



Cold Spring Harbor Perspectives in Biology

Prions

David W. Colby and Stanley B. Prusiner

Cold Spring Harb Perspect Biol 2011;3:a006833

References

This article cites 191 articles, 94 of which can be accessed free

<http://cshperspectives.cshlp.org/content/3/1/a006833.full.html#ref-list-1>

Email alerting service

Receive free email alerts when new articles cite this article - sign up in the box at the top right corner of the article or [click here](#)

Subject collections

Articles on similar topics can be found in the following collections

[Protein Homeostasis](#) (5 articles)

To subscribe to *Cold Spring Harbor Perspectives in Biology* go to:
<http://cshperspectives.cshlp.org/site/misc/subscribe.xhtml>

Prions

David W. Colby^{1*} and Stanley B. Prusiner^{1,2}

¹Institute for Neurodegenerative Diseases, University of California, San Francisco, San Francisco, California 94143

²Department of Neurology, University of California, San Francisco, San Francisco, California 94143

Correspondence: stanley@ind.ucsf.edu



The discovery of infectious proteins, denoted prions, was unexpected. After much debate over the chemical basis of heredity, resolution of this issue began with the discovery that DNA, not protein, from pneumococcus was capable of genetically transforming bacteria (Avery et al. 1944). Four decades later, the discovery that a protein could mimic viral and bacterial pathogens with respect to the transmission of some nervous system diseases (Prusiner 1982) met with great resistance. Overwhelming evidence now shows that Creutzfeldt–Jakob disease (CJD) and related disorders are caused by prions. The prion diseases are characterized by neurodegeneration and lethality. In mammals, prions reproduce by recruiting the normal, cellular isoform of the prion protein (PrP^C) and stimulating its conversion into the disease-causing isoform (PrP^{Sc}). PrP^C and PrP^{Sc} have distinct conformations: PrP^C is rich in α -helical content and has little β -sheet structure, whereas PrP^{Sc} has less α -helical content and is rich in β -sheet structure (Pan et al. 1993). The conformational conversion of PrP^C to PrP^{Sc} is the fundamental event underlying prion diseases. In this article, we provide an introduction to prions and the diseases they cause.

PRION PROTEIN ISOFORMS

PrP^{Sc}, an alternative or abnormal isoform of PrP, stimulates the conversion of PrP^C into nascent PrP^{Sc}; in the brain, accumulation of PrP^{Sc} causes neurodegeneration. In Syrian hamsters, PrP^C and PrP^{Sc} are both 209-residue proteins with two glycosylation sites and a glycosylphosphatidyl inositol (GPI) anchor (Fig. 1). PrP is posttranslationally processed to remove a 22-amino-acid, amino-terminal signal peptide and a 23-amino-acid, carboxy-terminal peptide, which directs addition of the GPI anchor that tethers the protein to the cell

membrane. No posttranslational modifications to the primary structure differentiate PrP^C from PrP^{Sc} (Stahl et al. 1993). Limited protease digestion of PrP^{Sc} often produces a smaller, protease-resistant molecule of approximately 142 amino acids, referred to as PrP 27–30 (Fig. 1). Under the same conditions, PrP^C and some forms of PrP^{Sc} are completely hydrolyzed. Although resistance to limited proteolysis has proved to be a convenient tool for detecting PrP^{Sc}, not all PrP^{Sc} molecules are resistant to protease digestion (Hsiao et al. 1994; Telling et al. 1996; Safar et al. 1998; Gambetti et al. 2008; Colby

*Current address: Department of Chemical Engineering, University of Delaware, Newark, Delaware 19716

Editors: Richard Morimoto, Jeffrey Kelly, and Dennis Selkoe

Additional Perspectives on Protein Homeostasis available at www.cshperspectives.org

Copyright © 2011 Cold Spring Harbor Laboratory Press; all rights reserved; doi: 10.1101/cshperspect.a006833

Cite this article as *Cold Spring Harb Perspect Biol* 2011;3:a006833

D.W. Colby and S.B. Prusiner

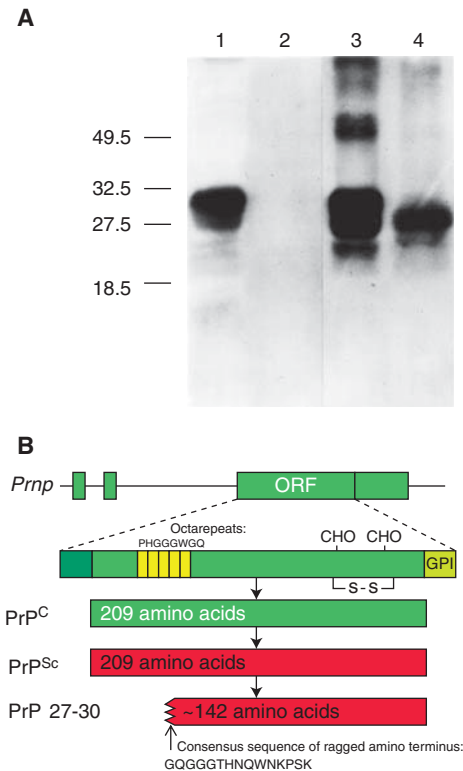


Figure 1. Prion protein isoforms. (A) Western immunoblot of brain homogenates from uninfected (lanes 1 and 2) and prion-infected (lanes 3 and 4) Syrian hamsters. Samples in lanes 2 and 4 were digested with 50 $\mu\text{g}/\mu\text{l}$ proteinase K for 30 min at 37°C, completely hydrolyzing PrP^C. Proteinase digestion cleaves ~67 amino acids from the amino terminus of PrP^{Sc} to generate PrP 27–30 (lane 4). Blot developed with anti-PrP polyclonal antiserum R073 (Serban et al. 1990). (B) Bar diagrams of the hamster *Prnp* gene and PrP isoforms. The *Prnp* ORF encodes a protein of 254 residues, which is shortened to 209 residues during posttranslational processing. PrP^{Sc} is an alternate conformation of PrP^C with identical primary structure. Limited proteolysis of PrP^{Sc} cleaves the amino terminus and produces PrP 27–30, composed of approximately 142 residues. Panel A, reprinted with permission, from Prusiner 2004.

et al. 2010); these protease-sensitive PrP^{Sc} forms are denoted sPrP^{Sc}. Furthermore, PrP^{Sc} from different species or prion strains may show different degrees of protease resistance.

In the presence of detergent, PrP 27–30 polymerizes into amyloid (McKinley et al. 1991). The tendency of prions to form amyloids has

also provided a useful means of prion detection (Colby et al. 2007); however, amyloid formation is a nonobligatory feature of prion disease (Wille et al. 2000). Prion rods formed by limited proteolysis and detergent extraction are indistinguishable from the filaments that aggregate to form PrP amyloid plaques in the CNS (DeArmond et al. 1985). Both the rods and the PrP amyloid filaments found in brain tissue show similar ultrastructural morphology and green-gold birefringence after staining with Congo red dye (Prusiner et al. 1983).

As in mammals, proteins with self-propagating conformations have been found in fungi; these fungal prions share many similarities with mammalian prions (Chien et al. 2004). Because of the ease of genetic manipulation and fast growth rates of fungi, fungal prion research has progressed at a rapid pace, often presaging discoveries in mammalian prion research. In yeast, alternative conformational states of the Ure2p and Sup35 proteins encipher the [URE3] and [PSI] phenotypes (Wickner 1994; Patino et al. 1996), respectively, whereas the Het-s protein enciphers the [HET-s] phenotype in *Podospora anserina* (Coustou et al. 1997). However, it is important to note that there are also many differences between yeast and mammalian prions—for example, yeast prions do not cause disease nor do they transmit from one mature cell to another.

THE PrP GENE

A chromosomal gene encodes PrP and is denoted *Prnp*, which is a member of the *Prn* gene family that also includes *Prnd*, encoding the doppel protein (Moore et al. 1999), and *Sprn*, encoding shadoo (Watts and Westaway 2007). In all known PrP genes from various species, the PrP open reading frame (ORF) is encoded within a single exon although the gene itself comprises two to three exons (Basler et al. 1986; Westaway et al. 1987; Hsiao et al. 1989; Gabriel et al. 1992). The other exons contain untranslated sequences including the promoter and termination sites. The PrP promoter contains multiple copies of GC-rich repeats—a canonical binding site for the transcription

factor Sp1 (McKnight and Tjian 1986), driving expression in many different tissues.

The alignment of the translated sequences from more than 40 PrP genes shows a striking degree of conservation between the mammalian sequences, suggesting the retention of some important function for PrP through evolution. However, variations in PrP sequences exist both between species and between individuals within species (Fig. 2), greatly affecting susceptibility to prion infection.

The shortest incubation times, or the interval between inoculation and clinical signs of disease, are achieved with intracerebral inoculation of prions with a sequence identical to that of the host animal; under these conditions, all animals develop prion disease within a narrow interval for a particular dose. When the donor prion originates from a species different from the host animal, and thus, the sequences differ between infecting PrP^{Sc} and host PrP^C, the incubation time can be prolonged and vary substantially between individual animals inoculated; often, many of the inoculated animals do not develop disease (Carlson et al. 1989; Telling et al. 1994; Telling et al. 1995; Tateishi et al. 1996). This phenomenon is referred to as the species barrier that was first noted by Ian Pattison (Pattison 1965).

HUMAN PRION DISEASES

Prion diseases occur as sporadic, genetic, and transmissible disease in humans (Table 1). Although infectious forms of prion disease are most well known to the general public, sporadic and heritable forms of the disease occur much more frequently in humans, with sporadic (s) CJD accounting for approximately 85% of cases. sCJD has no known cause although spontaneous misfolding of PrP^C into PrP^{Sc} is a leading hypothesis (Prusiner 1989; Hsiao et al. 1991a). Alternate hypotheses include somatic mutation of *PRNP*, undetected horizontal transmission (Gajdusek 1977), and infrequent amplification of low levels of PrP^{Sc} that are part of “normal” protein homeostasis. The brains of sCJD patients harbor infectious prions that are transmissible to experimental animals

(Gibbs et al. 1968; Brown et al. 1994). In humans, virtually all forms of prion disease feature neuropathological changes including vacuolation (resulting in the spongiform appearance of brain tissue), astrocytic gliosis, and PrP deposition. The morphology of vacuoles and PrP deposits varies depending on the prion strain and host, as do the regions of the brain affected.

To date, over 40 different mutations of the PrP gene have been shown to segregate with the heritable human prion diseases (Fig. 2). The resulting diseases have been classified as Gerstmann–Sträussler–Scheinker syndrome (GSS), familial (f) CJD, or fatal familial insomnia (FFI) according to the clinical symptoms, although all result from *PRNP* mutations. At the time when the discoveries were reported that fCJD and GSS could be transmitted to apes and monkeys, many still thought that scrapie, CJD, and related disorders were caused by slow viruses (Roos et al. 1973; Masters et al. 1981). Only the discovery that a proline-to-leucine mutation at codon 102 of the human PrP gene was genetically linked to some GSS pedigrees permitted the unprecedented conclusion that prion disease can have both genetic and infectious etiologies (Hsiao et al. 1989; Prusiner 1989). This mutation has been found in unrelated families from several countries (Doh-ura et al. 1989; Goldgaber et al. 1989; Kretzschmar et al. 1991), and other mutations causing GSS have since been identified (Dlouhy et al. 1992; Petersen et al. 1992; Poulter et al. 1992; Rosemann et al. 1998).

Likewise, several different mutations have also been discovered to cause fCJD. A repeat expansion in the amino-terminal region of PrP, which in the healthy population contains five repetitive sequences of eight residues each (octarepeats), has been genetically linked to fCJD. Insertions of two to nine additional octarepeats have been found in individuals within fCJD pedigrees (Owen et al. 1989; Goldfarb et al. 1991a). Molecular genetic investigations have revealed that Libyan and Tunisian Jews with fCJD have a PrP gene point mutation at codon 200, resulting in a glutamic acid-to-lysine substitution (Goldfarb et al. 1990a; Hsiao

D.W. Colby and S.B. Prusiner

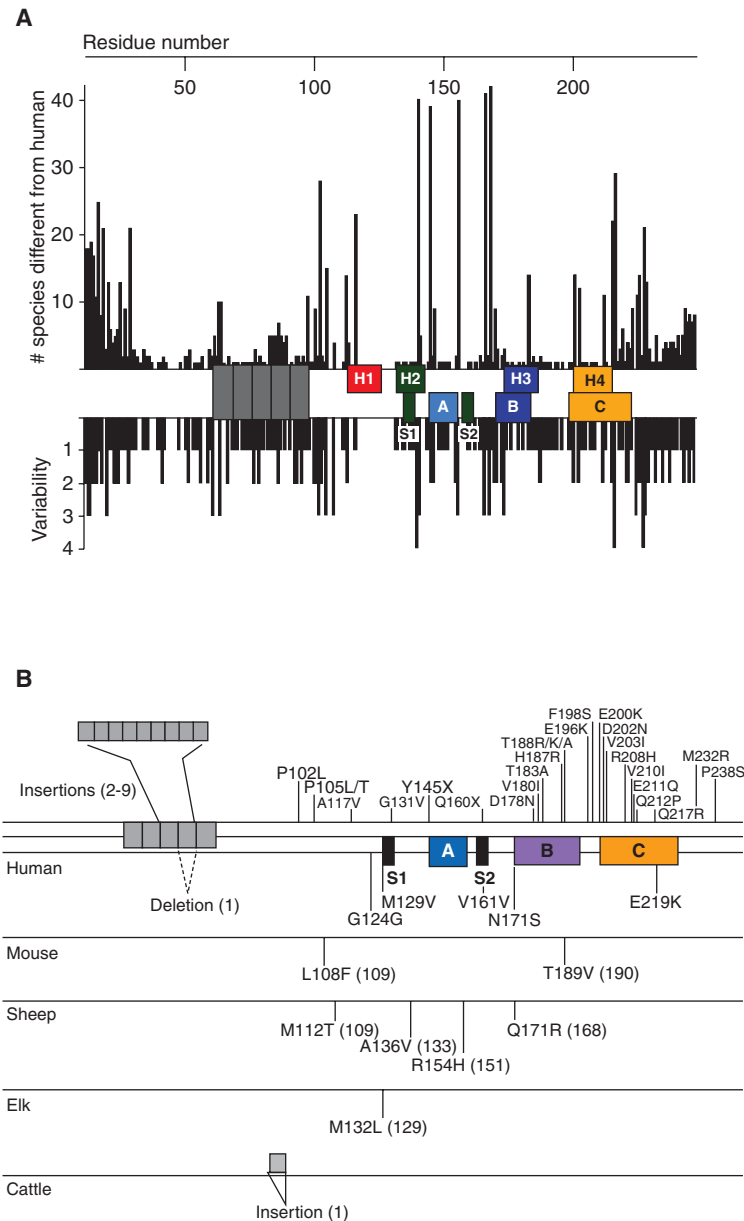


Figure 2. Variation of the prion protein gene. (A) Species variations of the prion protein gene. The x-axis represents the human PrP sequence, with the five octarepeats and H1–H4 regions of the putative secondary structure shown, as well as the three α -helices A, B, and C and the two β -strands S1 and S2 as determined by NMR. Vertical bars above the axis indicate the number of species that differ from the human sequence at each position. Below the axis, the length of the bars indicates the number of alternative amino acids at each position in the alignment. (B) PrP mutations causing inherited human prion disease (above the line) and PrP polymorphisms (below the line) found in humans, mice, sheep, elk, and cattle. Residue numbers in parentheses correspond to the human codons. Data in Panel A compiled by P. Bamorough and E.E. Cohen and reprinted, with permission, from Prusiner 2004.

Table 1. Prion diseases in humans and animals.

Disease	Host	Mechanism of pathogenesis
Kuru	humans (Fore people)	infection through ritualistic cannibalism
Iatrogenic CJD	humans	infection from prion-contaminated HGH, medical equipment, etc.
Variant CJD	humans	infection from bovine prions
Familial CJD	humans	germline mutations in the <i>PRNP</i> gene
GSS	humans	germline mutations in the <i>PRNP</i> gene
FFI	humans	germline mutations in the <i>PRNP</i> gene
Sporadic CJD	humans	somatic mutation or spontaneous conversion of PrP ^C to PrP ^{Sc}
sFI	humans	somatic mutation or spontaneous conversion of PrP ^C to PrP ^{Sc}
Scrapie	sheep	infection
BSE	cattle	infection or sporadic
TME	mink	infection with prions from sheep or cattle
CWD	deer, elk	infection
FSE	cats	infection with prion-contaminated bovine tissues or MBM
Exotic ungulate encephalopathy	greater kudu, nyala, oryx	infection with prion-contaminated MBM

et al. 1991b), a mutation that has since been identified in fCJD pedigrees in many locations (Goldfarb et al. 1990a; Goldfarb et al. 1990b; Bertoni et al. 1992).

The D178N mutation can cause either fCJD or FFI, depending on the polymorphism present at codon 129, where both methionine and valine are commonly found. D178N coupled with V129 produces fCJD, in which patients present with dementia and widespread deposition of PrP^{Sc} (Goldfarb et al. 1991c). If the disease mutation is coupled with M129, however, FFI results and patients present with a progressive sleep disorder that is ultimately fatal. Postmortem analysis of FFI brains revealed deposition of PrP^{Sc} confined largely to specific regions of the thalamus (Lugaresi et al. 1986; Gambetti et al. 1995).

Infectious forms of prion diseases include kuru, iatrogenic (i) CJD, and variant (v) CJD. Kuru in the highlands of New Guinea was transmitted by ritualistic cannibalism, as people in the region ate the brains of their dead relatives in an attempt to immortalize them (Glasse 1967; Alpers 1968; Gajdusek 1977). Iatrogenic transmissions include prion-tainted human growth hormone and gonadotropin, dura mater grafts, and transplants of corneas obtained from people who died of CJD (Koch et al.

1985; PHS 1997). In addition, CJD cases have been recorded after neurosurgical procedures in which ineffectively sterilized depth electrodes or instruments were used.

More than 200 teenagers and young adults have died of vCJD, mostly in Britain (Spencer et al. 2002; Will 2003). Both epidemiologic and experimental studies have built a convincing case that vCJD resulted from prions being transmitted from cattle with bovine spongiform encephalopathy (BSE, or “mad cow” disease) to humans through consumption of contaminated beef products (Chazot et al. 1996; Will et al. 1996; Cousens et al. 1997). Until recently, all of the vCJD-affected individuals were identified to express methionine homozygously at codon 129. A single case of vCJD in a patient heterozygous at codon 129 has been reported, raising the possibility of a second wave of “mad cow”-related deaths (Kaski et al. 2009).

PRION DISEASES OF ANIMALS

Prion diseases occur naturally in many mammals, including scrapie of sheep and goats, BSE, transmissible mink encephalopathy (TME), chronic wasting disease (CWD) of mule deer and elk, feline spongiform encephalopathy, and exotic ungulate encephalopathy (Table 1).

D.W. Colby and S.B. Prusiner

Unlike in humans, prion diseases in animals mainly occur as infectious disorders. As in humans, prion disease in animals is characterized by neuropathologic changes, including vacuolation, astrocytic gliosis, and PrP deposition.

Scrapie of sheep has been documented in Europe for hundreds of years. Despite efforts attempting to link scrapie to CJD, no evidence exists to establish a relationship (Chatelain et al. 1981). Polymorphisms in sheep PrP modulate susceptibility to scrapie, rendering some breeds more resistant to infection than others (Goldmann et al. 1991). As scrapie prions can persist in soil for years (Palsson 1979; Brown and Gajdusek 1991), selective breeding programs may be the most effective means to eradicate scrapie. In part because scrapie is not infectious for humans, hamster- and mouse-adapted scrapie strains, such as Sc237 and RML, are important laboratory tools for studying prions.

During the BSE epidemic in Britain, it was estimated that nearly one million cattle were infected with prions (Anderson et al. 1996; Nathanson et al. 1997). The mean incubation time for BSE is approximately 5 years. Most cattle were slaughtered between 2 and 3 years of age, and therefore, in a presymptomatic phase of infection (Stekel et al. 1996). BSE is a massive common-source epidemic caused by meat and bone meal (MBM) fed primarily to dairy cows (Wilesmith et al. 1991; Nathanson et al. 1997). MBM was prepared from the offal of sheep, cattle, pigs, and chickens as a high-protein nutritional supplement. In the late 1970s, the hydrocarbon-solvent extraction method used in the rendering of offal began to be abandoned, resulting in MBM with a much higher fat content (Wilesmith et al. 1991; Muller et al. 2007). It is now thought that this change allowed scrapie prions from sheep or low levels of bovine prions generated sporadically to survive the rendering process, resulting in the widespread infection of cattle. Changes in the methods used for feeding cattle have since eliminated the epidemic, although sporadic BSE cases arise occasionally.

Mule deer, white-tailed deer, and elk have been reported to develop CWD. As the only

prion disease identified in free-ranging animals, CWD appears to be far more communicable than other forms of prion disease. CWD was first described in 1967 and was reported to be a spongiform encephalopathy in 1978 on the basis of histopathology of the brain. Originally detected in the American West, CWD has spread across much of North America and has been reported also in South Korea. In captive populations, up to 90% of mule deer have been reported to be positive for prions (Williams and Young 1980). The incidence of CWD in cervids living in the wild has been estimated to be as high as 15% (Miller et al. 2000). The development of transgenic (Tg) mice expressing cervid PrP, and thus susceptible to CWD, has enhanced detection of CWD and the estimation of prion titers (Browning et al. 2004; Tamgüney et al. 2006). Shedding of prions in the feces, even in presymptomatic deer, has been identified as a likely source of infection for these grazing animals (Williams and Miller 2002; Tamgüney et al. 2009b). CWD has been transmitted to cattle after intracerebral inoculation, although the infection rate was low (4 of 13 animals [Hamir et al. 2001]). This finding raised concerns that CWD prions might be transmitted to cattle grazing in contaminated pastures.

TRANSGENIC MICE

The development of various lines of Tg mice has provided valuable insight on these disorders. Altering the expression level of PrP in Tg mice can lead to abnormalities in uninfected mice and strongly affects incubation times in prion-infected mice. Tg mice expressing different levels of wild-type (wt) PrP of the Syrian hamster (SHa) sequence showed incubation times following prion inoculation that were inversely proportional to the level of PrP expression (Prusiner et al. 1990). Older, uninoculated mice expressing high levels of SHaPrP, ovine PrP, or mouse PrP(F108,V189) developed neurological dysfunction that was distinct from prion disease (Westaway et al. 1994).

Mice with the *Prnp* gene knocked out, termed *Prnp*^{0/0} mice, have also been created; ablation of *Prnp* does not affect normal



development but renders mice resistant to prion disease (Büeler et al. 1992; Büeler et al. 1993; Prusiner et al. 1993; Manson et al. 1994). Altered synaptic behavior in the brains of *Prnp*^{0/0} mice was found in some studies (Collinge et al. 1994; Whittington et al. 1995) but not others (Herms et al. 1995; Lledo et al. 1996). Some early findings of dysfunction in *Prnp*^{0/0} mice were later attributed to abnormal expression of the doppel protein, which resulted from the technique used to ablate *Prnp* gene expression (Sakaguchi et al. 1996; Moore et al. 1999).

Tg mouse models of genetic forms of prion disease have been constructed, and several recapitulate key features of prion disease. Tg mice overexpressing high levels of mouse (Mo) PrP with a P→L substitution at position 101, which corresponds to the mutation causing GSS in humans, spontaneously develop neuropathology characteristic of prion disease and accumulate an abnormal isoform of PrP (Hsiao et al. 1990; Tremblay et al. 2004). Serial passage of brain homogenates to Tg mice expressing lower levels of the same transgene accelerated the onset of disease (Hsiao et al. 1994). As models of FFI and fCJD in humans, Tg mice expressing D178N coupled with M129 and V129, respectively, show behavioral abnormalities, misfolded PrP, and neuropathological changes (Dossena et al. 2008; Jackson et al. 2009). Mice overexpressing a novel set of mutations (S170N/N174T) engineered to alter the structure of PrP also developed disease, which transmitted to animals expressing wt PrP (Sigurdson et al. 2009).

PRION REPLICATION

Prion propagation requires conversion of PrP^C to PrP^{Sc}, thought to occur by a template-assisted process in which PrP^{Sc} acts as a template onto which PrP^C is refolded into the infectious conformation. The faithful replication of prion strains supports this theory. Evidence for this theory also comes from investigations of Tg mice expressing both SHaPrP and MoPrP, designated Tg(SHaPrP)*Prnp*^{+/+} mice (Prusiner et al. 1990). When these mice were inoculated

with prions originating from mice, MoPrP^C was recruited and converted into MoPrP^{Sc}. Inoculation of these mice with hamster prions resulted in the conversion of SHaPrP^C into SHaPrP^{Sc}. These findings indicate that molecules with the PrP sequence that is most well suited to adapt to the PrP^{Sc} template are selected for conversion.

PrP^C may need to enter a partially unfolded, intermediate state to interact with PrP^{Sc} and undergo conversion; this intermediate state is referred to as PrP* (Cohen et al. 1994). During *in vitro* conversion, PrP^C must be denatured either by GdnHCl (Kocisko et al. 1994; Kaneko et al. 1997b) or by sonication (Castilla et al. 2005). This denaturation is presumed to convert PrP^C into a PrP*-like molecule. The conversion of PrP^C to PrP^{Sc} may also require the assistance of one or more as-yet-unidentified cofactors, provisionally designated protein X. Presumably, protein X binds to PrP^C and enables it to interact with PrP^{Sc} for conversion. Overexpression of protein X would thus shorten incubation times for disease, whereas ablation of protein X would prolong or abolish prion disease. Many putative protein X genes have been identified, but transgenic knockouts for these genes have failed to alter incubation times substantially (Tamgüney et al. 2008). Several *in vitro* investigations have suggested that polyanions, including nucleic acids, may accelerate prion formation (Deleault et al. 2007; Wang et al. 2010) although this has not been shown in animals. For yeast prions, several protein chaperones that modulate prion states have been identified (Paushkin et al. 1997; Shorter and Lindquist 2008).

In mammalian cell cultures, prion accumulation was determined by the interplay between *de novo* prion formation, catabolism, cell division, and horizontal cell-to-cell transmission. Using a subline of neuroblastoma (N2a) cells, we studied the kinetics of prion propagation and found that cell division led to a predictable reduction in steady-state prion levels but not to complete clearance (Ghaemmaghami et al. 2007). Scrapie-infected N2a cells were capable of accumulating different steady-state levels of prions, dictated partly by the rate of cell

D.W. Colby and S.B. Prusiner

division. We also observed that prions in this subline of N2a cells were transmitted primarily from mother to daughter cells, rather than horizontal cell-to-cell transmission. Our kinetic results were modeled based on a mechanism that assumed a subpopulation of prions is capable of self-catalysis, and the levels of this subpopulation reached saturation in fully infected cells.

BIOLUMINESCENCE IMAGING

Because astrocytic gliosis marked by the deposition of fibrils composed of GFAP is a prominent feature of prion disease (DeArmond et al. 1987; Hwang et al. 2009), we investigated whether GFAP might be used as a surrogate marker for prions. To interrogate this posit, we inoculated prions into Tg mice expressing luciferase (*luc*) under the GFAP gene (*Gfap*) promoter, denoted Tg(*Gfap-luc*) mice (Tamgüney et al. 2009a). Weekly noninvasive, bioluminescence imaging (BLI) detected an increase in light emitted from the brains of Tg(*Gfap-luc*) mice at ~55 d after inoculation and ~62 d before neurologic deficits appeared (Fig. 3). To determine whether BLI could be used as a proxy bioassay for prion infectivity, we performed endpoint titrations of prions in Tg(*Gfap-luc*) mice. BLI bioassays were as or more sensitive than those determined by the onset of neurological dysfunction, and were completed in approximately half the time. These findings indicate that BLI is likely to be a suitable surrogate for measuring prion infectivity, and might be useful in the study of Tg mouse models for other neurodegenerative illnesses.

PrP AMYLOID

As mentioned earlier, amyloid plaques are a nonobligatory feature of prion diseases. Approximately 10% of sCJD cases whereas 70% of kuru cases show amyloid plaques; all vCJD cases show amyloid plaques surrounded by a halo of spongiform degeneration—such structures are called florid plaques (Klatzo et al. 1959; Will et al. 1996). In Tg(SHaPrP)*Prnp*^{+/+} mice expressing both MoPrP and SHaPrP,

amyloid plaques were found when hamster prions replicated but not when mouse prions replicated (Prusiner et al. 1990). These experimental studies showed unequivocally that amyloid plaques need not accompany prion replication. In earlier studies, the 87V prion strain that produced numerous amyloid plaques was isolated from Cheviot sheep with scrapie and resulted in amyloid when passaged in *Prnp*^{b/b} mice (Bruce et al. 1976; Jeffrey et al. 1994).

Importantly, ionizing radiation studies showed the target size for scrapie prions was ~55,000 Da regardless of the preparation (Bellinger-Kawahara et al. 1988). Fractions containing purified PrP 27–30 amyloid rods showed the same resistance to inactivation by X-rays as crude brain homogenates or PrP 27–30 dispersed into liposomes. Electron crystallography of purified PrP 27–30 amyloid rods identified two-dimensional (2D) crystals with a unit cell of 70 Å, which allowed sufficient space for a PrP 27–30 trimer assuming each protein contained a β-helix (Wille et al. 2002; Govaerts et al. 2004b; Wille et al. 2009b). Because each PrP 27–30 molecule is composed of approximately 140 amino acids, an infectious trimer is readily accommodated by the putative target size.

Although some investigators argue that mammalian prions multiply by a seeded polymerization process during which PrP^C is transformed into PrP^{Sc}, there is little evidence for such a process. More likely it is a template-assisted replication mechanism whereby the conformation of PrP^{Sc} is copied with a high degree of fidelity. As noted earlier, it seems likely that chaperone proteins feature in the formation of mammalian prions but none have been identified to date. Some investigators argue that yeast prions replicate through polymerization into amyloid fibers (Wickner et al. 1995; Speransky et al. 2001). The chaperone protein Hsp104 appears to enhance fungal prion replication by breaking the amyloid fibers to create more seeds for polymerization; in addition, there is evidence that other chaperones, including Hsp 40 and Hsp 70, participate in yeast prion replication (Shorter and Lindquist 2008).

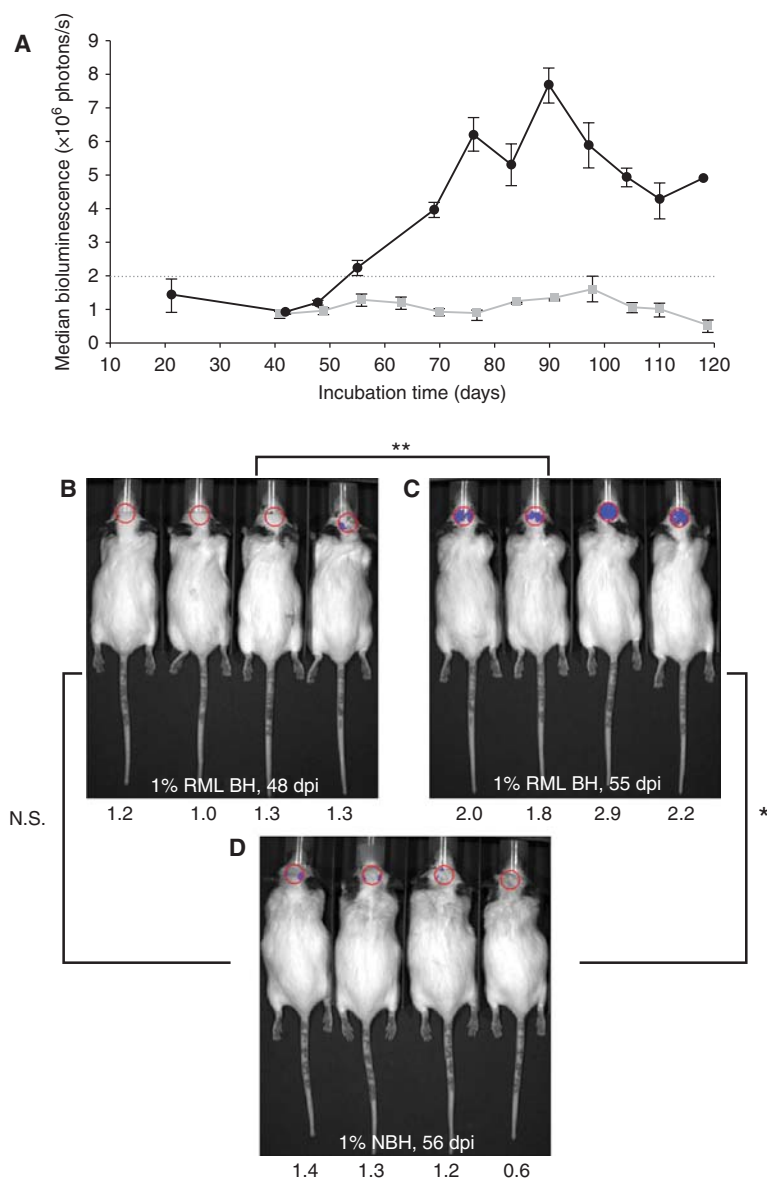


Figure 3. Bioluminescence in Tg(*Gfap-luc*) mice inoculated intracerebrally with RML prions ($n = 12$) indicated a reactive astrocytic gliosis. (A) Bioluminescence measured from the brains of prion-inoculated mice (black circles) began to increase at 55 d postinoculation (dpi). Bioluminescence in control Tg(*Gfap-luc*) mice inoculated with 1% normal brain homogenate (NBH) ($n = 4$, gray squares) remained low throughout the incubation period. (B–D) Photos of representative Tg(*Gfap-luc*) mice, with overlays of the circular area above the brain from which bioluminescence was quantified. Bioluminescence measured, $\times 10^6$ photons/s, from each mouse brain is shown below each image. The bioluminescence measured from the brains of prion-infected mice significantly increased (**, $P < 0.001$, Bonferroni *t* test) from 48 dpi (B) to 55 dpi (C). Similarly, bioluminescence measured from infected mice at 55 dpi (C) was also significantly (*, $P < 0.005$) greater than in control mice inoculated with NBH and imaged at 56 dpi (D). No significant difference (N.S., $P < 0.5$) was measured between RML-inoculated mice at 48 dpi (B) and control mice at 56 dpi (D). Based on this result, astrocytic gliosis was detectable at bioluminescence measurements $> 2.0 \times 10^6$ photons/s. Reprinted, with permission, from Tamgüney et al. 2009a.

D.W. Colby and S.B. Prusiner

CELL BIOLOGY OF PrP^{Sc} FORMATION

Prion-infected cell lines, including scrapie-infected neuroblastoma (ScN2a) cells, have been used to investigate the subcellular localization of PrP conversion. In scrapie-infected cells, PrP^C molecules are trafficked to the cell surface via their GPI anchor before conversion into PrP^{Sc} (Stahl et al. 1987; Borchelt et al. 1990; Caughey and Raymond 1991). PrP^C then appears to re-enter the cell through subcellular compartments, which are likely cholesterol-rich, detergent-insoluble membranes called caveolae-like domains (Gorodinsky and Harris 1995; Taraboulos et al. 1995; Vey et al. 1996; Kaneko et al. 1997a; Naslavsky et al. 1997). Within this cholesterol-rich, nonacidic compartment, GPI-anchored PrP^C can be either converted into PrP^{Sc} or partially degraded (Taraboulos et al. 1995; Peters et al. 2003). Subsequently, PrP^{Sc} is trimmed at the amino terminus in an acidic compartment in scrapie-infected cultured cells to form PrP 27–30 (Caughey et al. 1991a). In contrast, amino-terminal trimming of PrP^{Sc} is minimal in the brain, where little PrP 27–30 is found (McKinley et al. 1991).

STRUCTURAL FEATURES OF PrP^C AND PrP^{Sc}

Determining the structural features that differ between PrP^C and PrP^{Sc} will likely provide important insight into the pathogenic conversion of PrP^C into PrP^{Sc}. NMR structures of recombinant PrP from many different species have been solved over the past 15 years, representing the best estimate of the structure of PrP^C. All reveal a three alpha-helix bundle protein with two short antiparallel β -strands (Riek et al. 1996; James et al. 1997; Riek et al. 1998; Zahn et al. 2000) (Fig. 4). These well-folded structural elements are composed of the carboxyl terminus of the protein; the amino-terminal domain is highly flexible and lacks identifiable secondary structure under the experimental conditions employed (Donne et al. 1997). More recently, a crystal structure of PrP has been obtained, largely in agreement with the NMR structures (Antonyuk et al. 2009).

Because PrP^{Sc} is insoluble and forms aggregates with some degree of disorder, no successful attempts at crystallization or solution-based NMR have been reported. Investigations using solid-state NMR have been limited by the ability to produce labeled PrP^{Sc} and by the molecular size of PrP. However, key insights into the structure of PrP^{Sc} have been obtained through electron crystallography coupled with computational modeling (Govaerts et al. 2004a; Wille et al. 2009b) (Fig. 5). Isomorphous, 2D crystals were discovered by negative-stain electron microscopy. Such crystals were found both in preparations of PrP 27–30 and in preparations of a “miniprion” composed of 106 residues formed from discontinuous PrP segments (termed PrP^{Sc}106). Image processing allowed the extraction of limited structural information to 7-Å resolution. Models were generated based on known protein folds, constrained by space filling of the 2D crystals, the amount of β -sheet content measure by FTIR (Caughey et al. 1991b; Pan et al. 1993), the locations of the glycosylation sites, and the location of the deleted protein segments in PrP^{Sc}106 (Supattapone et al. 1999a). Only models including parallel β -helices as the key element could satisfy the constraints (Wille et al. 2002). Subsequent computational modeling identified trimeric, left-handed β -helices as the most likely substructure for PrP^{Sc} (Govaerts et al. 2004a). X-ray diffraction patterns obtained from PrP 27–30 fibers were consistent with this model (Wille et al. 2009a).

Given the evidence that distinct conformations of PrP result in different prion strains (see sections “De novo Generation of Prions” and “Prion Strains” below), it is perhaps better to speak of prion *structures* rather than a single structure. Whether the structural differences that encipher prion strains are subtle or more substantial remains to be determined.

DE NOVO GENERATION OF PRIONS

Refolding PrP into an infectious conformation *in vitro* has been considered by many to be final proof of the protein-only hypothesis. Many studies have advanced knowledge toward

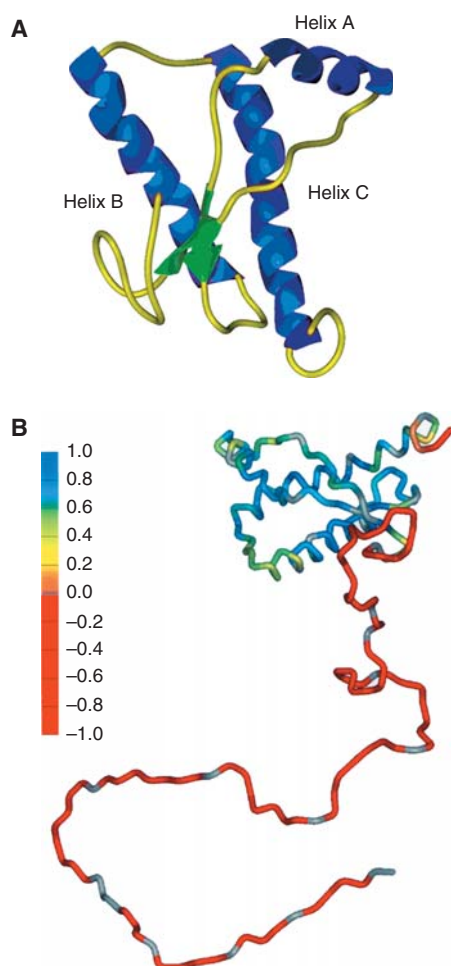


Figure 4. Structures of PrP^C. (A) NMR structure of Syrian hamster (SHa) recombinant (rec) PrP(90–231), which presumably resembles PrP^C. Blue, α -helices; yellow, loops; green, β -strands (James et al. 1997). (B) Schematic diagram showing degree of structure for entire PrP polypeptide chain based on $\{^1\text{H}\}$ - ^{15}N NOE data. Red, most flexible regions of the protein; blue, least flexible regions (James et al. 1997). Arbitrary structure is shown for residues 23–89. Reprinted, with permission, from Prusiner 2004.

achieving this goal. In Tg(PrP,P101L) mice, an experimental model of human GSS, prion disease was transmitted from high-expressing Tg(PrP,P101L) mice to Tg mice expressing low levels of MoPrP(P101L), which are far less susceptible to spontaneous prion disease (Hsiao et al. 1990; Hsiao et al. 1994; Nazor et al.

2005). Similar transmissions were later accomplished with a synthetic, 55-residue peptide carrying the same P→L mutation and folded into a β -rich structure (Kaneko et al. 2000; Tremblay et al. 2004).

Synthetic prions were formed by polymerization of recombinant MoPrP into amyloid fibers (Legname et al. 2004). Inoculation of PrP amyloid fibers into Tg9949 mice, which overexpress amino-terminally truncated PrP at 16–32 \times levels, led to the recovery of prions containing protease-resistant (r) PrP^{Sc} and to neuropathological changes typical of prion disease. The conformational stability of the resulting prion isolate, as measured by the GdnHCl concentration required to denature half of the sample ($[\text{GdnHCl}]_{1/2}$), was unusually high (~ 4.5 M), confirming the novelty of the prion strain generated (Legname et al. 2005). Subsequent serial passage of this isolate led to shortened incubation periods and a decrease in the conformational stability of the resulting prion isolate. Combining these data with those available for naturally occurring prion strains, it was found that the conformational stability of prions was directly proportional to the incubation period (Fig. 6) (Legname et al. 2006).

Based on the relationship between conformational stability and incubation period (Legname et al. 2006), the conditions used to refold recombinant PrP were altered to generate a spectrum of amyloids with different conformational stabilities. The amyloids were inoculated into mice that moderately overexpress full-length PrP (8 \times), resulting in distinguishable prion strains with incubation periods and conformational stabilities dictated by the stability of the recombinant PrP amyloid fibers (Colby et al. 2009). Amyloids with higher conformational stability resulted in prions with longer incubation periods, whereas amyloids of low conformational stability caused prion disease in shorter durations. Amyloids of intermediate stability enciphered intermediate incubation periods. This direct demonstration of the conformational basis of prion strain diversity provided further evidence that synthetic prions arise from the recombinant amyloid preparations, and not from the host or

D.W. Colby and S.B. Prusiner

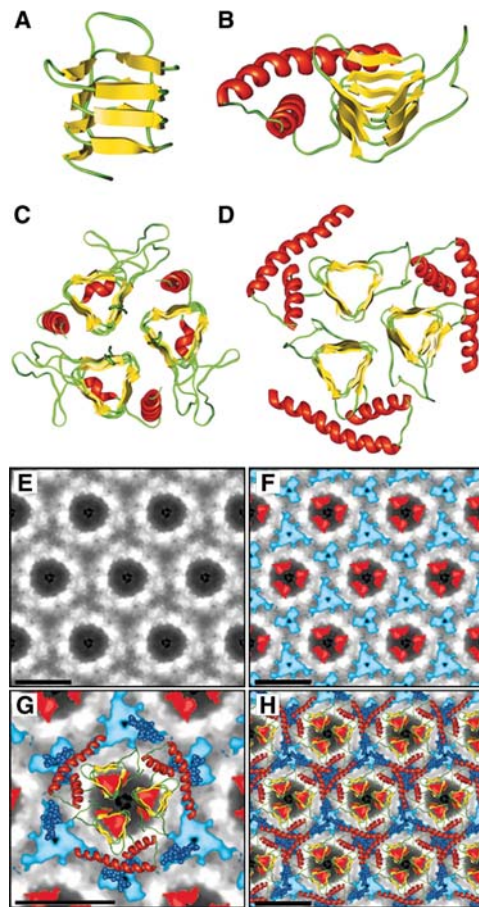


Figure 5. Structural models of PrP^{Sc}. (A) Residues 89–174 of PrP threaded into a left-handed β -helix based on UDP *N*-acetylglucosamine *O*-acyltransferase from *Escherichia coli* (PDB ID code 1LXA). (B) Model of the monomer of PrP 27–30 with the α -helical region (residues 177–227) as determined by NMR spectroscopy shown in red. (C) The crystal structure of the trimeric carbonic anhydrase from *Methanosarcina thermophila*. (D) Trimeric model of PrP 27–30 built by superimposing three monomeric models onto the structure shown in C. (E) Projection map of PrP 27–30 obtained by processing and averaging three independent 2D crystals of PrP 27–30. (F) Statistically significant differences between PrP 27–30 and PrP^{Sc}106 overlaid onto the projection map of PrP 27–30. The differences attributed to the internal deletion of PrP^{Sc}106 (residues 141–176) are shown in red; the differences in glycosylation between PrP 27–30 and PrP^{Sc}106 are shown in blue. (G) Superimposition of the trimeric left-handed model onto the EM maps. The trimeric left-handed α -helical model of PrP 27–30 is superimposed on a 1:1 scale with the electron crystallographic maps of PrP 27–30. (H) The scaled trimeric model was copied onto the neighboring units of the crystals to show the crystallographic packing suggested by the model. Bars in panels E–H represent 50 Å. Reprinted with permission, from Govaerts et al. 2004a.

from contamination. If prions were arising spontaneously in the host, one would expect the strain properties to be independent of the amyloid properties. Exhaustive negative controls also excluded spontaneous prion generation and contamination.

In other work, amyloid inoculation of Tg9949 mice overexpressing an amino-terminally truncated PrP resulted in novel, protease-sensitive, synthetic prions (Colby et al. 2010). Although these strains lacked protease resistance, they caused severe neuropathology and were serially transmissible both in Tg9949 mice and in Tg mice moderately overexpressing full-length PrP. Most, if not all, naturally occurring prions contain some fraction of PrP^{Sc} in a conformation that resists protease digestion (McKinley et al. 1983). This observation has led some researchers to equate protease resistance

with prion infectivity and pathogenesis. However, many naturally occurring prion strains also contain PrP^{Sc} in a conformation that is sensitive to protease digestion (Safar et al. 1998). The novel, protease-sensitive, synthetic prion strains showed that sPrP^{Sc} is both transmissible and pathogenic.

Synthetic prions have also been generated using sonication (Deleault et al. 2007; Barria et al. 2009; Wang et al. 2010). Infectivity was spontaneously generated in sonicated mixtures of polyanions combined with PrP^C, which was accompanied by copurified lipids (Deleault et al. 2007). Prions were generated in a similar fashion using brain homogenate as the substrate, rather than minimal components described earlier (Barria et al. 2009). Prions created in these studies using PrP^C or normal brain homogenate had titers that were sufficient

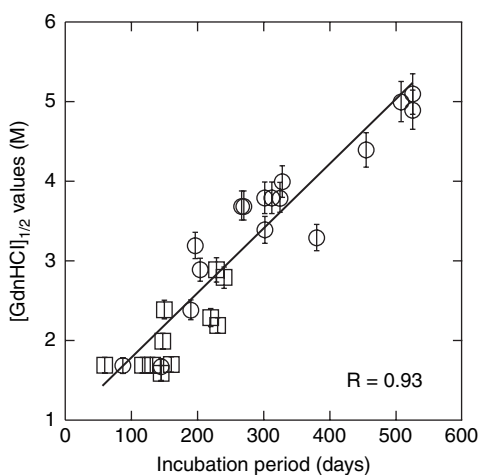


Figure 6. The conformational stability of prions is directly proportional to the length of the incubation time in mice. The $[\text{GdnHCl}]_{1/2}$ values for prions were plotted as a function of the incubation times. Synthetic prions (circles) in the brains of Tg9949, Tg4053, and non-Tg FVB mice were plotted with many naturally occurring prions passaged (squares) in both non-Tg and Tg mice. $R = 0.93$. Reprinted, with permission, from Legname et al. 2006.

to infect hamsters with prolonged incubation periods of 113 to 168 days, compared to incubation periods of approximately 70 days with some naturally occurring prion strains (Kimberlin and Walker 1977). Synthesis of high-titer prions from recombinant PrP was reported using sonication in the presence of lipids and RNA (Wang et al. 2010); the infectivity of these prions was comparable to naturally occurring strains.

Synthetic yeast prions have also been constructed. A recombinant fragment of the Sup35 NM protein fragment was polymerized into amyloid fibrils and introduced into yeast (Sparner et al. 2000). Similar studies have also been performed for the [HET-s] and [URE3] fungal prions (Maddelin et al. 2002; Brachmann et al. 2005).

PRION STRAINS

Naturally occurring prion strains have been isolated, each with a distinct incubation period and characteristic pathology; these traits are

often conserved on serial transmission (Dickinson and Meikle 1969; Fraser and Dickinson 1973). Because prions are composed only of protein and replicate using the PrP substrate present in the host, differences in prion strains cannot be attributed to genetic variability, which accounts for the existence of viral strains. Rather, prion strains arise from conformational variability—that is, PrP can assume several different, self-propagating conformations, each of which enciphers a distinct prion strain. Biochemical evidence (Bessen and Marsh 1994; Collinge et al. 1996; Telling et al. 1996; Peretz et al. 2001a) and recent studies with synthetic prions support this theory (Colby et al. 2009).

Studies with synthetic prions showed that the mouse synthetic prion (MoSP) strain 1 gradually adopted properties associated with naturally occurring prion strains such as RML, including short incubation times and low conformational stabilities (Ghaemmaghami et al., in prep.). These changes were accompanied by a structural transformation, as indicated by a shift in the molecular mass of the protease-resistant core of MoSP1 from approximately 19 kDa [MoSP1(2)] to 21 kDa [MoSP1(1)]. We found that MoSP1(1) and MoSP1(2) could be bred with fidelity when cloned in N2a cells but when present as a mixture, MoSP1(1) propagation led to the disappearance of MoSP1(2). In culture, the rate of this transformation could be modified by the culture media and the presence of polyamidoamines. These findings showed that prions exist as conformationally diverse populations and each strain can replicate with high fidelity. Competition and selection among the pool of strains provide a mechanism for prion transformation and adaptation (Li et al. 2010).

Yeast also show multiple prion strains. A recombinant Sup35 protein fragment refolded into two different conformations was shown to initiate two distinct [PSI^+] strain phenotypes on transduction into yeast (King and Diaz-Avalos 2004; Tanaka et al. 2004). The propagation rates for these synthetic yeast prion strains were coupled to their conformational stability (Tanaka et al. 2004), a finding that was later

D.W. Colby and S.B. Prusiner

extended to mammalian prion strains (Legname et al. 2006; Colby et al. 2009).

ENLARGING SPECTRUM OF PRION-LIKE DISEASES

The discovery that prions form amyloid prompted one of us to suggest that the common neurodegenerative diseases are also caused by prions (Prusiner 1984; Prusiner 2001) despite the inability to transmit such illnesses to monkeys and apes (Goudsmit et al. 1980). Brain extracts from either Alzheimer's patients or aged Tg mice expressing mutant APP injected into the brains of Tg mice expressing the amyloid precursor protein (APP) carrying the Swedish point mutation (Haass et al. 1995) accelerated the formation of A β amyloid plaques (Meyer-Luehmann et al. 2006; Eisele et al. 2009). Brain extracts from Tg mice expressing mutant tau injected into the brains of Tg mice expressing human wt tau produced aggregates of human tau (Clavaguera et al. 2009). Similar results were found for aggregated tau protein added to cultured cells, which induced the aggregation of nascent tau (Frost et al. 2009). These findings suggest that the tauopathies result from a prion-like process that induces hyperphosphorylation of tau followed by polymerization into filamentous aggregates. The production of hyperphosphorylated tau also appears to be stimulated by oligomers of the A β peptide, whereas amyloid fibrils comprised of A β are a much less efficient stimulus (Lambert et al. 1998). An expanded 44-mer polyglutamine repeat of a truncated huntingtin protein was found to stimulate aggregation of a "normal" 25 mer; this aggregated state could be maintained in cell culture over many generations, arguing for prion-like propagation of huntingtin aggregates (Ren et al. 2009). Patients suffering from Parkinson's disease who received fetal grafts of substantia nigral cells later showed aberrantly folded α -synuclein in Lewy bodies within the transplanted grafts, arguing that α -synuclein acted like a prion (Kordower et al. 2008; Li et al. 2008; Olanow and Prusiner 2009). Taken together, these findings argue that prion-like, self-propagating

states feature in many different, if not all, neurodegenerative diseases.

A general model of propagation of mammalian prion-like conformational states should include the following considerations (Table 2): First, when the precursor protein is converted to a prion, it undergoes posttranslational modification. Such changes generally result in the acquisition of a high β -sheet content. Proteolytic cleavage features in Alzheimer's disease (AD) (Glennner and Wong 1984; Masters et al. 1985) and hyperphosphorylation occurs in both AD and the tauopathies (Grundke-Iqbal et al. 1986; Lee et al. 1991). Second, the β -sheet-rich conformers form oligomers that are toxic to cells (Walsh and Selkoe 2007). Third, such oligomers are generally rendered less toxic when they polymerize into amyloid fibrils. Fourth, amyloid fibrils are sequestered into biological wastebaskets in the CNS where they are designated "plaques" in the extracellular space, and "tangles" or "bodies" within the cytoplasm of neurons. Inert PrP amyloid fibrils coalesce to form plaques in prion diseases whereas fibrils composed of the A β peptide form plaques in AD. Paired-helical filaments composed of hyperphosphorylated tau form neurofibrillary tangles in AD, whereas tau fibrils coalesce into deposits called Pick bodies in one of the frontotemporal dementias generally labeled Pick's disease. In other tauopathies, less well-formed tau aggregates have been

Table 2. Some characteristics of mammalian prions.

- When the precursor protein is converted to a prion, it undergoes posttranslational modification during which it becomes enriched in β -sheet structure.
- β -sheet-rich conformers form oligomers that are toxic to cells.
- Prion oligomers are generally rendered less toxic when they polymerize into amyloid fibrils.
- Amyloid fibrils are sequestered in biological wastebaskets such as plaques, tangles, or inclusion bodies.
- Mutations in specific proteins cause familial neurodegenerative diseases by facilitating conversion of the protein into the prion state.

identified inside cells. After α -synuclein acquires a high β -sheet content, it polymerizes into amyloid fibrils that coalesce in neurons to form Lewy bodies. Fifth, mutations in the corresponding proteins cause familial neurodegenerative diseases and facilitate conversion of the protein to its prion state. For example, over 40 mutations in PrP have been identified that cause GGS, fCJD, and FFI (Hsiao et al. 1989; Goldfarb et al. 1991b; Medori et al. 1992). Mutations in APP or presenilin (γ -secretase) that cleaves APP into A β cause familial AD (Goate et al. 1991), and duplication of the APP gene in Down's syndrome invariably causes AD (Goldgaber et al. 1987). Mutations in tau cause tauopathies (Hutton et al. 1998). Mutations in α -synuclein cause familial Parkinson's disease (Polymeropoulos et al. 1997); duplication or triplication of the α -synuclein gene also causes Parkinson's disease (Singleton et al. 2003).

Prions need not cause disease but may function as regulators of cell metabolism. In yeast, all of the prion proteins found to date have a CG-rich domain that adopts a β -sheet-rich conformation that polymerizes into amyloid. The Sup35 protein in the prion state causes a reduction in the fidelity of polypeptide chain termination during protein synthesis (Wickner et al. 2007). The *Aplysia* prion comprised of the cytoplasmic polyadenylation element binding (CPEB) protein appears to facilitate polyadenylation within limited regions of neuronal cells, such as dendrites, and has been suggested to function in long-term memory (Si et al. 2010).

TOWARD THERAPEUTICS FOR PRION DISEASES

Despite these advances in understanding prions and many of the neurodegenerative diseases, no treatment is currently available to halt the progression of any of these illnesses. Studies of prions in mice have elucidated several aspects of neurodegeneration that may prove useful in developing effective therapeutics. First, reduction of the precursor protein PrP^C prolongs the incubation time (Büeler et al. 1993; Prusiner

et al. 1993; Safar et al. 2005). Second, slowing prion formation by inhibiting of the formation of nascent PrP^{Sc} prolongs the incubation time (Kawasaki et al. 2007). Third, reducing the availability of PrP^C in cells or mice where prion infection has already been established allows for existing prions to be cleared (Enari et al. 2001; Peretz et al. 2001b; Safar et al. 2005). Fourth, enhancing the clearance of PrP^{Sc} provides an alternative route of action for therapeutic intervention (Supattapone et al. 1999b; Supattapone et al. 2001).

Blocking conversion of PrP^C to PrP^{Sc} would seem to be the most practical therapeutic approach, as the cellular pathogenesis of prion disease is downstream of this event and not well understood. Many compounds that inhibit conversion have been identified, including polysulfated anions, dextrans, Congo red dye, oligonucleotides, and cyclic tetrapyrroles (for reviews, see Trevitt and Collinge [2006]; Sim and Caughey [2009]; Silber [2010]). Effective treatment for prion disease is hampered by the difficulty of these and other putative therapeutics to access the CNS, and by the difficulty of identifying small molecules that can prevent the protein-protein interactions that result in propagation of alternatively folded protein isoforms. Studies with a phenylhydrazone revealed restricted efficacy for specific prion strains (Kawasaki et al. 2007) whereas studies with the drug quinacrine revealed the development of drug-resistant prions (Ghaemmaghami et al. 2009).

It seems likely that studies on therapeutics for prion diseases will inform the development of drugs that halt AD, the frontotemporal dementias, or Parkinson's disease; moreover, the lack of success in treating such diseases argues for new paradigms. Work on the prion diseases suggests that treatment for a limited time that reduces or interrupts the formation of nascent prions may be sufficient for the normal cellular clearance mechanisms to overtake the synthesis of new prions. Such an approach would argue for the development of drugs that can be administered for a short period of time instead of many years, which is the commonly held supposition.

D.W. Colby and S.B. Prusiner

REFERENCES

- Alpers MP. 1968. Kuru: implications of its transmissibility for the interpretation of its changing epidemiological pattern. In *The central nervous system: some experimental models of neurological diseases*. (ed. OT Bailey, DE Smith), pp. 234–251. Williams and Wilkins Company, Baltimore.
- Anderson RM, Donnelly CA, Ferguson NM, Woolhouse MEJ, Watt CJ, Udy HJ, MaWhinney S, Dunstan SP, Southwood TRE, Wilesmith JW, et al. 1996. Transmission dynamics and epidemiology of BSE in British cattle. *Nature* **382**: 779–788.
- Antonyuk SV, Trevitt CR, Strange RW, Jackson GS, Sangar D, Batchelor M, Cooper S, Fraser C, Jones S, Georgiou T, et al. 2009. Crystal structure of human prion protein bound to a therapeutic antibody. *Proc Natl Acad Sci* **106**: 2554–2558.
- Avery OT, MacLeod CM, McCarty M. 1944. Studies on the chemical nature of the substance inducing transformation of pneumococcal types. Induction of transformation by a deoxyribonucleic acid fraction isolated from pneumococcus type III. *J Exp Med* **79**: 137–158.
- Barria MA, Mukherjee A, Gonzalez-Romero D, Morales R, Soto C. 2009. *De novo* generation of infectious prions *in vitro* produces a new disease phenotype. *PLoS Pathog* **5**: e1000421.
- Basler K, Oesch B, Scott M, Westaway D, Wälchli M, Groth DF, McKinley MP, Prusiner SB, Weissmann C. 1986. Scrapie and cellular PrP isoforms are encoded by the same chromosomal gene. *Cell* **46**: 417–428.
- Bellinger-Kawahara CG, Kempner E, Groth DF, Gabizon R, Prusiner SB. 1988. Scrapie prion liposomes and rods exhibit target sizes of 55,000 Da. *Virology* **164**: 537–541.
- Bertoni JM, Brown P, Goldfarb LG, Rubenstein R, Gajdusek DC. 1992. Familial Creutzfeldt-Jakob disease (codon 200 mutation) with supranuclear palsy. *JAMA* **268**: 2413–2415.
- Bessen RA, Marsh RF. 1994. Distinct PrP properties suggest the molecular basis of strain variation in transmissible mink encephalopathy. *J Virol* **68**: 7859–7868.
- Borchelt DR, Scott M, Taraboulos A, Stahl N, Prusiner SB. 1990. Scrapie and cellular prion proteins differ in their kinetics of synthesis and topology in cultured cells. *J Cell Biol* **110**: 743–752.
- Brachmann A, Baxa U, Wickner RB. 2005. Prion generation *in vitro*: amyloid of Ure2p is infectious. *EMBO J* **24**: 3082–3092.
- Brown P, Gajdusek DC. 1991. Survival of scrapie virus after 3 years' interment. *Lancet* **337**: 269–270.
- Brown P, Gibbs CJ Jr, Rodgers-Johnson P, Asher DM, Sulima MP, Bacote A, Goldfarb LG, Gajdusek DC. 1994. Human spongiform encephalopathy: the National Institutes of Health series of 300 cases of experimentally transmitted disease. *Ann Neurol* **35**: 513–529.
- Browning SR, Mason GL, Seward T, Green M, Eliason GA, Mathiason C, Miller MW, Williams ES, Hoover E, Telling GC. 2004. Transmission of prions from mule deer and elk with chronic wasting disease to transgenic mice expressing cervid PrP. *J Virol* **78**: 13345–13350.
- Bruce ME, Dickinson AG, Fraser H. 1976. Cerebral amyloidosis in scrapie in the mouse: Effect of agent strain and mouse genotype. *Neuropathol Appl Neurobiol* **2**: 471–478.
- Büeler H, Aguzzi A, Sailer A, Greiner R-A, Autenried P, Aguet M, Weissmann C. 1993. Mice devoid of PrP are resistant to scrapie. *Cell* **73**: 1339–1347.
- Büeler H, Fisher M, Lang Y, Bluethmann H, Lipp H-P, DeArmond SJ, Prusiner SB, Aguet M, Weissmann C. 1992. Normal development and behaviour of mice lacking the neuronal cell-surface PrP protein. *Nature* **356**: 577–582.
- Carlson GA, Westaway D, DeArmond SJ, Peterson-Torchia M, Prusiner SB. 1989. Primary structure of prion protein may modify scrapie isolate properties. *Proc Natl Acad Sci* **86**: 7475–7479.
- Castilla J, Saa P, Hetz C, Soto C. 2005. *In vitro* generation of infectious scrapie prions. *Cell* **121**: 195–206.
- Caughey B, Raymond GJ. 1991. The scrapie-associated form of PrP is made from a cell surface precursor that is both protease- and phospholipase-sensitive. *J Biol Chem* **266**: 18217–18223.
- Caughey BW, Dong A, Bhat KS, Ernst D, Hayes SE, Caughey WS. 1991b. Secondary structure analysis of the scrapie-associated protein PrP 27–30 in water by infrared spectroscopy. *Biochemistry* **30**: 7672–7680.
- Caughey B, Raymond GJ, Ernst D, Race RE. 1991a. N-terminal truncation of the scrapie-associated form of PrP by lysosomal protease(s): Implications regarding the site of conversion of PrP to the protease-resistant state. *J Virol* **65**: 6597–6603.
- Chatelain J, Cathala F, Brown P, Raharison S, Court L, Gajdusek DC. 1981. Epidemiologic comparisons between Creutzfeldt-Jakob disease and scrapie in France during the 12-year period 1968–1979. *J Neurol Sci* **51**: 329–337.
- Chazot G, Broussolle E, Lapras CI, Blättler T, Aguzzi A, Kopp N. 1996. New variant of Creutzfeldt-Jakob disease in a 26-year-old French man. *Lancet* **347**: 1181.
- Chien P, Weissman JS, DePace AH. 2004. Emerging principles of conformation-based prion inheritance. *Annu Rev Biochem* **73**: 617–656.
- Clavaguera F, Bolmont T, Crowther RA, Abramowski D, Frank S, Probst A, Fraser G, Stalder AK, Beibel M, Staufenbiel M, et al. 2009. Transmission and spreading of tauopathy in transgenic mouse brain. *Nat Cell Biol* **11**: 909–913.
- Cohen FE, Pan K-M, Huang Z, Baldwin M, Fletterick RJ, Prusiner SB. 1994. Structural clues to prion replication. *Science* **264**: 530–531.
- Colby DW, Giles K, Legname G, Wille H, Baskakov IV, DeArmond SJ, Prusiner SB. 2009. Design and construction of diverse mammalian prion strains. *Proc Natl Acad Sci* **106**: 20417–20422.
- Colby DW, Wain R, Baskakov IV, Legname G, Palmer CG, Nguyen H-OB, Lemus A, Cohen FE, DeArmond SJ, Prusiner SB. 2010. Protease-sensitive synthetic prions. *PLoS Pathog* **6**: e1000736.
- Colby DW, Zhang Q, Wang S, Groth D, Legname G, Riesner D, Prusiner SB. 2007. Prion detection by an amyloid seeding assay. *Proc Natl Acad Sci* **104**: 20914–20919.



- Collinge J, Sidle KCL, Meads J, Ironside J, Hill AF. 1996. Molecular analysis of prion strain variation and the aetiology of "new variant" CJD. *Nature* **383**: 685–690.
- Collinge J, Whittington MA, Sidle KC, Smith CJ, Palmer MS, Clarke AR, Jefferys JGR. 1994. Prion protein is necessary for normal synaptic function. *Nature* **370**: 295–297.
- Cousens SN, Vynnycky E, Zeidler M, Will RG, Smith PG. 1997. Predicting the CJD epidemic in humans. *Nature* **385**: 197–198.
- Coustou V, Deleu C, Saupe S, Begueret J. 1997. The protein product of the *het-s* heterokaryon incompatibility gene of the fungus *Podospora anserina* behaves as a prion analog. *Proc Natl Acad Sci* **94**: 9773–9778.
- DeArmond SJ, McKinley MP, Barry RA, Braunfeld MB, McColloch JR, Prusiner SB. 1985. Identification of prion amyloid filaments in scrapie-infected brain. *Cell* **41**: 221–235.
- DeArmond SJ, Mobley WC, DeMott DL, Barry RA, Beckstead JH, Prusiner SB. 1987. Changes in the localization of brain prion proteins during scrapie infection. *Neurology* **37**: 1271–1280.
- Deleault NR, Harris BT, Rees JR, Supattapone S. 2007. Formation of native prions from minimal components in vitro. *Proc Natl Acad Sci* **104**: 9741–9746.
- Dickinson AG, Meikle VMH. 1969. A comparison of some biological characteristics of the mouse-passaged scrapie agents, 22A and ME7. *Genet Res* **13**: 213–225.
- Dlouhy SR, Hsiao K, Farlow MR, Foroud T, Conneally PM, Johnson P, Prusiner SB, Hodes ME, Ghetti B. 1992. Linkage of the Indiana kindred of Gerstmann-Sträussler-Scheinker disease to the prion protein gene. *Nat Genet* **1**: 64–67.
- Doh-ura K, Tateishi J, Sasaki H, Kitamoto T, Sakaki Y. 1989. Pro- \rightarrow Leu change at position 102 of prion protein is the most common but not the sole mutation related to Gerstmann-Sträussler syndrome. *Biochem Biophys Res Commun* **163**: 974–979.
- Donne DG, Viles JH, Groth D, Mehlhorn I, James TL, Cohen FE, Prusiner SB, Wright PE, Dyson HJ. 1997. Structure of the recombinant full-length hamster prion protein PrP(29-231): the N terminus is highly flexible. *Proc Natl Acad Sci* **94**: 13452–13457.
- Dossena S, Imeri L, Mangieri M, Garofoli A, Ferrari L, Senatore A, Restelli E, Balducci C, Fiordaliso F, Salio M, et al. 2008. Mutant prion protein expression causes motor and memory deficits and abnormal sleep patterns in a transgenic mouse model. *Neuron* **60**: 598–609.
- Eisele YS, Bolmont T, Heikenwalder M, Langer F, Jacobson LH, Yan ZX, Roth K, Aguzzi A, Staufenbiel M, Walker LC, et al. 2009. Induction of cerebral β -amyloidosis: Intracerebral versus systemic $A\beta$ inoculation. *Proc Natl Acad Sci* **106**: 12926–12931.
- Enari M, Flechsig E, Weissmann C. 2001. Scrapie prion protein accumulation by scrapie-infected neuroblastoma cells abrogated by exposure to a prion protein antibody. *Proc Natl Acad Sci* **98**: 9295–9299.
- Fraser H, Dickinson AG. 1973. Scrapie in mice. Agent-strain differences in the distribution and intensity of grey matter vacuolation. *J Comp Pathol* **83**: 29–40.
- Frost B, Jacks RL, Diamond MI. 2009. Propagation of tau misfolding from the outside to the inside of a cell. *J Biol Chem* **284**: 12845–12852.
- Gabriel J-M, Oesch B, Kretzschmar H, Scott M, Prusiner SB. 1992. Molecular cloning of a candidate chicken prion protein. *Proc Natl Acad Sci* **89**: 9097–9101.
- Gajdusek DC. 1977. Unconventional viruses and the origin and disappearance of kuru. *Science* **197**: 943–960.
- Gambetti P, Dong Z, Yuan J, Xiao X, Zheng M, Alshekhlee A, Castellani R, Cohen M, Barria MA, Gonzalez-Romero D, et al. 2008. A novel human disease with abnormal prion protein sensitive to protease. *Ann Neurol* **63**: 697–708.
- Gambetti P, Parchi P, Petersen RB, Chen SG, Lugaresi E. 1995. Fatal familial insomnia and familial Creutzfeldt-Jakob disease: Clinical, pathological and molecular features. *Brain Pathol* **5**: 43–51.
- Ghaemmaghami S, Ahn M, Lessard P, Giles K, Legname G, DeArmond SJ, Prusiner SB. 2009. Continuous quina-craine treatment results in the formation of drug-resistant prions. *PLoS Pathog* **5**: e1000673.
- Ghaemmaghami S, Phuan PW, Perkins B, Ullman J, May BC, Cohen FE, Prusiner SB. 2007. Cell division modulates prion accumulation in cultured cells. *Proc Natl Acad Sci* **104**: 17971–17976.
- Gibbs CJ Jr, Gajdusek DC, Asher DM, Alpers MB, Beck E, Daniel PM, Matthews WB. 1968. Creutzfeldt-Jakob disease (spongiform encephalopathy): Transmission to the chimpanzee. *Science* **161**: 388–389.
- Glasse R. 1967. Cannibalism in the kuru region of New Guinea. *Trans NY Acad Sci [Ser 2]* **29**: 748–754.
- Glenner GG, Wong CW. 1984. Alzheimer's disease: Initial report of the purification and characterization of a novel cerebrovascular amyloid protein. *Biochem Biophys Res Commun* **120**: 885–890.
- Goate A, Chartier-Harlin M-C, Mullan M, Brown J, Crawford F, Fidani L, Giuffra L, Haynes A, Irving N, James L, et al. 1991. Segregation of a missense mutation in the amyloid precursor protein gene with familial Alzheimer's disease. *Nature* **349**: 704–706.
- Goldfarb LG, Brown P, McCombie WR, Goldgaber D, Swergold GD, Wills PR, Cervenakova L, Baron H, Gibbs CJ, Gajdusek DC. 1991a. Transmissible familial Creutzfeldt-Jakob disease associated with five, seven, and eight extra octapeptide coding repeats in the *Prnp* gene. *Proc Natl Acad Sci* **88**: 10926–10930.
- Goldfarb LG, Brown P, Mitrova E, Cervenakova L, Goldin L, Korczyn AD, Chapman J, Galvez S, Cartier L, Rubenstein R, et al. 1991b. Creutzfeldt-Jacob disease associated with the *Prnp* codon 200^{Lys} mutation: An analysis of 45 families. *Eur J Epidemiol* **7**: 477–486.
- Goldfarb LG, Haltia M, Brown P, Nieto A, Kovanen J, McCombie WR, Trapp S, Gajdusek DC. 1991c. New mutation in scrapie amyloid precursor gene (at codon 178) in Finnish Creutzfeldt-Jakob kindred. *Lancet* **337**: 425.
- Goldfarb LG, Korczyn AD, Brown P, Chapman J, Gajdusek DC. 1990a. Mutation in codon 200 of scrapie amyloid precursor gene linked to Creutzfeldt-Jakob disease in Sephardic Jews of Libyan and non-Libyan origin. *Lancet* **336**: 637–638.

D.W. Colby and S.B. Prusiner

- Goldfarb LG, Mitrova E, Brown P, Toh BH, Gajdusek DC. 1990b. Mutation in codon 200 of scrapie amyloid protein gene in two clusters of Creutzfeldt-Jakob disease in Slovakia. *Lancet* **336**: 514–515.
- Goldgaber D, Goldfarb LG, Brown P, Asher DM, Brown WT, Lin S, Teener JW, Feinstein SM, Rubenstein R, Kascsak RJ, et al. 1989. Mutations in familial Creutzfeldt-Jakob disease and Gerstmann-Sträussler-Scheinker's syndrome. *Exp Neurol* **106**: 204–206.
- Goldgaber D, Lerman MI, McBride OW, Saffiotti U, Gajdusek DC. 1987. Characterization and chromosomal localization of a cDNA encoding brain amyloid of Alzheimer's disease. *Science* **235**: 877–880.
- Goldmann W, Hunter N, Benson G, Foster JD, Hope J. 1991. Different scrapie-associated fibril proteins (PrP) are encoded by lines of sheep selected for different alleles of the *Sip* gene. *J Gen Virol* **72**: 2411–2417.
- Gorodinsky A, Harris DA. 1995. Glycolipid-anchored proteins in neuroblastoma cells form detergent-resistant complexes without caveolin. *J Cell Biol* **129**: 619–627.
- Goudsmit J, Morrow CH, Asher DM, Yanagihara RT, Masters CL, Gibbs CJ Jr, Gajdusek DC. 1980. Evidence for and against the transmissibility of Alzheimer's disease. *Neurology* **30**: 945–950.
- Govaerts C, Wille H, Prusiner SB, Cohen FE. 2004a. Evidence for assembly of prions with left-handed β -helices into trimers. *Proc Natl Acad Sci* **101**: 8342–8347.
- Govaerts C, Wille H, Prusiner SB, Cohen FE. 2004b. Structural studies of prion proteins. In *Prion biology and diseases* (ed. SB Prusiner), pp. 243–282. Cold Spring Harbor Laboratory Press, Cold Spring Harbor.
- Grundke-Iqbal I, Iqbal K, Tung Y-C, Quinlan M, Wisniewski HM, Binder LI. 1986. Abnormal phosphorylation of the microtubule-associated protein (tau) in Alzheimer cytoskeletal pathology. *Proc Natl Acad Sci* **83**: 4913–4917.
- Haass C, Lemere CA, Capell A, Citron M, Seubert P, Schenk D, Lannfelt L, Selkoe DJ. 1995. The Swedish mutation causes early-onset Alzheimer's disease by β -secretase cleavage within the secretory pathway. *Nat Med* **1**: 1291–1296.
- Hamir AN, Cutlip RC, Miller JM, Williams ES, Stack MJ, Miller MW, O'Rourke KI, Chaplin MJ. 2001. Preliminary findings on the experimental transmission of chronic wasting disease agent of mule deer to cattle. *J Vet Diagn Invest* **13**: 91–96.
- Hermes JW, Kretschmar HA, Titz S, Keller BU. 1995. Patch-clamp analysis of synaptic transmission to cerebellar Purkinje cells of prion protein knockout mice. *Eur J Neurosci* **7**: 2508–2512.
- Hsiao K, Baker HF, Crow TJ, Poulter M, Owen F, Terwilliger JD, Westaway D, Ott J, Prusiner SB. 1989. Linkage of a prion protein missense variant to Gerstmann-Sträussler syndrome. *Nature* **338**: 342–345.
- Hsiao KK, Cass C, Schellenberg GD, Bird T, Devine-Gage E, Wisniewski H, Prusiner SB. 1991b. A prion protein variant in a family with the telencephalic form of Gerstmann-Sträussler-Scheinker syndrome. *Neurology* **41**: 681–684.
- Hsiao KK, Groth D, Scott M, Yang S-L, Serban H, Rapp D, Foster D, Torchia M, DeArmond SJ, Prusiner SB. 1994. Serial transmission in rodents of neurodegeneration from transgenic mice expressing mutant prion protein. *Proc Natl Acad Sci* **91**: 9126–9130.
- Hsiao K, Meiner Z, Kahana E, Cass C, Kahana I, Avrahami D, Scarlato G, Abramsky O, Prusiner SB, Gabizon R. 1991a. Mutation of the prion protein in Libyan Jews with Creutzfeldt-Jakob disease. *N Engl J Med* **324**: 1091–1097.
- Hsiao KK, Scott M, Foster D, Groth DF, DeArmond SJ, Prusiner SB. 1990. Spontaneous neurodegeneration in transgenic mice with mutant prion protein. *Science* **250**: 1587–1590.
- Hutton M, Lendon CL, Rizzu P, Baker M, Froelich S, Houlden H, Pickering-Brown S, Chakraverty S, Isaacs A, Grover A, et al. 1998. Association of missense and 5'-splice-site mutations in τ with the inherited dementia FTDP-17. *Nature* **393**: 702–705.
- Hwang D, Lee IY, Yoo H, Gehlenborg N, Cho JH, Petritis B, Baxter D, Pitsstick R, Young R, Spicer D, et al. 2009. A systems approach to prion disease. *Mol Syst Biol* **5**: 252.
- Jackson WS, Borkowski AW, Faas H, Steele AD, King OD, Watson N, Jasanoff A, Lindquist S. 2009. Spontaneous generation of prion infectivity in fatal familial insomnia knockin mice. *Neuron* **63**: 438–450.
- James TL, Liu H, Ulyanov NB, Farr-Jones S, Zhang H, Donne DG, Kaneko K, Groth D, Mehlhorn I, Prusiner SB, et al. 1997. Solution structure of a 142-residue recombinant prion protein corresponding to the infectious fragment of the scrapie isoform. *Proc Natl Acad Sci* **94**: 10086–10091.
- Jeffrey M, Goodsir CM, Bruce M, McBride PA, Scott JR, Halliday WG. 1994. Correlative light and electron microscopy studies of PrP localisation in 87V scrapie. *Brain Res* **656**: 329–343.
- Kaneko K, Ball HL, Wille H, Zhang H, Groth D, Torchia M, Tremblay P, Safar J, Prusiner SB, DeArmond SJ, et al. 2000. A synthetic peptide initiates Gerstmann-Sträussler-Scheinker (GSS) disease in transgenic mice. *J Mol Biol* **295**: 997–1007.
- Kaneko K, Vey M, Scott M, Pilkuhn S, Cohen FE, Prusiner SB. 1997a. COOH-terminal sequence of the cellular prion protein directs subcellular trafficking and controls conversion into the scrapie isoform. *Proc Natl Acad Sci* **94**: 2333–2338.
- Kaneko K, Wille H, Mehlhorn I, Zhang H, Ball H, Cohen FE, Baldwin MA, Prusiner SB. 1997b. Molecular properties of complexes formed between the prion protein and synthetic peptides. *J Mol Biol* **270**: 574–586.
- Kaski D, Mead S, Hyare H, Cooper S, Jampana R, Overell J, Knight R, Collinge J, Rudge P. 2009. Variant CJD in an individual heterozygous for *PRNP* codon 129. *Lancet* **374**: 2128.
- Kawasaki Y, Kawagoe K, Chen CJ, Teruya K, Sakasegawa Y, Doh-ura K. 2007. Orally administered amyloidophilic compound is effective in prolonging the incubation periods of animals cerebrally infected with prion diseases in a prion strain-dependent manner. *J Virol* **81**: 12889–12898.
- Kimberlin R, Walker C. 1977. Characteristics of a short incubation model of scrapie in the golden hamster. *J Gen Virol* **34**: 295–304.
- King CY, Diaz-Avalos R. 2004. Protein-only transmission of three yeast prion strains. *Nature* **428**: 319–323.



- Klatzo I, Gajdusek DC, Zigas V. 1959. Pathology of kuru. *Lab Invest* **8**: 799–847.
- Koch TK, Berg BO, DeArmond SJ, Gravina RF. 1985. Creutzfeldt-Jakob disease in a young adult with idiopathic hypopituitarism. Possible relation to the administration of cadaveric human growth hormone. *N Engl J Med* **313**: 731–733.
- Kocisko DA, Come JH, Priola SA, Chesebro B, Raymond GJ, Lansbury PT Jr, Caughey B. 1994. Cell-free formation of protease-resistant prion protein. *Nature* **370**: 471–474.
- Kordower JH, Chu Y, Hauser RA, Freeman TB, Olanow CW. 2008. Lewy body-like pathology in long-term embryonic nigral transplants in Parkinson's disease. *Nat Med* **14**: 504–506.
- Kretzschmar HA, Honold G, Seitelberger F, Feucht M, Wessely P, Mehraein P, Budka H. 1991. Prion protein mutation in family first reported by Gerstmann, Strüssler, and Scheinker. *Lancet* **337**: 1160.
- Lambert MP, Barlow AK, Chromy BA, Edwards C, Freed R, Liosatos M, Morgan TE, Rozovsky I, Trommer B, Viola KL, et al. 1998. Diffusible, nonfibrillar ligands derived from A β 1–42 are potent central nervous system neurotoxins. *Proc Natl Acad Sci* **95**: 6448–6453.
- Lee VM-Y, Balin BJ, Orvos IJ, Trojanowski JQ. 1991. A68: a major subunit of paired helical filaments and derivatized forms of normal tau. *Science* **251**: 645–678.
- Legname G, Baskakov IV, Nguyen H-OB, Riesner D, Cohen FE, DeArmond SJ, Prusiner SB. 2004. Synthetic mammalian prions. *Science* **305**: 673–676.
- Legname G, Nguyen H-OB, Baskakov IV, Cohen FE, DeArmond SJ, Prusiner SB. 2005. Strain-specified characteristics of mouse synthetic prions. *Proc Natl Acad Sci* **102**: 2168–2173.
- Legname G, Nguyen H-OB, Peretz D, Cohen FE, DeArmond SJ, Prusiner SB. 2006. Continuum of prion protein structures enciphers a multitude of prion isolate-specified phenotypes. *Proc Natl Acad Sci* **103**: 19105–19110.
- Li J, Browning S, Mahal SP, Oelschlegel AM, Weissmann C. 2010. Darwinian evolution of prions in cell culture. *Science* **327**: 869–872.
- Li JY, Englund E, Holton JL, Soulet D, Hagell P, Lees AJ, Lashley T, Quinn NP, Rehnrcrona S, Bjorklund A, et al. 2008. Lewy bodies in grafted neurons in subjects with Parkinson's disease suggest host-to-graft disease propagation. *Nat Med* **14**: 501–503.
- Lledo P-M, Tremblay P, DeArmond SJ, Prusiner SB, Nicoll RA. 1996. Mice deficient for prion protein exhibit normal neuronal excitability and synaptic transmission in the hippocampus. *Proc Natl Acad Sci* **93**: 2403–2407.
- Lugaresi E, Medori R, Montagna P, Baruzzi A, Cortelli P, Lugaresi A, Tinuper P, Zucconi M, Gambetti P. 1986. Fatal familial insomnia and dysautonomia with selective degeneration of thalamic nuclei. *N Engl J Med* **315**: 997–1003.
- Maddelein ML, Dos Reis S, Duvezin-Caubet S, Coulyar-Salin B, Saupe SJ. 2002. Amyloid aggregates of the HET-s prion protein are infectious. *Proc Natl Acad Sci* **99**: 7402–7407.
- Manson JC, Clarke AR, Hooper ML, Aitchison L, McConnell I, Hope J. 1994. 129/Ola mice carrying a null mutation in PrP that abolishes mRNA production are developmentally normal. *Mol Neurobiol* **8**: 121–127.
- Masters CL, Gajdusek DC, Gibbs CJ Jr. 1981. Creutzfeldt-Jakob disease virus isolations from the Gerstmann-Sträussler syndrome. *Brain* **104**: 559–588.
- Masters CL, Simms G, Weinman NA, Multhaup G, McDonald BL, Beyreuther K. 1985. Amyloid plaque core protein in Alzheimer disease and Down syndrome. *Proc Natl Acad Sci* **82**: 4245–4249.
- McKinley MP, Bolton DC, Prusiner SB. 1983. A protease-resistant protein is a structural component of the scrapie prion. *Cell* **35**: 57–62.
- McKinley MP, Meyer RK, Kenaga L, Rahbar F, Cotter R, Serban A, Prusiner SB. 1991. Scrapie prion rod formation in vitro requires both detergent extraction and limited proteolysis. *J Virol* **65**: 1340–1351.
- McKnight S, Tjian R. 1986. Transcriptional selectivity of viral genes in mammalian cells. *Cell* **46**: 795–805.
- Medori R, Tritschler H-J, LeBlanc A, Villare F, Manetto V, Chen HY, Xue R, Leal S, Montagna P, Cortelli P, et al. 1992. Fatal familial insomnia, a prion disease with a mutation at codon 178 of the prion protein gene. *N Engl J Med* **326**: 444–449.
- Meyer-Luehmann M, Coomaraswamy J, Bolmont T, Kaeser S, Schaefer C, Kilger E, Neuenschwander A, Abramowski D, Frey P, Jaton AL, et al. 2006. Exogenous induction of cerebral β -amyloidogenesis is governed by agent and host. *Science* **313**: 1781–1784.
- Miller MW, Williams ES, McCarty CW, Spraker TR, Kreeger TJ, Larsen CT, Thorne ET. 2000. Epizootiology of chronic wasting disease in free-ranging cervids in Colorado and Wyoming. *J Wildl Dis* **36**: 676–690.
- Moore RC, Lee IY, Silverman GL, Harrison PM, Strome R, Heinrich C, Karunaratne A, Pasternak SH, Chishti MA, Liang Y, et al. 1999. Ataxia in prion protein (PrP)-deficient mice is associated with upregulation of the novel PrP-like protein doppel. *J Mol Biol* **292**: 797–817.
- Muller H, Stitz L, Wille H, Prusiner SB, Riesner D. 2007. Influence of water, fat, and glycerol on the mechanism of thermal prion inactivation. *J Biol Chem* **282**: 35855–35867.
- Naslavsky N, Stein R, Yanai A, Friedlander G, Taraboulos A. 1997. Characterization of detergent-insoluble complexes containing the cellular prion protein and its scrapie isoform. *J Biol Chem* **272**: 6324–6331.
- Nathanson N, Wilesmith J, Griot C. 1997. Bovine spongiform encephalopathy (BSE): cause and consequences of a common source epidemic. *Am J Epidemiol* **145**: 959–969.
- Nazor KE, Kuhn F, Seward T, Green M, Zwald D, Purro M, Schmid J, Biffiger K, Power AM, Oesch B, et al. 2005. Immunodetection of disease-associated mutant PrP, which accelerates disease in GSS transgenic mice. *EMBO J* **24**: 2472–2480.
- Olanow CW, Prusiner SB. 2009. Is Parkinson's disease a prion disorder? *Proc Natl Acad Sci* **106**: 12571–12572.
- Owen F, Poulter M, Lofthouse R, Collinge J, Crow TJ, Risby D, Baker HF, Ridley RM, Hsiao K, Prusiner SB. 1989. Insertion in prion protein gene in familial Creutzfeldt-Jakob disease. *Lancet* **333**: 51–52.

D.W. Colby and S.B. Prusiner

- Palsson PA. 1979. Rida (scrapie) in Iceland and its epidemiology. In *Slow transmissible diseases of the nervous system*, Vol. 1 (ed. SB Prusiner, WJ Hadlow), pp. 357–366. Academic Press, New York.
- Pan K-M, Baldwin M, Nguyen J, Gasset M, Serban A, Groth D, Mehlhorn I, Huang Z, Fletterick RJ, Cohen FE, et al. 1993. Conversion of α -helices into β -sheets features in the formation of the scrapie prion proteins. *Proc Natl Acad Sci* **90**: 10962–10966.
- Patino MM, Liu J-J, Glover JR, Lindquist S. 1996. Support for the prion hypothesis for inheritance of a phenotypic trait in yeast. *Science* **273**: 622–626.
- Pattison IH. 1965. Experiments with scrapie with special reference to the nature of the agent and the pathology of the disease. In *Slow, latent and temperate virus infections, NINDB Monograph 2*, (ed. DC Gajdusek, CJ Jr Gibbs, MP Alpers), pp. 249–257. U.S. Government Printing, Washington, D.C.
- Paushkin SV, Kushnirov VV, Smirnov VN, Ter-Avanesyan MD. 1997. In vitro propagation of the prion-like state of yeast Sup35 protein. *Science* **277**: 381–383.
- Peretz D, Scott M, Groth D, Williamson A, Burton D, Cohen FE, Prusiner SB. 2001a. Strain-specified relative conformational stability of the scrapie prion protein. *Protein Sci* **10**: 854–863.
- Peretz D, Williamson RA, Kaneko K, Vergara J, Leclerc E, Schmitt-Ulms G, Mehlhorn IR, Legname G, Wormald MR, Rudd PM, et al. 2001b. Antibodies inhibit prion propagation and clear cell cultures of prion infectivity. *Nature* **412**: 739–743.
- Peters PJ, Mironov A, Peretz D, van Donselaar E, Leclerc E, Erpel S, DeArmond SJ, Burton DR, Williamson RA, Vey M, et al. 2003. Trafficking of prion proteins through a caveolae-mediated endosomal pathway. *J Cell Biol* **162**: 703–717.
- Petersen RB, Tabaton M, Berg L, Schrank B, Torack RM, Leal S, Julien J, Vital C, Deleplanque B, Pendlebury WW, et al. 1992. Analysis of the prion protein gene in thalamic dementia. *Neurology* **42**: 1859–1863.
- Public Health Service. 1997. Report on human growth hormone and Creutzfeldt-Jakob disease. Public Health Service Interagency Coordinating Committee, Washington, DC. **14**: 1–11.
- Polymeropoulos MH, Lavedan C, Leroy E, Ide SE, Dehejia A, Dutra A, Pike B, Root H, Rubenstein J, Boyer R, et al. 1997. Mutation in the α -synuclein gene identified in families with Parkinson's disease. *Science* **276**: 2045–2047.
- Poulter M, Baker HF, Frith CD, Leach M, Lofthouse R, Ridley RM, Shah T, Owen E, Collinge J, Brown G, et al. 1992. Inherited prion disease with 144 base pair gene insertion. 1. Genealogical and molecular studies. *Brain* **115**: 675–685.
- Prusiner SB. 1982. Novel proteinaceous infectious particles cause scrapie. *Science* **216**: 136–144.
- Prusiner SB. 1984. Some speculations about prions, amyloid, and Alzheimer's disease. *N Engl J Med* **310**: 661–663.
- Prusiner SB. 1989. Creutzfeldt-Jakob disease and scrapie prions. *Alzheimer Dis Assoc Disord* **3**: 52–78.
- Prusiner SB. 2001. Shattuck Lecture—Neurodegenerative diseases and prions. *N Engl J Med* **344**: 1516–1526.
- Prusiner SB. 2004. An introduction to prion biology and diseases. In *Prion Biology and Diseases*, (ed. SB Prusiner), pp. 1–87. Cold Spring Harbor Laboratory Press, Cold Spring Harbor.
- Prusiner SB, Groth D, Serban A, Koehler R, Foster D, Torchia M, Burton D, Yang S-L, DeArmond SJ. 1993. Ablation of the prion protein (PrP) gene in mice prevents scrapie and facilitates production of anti-PrP antibodies. *Proc Natl Acad Sci* **90**: 10608–10612.
- Prusiner SB, McKinley MP, Bowman KA, Bolton DC, Bendheim PE, Groth DE, Glenner GG. 1983. Scrapie prions aggregate to form amyloid-like birefringent rods. *Cell* **35**: 349–358.
- Prusiner SB, Scott M, Foster D, Pan K-M, Groth D, Mirenda C, Torchia M, Yang S-L, Serban D, Carlson GA, et al. 1990. Transgenic studies implicate interactions between homologous PrP isoforms in scrapie prion replication. *Cell* **63**: 673–686.
- Ren PH, Lauckner JE, Kachirskaja I, Heuser JE, Melki R, Kopito RR. 2009. Cytoplasmic penetration and persistent infection of mammalian cells by polyglutamine aggregates. *Nat Cell Biol* **11**: 219–225.
- Riek R, Hornemann S, Wider G, Billeter M, Glockshuber R, Wüthrich K. 1996. NMR structure of the mouse prion protein domain PrP(121–231). *Nature* **382**: 180–182.
- Riek R, Wider G, Billeter M, Hornemann S, Glockshuber R, Wüthrich K. 1998. Prion protein NMR structure and familial human spongiform encephalopathies. *Proc Natl Acad Sci* **95**: 11667–11672.
- Roos R, Gajdusek DC, Gibbs CJ Jr. 1973. The clinical characteristics of transmissible Creutzfeldt-Jakob disease. *Brain* **96**: 1–20.
- Rosenmann H, Vardi J, Finkelstein Y, Chapman J, Gabizon R. 1998. Identification in Israel of 2 Jewish Creutzfeldt-Jakob disease patients with a 178 mutation at their PrP gene. *Acta Neurol Scand* **97**: 184–187.
- Safar JG, DeArmond SJ, Kociuba K, Deering C, Didorenko S, Bouzamondo-Bernstein E, Prusiner SB, Tremblay P. 2005. Prion clearance in bigenic mice. *J Gen Virol* **86**: 2913–2923.
- Safar J, Wille H, Itri V, Groth D, Serban H, Torchia M, Cohen FE, Prusiner SB. 1998. Eight prion strains have PrP^{Sc} molecules with different conformations. *Nat Med* **4**: 1157–1165.
- Sakaguchi S, Katamine S, Nishida N, Moriuchi R, Shigematsu K, Sugimoto T, Nakatani A, Kataoka Y, Houtani T, Shirabe S, et al. 1996. Loss of cerebellar Purkinje cells in aged mice homozygous for a disrupted PrP gene. *Nature* **380**: 528–531.
- Serban D, Taraboulos A, DeArmond SJ, Prusiner SB. 1990. Rapid detection of Creutzfeldt-Jakob disease and scrapie prion proteins. *Neurology* **40**: 110–117.
- Shorter J, Lindquist S. 2008. Hsp104, Hsp70 and Hsp40 interplay regulates formation, growth and elimination of Sup35 prions. *EMBO J* **27**: 2712–2724.
- Si K, Choi YB, White-Grindley E, Majumdar A, Kandel ER. 2010. Aplysia CPEB can form prion-like multimers in sensory neurons that contribute to long-term facilitation. *Cell* **140**: 421–435.



- Sigurdson CJ, Nilsson KP, Hornemann S, Heikenwalder M, Manco G, Schwarz P, Ott D, Rulicke T, Liberski PP, Julius C, et al. 2009. *De novo* generation of a transmissible spongiform encephalopathy by mouse transgenesis. *Proc Natl Acad Sci* **106**: 304–309.
- Silber BM. 2010. Driving drug discovery: the fundamental role of academic labs. *Sci Transl Med* **2**: 30cm16.
- Sim VL, Caughey B. 2009. Recent advances in prion chemotherapeutics. *Infect Disord Drug Targets* **9**: 81–91.
- Singleton AB, Farrer M, Johnson J, Singleton A, Hague S, Kachergus J, Hulihan M, Peuralinna T, Dutra A, Nussbaum R, et al. 2003. α -Synuclein locus triplication causes Parkinson's disease. *Science* **302**: 841.
- Sparrer HE, Santoso A, Szoka FC Jr, Weissman JS. 2000. Evidence for the prion hypothesis: Induction of the yeast [PSI⁺] factor by in vitro-converted Sup35 protein. *Science* **289**: 595–599.
- Spencer MD, Knight RS, Will RG. 2002. First hundred cases of variant Creutzfeldt-Jakob disease: Retrospective case note review of early psychiatric and neurological features. *BMJ* **324**: 1479–1482.
- Speransky VV, Taylor KL, Edsdes HK, Wickner RB, Steven AC. 2001. Prion filament networks in [URE3] cells of *Saccharomyces cerevisiae*. *J Cell Biol* **153**: 1327–1336.
- Stahl N, Baldwin MA, Teplow DB, Hood L, Gibson BW, Burlingame AL, Prusiner SB. 1993. Structural analysis of the scrapie prion protein using mass spectrometry and amino acid sequencing. *Biochemistry* **32**: 1991–2002.
- Stahl N, Borchelt DR, Hsiao K, Prusiner SB. 1987. Scrapie prion protein contains a phosphatidylinositol glycolipid. *Cell* **51**: 229–240.
- Stekel DJ, Nowak MA, Southwood TRE. 1996. Prediction of future BSE spread. *Nature* **381**: 119.
- Supattapone S, Bosque P, Muramoto T, Wille H, Aagaard C, Peretz D, Nguyen H-OB, Heinrich C, Torchia M, Safar J, et al. 1999a. Prion protein of 106 residues creates an artificial transmission barrier for prion replication in transgenic mice. *Cell* **96**: 869–878.
- Supattapone S, Nguyen H-OB, Cohen FE, Prusiner SB, Scott MR. 1999b. Elimination of prions by branched polyamines and implications for therapeutics. *Proc Natl Acad Sci* **96**: 14529–14534.
- Supattapone S, Wille H, Uyechi L, Safar J, Tremblay P, Szoka FC, Cohen FE, Prusiner SB, Scott MR. 2001. Branched polyamines cure prion-infected neuroblastoma cells. *J Virol* **75**: 3453–3461.
- Tamgüney G, Francis KP, Giles K, Lemus A, DeArmond SJ, Prusiner SB. 2009a. Measuring prions by bioluminescence imaging. *Proc Natl Acad Sci* **106**: 15002–15006.
- Tamgüney G, Giles K, Bouzamondo-Bernstein E, Bosque PJ, Miller MW, Safar J, DeArmond SJ, Prusiner SB. 2006. Transmission of elk and deer prions to transgenic mice. *J Virol* **80**: 9104–9114.
- Tamgüney G, Giles K, Glidden DV, Lessard P, Wille H, Tremblay P, Groth DF, Yehiely F, Korh C, Moore RC, et al. 2008. Genes contributing to prion pathogenesis. *J Gen Virol* **89**: 1777–1788.
- Tamgüney G, Miller MW, Wolfe LL, Sirochman TM, Glidden DV, Palmer C, Lemus A, DeArmond SJ, Prusiner SB. 2009b. Asymptomatic deer excrete infectious prions in faeces. *Nature* **461**: 529–532.
- Tanaka M, Chien P, Naber N, Cooke R, Weissman JS. 2004. Conformational variations in an infectious protein determine prion strain differences. *Nature* **428**: 323–328.
- Taraboulos A, Scott M, Semenov A, Avrahami D, Laszlo L, Prusiner SB. 1995. Cholesterol depletion and modification of COOH-terminal targeting sequence of the prion protein inhibits formation of the scrapie isoform. *J Cell Biol* **129**: 121–132.
- Tateishi J, Kitamoto T, Hoque MZ, Furukawa H. 1996. Experimental transmission of Creutzfeldt-Jakob disease and related diseases to rodents. *Neurology* **46**: 532–537.
- Telling GC, Parchi P, DeArmond SJ, Cortelli P, Montagna P, Gabizon R, Mastrianni J, Lugaresi E, Gambetti P, Prusiner SB. 1996. Evidence for the conformation of the pathologic isoform of the prion protein enciphering and propagating prion diversity. *Science* **274**: 2079–2082.
- Telling GC, Scott M, Hsiao KK, Foster D, Yang S-L, Torchia M, Sidle KCL, Collinge J, DeArmond SJ, Prusiner SB. 1994. Transmission of Creutzfeldt-Jakob disease from humans to transgenic mice expressing chimeric human-mouse prion protein. *Proc Natl Acad Sci* **91**: 9936–9940.
- Telling GC, Scott M, Mastrianni J, Gabizon R, Torchia M, Cohen FE, DeArmond SJ, Prusiner SB. 1995. Prion propagation in mice expressing human and chimeric PrP transgenes implicates the interaction of cellular PrP with another protein. *Cell* **83**: 79–90.
- Tremblay P, Ball HL, Kaneko K, Groth D, Hegde RS, Cohen FE, DeArmond SJ, Prusiner SB, Safar JG. 2004. Mutant PrP^{Sc} conformers induced by a synthetic peptide and several prion strains. *J Virol* **78**: 2088–2099.
- Trevitt CR, Collinge J. 2006. A systematic review of prion therapeutics in experimental models. *Brain* **129**: 2241–2265.
- Vey M, Pilkuhn S, Wille H, Nixon R, DeArmond SJ, Smart EJ, Anderson RG, Taraboulos A, Prusiner SB. 1996. Subcellular colocalization of the cellular and scrapie prion proteins in caveolae-like membranous domains. *Proc Natl Acad Sci* **93**: 14945–14949.
- Walsh DM, Selkoe DJ. 2007. A β oligomers - a decade of discovery. *J Neurochem* **101**: 1172–1184.
- Wang F, Wang X, Yuan C-G, Ma J. 2010. Generating a prion with bacterially expressed recombinant prion protein. *Science* **327**: 1132–1135.
- Watts JC, Westaway D. 2007. The prion protein family: diversity, rivalry, and dysfunction. *Biochim Biophys Acta* **1772**: 654–672.
- Westaway D, DeArmond SJ, Cayetano-Canlas J, Groth D, Foster D, Yang S-L, Torchia M, Carlson GA, Prusiner SB. 1994. Degeneration of skeletal muscle, peripheral nerves, and the central nervous system in transgenic mice overexpressing wild-type prion proteins. *Cell* **76**: 117–129.
- Westaway D, Goodman PA, Mirenda CA, McKinley MP, Carlson GA, Prusiner SB. 1987. Distinct prion proteins in short and long scrapie incubation period mice. *Cell* **51**: 651–662.
- Whittington MA, Sidle KCL, Gowland I, Meads J, Hill AF, Palmer MS, Jefferys JGR, Collinge J. 1995. Rescue of neurophysiological phenotype seen in PrP null mice by transgene encoding human prion protein. *Nat Genet* **9**: 197–201.

D.W. Colby and S.B. Prusiner

- Wickner RB. 1994. [URE3] as an altered URE2 protein: Evidence for a prion analog in *Saccharomyces cerevisiae*. *Science* **264**: 566–569.
- Wickner RB, Masison DC, Edskes HK. 1995. [PSI] and [URE3] as yeast prions. *Yeast* **11**: 1671–1685.
- Wickner RB, Edskes HK, Shewmaker F, Nakayashiki T. 2007. Prions of fungi: Inherited structures and biological roles. *Nat Rev Microbiol* **5**: 611–618.
- Wilesmith JW, Ryan JBM, Atkinson MJ. 1991. Bovine spongiform encephalopathy: Epidemiologic studies on the origin. *Vet Rec* **128**: 199–203.
- Will RG. 2003. Acquired prion disease: Iatrogenic CJD, variant CJD, kuru. *Br Med Bull* **66**: 255–265.
- Will RG, Ironside JW, Zeidler M, Cousens SN, Estibeiro K, Alperovitch A, Poser S, Pocchiari M, Hofman A, Smith PG. 1996. A new variant of Creutzfeldt-Jakob disease in the UK. *Lancet* **347**: 921–925.
- Wille H, Prusiner SB, Cohen FE. 2000. Scrapie infectivity is independent of amyloid staining properties of the N-terminally truncated prion protein. *J Struct Biol* **130**: 323–338.
- Wille H, Bian W, McDonald M, Kendall A, Colby DW, Bloch L, Ollesch J, Boronvinskiy AL, Cohen FE, Prusiner SB, et al. 2009a. Natural and synthetic prion structure from X-ray fiber diffraction. *Proc Natl Acad Sci* **106**: 16990–16995.
- Wille H, Michelitsch MD, Guénebaut V, Supattapone S, Serban A, Cohen FE, Agard DA, Prusiner SB. 2002. Structural studies of the scrapie prion protein by electron crystallography. *Proc Natl Acad Sci* **99**: 3563–3568.
- Wille H, Shanmugam M, Murugesu M, Ollesch J, Stubbs G, Long JR, Safar JG, Prusiner SB. 2009b. Surface charge of polyoxometalates modulates polymerization of the scrapie prion protein. *Proc Natl Acad Sci* **106**: 3740–3745.
- Williams ES, Young S. 1980. Chronic wasting disease of captive mule deer: A spongiform encephalopathy. *J Wildl Dis* **16**: 89–98.
- Williams ES, Miller MW. 2002. Chronic wasting disease in deer and elk in North America. *Rev Sci Tech* **21**: 305–316.
- Zahn R, Liu A, Lührs T, Riek R, von Schroetter C, López García F, Billeter M, Calzolari L, Wider G, Wüthrich K. 2000. NMR solution structure of the human prion protein. *Proc Natl Acad Sci* **97**: 145–150.

