation periods. Therefore, asymptomatic animals can have significant infectious titers in the brain and other tissues. However, subclinical forms of prion infection that are distinct from preclinical forms have now been recognized in which animals become asymptomatic carriers of infectivity and do not develop clinical disease during a normal lifespan. Such carrier states are well recognized in other

infectious diseases. The high incidence of subclinical prion infection seen in BSE- and vCJD-inoculated transgenic mice expressing human PrP, together with other recent data demonstrating extensive subclinical prion infection in other rodent models of prion disease indicate that current definitions of transmission barriers, conventionally assessed on the basis of occurrence of clinical disease in inoculated animals, must be reassessed (Collinge & Clarke, 2007). Subclinical or carrier states of prion disease have major implications for public health, most notably iatrogenic transmission from apparently healthy individuals.

The mechanism of prion-mediated neurodegeneration is unknown

Various hypotheses have been proposed to explain the mechanism of spongiform change and neuronal cell loss. Two hypotheses are: direct neurotoxic effects from a region of the prion protein encompassing residues 106–126 and increased oxidative stress in neurons as a result of PrP^{C} depletion since PrP^{C} has been proposed to function as an antioxidant molecule. However, both hypotheses are controversial. It has also been suggested that PrP^{C} plays a role in regulating apoptosis, with disturbance of normal cellular levels of PrP during infection leading to cell death. Although PrP^{C} expression is required for susceptibility to the disease, a number of observations argue that PrP^{Sc} and indeed prions (whether or not they are identical) may not themselves be highly neurotoxic (Collinge & Clarke, 2007). Prion diseases in which PrP^{Sc} is barely or not detectable have been described, and different strains of prions are known to differ in their degree of protease resistance (Collinge, 2001). Mice with reduced levels of PrP^{C} expression have extremely high levels of PrP^{Sc} and prions in the brain, and yet remain well for several months after their wild-type counterparts succumb. Conversely, transgenic mice, with high levels of murine PrP^{C}, have short incubation periods and yet produce low levels of PrP^{Sc} after inoculation with mouse prions. In addition, wild-type brain grafts producing high levels of PrP^{Sc} do not damage adjacent tissue in *Prnp*$^{o/o}$ mice. The demonstration that animals with subclinical prion infection can harbor high levels of prion infectivity and detectable PrP^{Sc} without exhibiting any clinical signs of prion disease challenges our understanding of the pathogenic mechanisms involved in these diseases (Collinge, 2001; Collinge & Clarke, 2007). A possible explanation is that PrP^{Sc} is itself relatively inert, but toxicity resides in a smaller, labile, oligomeric PrP species (named PrP^{L} for lethal), generated as an intermediate or side product during prion propagation (Collinge & Clarke, 2007). Neurotoxicity may require a critical PrP^{L} concentration that is reached during conventional infections, but the slower kinetic of increase in infective titer in the subclinically infected mice may mean that toxic PrP^{L} levels are not reached (Collinge & Clarke, 2007). Support for this hypothesis has been demonstrated by depleting endogenous neuronal PrP^{C} in mice with established neuroinvasive prion infection (Mallucci et al., 2003). This depletion of PrP^{C} reverses early spongiform change and prevents neuronal loss and progression to clinical prion disease despite the accumulation of extra-neuronal PrP^{Sc} to levels seen in terminally ill wild-type mice (Mallucci et al., 2003). These data establish that propagation of non-neuronal PrP^{Sc} can be tolerated, and that arresting the continued conversion of PrP^{C} to PrP^{Sc} within neurons during scrapie infection prevents prion neurotoxicity.

FUTURE PERSPECTIVES

Prion diseases appear to be disorders of protein conformation, and elucidating their precise molecular mechanisms may be of very wide significance. It is of considerable interest that many of the commoner neurodegenerative diseases, such as Alzheimer's disease, Parkinson's disease and Huntington's disease, are also associated with abnormal protein aggregates. The apparent ability of a single polypeptide chain to encode information and specify distinctive phenotypes is unprecedented, and it seems unlikely that evolution will not have used this mechanism in many other ways. While the protein-only hypothesis of prion propagation is supported by compelling experimental data, and now appears able to encompass the phenomenon of prion strain diversity, detailed structural studies remain problematic. Systematic production of high-titer synthetic prions from recombinant PrP remains a critical future goal that will allow the structure of the infectious prion to be understood in atomic detail.

THE SEEDING AND TRANSMISSIBILITY OF TAU, Aβ AND SYNUCLEIN AGGREGATES

Scott T. Brady

One of the most striking and surprising aspects of prion diseases was the transmissibility of disease through a proteinaceous infectious particle (see main text). For many years, this property was thought to be unique to the prion protein gene product, but recent studies suggest that a more general principle may be involved in the mechanisms of prion transmissibility. Indeed, the evidence is mounting that some non-prion protein aggregates can be transmitted from one cell to a neighboring cell (Cushman et al., Frost & Diamond, 2010; Goedert et al., 2010; Lee et al., 2010), and these events may play a role in pathogenesis for diseases that include Alzheimer's (AD) and Parkinson's (PD) among others. Although none of these diseases exhibit infectivity in the same sense as prions, these mechanisms could contribute to the temporospatial spreading of pathology often observed in these diseases (Goedert et al., 2010).

THE SEEDING AND TRANSMISSIBILITY OF TAU, Aβ AND SYNUCLEIN AGGREGATES (*cont'd*)

Tau, Aβ42 peptide and synuclein are all polypeptides with multiple, dynamic conformations, but they can become locked into specific conformations that act as self-templates for higher-order aggregates (Lee et al., 2010). This is a property shared with the prion protein and is thought to be an essential element of pathogenicity in prion diseases as well as in AD and PD. Specific conformations of tau, Aβ or synuclein appear to be associated with neurotoxicity. For example, filamentous forms of tau activate GSK3 kinase, a property that is not shared with soluble monomeric tau (Lapointe et al., 2009). Extracellular aggregates of tau are taken up by cells in culture and can seed formation of similar aggregates (Frost et al., 2010; Goedert et al., 2010).

Aβ42 peptides exist in unaggregated, oligomeric or filamentous immunologically distinct forms *in vivo* (Sakono & Zako, 2010). Several lines of evidence suggest that oligomeric forms of Aβ42 are significantly more toxic. For example, oligomeric Aβ42, but not unaggregated or fibrillar forms, inhibit fast axonal transport and produce synaptic failure (Moreno et al., 2009; Pigino et al., 2009). Remarkably, both intracerebral and peripheral injection of Aβ42 amyloid can lead to amyloidosis and tau pathology in vulnerable cells expressing human APP and tau (Frost & Diamond, 2010; Goedert et al., 2010).

Finally, synuclein fibril formation can be nucleated by existing oligomers or fibrils (Frost et al., 2010) much like tau or Aβ42. Studies with neurons in culture expressing mutant synuclein associated with familial forms of PD indicate that synuclein aggregates can be released from cells and taken up by others, leading to Lewy-type pathology (Cushman et al., 2010; Frost et al., 2010). Moreover, patients who have received mesencephalic dopaminergic neuron grafts can exhibit host-to-graft spreading of Lewy pathology (Goedert et al., 2010).

The evidence is compelling that such transmissibility can occur with tau, Aβ and synuclein aggregates. Rather less certain is the extent to which this pathway contributes to the disease process in human patients. Studies that show elevated tau and synuclein conformers in cerebrospinal fluid of patients appear to correlate with neuropathology (Blennow et al., 2010; Eller & Williams, 2009) and these elevated levels are increasingly used as biomarkers for diagnostic purposes. Such findings indicate that disease-associated forms of tau and synuclein can be released into the extracellular environment, but more work is needed to determine whether this release is a consequence of neurodegeneration or whether it contributes to the spread of pathology.

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