

# The Prion Diseases

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**The human prion diseases are fatal neurodegenerative maladies that may present as sporadic, genetic, or infectious illnesses. The sporadic form is called Creutzfeldt-Jakob disease (CJD) while the inherited disorders are called familial (f) CJD, Gerstmann-Sträussler-Scheinker (GSS) disease and fatal familial insomnia (FFI). Prions are transmissible particles that are devoid of nucleic acid and seem to be composed exclusively of a modified protein (PrP<sup>Sc</sup>). The normal, cellular PrP (PrP<sup>C</sup>) is converted into PrP<sup>Sc</sup> through a posttranslational process during which it acquires a high  $\beta$ -sheet content. In fCJD, GSS, and FFI, mutations in the PrP gene located on the short arm of chromosome 20 are the cause of disease. Considerable evidence argues that the prion diseases are disorders of protein conformation.**

*Abbreviations.* Prions are defined as proteinaceous infectious particles that lack nucleic acid. PrP<sup>C</sup> is the cellular prion protein; PrP<sup>Sc</sup> is the pathologic isoform. NH<sub>2</sub>-terminal truncation during limited proteolysis of PrP<sup>Sc</sup> produces PrP 27-30. CJD, Creutzfeldt-Jakob disease; sCJD, sporadic CJD; fCJD, familial CJD; iCJD, iatrogenic CJD; vCJD, variant CJD; GSS, Gerstmann-Sträussler-Scheinker disease; FFI, fatal familial insomnia; BSE, bovine spongiform encephalopathy; SHa, Syrian hamster; Tg, transgenic; HGH, human growth hormone; MBM, meat and bone meal; TME, transmissible mink encephalopathy; CWD, chronic wasting disease; FSE, feline spongiform encephalopathy.

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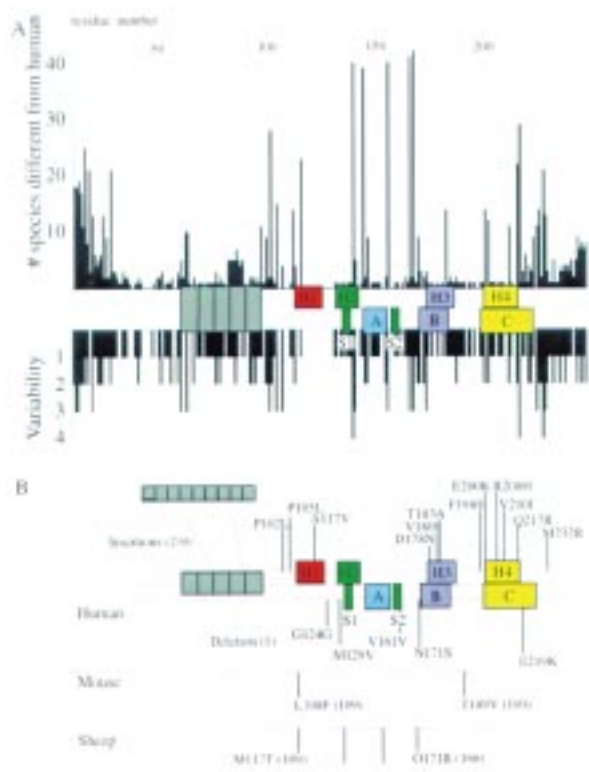
Several fatal illnesses, often referred to as the prion diseases and including scrapie of sheep, bovine spongiform encephalopathy (BSE), as well as Creutzfeldt-Jakob disease (CJD) Gerstmann-Sträussler-Scheinker (GSS) disease, and fatal familial insomnia (FFI) of humans, are caused by the accumulation 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) (132). The conversion of PrP<sup>C</sup> (the normal cellular protein) into PrP<sup>Sc</sup> (the abnormal disease-causing isoform) involves a conformation change whereby the  $\alpha$ -helical content diminishes and the amount of  $\beta$ -sheet increases (120). This structural transition is accompanied by profound changes in the properties of the protein: PrP<sup>C</sup> is soluble in non-denaturing detergents, PrP<sup>Sc</sup> is not (113); and PrP<sup>C</sup> is readily digested by proteases, whereas PrP<sup>Sc</sup> is partially resistant (119).

Investigations of the prion diseases have taken on new significance with the reports of 20 cases of an atypical, variant CJD (vCJD) in three teenagers and 17 adults (6, 15, 34, 42, 173). All of these cases have been reported from Great Britain and France, to date. It now seems possible that bovine prions from "mad cows" passed to humans through the consumption of tainted beef products.

## **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 post-mortem examination, they usually show spongiform degeneration and astrocytic gliosis under the microscope. The human prion diseases can present as sporadic, genetic, or infectious disorders (131) (Table 1A).

Sporadic forms of prion disease comprise most cases of CJD and possibly a few cases of Gerstmann-Sträussler-Scheinker disease (GSS) (64, 77, 109). In these patients, mutations of the PrP gene are not found. How prions causing disease arise in patients with sporadic forms is unknown; hypotheses include horizontal



**Figure 1.** Species variations and mutations of the prion protein gene. (A) Species variations. The x-axis represents the human PrP sequence, with the five octarepeats and H1-H4 shown as well as the three  $\alpha$ -helices A, B, and C and the two  $\beta$ -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 onset as well as the phenotype of disease. Data were compiled by Paul Bamorough and Fred E. Cohen.

transmission of prions from humans or animals (61), somatic mutation of the PrP gene, and spontaneous conversion of PrP<sup>C</sup> into PrP<sup>Sc</sup> (78, 131). Numerous attempts to establish an infectious link between sporadic CJD and a preexisting prion disease in animals or humans have been unrewarding (13, 16, 41, 74, 107).

Twenty different mutations in the human PrP gene resulting in nonconservative substitutions have been found that segregate with the inherited prion diseases, to date (Figure 1). Familial CJD cases suggested that genetic factors might influence pathogenesis (111, 144, 156), but this was difficult to reconcile with the trans-

Disease	Host	Mechanism of Pathogenesis
A. Kuru	Fore people	Infection through ritualistic cannibalism
Iatrogenic CJD*	Humans	Infection from prion-contaminated HGH, dura mater grafts, etc.
Variante CJD	Humans	Infection from bovine prions?
Familial CJD	Humans	Germline mutations in PrP gene
GSS	Humans	Germline mutations in PrP gene
FFI	Humans	Germline mutation in PrP gene (D178N, M129)
Sporadic CJD	Humans	Somatic mutation or spontaneous conversion of PrP <sup>C</sup> into PrP <sup>Sc</sup> ?
B. Scrapie	Sheep	Infection in genetically susceptible sheep
BSE	Cattle	Infection with prion-contaminated MBM
TME	Mink	Infection with prions from sheep or cattle
CWD	Mule deer, elk	Unknown
FSE	Cats	Infection with prion-contaminated MBM
exotic ungulate encephalopathy	Greater kudu, nyala, onyx	Infection with prion-contaminated MBM

\*See abbreviations list for definitions.

**Table 1.** The prion diseases.

missibility of fCJD and GSS (108). The discovery of genetic linkage between the PrP gene and scrapie incubation times in mice (27) raised the possibility that mutation might feature in the hereditary human prion diseases. The P102L mutation was the first PrP mutation to be genetically linked to CNS dysfunction in GSS (Figure 1B) (77) and has since been found in many GSS families throughout the world (52, 67, 101). 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 (51, 60, 129, 130). One of these mutations at PrP codon 178 causes FFI.

FFI is arguably the most fascinating of the human prion diseases (106, 110). While the D178N mutation causes the disease, the phenotype is modified by the polymorphic residue at position 129 (66). When codon 129 encodes a Met residue on the mutant allele, FFI is seen in patients. However, if PrP position 129 is a Val residue, then the inherited prion disease caused by the D178N mutation presents as a dementia and is called fCJD(D178N,V129). Virtually every aspect of FFI is discussed in the pages that follow.

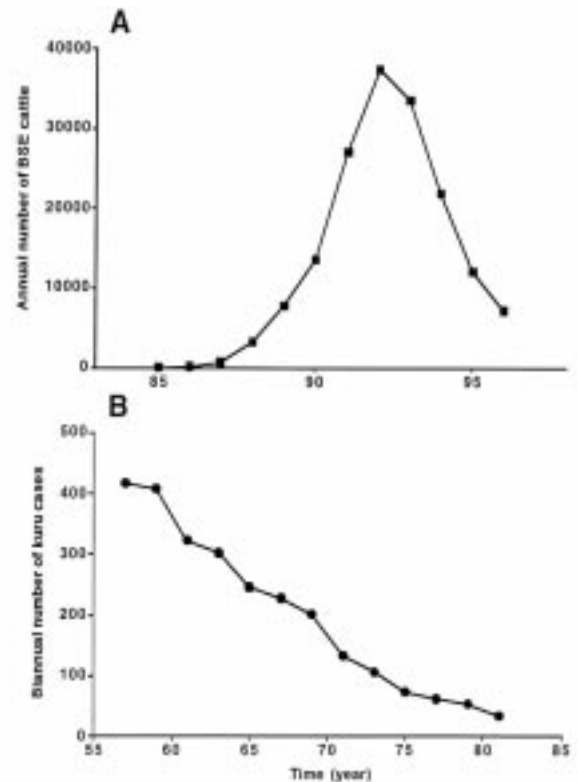
Transgenic (Tg) mouse (Mo) studies confirmed that mutations of the PrP gene can cause neurodegeneration. The P102L mutation of GSS was introduced into the MoPrP gene and five lines of Tg(MoPrP-P101L) mice expressing high levels of mutant PrP developed CNS degeneration consisting of widespread vacuolation of

A) Influence of prion species and strains on transmission across a species barrier*			
Inoculum	Host	Sc237 Incubation time / (days ± SEM) (n/no)	139H Incubation time / (days ± SEM) (n/no)
SHa	SHa	77 ± 1 (48/48)	167 ± 1 (94/94)
SHa	non-Tg mice	>700 (0/9)	499 ± 15 (11/11)
SHa	Tg(SHaPrP) <sup>81</sup> / FVB mice	75 ± 2 (22/22)	110 ± 2 (19/19)
SHa	Tg(SHaPrP) <sup>81</sup> / Prnp <sup>0/0</sup> mice	54 ± 1 (9/9)	65 ± 1 (15/15)
*data from (75, 135, 137) (D. Groth and S.B. Prusiner, unpublished data).			
B) Evidence for protein X from transmission studies of human prions†			
Inoculum	Host	MoPrP gene	Incubation Time (days ± SEM) (n/no)
sCJD	Tg(HuPrP)	Prnp <sup>+/+</sup>	721 (1/10)
sCJD	Tg(HuPrP)Prnp <sup>0/0</sup>	Prnp <sup>0/0</sup>	263 ± 2 (6/6)
sCJD	Tg(MHu2M)	Prnp <sup>+/+</sup>	238 ± 3 (8/8)
sCJD	Tg(MHu2M)Prnp <sup>0/0</sup>	Prnp <sup>0/0</sup>	191 ± 3 (10/10)
†data with inoculum RG from (163).			

**Table 2.** Foreign prion transmission in transgenic mice

the neuropil, astrocytic gliosis, and PrP amyloid plaques (79, 80, 160). Brain extracts prepared from spontaneously ill Tg(MoPrP-P101L) mice transmitted CNS degeneration to Tg196 mice (79, 160). The Tg196 mice do not develop spontaneous disease but express low levels of the mutant transgene MoPrP-P101L and are deficient for mouse PrP (Prnp<sup>0/0</sup>) (24). These studies, combined with the transmission of prions from patients who died of GSS to apes and monkeys (108) or to Tg(MHu2M-P101L) mice (163) demonstrate that prions are generated de novo by mutations in PrP. Additionally, an artificial set of mutations in a PrP transgene consisting of A113V, A115V, and A118V produced neurodegeneration in neonatal mice; brain extracts from these mice transmitted disease to hamsters and Tg mice expressing a chimeric SHa/Mo PrP (151).

The infectious prion diseases include kuru of the Fore people in New Guinea where prions were transmitted by ritualistic cannibalism (1, 2, 61). With the cessation of cannibalism at the urging of missionaries, kuru began to decline long before it was known to be transmissible (Figure 2). Sources of prions causing infectious CJD include improperly sterilized depth electrodes, transplanted corneas, human growth hormone (HGH), and gonadotrophin derived from cadaveric pituitaries, and dura mater grafts (17). Over 90 young adults have developed CJD after treatment with cadaveric HGH; the incubation periods range from 3 to more than 20 years (12, 40). Dura mater grafts implanted during neurosur-



**Figure 2.** Disappearance of kuru and the BSE epidemic. (A) Number of annual cases of BSE in cattle in Great Britain. (B) Number of annual cases of kuru in Papua New Guinea. Data compiled by for BSE by John Wilesmith at the Central Veterinary Laboratory at Weybridge, UK and for kuru by Michael Alpers at the Institute for Human Disease, Goroka, PNG.

gical procedures seem to have caused more than 60 cases of CJD; these incubation periods range from one to more than 14 years (32, 55, 102).

The transmission of prions from one species to another is generally accompanied by a prolongation of the incubation time compared to transmissions where the host species is unchanged. This prolongation is often referred to as the “species barrier” (124, 125). 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<sup>C</sup> and facilitates PrP<sup>Sc</sup> formation. This factor is likely to be a protein, hence the provisional designation protein X (92, 163). The prion donor is the last mammal in which the prion was passaged and its PrP sequence represents the “species” of the prion. The

Inoculum	Host Species	Host PrP Genotype	Incubation time (days $\pm$ SEM) (n/no)	PrP <sup>Sc</sup> (kD)
None	Human	FFI(D178N,M129)		19
FFI	Mouse	Tg(MHu2M)	206 $\pm$ 7 (7/7)	19
FFI $\rightarrow$ Tg(MHu2M)	Mouse	Tg(MHu2M)	136 $\pm$ 1 (6/6)	19
None	Human	ICJD(E200K)		21
ICJD	Mouse	Tg(MHu2M)	170 $\pm$ 2 (10/10)	21
ICJD $\rightarrow$ Tg(MHu2M)	Mouse	Tg(MHu2M)	167 $\pm$ 3 (15/15)	21

\*data from (161) (G. Telling et al., in preparation).

**Table 3.** Distinct prion strains generated in humans with inherited prion diseases and transmitted to transgenic mice\*

strain of prion, which seems to be enciphered in the conformation of PrP<sup>Sc</sup>, conspires with the PrP sequence, which is specified by the recipient, to determine the tertiary structure of nascent PrP<sup>Sc</sup>. These principles are demonstrated by studies on the transmission of Syrian hamster (SHa) prions to mice showing that expression of a SHaPrP transgene in mice abrogated the species barrier (Table 2A) (148). Besides the PrP sequence, the strain of prion modified transmission of SHa prions to mice (Table 2A) (75, 97).

Protein X was postulated to explain the results on the transmission of human (Hu) prions to Tg mice expressing either HuPrP or a chimeric Hu/MoPrP designated MHu2M. Tg mice expressing Hu and MoPrP were resistant to prions while mice expressing only HuPrP or chimeric MHu2M were susceptible (Table 2B) (162, 163). We produced mice expressing only HuPrP by crossing the Tg(HuPrP) mice with Prnp<sup>0/0</sup> mice. These studies showed that MoPrP<sup>C</sup> prevented the conversion of HuPrP<sup>C</sup> into PrP<sup>Sc</sup> but had little effect on the conversion of MHu2M into PrP<sup>Sc</sup>. We interpreted these results in terms of MoPrP<sup>C</sup> binding to another Mo protein with a higher affinity than to a foreign protein such as HuPrP<sup>C</sup>. We postulated that we had not seen this effect in Tg(SHaPrP) mice (Table 2A) because SHaPrP is more closely related to MoPrP than HuPrP is. In addition, MoPrP<sup>C</sup> had little effect on the formation of PrP<sup>Sc</sup> from MHu2M (Table 2B) because the COOH-termini of MoPrP and MHu2M are identical in amino acid sequence.

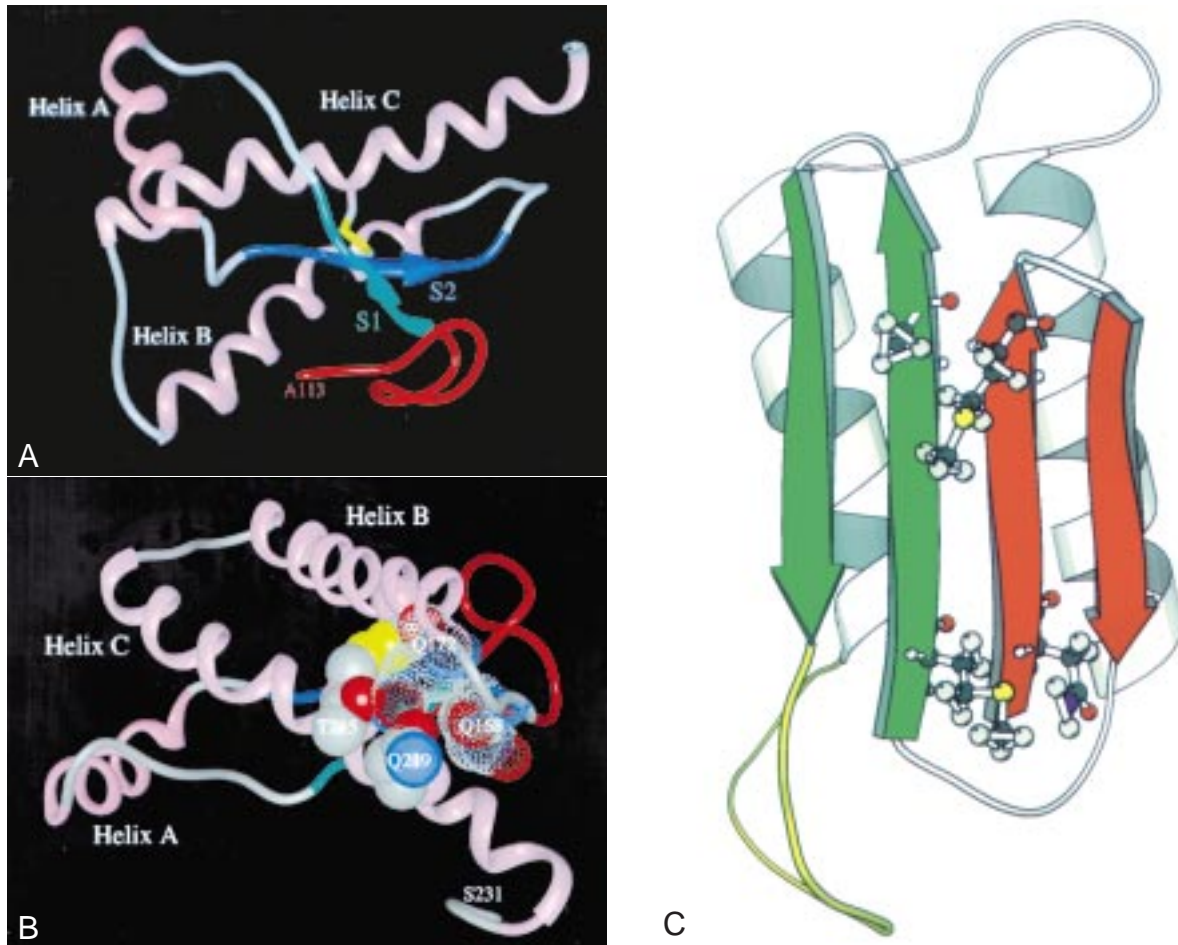
#### Molecular attributes of prions.

PrP<sup>Sc</sup> is the major, and very probably the only, component of the infectious prion particle. PrP<sup>Sc</sup> formation is a posttranslational process involving only a conformational change in PrP<sup>C</sup> (14, 29, 120). Molecular modeling studies predicted that PrP<sup>C</sup> is a four-helix bundle protein containing four regions of secondary structure denoted H1, H2, H3, and H4 (Figure 1) (63, 81). Fourier trans-

form infrared (FTIR) and circular dichroism (CD) studies showed that PrP<sup>C</sup> contains about 40%  $\alpha$ -helix and little  $\beta$ -sheet, consistent with the structural predictions (120, 128). Subsequent NMR studies of a synthetic PrP peptide containing residues 90-145 provided good evidence for H1 (174). This peptide contains the residues 113-128 that are most highly conserved among all species studied (Figure 1A) (5, 81, 147). When the peptide is extended to include  $\alpha$ -helix A (Figure 3A), this forms the central domain of PrP<sup>C</sup> (approximately residues 95-170) that binds to PrP<sup>Sc</sup> during formation of nascent PrP<sup>Sc</sup> (149). It is this domain that shows more homology between cattle and humans than between sheep and humans raising the possibility that prion transmission from cattle to humans may occur more readily than from sheep to humans (100).

The NMR structure of an  $\alpha$ -helical form of a recombinant (r) PrP containing residues 90-231 and corresponding to SHaPrP 27-30 presumably resembles that of PrP<sup>C</sup> (90, 112, 175). Residues 90-112 are not shown since marked conformational heterogeneity was found in this region while residues 113-126 constitute the conserved hydrophobic region that also displays some structural plasticity (175) (Figure 3A). The NH<sub>2</sub>-terminal domain of PrP<sup>C</sup> is thought to form the interface where PrP<sup>Sc</sup> binds while the COOH-terminal region appears to contain the site for protein X binding (Figure 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 121-231 (11, 140), substantial differences were found. For example, the loop at the NH<sub>2</sub>-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 extends only from 200 to 217 in MoPrP(121-231). The loop and the COOH-terminal portion of helix C are particularly important since they form the site to which protein X





**Figure 3.** Structures of prion proteins. (A) NMR structure of Syrian hamster (SHa) recombinant (r) PrP(90-231). Presumably, the structure of the  $\alpha$ -helical form of rPrP(90-231) resembles that of PrP<sup>C</sup>. rPrP(90-231) is viewed from the interface where PrP<sup>Sc</sup> is thought to bind to PrP<sup>C</sup>. The color scheme is:  $\alpha$ -helices A (residues 144-157), B (172-193), and C (200-227) in pink; disulfide between Cys179 and Cys214 in yellow; conserved hydrophobic region composed of residues 113-126 in red; loops in gray; residues 129-134 in green encompassing strand S1 and residues 159-165 in blue encompassing strand S2; the arrows span residues 129-131 and 161-163, as these show a closer resemblance to  $\beta$ -sheet (90). (B) NMR structure of rPrP(90-231) is viewed from the interface where protein X is thought to bind to PrP<sup>C</sup>. 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 165-171 and at the end of helix B (Gln168 and Gln172 with a low density van der Waals rendering) while others are on the surface of helix C (Thr215 and Gln219 with a high density van der Waals rendering) (92). (C) Plausible model for the tertiary structure of human PrP<sup>Sc</sup> (82). Color scheme is: S1  $\beta$ -strands are 108-113 and 116-122 in red; S2  $\beta$ -strands are 128-135 and 138-144 in green;  $\alpha$ -helices H3 (residues 178-191) and H4 (residues 202-218) in gray; loop (residues 142-177) in yellow. Four residues implicated in the species barrier are shown in ball-and-stick form (Asn 108, Met 112, Met 129, Ala 133).

binds (Figure 3B) (92). Whether the differences between the two recombinant PrP fragments are due to (i) their different lengths, (ii) species specific differences in sequences, or (iii) the conditions used for solving the structures remains to be determined.

Recent NMR studies of full length MoPrP(23-231) and SHaPrP(29-231) have shown that the NH<sub>2</sub>-termini are highly flexible and lack identifiable secondary structure under the experimental conditions employed (53,

141). Studies of SHaPrP(29-231) indicate transient interactions between the COOH-terminal end of helix B and the highly flexible, NH<sub>2</sub>-terminal random-coil containing the octarepeats (residues 29-125) (53); such interactions were not reported for MoPrP(23-231) (141). Tertiary structure of the NH<sub>2</sub>-terminus is of considerable interest since it is within this region of PrP that a profound conformational change occurs during the formation of PrP<sup>Sc</sup> as described below (127).

Models of PrP<sup>Sc</sup> suggested that formation of the disease causing isoform involves refolding of the NH<sub>2</sub>-terminal helices (H1 and H2) into  $\beta$ -sheets (82); the single disulfide bond joining COOH-terminal helices would remain intact since the disulfide is required for PrP<sup>Sc</sup> formation (Figure 3C) (115, 165). The high  $\beta$ -sheet content of PrP<sup>Sc</sup> was predicted from the ability of PrP 27-30 to polymerize into amyloid fibrils (136). Subsequent optical spectroscopy confirmed the presence of  $\beta$ -sheet in both PrP<sup>Sc</sup> and PrP 27-30 (30, 62, 120, 145). Deletion of each of the regions of putative secondary structure in PrP, except for the NH<sub>2</sub>-terminal 66 amino acids (residues 23 - 88) (56, 143) and the 36 amino acid loop (Mo residues 141 to 176) between H2 and H3, prevented formation of PrP<sup>Sc</sup> as measured in scrapie-infected cultured neuroblastoma cells (115). With  $\alpha$ -PrP Fabs selected from phage display libraries and two monoclonal antibodies derived from hybridomas, the major conformational change that occurs during conversion of PrP<sup>C</sup> into PrP<sup>Sc</sup> has been localized to residues 90-112 (127). While these results indicate that PrP<sup>Sc</sup> formation involves primarily a conformational change at the NH<sub>2</sub>-terminus, mutations causing inherited prion diseases have been found throughout the protein (Figure 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 (81, 140, 174).

#### **Prion diversity enciphered in the conformation of PrP<sup>Sc</sup>.**

The existence of prion strains has posed a conundrum as to how biological information can be enciphered in any molecule other than nucleic acid (21, 49, 50, 94, 95, 139, 167). Prions from cattle, nyala, kudu, and domestic cats were inoculated into C57BL, VM, and F1(C57BL x VM) mice for "strain typing" (20, 48, 49); all of these prions gave the same distribution of incubation times, suggesting that they all originated in cattle (19). Whether prions from humans with vCJD will give similar incubation times is unknown.

The typing of prion strains in C57BL, VM, and F1(C57BL x VM) mice began with isolates from sheep with scrapie. The prototypic strains called Me7 and 22A gave incubation times of ~150 and ~400 days in C57BL mice, respectively (20, 48, 49). The PrPs of C57BL and IlnJ (and later VM) mice differ at two residues and control incubation times (25, 26, 168). Besides incubation times, profiles of spongiform change have been used to characterize prion strains (58), but recent studies with PrP transgenes argue that such profiles are not an intrinsic feature of strains (28, 46).

Until recently, support for the hypothesis that the tertiary structure of PrP<sup>Sc</sup> enciphers strain-specific information (132) was minimal except for the DY strain isolated from mink with transmissible encephalopathy (8-10). PrP<sup>Sc</sup> in DY prions showed diminished resistance to proteinase K digestion and extended truncation of the NH<sub>2</sub>-terminus. The DY strain presented a puzzling anomaly since other prion strains exhibiting similar incubation times did not show this aberrant behavior of PrP<sup>Sc</sup>. Also notable was the generation of new strains during passage of prions through animals with different PrP genes (97, 150).

Persuasive evidence for strain-specific information being enciphered in the tertiary structure of PrP<sup>Sc</sup> has come from transmission of two different inherited human prion diseases to mice expressing a chimeric MHu2M PrP transgene (161). In FFI, the protease-resistant fragment of PrP<sup>Sc</sup> after deglycosylation has an M<sub>r</sub> of 19 kD; whereas, in other inherited and most sporadic prion diseases, it is 21 kD (Table 3) (114, 121). This difference in molecular size was shown to be due to different sites of proteolytic cleavage at the NH<sub>2</sub>-termini of the two human PrP<sup>Sc</sup> molecules reflecting different tertiary structures (114). Extracts from the brains of FFI patients transmitted disease to mice expressing a chimeric MHu2M PrP gene about 200 days after inoculation and induced formation of the 19 kD PrP<sup>Sc</sup>; whereas, fCJD(E200K) and sCJD produced the 21 kD PrP<sup>Sc</sup> in mice expressing the same transgene (161). On second passage, Tg(MHu2M) mice inoculated with FFI prions showed an incubation time of ~130 days and a 19 kD PrP<sup>Sc</sup> while those inoculated with fCJD(E200K) prions exhibited an incubation time of ~170 days and a 21 kD PrP<sup>Sc</sup>. These findings argue that PrP<sup>Sc</sup> acts as a template for the conversion of PrP<sup>C</sup> into nascent PrP<sup>Sc</sup>. Imparting the size of the protease-resistant fragment of PrP<sup>Sc</sup> through conformational templating provides a mechanism for both the generation and propagation of prion strains.

Besides incubation times, profiles of spongiform change have been used to characterize prion strains (58), but recent studies with PrP transgenes argue that such profiles are not an intrinsic feature of strains (28, 46). The mechanism by which prion strains modify the pattern of spongiform degeneration was perplexing since earlier investigations had shown that PrP<sup>Sc</sup> deposition precedes neuronal vacuolation and reactive gliosis. When FFI prions were inoculated into Tg(MHu2M) mice, PrP<sup>Sc</sup> was confined largely to the thalamus as is the case for FFI in humans (110, 161). In contrast, fCJD(E200K) prions inoculated into Tg(MHu2M) mice produced widespread deposition of PrP<sup>Sc</sup> throughout the

cortical mantle and many of the deep structures of the CNS as is seen in fCJD(E200K) of humans. To examine whether the diverse patterns of PrP<sup>Sc</sup> deposition are influenced by Asn-linked glycosylation of PrP<sup>C</sup>, we constructed Tg mice expressing PrPs mutated at one or both of the Asn-linked glycosylation consensus sites (46). These mutations resulted in aberrant neuroanatomic topologies of PrP<sup>C</sup> within the CNS, whereas pathologic point mutations adjacent to the consensus sites did not alter the distribution of PrP<sup>C</sup>. Tg mice with mutation of the second PrP glycosylation site exhibited prion incubation times of >500 days and unusual patterns of PrP<sup>Sc</sup> deposition. These findings raise the possibility that glycosylation can modify the conformation of PrP<sup>C</sup> and affect its affinity for a particular conformer of PrP<sup>Sc</sup>, which results in specific patterns of PrP<sup>Sc</sup> deposition; such interactions between PrP<sup>Sc</sup> and PrP<sup>C</sup> are likely to determine the rate of nascent PrP<sup>Sc</sup> formation.

#### **The bovine spongiform encephalopathy epidemic.**

Understanding prion strains and the species barrier is paramount with respect to the BSE epidemic in Britain, in which it is estimated that almost one million cattle were infected with prions (3, 117). The mean incubation time for BSE is about 5 years. Most cattle therefore did not manifest disease since they were slaughtered between 2 and 3 years of age (155). Nevertheless, more than 160,000 cattle, primarily dairy cows, have died of BSE over the past decade (Figure 2A) (3). BSE is a massive common source epidemic caused by meat and bone meal (MBM) fed primarily to dairy cows (117, 172). 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 (172). 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, bovine prions were present at low levels prior to 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. Against the latter hypothesis is the widespread geographical distribution throughout England of the initial 17 cases of BSE, which occurred almost simultaneously (96, 117, 171).

The origin of the bovine prions causing BSE cannot be determined by examining the amino acid sequence of PrP<sup>Sc</sup> in cattle with BSE since the PrP<sup>Sc</sup> 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 7 or 8 positions (68, 69, 134). In contrast to the many PrP polymorphisms found in sheep, only one PrP polymorphism has been found in cattle. Though most bovine PrP alleles encode five octarepeats, some encode six. PrP alleles encoding six octarepeats do not seem to be overrepresented in BSE (Figure 1B) (86).

Brain extracts from BSE cattle cause disease in cattle, sheep, mice, pigs, and mink after intracerebral inoculation (18, 22, 44, 45, 59), but prions in brain extracts from sheep with scrapie fed to cattle produced illness substantially different from BSE (142). The annual incidence of sheep with scrapie in Britain over the past two decades has remained relatively low (J. Wilesmith, unpublished data). 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 (Figure 2A) (3), reminiscent of the disappearance of kuru in the Fore people of New Guinea (1, 61) (Figure 2B).

Although many plans have been offered for the culling of older cattle in order to minimize the spread of BSE (3), 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, but immunoassays for PrP<sup>Sc</sup> in the brainstems of cattle might provide a reasonable approach to establishing the incidence of subclinical BSE in cattle entering the human food chain (72, 76, 99, 134, 153, 157). Determining how early in the incubation period PrP<sup>Sc</sup> can be detected by immunological methods is now possible since a reliable, sensitive, and relatively rapid bioassay has been created by expressing the BoPrP gene in Tg mice (152). Prior to development of Tg(BoPrP)Prnp<sup>0/0</sup> mice, non-Tg mice inoculated intracerebrally with BSE brain extracts required more than a year to develop disease (22, 57, 104, 159). Depending on the titer of the inoculum, the structures of PrP<sup>C</sup> and PrP<sup>Sc</sup>, and the structure of protein X, the number of inoculated animals developing disease can vary over a wide range (Table 2). Some investigators have stated that transmission of BSE to mice is quite variable with incubation periods exceeding one year (104) while others report low prion titers in BSE brain homogenates (57, 159) compared to rodent brain (54, 83, 93, 133).

#### **Transmission of bovine prions to humans?**

Cases of vCJD in the UK and France raise the possibility that BSE has been transmitted to humans (6, 15,

22, 34, 42, 173). All but one of the 25 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. These atypical neuropathologic changes have not been seen in CJD cases in the United States, Australia, and Japan (31, 89). Both macaque monkeys and marmosets developed neurologic disease several years after inoculation with bovine prions (4), but only the macaques exhibited numerous PrP plaques similar to those found in vCJD (103) (R. Ridley and H. Baker, unpublished data).

If the current cases of vCJD are due to bovine prions, some investigators argue that the exposure may have occurred prior to the specified bovine offals ban of November 1989 that prohibited human consumption of CNS and lymphoid tissues from cattle older than 6 months of age. This legislation was based upon studies in sheep showing that the highest titers of scrapie prions are found in these tissues (73). Only now with the development of Tg(BoPrP)Prnp<sup>0/0</sup> mice (152) can the efficacy of the offal ban be determined.

Attempts to predict the future number of cases of vCJD, assuming exposure to bovine prions prior to the offal ban, have been uninformative because so few cases of vCJD have occurred (42). The finding of only 14 new cases in the past 18 months since the first 11 were announced raises questions as to the origin of vCJD. It is noteworthy that epidemiological studies over the past three decades have failed to find evidence for transmission of sheep prions to humans (16, 41, 74, 107). Are we at the beginning of a human prion disease epidemic in Britain like those seen for BSE and kuru (Figure 2) or will the number of vCJD cases remain small as seen with iCJD caused by cadaveric HGH (12, 40)? Until more time passes, assessing the magnitude of vCJD will not be possible (38, 42, 138).

#### **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 (33, 154). Although we have no way of predicting the number of individuals who may develop neurologic dysfunction from bovine prions in the future (42), seeking an effective therapy now seems most prudent. Interfering with the conversion of PrP<sup>C</sup> into PrP<sup>Sc</sup> would

seem to be the most attractive therapeutic target (37). Either stabilizing the structure of PrP<sup>C</sup> by binding a drug or modifying the action of protein X, which might function as a molecular chaperone (Figure 3), are reasonable strategies. Whether it is more efficacious to design a drug that binds to PrP<sup>C</sup> at the protein X binding site or one that mimics the structure of PrP<sup>C</sup> with basic polymorphic residues that seem to prevent scrapie and CJD remains to be determined. Since PrP<sup>Sc</sup> formation seems limited to caveolae-like domains (71, 91, 116, 158, 166), drugs designed to inhibit this process need not penetrate the cytosol of cells but they do need to be able to enter the CNS. Alternatively, drugs that destabilize the structure of PrP<sup>Sc</sup> 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 R/R polymorphism at position 171 seem resistant to scrapie (7, 36, 70, 84, 85, 87, 88, 118, 169); presumably, this was the genetic basis of Parry's scrapie eradication program in Great Britain 30 years ago (122, 123). A more effective approach using dominant negatives for producing prion resistant domestic animals including sheep and cattle is probably the expression of PrP transgenes encoding R171 as well as additional basic residues at the protein X binding site (Fig. 3B) (92). Such an approach can be readily evaluated in Tg mice and once 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 (23, 135), they might suffer some deleterious effects from ablation of the PrP gene (39, 105, 146, 164).

Understanding how PrP<sup>C</sup> unfolds and refolds into PrP<sup>Sc</sup> has implications not only for interfering with the pathogenesis of prion diseases but may open new approaches to deciphering the causes of and to 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 another fungus have been ascribed to prion-like changes in protein conformation (35, 43, 47, 65, 98, 126, 170). 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 3), indicate that much more attention to these fatal disorders of protein conformation is urgently needed.



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