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Prion Diseases and the BSE Crisis

Stanley B. Prusiner

Bovine spongiform encephalopathy (BSE) and human Creutzfeldt-Jakob disease (CJD) are among the most notable central nervous system degenerative disorders caused by prions. CJD may present as a sporadic, genetic, or infectious illness. Prions are transmissible particles that are devoid of nucleic acid and seem to be composed exclusively of a modified protein (PrP^{Sc}). The normal, cellular prion protein (PrP^{C}) is converted into PrP^{Sc} through a posttranslational process during which it acquires a high β-sheet content. It is thought that BSE is a result of cannibalism in which faulty industrial practices produced prion-contaminated feed for cattle. There is now considerable concern that bovine prions may have been passed to humans, resulting in a new form of CJD.

During the past two decades, a previously unknown mechanism of disease has been described in humans and animals. Several fatal illnesses, often referred to as the prion diseases and including scrapie of sheep, BSE, and CJD of humans, are caused by the accumulation of a posttranslationally modified cellular protein. Indeed, the hallmark of all prion diseases—whether sporadic, dominantly inherited, or acquired by infection—is that they involve the aberrant metabolism and resulting accumulation of the prion protein (Table 1) (*1, 2*). The conversion of PrP^{C} (the normal cellular protein) into PrP^{Sc} (the abnormal disease-causing isoform) involves a conformation change whereby the α-helical content diminishes and the amount of β sheet increases (*3*). This structural transition is accompanied by profound changes in the properties of the protein: PrP^{C} is soluble in nondenaturing detergents, whereas PrP^{Sc} is not (*4*); and PrP^{C} is readily digested by proteases, whereas PrP^{Sc} is partially resistant (*5*).

Investigations of the prion diseases have taken on new importance with the reports of 20 cases of an atypical, variant CJD (vCJD) in 3 teenagers and 17 adults (*6, 7*). All of these cases have been reported from Great Britain and France to date. It now seems possible that bovine prions from "mad cows" were passed to humans through the consumption of tainted beef products. In this article, I discuss the information on prions with respect to the origins of BSE and vCJD. I raise the possibility that a particular conformation of bovine PrP^{Sc} was selected for heat resistance during the manufacture of meat and bone meal (MBM), thought to be the source of prions responsible for BSE. I also address the issue of preventing prion diseases and developing therapeutic approaches.

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Human Prion Diseases

Most humans afflicted with prion disease present with a rapidly progressive dementia but some manifest a cerebellar ataxia. Although the brains of patients appear grossly normal upon postmortem examination, they usually show spongiform degeneration and astrocytic gliosis under the microscope. The human prion diseases can present as sporadic, genetic, or infectious disorders (*8*) (Table 1).

Sporadic forms of prion disease comprise most cases of CJD and possibly a few cases of Gerstmann-Sträussler-Scheinker disease (GSS) (*9, 10*). In these patients, mutations of the PrP gene are not found. It is not known how disease-causing prions arise in patients with sporadic forms; hypotheses include horizontal transmission of prions from humans or animals (*11*), somatic mutation of the PrP gene, and spontaneous conversion of PrP^{C} into PrP^{Sc} (*8, 12*). Numerous attempts to establish an infectious link between sporadic CJD and a preexisting prion disease in animals or humans have been unrewarding (*13, 14*).

To date, 20 different mutations in the human PrP gene, resulting in nonconservative substitutions, have been found that segregate with the inherited prion diseases (Fig. 1). Familial CJD (fCJD) cases suggested that genetic factors might influence pathogenesis (*15*), but this was difficult to reconcile with the transmissibility of fCJD and GSS (*16*). The discovery of genetic linkage between the PrP gene and scrapie incubation times in mice (*17*) raised the possibility that mutation might be an aspect of the hereditary human prion diseases. The $Pro^{102} \rightarrow$ Leu (P102L) mutation was the first PrP mutation to be genetically linked to central nervous system (CNS) dysfunction in GSS (Fig. 1B) (*10*) and has since been found in many GSS families throughout the world (*18*). Indeed, a mutation in the protein-coding region of the PrP gene has been found in all reported kindreds with inherited human prion disease; besides the P102L mutation, genetic linkage has been established for four other mutations (*19*).

Transgenic (Tg) studies confirmed that mutations of the PrP gene can cause neurodegeneration. The P102L mutation of GSS was introduced into the mouse PrP (MoPrP) gene, and five lines of Tg(MoPrP-P101L) mice expressing large amounts of mutant PrP developed CNS degeneration consisting of widespread vacuolation of the neuropil, astrocytic gliosis, and PrP amyloid plaques (*20, 21*). Brain extracts prepared from spontaneously ill Tg(MoPrP-P101L) mice transmitted

Table 1. The prion diseases.

Disease	Mechanism of pathogenesis
Human diseases	
Kuru (Fore people)	Infection through ritualistic cannibalism
Iatrogenic Creutzfeldt-Jakob disease	Infection from prion-contaminated HGH, dura mater grafts, and so forth
Variant Creutzfeldt-Jakob disease	Infection from bovine prions?
Familial Creutzfeldt-Jakob disease	Germline mutations in PrP gene
Gerstmann-Sträussler-Scheinker disease	Germline mutations in PrP gene
Fatal familial insomnia	Germline mutation in PrP gene (D178N and M129)
Sporadic Creutzfeldt-Jakob disease	Somatic mutation or spontaneous conversion of PrP^{C} into PrP^{Sc}?
Animal diseases	
Scrapie (sheep)	Infection in genetically susceptible sheep
Bovine spongiform encephalopathy (cattle)	Infection with prion-contaminated MBM
Transmissible mink encephalopathy (mink)	Infection with prions from sheep or cattle
Chronic wasting disease (mule deer, elk)	Unknown
Feline spongiform encephalopathy (cats)	Infection with prion-contaminated MBM
Exotic ungulate encephalopathy (greater kudu, nyala, oryx)	Infection with prion-contaminated MBM

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CNS degeneration to Tg196 mice (*21*). Although the Tg196 mice did not develop spontaneous disease, they expressed small amounts of the protein encoded by the mutant transgene MoPrP-P101L and were deficient for MoPrP ($Prnp^{0/0}$) (*22*). Prions from patients who died of GSS could be transmitted to apes and monkeys (*16*) or to Tg(MHu2M-P101L) mice (MHu2M designates a chimeric human-mouse PrP) (*23*, *24*). Together, these results demonstrate that prions are generated de novo by mutations in PrP. Additionally, an artificial set of mutations in a PrP transgene (consisting of $Ala^{113} \rightarrow Val$, $Ala^{115} \rightarrow Val$, and $Ala^{118} \rightarrow Val$) produced neurodegeneration in neonatal mice; brain extracts from these mice transmitted disease to hamsters and Tg mice expressing a chimeric Syrian hamster–mouse PrP (*25*).

The infectious prion diseases include kuru of the Fore people in New Guinea, where prions were transmitted by ritualistic cannibalism (*11*, *26*, *27*). With the cessation of cannibalism at the urging of missionaries, kuru began to decline long before it was known to be transmissible (Fig. 2). Sources of prions causing infectious CJD include improperly sterilized depth electrodes, transplanted corneas, human growth hormone (HGH) and gonadotropin derived from cadaveric pituitaries, and dura mater grafts (*28*). More than 90 young adults have developed CJD after treatment with cadaveric HGH, with incubation periods ranging from 3 years to more than 20 years (*29*). Dura mater grafts implanted during neurosurgical procedures seem to have caused more than 60 cases of CJD, with incubation periods ranging from 1 year to more than 14 years (*30*).

The transmission of prions from one species to another is generally accompanied by a prolongation of the incubation time relative to transmissions where the host species is the same. This prolongation is often referred to as the "species barrier" (*31*). From studies with Tg mice, three factors have been identified that contribute to the species barrier: (i) the difference in PrP sequences between the prion donor and recipient, (ii) the strain of prion, and (iii) the species specificity of protein X, a factor defined by molecular genetic studies that binds to PrP^C and facilitates PrP^{Sc} formation. This factor is likely to be a protein, hence the provisional designation protein X

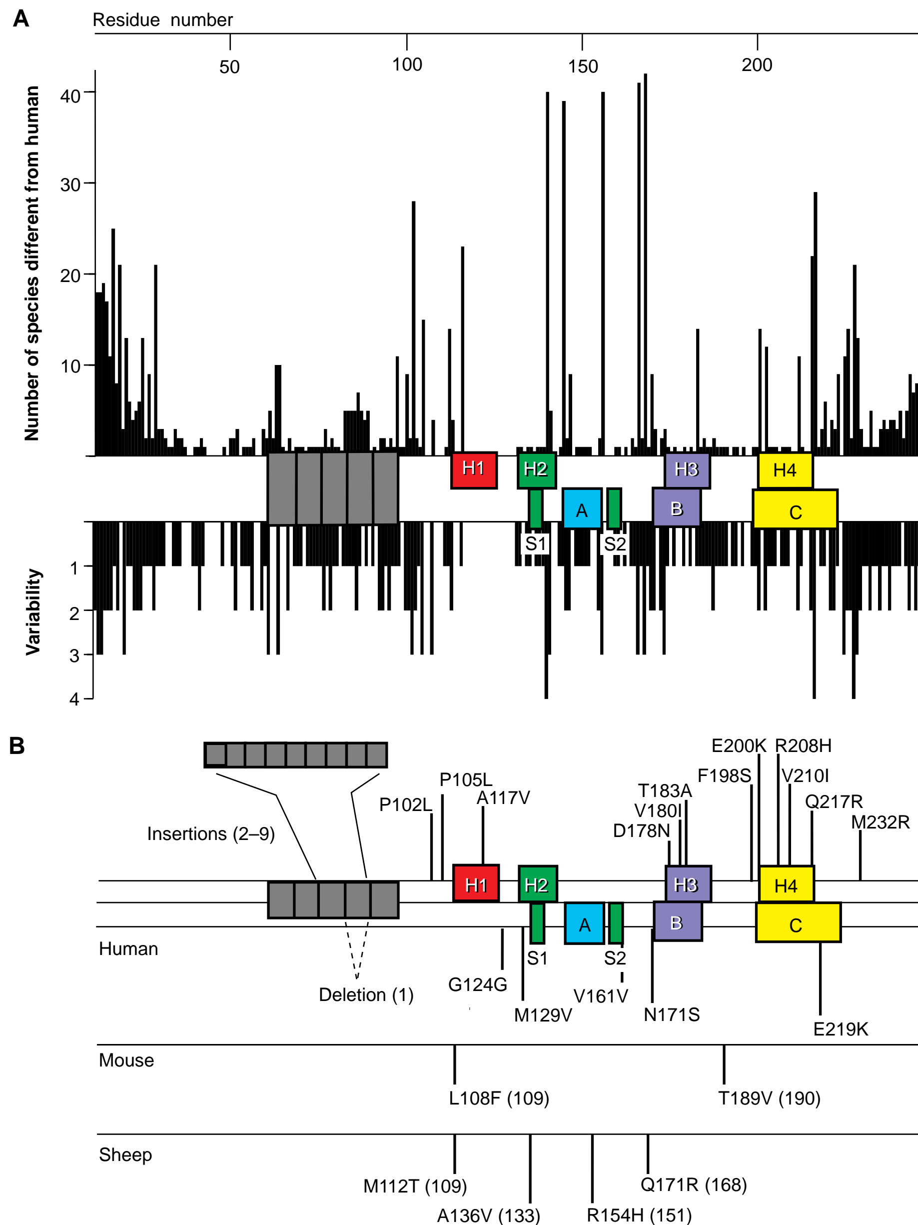

Fig. 1. Species variations and mutations of the gene encoding the prion protein. (**A**) Species variations. The *x* axis represents the human PrP sequence, showing the five octarepeats and H1 through H4 as well as the three α helices A, B, and C and the two β strands S1 and S2. Vertical bars above the 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**) Mutations causing inherited human prion disease and polymorphisms in human, mouse, and sheep. Above the line of the human sequence are mutations that cause prion disease. Below the lines are polymorphisms, some but not all of which are known to influence the phenotype of disease. Parentheses indicate corresponding human codons. [Data compiled by P. Bamborough and F. E. Cohen]

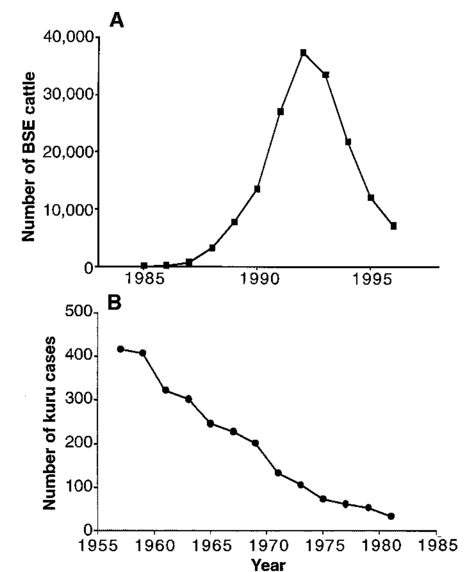

Fig. 2. Disappearance of kuru and the BSE epidemic. (**A**) Number of annual cases of BSE in cattle in Great Britain; (**B**) number of biannual cases of kuru in Papua New Guinea. Data compiled for BSE by J. Wilesmith at the Central Veterinary Laboratory, Weybridge, United Kingdom, and for kuru by M. Alpers at the Institute for Human Disease, Goroka, Papua New Guinea.

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(23, 32). The prion donor is the last mammal in which the prion was passaged, and its PrP sequence represents the "species" of the prion. The strain of prion, which seems to be enciphered in the conformation of PrP^{Sc}, conspires with the PrP sequence (which is specified by the recipient) to determine the tertiary structure of nascent PrP^{Sc}. These principles are demonstrated by studies on the transmission of Syrian hamster prions to mice, which showed that expression of a Syrian hamster PrP (SHaPrP) transgene in mice abrogated the species barrier (Table 2) (33). Besides the PrP sequence, the strain of prion also modified the transmission of SHa prions to mice (Table 2) (34, 35).

Protein X was postulated to explain the results of studies on the transmission of prions to Tg mice expressing either human PrP (HuPrP) or MHu2M. Transgenic mice expressing HuPrP and MoPrP were resistant to prions, whereas mice expressing only HuPrP or chimeric MHu2M were susceptible (Table 3) (23, 36). We produced mice expressing only HuPrP by crossing the Tg(HuPrP) mice with $Prnp^{0/0}$ mice. These studies showed that $MoPrP^C$ prevented the conversion of $HuPrP^C$ into PrP^{Sc} but had little effect on the conversion of MHu2M into PrP^{Sc}. We interpreted these results in terms of $MoPrP^C$ binding to another mouse protein with a higher affinity than to a foreign protein such as $HuPrP^C$. We postulated that we had not seen this effect in Tg(SHaPrP) mice (Table 2) because SHaPrP is more closely related to MoPrP than is HuPrP. In addition, $MoPrP^C$ had little effect on the formation of PrP^{Sc} from MHu2M (Table 3) because the COOH-termini of MoPrP and MHu2M are identical in amino acid sequence.

Characteristics of Prions

PrP^{Sc} is the major, and very probably the only, component of the infectious prion particle. PrP^{Sc} formation is a posttranslational process involving only a conformational change in PrP^C (3, 37). Molecular modeling studies predicted that PrP^C is a four-helix bundle protein containing four regions of secondary structure, denoted H1 through H4 (Fig. 1) (38, 39). Fourier transform infrared (FTIR) and circular dichroism (CD) studies showed that PrP^C contains about 40% α helix and little β sheet, consistent with the structural predictions (3, 40). Subsequent nuclear magnetic resonance (NMR) studies of a synthetic PrP peptide containing residues 90 to 145 provided good evidence for H1 (41). This peptide contains residues 113 to 128, the most highly conserved residues among all species studied (Fig. 1A) (39, 42). When the peptide is extended to include α helix A (Fig. 3A), this forms the central domain of PrP^C (approximately residues 95 to 170) that binds to PrP^{Sc} during the formation of nascent PrP^{Sc} (43). This domain shows higher homology between cattle and humans than between sheep and humans, which raises the possibility that prion transmission from cattle to humans may occur more readily than from sheep to humans (44).

The NMR structure of an α-helical form of a recombinant PrP (rPrP), containing residues 90 to 231 and corresponding to SHaPrP 27–30 (1), presumably resembles that of PrP^C (45–47). Residues 90 to 112 are not shown because marked conformational heterogeneity was found in this region, whereas residues 113 to 126 constitute the conserved hydrophobic region that also displays some structural plasticity (46) (Fig. 3A). The NH_2-terminal domain of PrP^C is thought to form the interface where PrP^{Sc} binds, whereas the COOH-terminal region appears to contain the site for protein X binding (Fig. 3B). Although some features of the structure of rPrP(90–231) are similar to those reported earlier for a smaller recombinant MoPrP fragment containing residues 121 to 231 (48, 49), substantial differences were found. For example, the loop at the NH_2-terminus of helix B is well defined in rPrP(90–231) but is disordered in MoPrP(121–231); in addition, helix C is composed of residues 200 to 227 in rPrP(90–231) but encompasses only residues 200 to 217 in MoPrP(121–231). The loop and the COOH-terminal portion of helix C are particularly important because they form the site to which protein X binds (Fig. 3B) (32). It is not yet known whether the differences between the two recombinant PrP fragments are attributable to their different lengths, to species-specific differences in sequences, or to the conditions used for solving the structures.

Recent NMR studies of full-length MoPrP(23–231) and SHaPrP(29–231) have shown that the NH_2-termini are highly flexible and lack identifiable secondary structure under the experimental conditions used (50, 51). Studies of SHaPrP(29–231) indicate transient interactions between the COOH-terminal end of helix B and the highly flexible NH_2-terminal random coil containing the octarepeats (residues 29 to 125) (51); such interactions were not reported for MoPrP-(23–231) (50). The tertiary structure of the NH_2-terminus is of considerable interest because it is within this region of PrP that a profound conformational change occurs during the formation of PrP^{Sc}, as described below (59).

Models of PrP^{Sc} suggested that formation of the disease-causing isoform involves refolding of the NH_2-terminal helices (H1 and H2) into β sheets (52); the single disulfide bond joining COOH-terminal helices would remain intact because the disulfide is required for PrP^{Sc} formation (Fig. 3C) (53, 54). The high β-sheet content of PrP^{Sc} was predicted from the ability of PrP 27–30 to polymerize into amyloid fibrils (55). Subsequent optical spectroscopy confirmed the presence of β

Table 2. Influence of prion species and strains on transmission across a species barrier in Tg mice [inoculum, SHa; data from (35, 104, 109)].

Host	Prion strain and inoculation time			
	Sc237		139H	
	Days (± SEM)	n/n_0	Days (± SEM)	n/n_0
SHa	77 ± 1	48/48	167 ± 1	94/94
Non-Tg mice	>700	0/9	499 ± 15	11/11
Tg(SHaPrP)81/ FVB mice	75 ± 2	22/22	110 ± 2	19/19
Tg(SHaPrP)81/ $Prnp^{0/0}$ mice	54 ± 1	9/9	65 ± 1	15/15

Table 3. Evidence for protein X from studies of human prion transmission in Tg mice [inoculum, sCJD; data with inoculum RG from (23)].

Host	MoPrP gene	Incubation time	
		Days (± SEM)	n/n_0
Tg(HuPrP)	$Prnp^{+/+}$	721	1/10
Tg(HuPrP)$Prnp^{0/0}$	$Prnp^{0/0}$	263 ± 2	6/6
Tg(MHu2M)	$Prnp^{+/+}$	238 ± 3	8/8
Tg(MHu2M)$Prnp^{0/0}$	$Prnp^{0/0}$	191 ± 3	10/10

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sheet in both PrP^{Sc} and PrP 27–30 (*3, 56*). Deletion of each of the regions of putative secondary structure in PrP, except for the NH_2-terminal 66 amino acids (residues 23 to 88) (*57, 58*) and the 36–amino acid loop (mouse residues 141 to 176) between H2 and H3, prevented formation of PrP^{Sc} as measured in scrapie-infected cultured neuroblastoma cells (*54*). With the use of α-PrP Fabs selected from phage display libraries and two monoclonal antibodies derived from hybridomas, the major conformational change that occurs during conversion of PrP^{C} into PrP^{Sc} has been localized to residues 90 to 112 (59). Although these results indicate that PrP^{Sc} formation primarily involves a conformational change at the NH_2-terminus, mutations causing inherited prion diseases have been found throughout the protein (Fig. 1B). Interestingly, all of the known point mutations in PrP occur either within or adjacent to regions of putative secondary structure in PrP and, as such, appear to destabilize the structure of PrP (*39, 41, 48*).

PrP^{Sc} Conformation Enciphers Prion Diversity

The existence of prion strains has posed a conundrum as to how biological information can be enciphered in any molecule other than nucleic acid (*60, 61*). Prions from cattle, nyala, kudu, and domestic cats were inoculated into C57BL, VM, and F_1(C57BL × VM) mice for "strain typing" (*60, 62*); all of these prions gave the same distribution of incubation times, which suggests that they all originated in cattle (*63*). Whether prions from humans with vCJD will give similar incubation times is unknown.

The typing of prion strains in C57BL, VM, and F_1(C57BL × VM) mice began with isolates from sheep with scrapie. The prototypic strains Me7 and 22A gave incubation times of ~150 and ~400 days, respectively, in C57BL mice (*60, 62*). The PrPs of C57BL and IlnJ mice (later shown to be genetically identical to VM mice) differ at two residues and control incubation times (Fig. 1B) (*64*). Besides incubation times, profiles of spongiform change have been used to characterize prion strains (*65*), but recent studies with PrP transgenes imply that such profiles are not an intrinsic feature of strains (*66*).

Until recently, support for the hypothesis that the tertiary structure of PrP^{Sc} enciphers strain-specific information (*2*) was minimal, except for the DY strain isolated from mink with transmissible encephalopathy (*67*). PrP^{Sc} in DY prions showed diminished resistance to proteinase K digestion and greater truncation of the NH_2-terminus. The DY strain presented a puzzling anomaly because other prion strains exhibiting similar incubation times did not show this aberrant behavior of PrP^{Sc} (*68*). Also notable was the generation of new strains during passage of prions through animals with different PrP genes (*34, 68*).

The transmission of two different inherited human prion diseases to mice expressing a chimeric MHu2M PrP transgene (*24*) has provided persuasive evidence for the enciphering of strain-specific information in the tertiary structure of PrP^{Sc}. In fatal familial insomnia (FFI), the protease-resistant fragment of PrP^{Sc} after deglycosylation has a relative molecular mass of 19 kD, whereas in other inherited and most sporadic prion diseases it is 21 kD (Table 4) (*69, 70*). This difference in molecular size was shown to be attributable to different sites of proteolytic cleavage at the NH_2-termini of the two human PrP^{Sc} molecules, reflecting different tertiary structures (*69*). Extracts from the brains of FFI patients transmitted disease to mice expressing a chimeric MHu2M PrP gene ~200 days after inoculation and induced formation of the 19-kD PrP^{Sc}, whereas fCJD(E200K) and sporadic CJD produced the 21-kD PrP^{Sc} in mice expressing the same transgene (*24*). On second passage, Tg(MHu2M) mice inoculated with FFI prions showed an incubation time of ~130 days and a 19-kD PrP^{Sc}, whereas those inoculated with fCJD(E200K) prions exhibited an incubation time of ~170 days and a 21-kD PrP^{Sc}. These findings imply that PrP^{Sc} acts as a template for the conversion of PrP^{C} into nascent PrP^{Sc}. Imparting the size of the protease-resistant fragment of PrP^{Sc} through conformational templating provides a mechanism for both the generation and propagation of prion strains.

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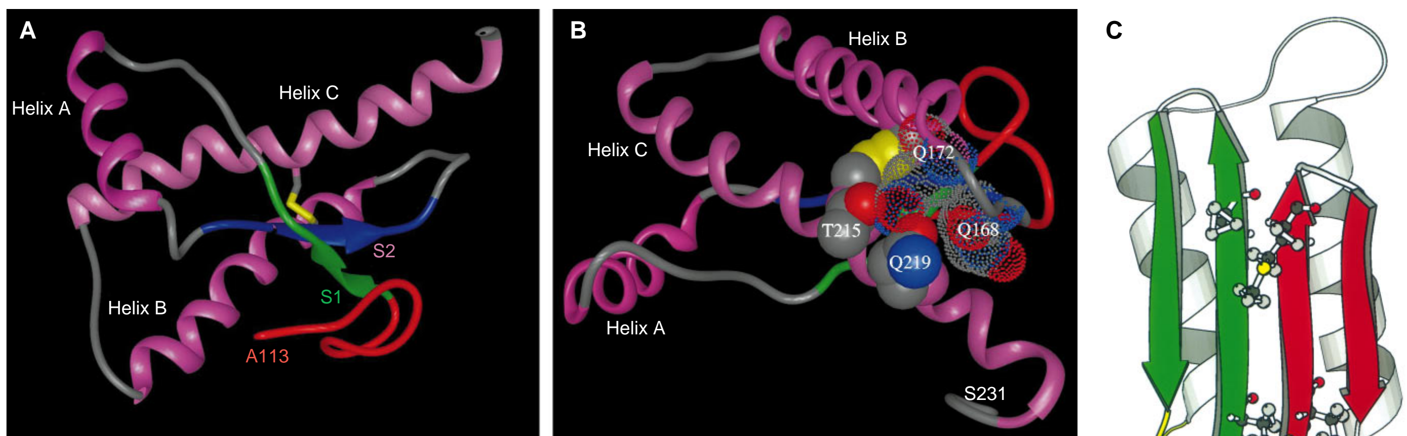

Fig. 3. Structures of prion proteins. (**A**) NMR structure of Syrian hamster rPrP(90–231). Presumably, the structure of the α-helical form of rPrP(90–231) resembles that of PrP^{C}. rPrP(90–231) is viewed from the interface where PrP^{Sc} is thought to bind to PrP^{C}. Color code: pink, α helices A (residues 144 to 157), B (172 to 193), and C (200 to 227); yellow, disulfide between Cys^{179} and Cys^{214}; red, conserved hydrophobic region (composed of residues 113 to 126); gray, loops; green, residues 129 to 134 encompassing strand S1; and blue, residues 159 to 165 encompassing strand S2. The arrows span residues 129 to 131 and 161 to 163, which show a closer resemblance to β sheet (*47*). (**B**) NMR structure of rPrP(90–231), viewed from the interface where protein X is thought to bind to PrP^{C}. Protein X appears to bind to the side chains of residues that form a discontinuous epitope: some amino acids are in the loop composed of residues 165 to 171 and at the end of helix B (Gln^{168} and Gln^{172} with a low-density van der Waals rendering), while others are on the surface of helix C (Thr^{215} and Gln^{219} with a high-density van der Waals rendering) (*32*). Images in (A) and (B) were generated with Midasplus. (**C**) Plausible model for the tertiary structure of human PrP^{Sc} (*52*). Color code: red, S1 β strands (residues 108 to 113 and 116 to 122); green, S2 β strands (residues 128 to 135 and 138 to 144); gray, α helices H3 (residues 178 to 191) and H4 (residues 202 to 218); and yellow, loop (residues 142 to 177). Four residues implicated in the species barrier (Asn^{108}, Met^{112}, Met^{129}, and Ala^{133}) are shown in ball-and-stick form (color code: dark gray, carbon; light gray, hydrogen; blue, nitrogen; red, oxygen; and yellow, sulfur).

Bovine Spongiform Encephalopathy

Understanding prion strains and the species barrier is paramount with respect to the BSE epidemic in Great Britain, where almost 1 million cattle are estimated to have been infected with prions (*71*). The mean incubation time for BSE is about 5 years. Most cattle were slaughtered between 2 and 3 years of age and therefore did not manifest disease (*72*). Nevertheless, more than 160,000 cattle, primarily dairy cows, have died of BSE over the past decade (Fig. 2A) (*71*). BSE is a massive common-source epidemic that may be caused by MBM fed primarily to dairy cows (*73*). The 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 (*73*). It is now thought that this change in the rendering process allowed scrapie prions from sheep to survive rendering and to be passed into cattle. Alternatively, some bovine prions may have been present before modification of the rendering process, and, with the processing change, survived in sufficient numbers to initiate the BSE epidemic when inoculated back into cattle orally through MBM. The latter hypothesis is inconsistent with the widespread geographical distribution throughout England of the initial 17 cases of BSE, which occurred almost simultaneously (*74*).

The origin of the bovine prions causing BSE cannot be determined by examining the amino acid sequence of PrP^{Sc} in cattle with BSE, because the PrP^{Sc} in these animals has the bovine sequence whether the initial prions in MBM came from cattle or sheep. The bovine PrP sequence differs from that of sheep at seven or eight positions (*75, 76*). In contrast to the many PrP polymorphisms found in sheep, only one PrP polymorphism has been found in cattle. Although most bovine PrP alleles encode five octarepeats, some encode six. PrP alleles encoding six octarepeats do not seem to be overrepresented in BSE (Fig. 1B) (*77*).

Brain extracts from BSE cattle cause disease in cattle, sheep, mice, pigs, and mink after intracerebral inoculation (*78*), but prions in brain extracts from sheep with scrapie fed to cattle produced illness substantially different from BSE (*79*). The annual incidence of sheep with scrapie in Great Britain over the past two decades has remained relatively low (*80*). In July 1988, the practice of feeding MBM to sheep and cattle was banned. Recent statistics argue that the epidemic is now disappearing as a result of this ruminant feed ban (Fig. 2A) (*71*), reminiscent of the disappearance of kuru in the Fore people of New Guinea (*11, 27*) (Fig. 2B).

Although many plans have been offered for the culling of older cattle to minimize the spread of BSE (*71*), it seems more important to monitor the frequency of prion disease in cattle as they are slaughtered for human consumption. No reliable, specific test for prion disease in live animals is available (*81*), but immunoblotting of the brainstems of cattle for PrP^{Sc} might provide a reasonable approach to establishing the incidence of subclinical BSE in cattle entering the human food chain (*76, 82*).

Determining how early in the incubation period PrP^{Sc} can be detected by immunological methods is complicated by the lack of a reliable, sensitive, and relatively rapid bioassay. Mice inoculated intracerebrally with BSE brain extracts require more than a year to develop disease (*83–85*). The number of inoculated animals developing disease can vary over a wide range, depending on the titer of the inoculum, the structures of PrP^C and PrP^{Sc}, and the structure of protein X (Table 2). Some investigators have stated that transmission of BSE to mice is quite variable, with incubation periods exceeding 1 year (*85*), while others report a low prion titer of $10^{2.7}$ ID_{50} units per milliliter of 10% BSE brain homogenate (*83*) compared with 10^7 to 10^9 ID_{50} units per milliliter in rodent brain (*86*). Such problems with the measurement of bovine prions demonstrate the urgent need for Tg mice that are highly susceptible to bovine prions.

Have Bovine Prions Been Transmitted to Humans?

Cases of vCJD in Great Britain and France raise the possibility that BSE has been transmitted to humans (*6, 7*). All but one of the 20 vCJD patients are 40 years of age or younger; the only other group of young CJD patients are those who received pituitary HGH during childhood. The neuropathology of vCJD patients is unusual, with numerous PrP amyloid plaques surrounded by intense spongiform degeneration (Fig. 4). These atypical neuropathologic changes have not been seen in CJD cases in the United States, Australia, and Japan (*87*). Macaque monkeys and marmosets both developed neurologic disease several years after inoculation with bovine prions (88), but only the macaques exhibited numerous PrP plaques similar to those found in vCJD (89).

If the current cases of vCJD are caused by bovine prions, then the exposure must have occurred before the specified bovine offals ban of November 1989 that prohibited human consumption of CNS and lym-

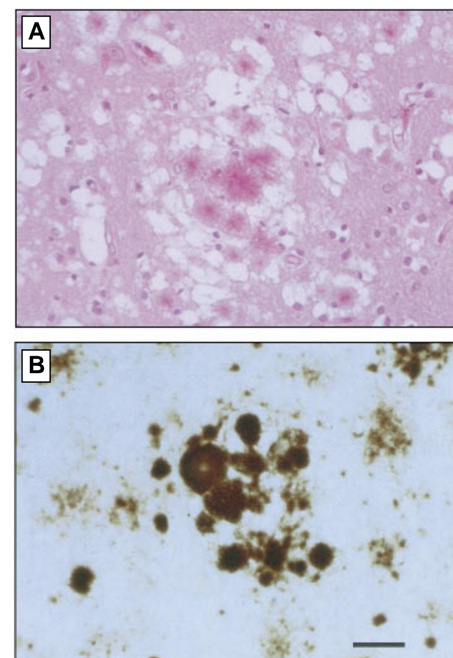

Fig. 4. Histopathology of vCJD in Great Britain. (**A**) Section from frontal cortex stained by the periodic acid–Schiff (PAS) method, showing a field with aggregates of plaques surrounded by spongiform degeneration. (**B**) Multiple plaques and amorphous deposits are PrP-immunopositive. Scale bar, 50 μm. Photomicrographs prepared by S. J. DeArmond.

Table 4. Distinct prion strains generated in humans with inherited prion diseases and transmitted to Tg mice [data from (*24, 110*)].

Inoculum	Host species	Host PrP genotype	Incubation time: Days (± SEM)	Incubation time: n/n_0	PrP^{Sc} (kD)
None	Human	FFI(D178N, M129)			19
FFI	Mouse	Tg(MHu2M)	206 ± 7	7/7	19
FFI → Tg(MHu2M)	Mouse	Tg(MHu2M)	136 ± 1	6/6	19
None	Human	fCJD(E200K)			21
fCJD	Mouse	Tg(MHu2M)	170 ± 2	10/10	21
fCJD → Tg(MHu2M)	Mouse	Tg(MHu2M)	167 ± 3	15/15	21

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phoid tissues from cattle older than 6 months of age. This legislation was based on studies showing that the highest titers of scrapie prions are found in these tissues in sheep (*90*). Because the bioassay for bovine prions in mice is so insensitive (*83*), the abundance of prions in bovine muscle remains unknown. If the distribution of bovine prions proves to be different from that presumed for sheep, then assumptions about the efficacy of the offal ban will need to be reassessed.

Attempts to predict the future number of cases of vCJD assuming exposure to bovine prions before the 1989 offal ban have been uninformative, because so few cases of vCJD have occurred (*7*). The finding of only 9 new cases in the past 15 months since the first 11 cases were announced raises questions as to the origin of vCJD. Epidemiological studies over the past three decades have failed to find evidence for transmission of sheep prions to humans (*14*). Are we at the beginning of a human prion disease epidemic in Great Britain like those seen for BSE and kuru (Fig. 2), or will the number of vCJD cases remain small, as seen with iatrogenic CJD caused by cadaveric HGH (*29*)? Until more time passes, assessing the magnitude of vCJD will not be possible (*7, 91, 92*).

Was a particular conformation of bovine PrP^{Sc} selected for heat resistance during the rendering process and then reselected multiple times as cattle infected by ingesting prion-contaminated MBM were slaughtered and their offal rendered into more MBM? Recent studies of PrP^{Sc} from the brains of patients who died of vCJD show a pattern of PrP glycoforms different from those found for sporadic or iatrogenic CJD (*93*). However, the utility of measuring PrP glycoforms is questionable in trying to relate BSE to vCJD (*94*) because PrP^{Sc} is formed after the protein is glycosylated (*37*) and enzymatic deglycosylation of PrP^{Sc} requires denaturation (*95*). Alternatively, it may be possible to establish a relation between the conformations of PrP^{Sc} from cattle with BSE and those from humans with vCJD by using Tg mice, as was done for strains generated in the brains of patients with FFI or fCJD (*24*).

It is also of interest to ask whether a particular strain of human prions was selected during ritualistic cannibalism among the Fore peoples of New Guinea when they cooked the brains of their dead relatives before eating them, or whether a strain was selected during the purification of cadaveric HGH. The uniform constellation of clinical signs of kuru and iatrogenic CJD caused by contaminated HGH contrasts with those found in other forms of prion disease (*28, 96*). Because the methods of preparation and the precise handling of brain tissue among the Fore are not well documented (*11, 26, 97*), such speculation may prove difficult to substantiate.

Prevention and Therapeutics for Prion Diseases

As our understanding of prion propagation increases, it should be possible to design effective therapeutics. Because people at risk for inherited prion diseases can now be identified decades before neurologic dysfunction is evident, the development of an effective therapy for these fully penetrant disorders is imperative (*98*). Although we have no way of predicting the number of individuals who may develop neurologic dysfunction from bovine prions in the future (*7*), it seems prudent to seek an effective therapy now.

Interfering with the conversion of PrP^{C} into PrP^{Sc} would seem to be the most attractive therapeutic target (*99*). One reasonable therapeutic strategy would be to stabilize the structure of PrP^{C} by binding a drug; another would be to modify the action of protein X, which might function as a molecular chaperone (Fig. 3). It remains to be determined whether a drug that binds to PrP^{C} at the protein X binding site would be more efficacious than a drug that mimics the structure of PrP^{C} with basic polymorphic residues that seem to prevent scrapie and CJD. Because PrP^{Sc} formation seems limited to caveolae-like domains (*100*), drugs designed to inhibit this process need not penetrate the cytosol of cells, but they must be able to enter the CNS. Alternatively, drugs that destabilize the structure of PrP^{Sc} might also prove useful.

The production of domestic animals that do not replicate prions may also be important with respect to preventing prion disease. Sheep encoding the Arg/Arg polymorphism at position 171 seem resistant to scrapie (Fig. 1B) (*101*); presumably, this was the genetic basis of Parry's scrapie eradication program in Great Britain 30 years ago (*102*). The use of dominant negatives to produce prion-resistant domestic animals, including sheep and cattle, through the expression of PrP transgenes encoding Arg^{171} as well as additional basic residues at the protein X binding site (Fig. 3B) (*32*) is likely a more effective approach. Such an approach can be readily evaluated in Tg mice, and, if shown to be effective, it could be instituted by artificial insemination of sperm from males homozygous for the transgene. Less practical is the production of PrP-deficient cattle and sheep. Although such animals would not be susceptible to prion disease (*103, 104*), they might suffer some deleterious effects from ablation of the PrP gene (*105*).

Understanding how PrP^{C} unfolds and refolds into PrP^{Sc} not only has implications for interfering with the pathogenesis of prion diseases, but may open new approaches to deciphering the causes of and developing effective therapies for the more common neurodegenerative diseases, including Alzheimer's disease, Parkinson's disease, and amyotrophic lateral sclerosis (ALS). In addition, two different stable metabolic states in yeast and one in a fungus have been ascribed to prion-like changes in protein conformation (*106–108*). Indeed, the expanding list of prion diseases and their novel modes of pathogenesis (Table 1), as well as the unprecedented mechanisms of prion propagation and information transfer (Table 4), indicate that much more attention to these fatal disorders of protein conformation is urgently needed.

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1. Prions are defined as proteinaceous infectious particles that lack nucleic acid. PrP^{C} is the cellular prion protein; PrP^{Sc} is the pathologic isoform. Amino-terminal truncation during limited proteolysis of PrP^{Sc} produces PrP 27–30 (so named because this protease-resistant core of PrP^{Sc} migrates at ~27 to 30 kD).
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