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GENETICS OF PRIONS

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ABSTRACT

Prions are unprecedented infectious pathogens that cause a group of invariably fatal, neurodegenerative diseases by an entirely novel mechanism. Prion diseases may present as genetic, infectious, or sporadic disorders, all of which involve modification of the prion protein (PrP). The human prion disease Creutzfeldt-Jakob disease (CJD) generally presents as a progressive dementia, whereas scrapie of sheep and bovine spongiform encephalopathy (BSE) are manifest as ataxic illnesses. Prions are devoid of nucleic acid and seem to be composed exclusively of a modified isoform of PrP designated PrP^{Sc}. The normal, cellular PrP designated PrP^{C} is converted into PrP^{Sc} through a process whereby some of its α-helical structure is converted into β-sheet. The species of a particular prion is encoded by the sequence of the chromosomal PrP gene of the mammals in which it last replicated. In contrast to pathogens with a nucleic acid genome, prions encipher strain-specific properties in the tertiary structure of PrP^{Sc}. Transgenetic studies argue that PrP^{Sc} acts as a template upon which PrP^{C} is refolded into a nascent PrP^{Sc} molecule through a process facilitated by another protein.

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In this review, we describe the molecular genetics of prions. We do not review the structural biology of prion proteins (PrPs), since a large body of information on this subject has accumulated, and it has been and will be reported in other review articles. Instead, we focus here on how information is encoded and enciphered within the infectious prion particle. We used the terms encoded and enciphered for two different processes involved in prion replication. Encoding of biological information refers to the sequence of DNA that codes for a protein, whereas enciphering refers to the conformation of PrP^{Sc} that determines the properties of a particular prion. We recognize that the primary structure as well as posttranslational chemical modifications determine the tertiary structure of PrP^{C} but we argue that the conformation of PrP^{C} is modified by PrP^{Sc} as it is refolded into a nascent molecule of PrP^{Sc} (196).

The hallmark common to all prion diseases, whether sporadic, dominantly inherited, or acquired by infection, is that they involve the aberrant metabolism of the prion protein (156). The conversion of cellular PrP, designated PrP^{C}, into the disease isoform PrP^{Sc} involves a conformation change whereby the α-helical content diminishes and the amount of β-sheet increases (146). This structural transition is accompanied by profound changes in the physicochemical properties of the PrP. While PrP^{C} is soluble in nondenaturing detergents, PrP^{Sc} is not (132). PrP^{C} is readily digested by proteases, whereas PrP^{Sc} is partially resistant (139).

THE PRION CONCEPT

For many years, the prion diseases were thought to be caused by slow-acting viruses. These diseases were often referred to as slow virus diseases, transmissible spongiform encephalopathies, or unconventional viral diseases (60, 61, 183). Considerable effort was expended searching for the "scrapie virus"; yet none was found either with respect to the discovery of a virus-like particle or a genome composed of RNA or DNA. This situation posed an interesting conundrum, with many hypotheses about the composition of the scrapie agent set forth.

The development of an effective purification protocol that enriched fractions for scrapie infectivity was made possible by the development of a greatly improved bioassay (163). The availability of fractions enriched for infectivity led to the prion hypothesis and the discovery of the prion protein (13, 157, 158). The titers of scrapie agent in partially purified fractions from infected Syrian hamster (SHa) brain were found to decrease when exposed to procedures that modify or hydrolyze proteins (157, 165). Conversely, scrapie infectivity was unaltered by procedures that modify or hydrolyze nucleic acids. The inability to demonstrate any dependence of infectivity on a polynucleotide (157) was in agreement with earlier studies reporting the extreme resistance of infectivity to UV irradiation at 254 nm (2).

Based on foregoing findings, the term "prion" was introduced to distinguish the **pro**teinaceous **in**fectious particles that cause scrapie from both viroids and viruses (157). Perhaps, the best current working definition of a prion is a proteinaceous infectious particle that lacks nucleic acid. A wealth of data support the theory that scrapie prions may be composed only of a protein that adopts an abnormal conformation (146, 189).

PRION PROTEIN ISOFORMS

The discovery of PrP transformed research on scrapie and related diseases (13, 158). It provided a molecular marker that was subsequently shown to be specific for these illnesses as well as the major, and possibly the only, component of the infectious prion particle.

The use of proteases to enrich fractions for scrapie infectivity created a problem when the NH_2-terminal sequence of the protease-resistant core of PrP^{Sc} denoted PrP 27–30 was determined (162). The ragged NH_2-terminus of PrP 27–30 yielded three sets of signals in almost every cycle of the Edman degradation. Only once these signals were properly interpreted and placed in correct register could a unique sequence be assigned for the NH_2-terminus of PrP 27–30. The determination of amino acid sequence of the NH_2-terminus of PrP 27–30 made possible subsequent molecular cloning studies of the PrP gene (30, 139).

The finding that PrP mRNA levels were similar in normal uninfected and scrapie-infected tissues caused some investigators to argue that PrP 27–30 was not related to the infectious prion particle (30). An alternative interpretation prompted a search for a protease-sensitive prion protein, which was found, designated PrP^C, and shown to be soluble in nondenaturing detergents, unlike PrP 27–30 (132, 139). Deduced amino acid sequences from PrP cDNA as well as immunoblotting studies revealed that PrP 27–30 was NH_2-terminally truncated and was derived from the larger PrP^{Sc} molecule (5, 120, 132, 139, 162).

In general, $\sim 10^5$ PrP^{Sc} molecules correspond to one ID_{50} unit of prions. PrP^{Sc} is probably best defined as the abnormal isoform of the prion protein that stimulates conversion of PrP^C into nascent PrP^{Sc} and that accumulates and causes disease. Although resistance to limited proteolysis has proved to be a convenient tool for detecting PrP^{Sc}, it has not proven to be useful in all studies (88, 195). Some investigators equate protease resistance with PrP^{Sc}, and this erroneous view has been compounded by the use of the term "PrP-res" (23).

Although insolubility as well as protease resistance were used in our initial studies to differentiate PrP^{Sc} from PrP^C (132), subsequent investigations have shown that these properties are only surrogate markers, as are high β-sheet content and polymerization into amyloid (64, 128, 136, 164, 168, 175). Only when these surrogate markers are present are they useful; certainly, their absence does not establish sterility for prion infectivity. PrP^{Sc} is usually not detected by immunoblotting if a sample contains less than 10^5 ID_{50} units/ml of prions (114); furthermore, PrP^{Sc} from different species may exhibit different degrees of protease resistance. For example, mice can harbor prion infectivity at levels below those that allow detection of protease-resistant PrP^{Sc} by immunoblotting while dying of causes unrelated to prions. Yet, the low levels of prion infectivity were demonstrated by the transmission of BSE prions to mice, with subsequent detection of PrP^{Sc} on second passage (116).

In our experience, the method of sample preparation from scrapie-infected brain also influences the sensitivity of PrP^{Sc} immunodetection, in part because PrP^{Sc} is not uniformly distributed in the brain (41, 191). Detection of PrP^{Sc} has also proved problematic when partially purified preparations (40, 217) have been used instead of crude homogenates; attempts to confirm such findings have failed (96, 127).

PrP GENE

PrP Gene Structure and Organization

The entire open reading frame (ORF) of all known mammalian and avian PrP genes resides within a single exon (5, 59, 85, 207), which eliminates the possibility that PrP^{Sc} arises from alternative RNA splicing (5, 207, 208). The two

exons of the Syrian hamster (SHa) PrP gene are separated by a 10-kb intron: Exon 1 encodes a portion of the 5′ untranslated leader sequence, while exon 2 encodes the ORF and 3′ untranslated region (5). Recently, a low abundance SHaPrP mRNA containing an additional small exon in the 5′ untranslated region was discovered that is encoded by the SHaPrP gene (117). The mouse (Mo), sheep, and rat PrP genes contain three exons, with exon 3 analogous to exon 2 of the hamster (174, 205, 208, 209). The promoters of both the SHa and MoPrP genes contain multiple copies of G-C rich repeats and are devoid of TATA boxes. These G-C nonamers represent a motif that may function as a canonical binding site for the transcription factor Sp1 (129). Mapping of PrP genes to the short arm of Hu chromosome 20 and to the homologous region of Mo chromosome 2 argues for the existence of PrP genes prior to the speciation of mammals (169, 186).

Expression of the PrP Gene

Although PrP mRNA is constitutively expressed in the brains of adult animals (30, 139), it is highly regulated during development. In the septum, concentrations of PrP mRNA and choline acetyltransferase were found to increase in parallel during development (134). In other brain regions, PrP gene expression occurred at an earlier age. In situ hybridization studies show that the highest concentrations of PrP mRNA are found in neurons (107).

Since no antibodies are currently available that distinguish PrP^{C} from PrP^{Sc}, and vice versa, PrP^{C} is generally measured in tissues from uninfected control animals where no PrP^{Sc} is found. PrP^{Sc} must be measured in tissues of infected animals but after PrP^{C} has been hydrolyzed by digestion with a proteolytic enzyme. PrP^{C} expression in brain was defined by standard immunohistochemistry (41) and by histoblotting in the brains of uninfected controls (191). Immunostaining of PrP^{C} in the SHa brain was most intense in the stratum radiatum and stratum oriens of the CA1 region of the hippocampus and was virtually absent from the granule cell layer of the dentate gyrus and the pyramidal cell layer throughout Ammon's horn. PrP^{Sc} staining was minimal in these regions, which were intensely stained for PrP^{C}. A similar relationship between PrP^{C} and PrP^{Sc} was found in the amygdala. In contrast, PrP^{Sc} accumulated in the medial habenular nucleus, the medial septal nuclei, and the diagonal band of Broca; these areas were virtually devoid of PrP^{C}. In the white matter, bundles of myelinated axons contained PrP^{Sc} but were devoid of PrP^{C}. These findings suggest that prions are transported along axons, in agreement with earlier findings in which scrapie infectivity was found to migrate in a pattern consistent with retrograde transport (54, 96, 100). While the rate of PrP^{Sc} synthesis appears to be a function of the level of PrP^{C} expression in Tg mice, the level to which PrP^{Sc} accumulates appears to be independent of PrP^{C} concentration (166).

Overexpression of wtPrP Transgenes

Mice were constructed expressing different levels of the wild-type (wt) SHaPrP transgene. Inoculation of these Tg(SHaPrP) mice with SHa prions demonstrated abrogation of the species barrier, resulting in abbreviated incubation times due to a nonstochastic process (166). The length of the incubation time after inoculation with SHa prions was inversely proportional to the concentration of $SHaPrP^C$ in the brains of Tg(SHaPrP) mice (166). Bioassays of brain extracts from clinically ill Tg(SHaPrP) mice inoculated with Mo prions revealed that only Mo prions but no SHa prions were produced. Conversely, inoculation of Tg(SHaPrP) mice with SHa prions led only to the synthesis of SHa prions.

During transgenetic studies, we discovered that uninoculated older mice harboring high copy-numbers of wtPrP transgenes derived from Syrian hamsters, sheep, and $Prnp^b$ mice spontaneously developed truncal ataxia, hind-limb paralysis, and tremors (205). These Tg mice exhibited a profound necrotizing myopathy involving skeletal muscle, a demyelinating polyneuropathy, and focal vacuolation of the CNS. Development of disease was dependent on transgene dosage. For example, Tg($SHaPrP^{+/+}$)7 mice homozygous for the SHaPrP transgene array regularly developed disease between 400 and 600 days of age, while hemizygous Tg($SHaPrP^{+/0}$)7 mice also developed disease, but after $>$650 days.

PrP Gene Dosage Controls the Length of the Scrapie Incubation Time

Incubation times have been extensively used to distinguish prion strains inoculated into sheep, goats, mice, and hamsters (46). A major determinant of scrapie incubation periods in mice, *Prn-i*, was found to be either congruent with or closely linked to the PrP gene, *Prnp* (207). Although the amino acid substitutions in PrP that distinguish $Prnp^a$ from $Prnp^b$ mice argued for the congruency of *Prnp* and *Prn-i*, experiments with $Prnp^a$ mice expressing $Prnp^b$ transgenes demonstrated a "paradoxical" shortening of incubation times (208). We had predicted that these Tg mice would exhibit a prolongation of the incubation time after inoculation with RML prions based on ($Prnp^a \times Prnp^b$) F1 mice, which do exhibit long incubation times. We described those findings as "paradoxical shortening" because we and others had believed for many years that long incubation times are dominant traits (21, 46). From studies of congenic and transgenic mice expressing different numbers of the *a* and *b* alleles of *Prnp,* we learned that these findings were not paradoxical; indeed, they result from increased PrP gene dosage (20). When the RML isolate was inoculated into congenic and transgenic mice, increasing the number of copies of the *a* allele was found to be the major determinant in reducing the incubation time; however, increasing the number of copies of the *b* allele also reduced the incubation time, but not to the same extent as that seen with the *a* allele.

PrP Deficient Mice

Ablation of the PrP gene ($Prnp^{0/0}$) in mice did not affect development of these animals (18, 123). In fact, they generally remain healthy at almost two years, except for one report (177). Although brain slices from $Prnp^{0/0}$ mice were reported to show altered synaptic behavior (36, 210), these results could not be confirmed by others (82, 119).

$Prnp^{0/0}$ mice are resistant to prions (17, 161). $Prnp^{0/0}$ mice were sacrificed 5, 60, 120, and 315 days after inoculation with RML prions and brain extracts bioassayed in CD-1 Swiss mice. Except for residual infectivity from the inoculum detected at 5 days after inoculation, no infectivity was detected in the brains of $Prnp^{0/0}$ mice (161). One group of investigators found that $Prnp^{0/0}$ mice inoculated with RML prions and sacrificed 20 weeks later had $10^{3.6}$ ID_{50} units/ml of homogenate by bioassay (17). Other investigators have used this report to argue that prion infectivity replicates in the absence of PrP (29, 116). Neither we nor the authors of the initial report (176) could confirm the finding of prion infectivity in $Prnp^{0/0}$ mice (161).

$Prnp^{0/0}$ mice crossed with Tg(SHaPrP) mice were rendered susceptible to SHa prions but remained resistant to Mo prions (17, 161). Since the absence of PrP^C expression does not provoke disease, it is likely that scrapie and other prion diseases are a consequence of PrP^{Sc} accumulation rather than an inhibition of PrP^C function (18). Such an interpretation is consistent with the dominant inheritance of familial prion diseases.

Mice heterozygous ($Prnp^{0/+}$) for ablation of the PrP gene had prolonged incubation times when inoculated with Mo prions (19, 161). The $Prnp^{0/+}$ mice developed signs of neurologic dysfunction at 400–460 days after inoculation. These findings are in accord with studies on Tg(SHaPrP) mice in which increased SHaPrP expression was accompanied by diminished incubation times (166).

Since $Prnp^{0/0}$ mice do not express PrP^C, we reasoned that they might more readily produce α-PrP antibodies. $Prnp^{0/0}$ mice immunized with Mo or SHa prion rods produced α-PrP antisera that bound Mo, SHa, and Hu PrP (161, 216). These findings contrast with earlier studies in which α-MoPrP antibodies could not be produced in mice, presumably because the mice had been rendered tolerant by the presence of $MoPrP^C$ (4, 98, 171). That $Prnp^{0/0}$ mice readily produce α-PrP antibodies is consistent with the hypothesis that the lack of an immune response in prion diseases is due to the fact that PrP^C and PrP^{Sc} share many epitopes.

SPECIES VARIATIONS IN THE PrP SEQUENCE

The human PRNP gene contains a single open reading frame encoding a protein of 253 amino acids (108). This protein is posttranslationally processed to

remove a 22–amino acid NH_2-terminal signal peptide. At the COOH terminus, 23 residues are also removed during the addition of a glycophosphatidyl inositol (GPI) moiety that anchors the protein to the cell membrane (188). Also contributing to the mass of the protein are two Asn side chains linked to large oligosaccharides, with multiple structures that have been shown to be complex and diverse (52). Although many species variants of PrP have now been sequenced (178), most have been of primates, and only the chicken sequence is greatly different from the human (59, 79). This makes it difficult to draw conclusions about the functional importance of many of the individual residues in the protein, because the short evolutionary distance between sequences means that many residues are conserved. The alignment of the 44 PrP sequences shows a striking degree of conservation between the mammalian sequences and is suggestive of the retention of some important function through evolution (Figure 1*A*).

The COOH-terminal 120 amino acids contain two conserved disulphide-bonded cysteines and a sequence that marks for the addition of the GPI anchor. With the exception of the chicken sequence, amino acid conservation is fairly high. The NH_2-terminal amino acids contain some very unusual features, which are discussed below.

NH_2-Terminal Sequence Repeats

The NH_2-terminal domain of mammalian PrP contains five copies of a P(H/Q) GGG(G)WGQ octarepeat sequence, occasionally more, as in the case of one sequenced bovine allele that has six copies (78, 159). These repeats are remarkably conserved between species, implying a functionally important role. The chicken sequence is notably different in this area and contains a different repeat, PGYP(H/Q)N (59, 79). Although insertions of extra repeats have been found in patients with familial disease, naturally occurring deletions of single octarepeats do not appear to cause disease, and deletion of all these repeats does not prevent PrP^C from undergoing a conformational transition into PrP^{Sc} (53, 172).

Conserved Ala-Gly Region

The other region of notable conservation is in the sequence following the COOH terminus of the last octarepeat. This is the unusual Gly- and Ala-rich region, from A113 to Y128. From an evolutionary standpoint, it is striking to see such conservation in such a nondescript sequence, containing only small, borderline-hydrophobic residues (Figure 1*A*). Although no differences between species have been found in this part of the sequence, a single point mutation A117V is linked to Gerstmann-Sträussler-Scheinker disease (GSS) (87). While the degree of conservation suggests a functional or structural importance, this region also appears to be of great importance to prion interconversion.

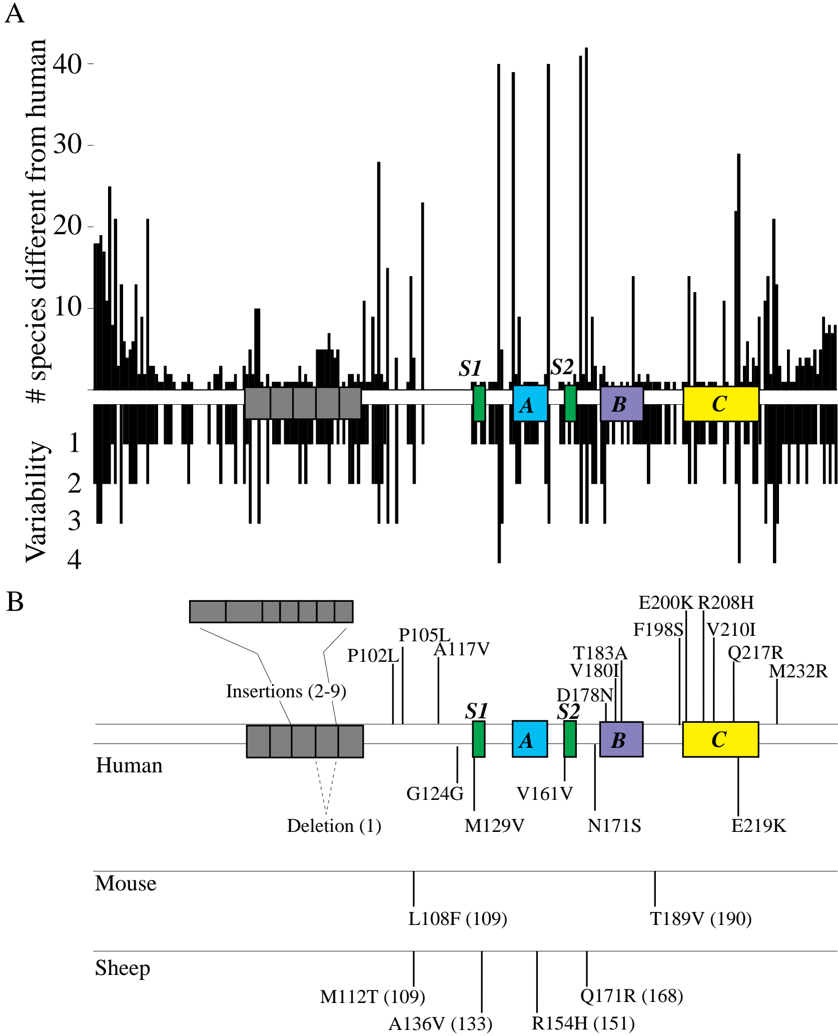

Figure 1 Species variations and mutations of the prion protein gene. (*A*) Species variations. The x-axis represents the human PrP sequence, with the five octarepeats, the three α-helices HA, HB, and HC, and the two β-strands S1 and S2 as shown. Vertical bars above the axis indicate the number of species 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 and phenotype of disease. Data were compiled by Paul Bamborough and Fred E. Cohen.

Other sequences exist in the database with similarities to this part of PrP, but it is difficult to assess their importance. Many of them are in sequences with long stretches of alanine and glycine, which do not resemble proteins with compositions typical of the globular proteins of the structural databank, and probably carry out a structural role for which a repetitive composition is required. A search through the SWISSPROT database reveals 1943 examples of eightfold stretches of sequence containing only alanine and glycine, out of 52,205 sequences (many sequences contain more than one such region). There are 4696 examples of runs of eight residues containing six or more alanines. Looking for similarities to the longer PrP sequence, AGAAAAGAVVGGLGG, the database contains 5111 examples of 15-mers containing 12 or more alanines or glycines. Such stretches of sequence are clearly not uncommon, but are rarely so well conserved as in PrP. It is interesting to speculate about why such a sequence, containing so many side chains with no chemical functionality or even very high hydrophobicity, should apparently be subject to strong evolutionary constraints. The sequence gives the appearance of having been selected for its properties of flexibility and, perhaps, for the ability to undergo conformational change.

Similarity has previously been noted to be part of the spider dragline silk protein sequence (218), which appears to be important because of its ability to form alanine-rich β-sheet aggregates (184). This is consistent with the assembly of PrP 27–30 into amyloid polymers that are rich in β-sheet. However, the silk sequence contains many such Ala-rich repeats, and therefore the pairings between strands are likely to be mostly intramolecular. This contrasts with PrP 27–30, in which only one Ala-rich section exists where intermolecular strand pairings are likely to play a greater role. FTIR spectroscopy showed that spider silk is predominantly antiparallel β-sheet, but that α-helical structure is induced by tension.

Prion Species Barrier and Protein X

The passage of prions between species is often a stochastic process, almost always characterized by prolonged incubation times during the first passage in the new host (151). Prions synthesized de novo reflect the sequence of the host PrP gene and not that of the PrP^{Sc} molecules in the inoculum (12). On subsequent passage in a homologous host, the incubation time shortens to that recorded for all subsequent passages. The species barrier concept is of practical importance in assessing the risk for humans of developing CJD after consumption of scrapie-infected lamb or BSE-infected beef (38). Attempts to abrogate the prion species barrier between humans and mice by using an approach similar to that described for the abrogation of the species barrier between Syrian hamsters and mice were initially unsuccessful. Mice expressing

HuPrP transgenes did not develop signs of CNS dysfunction more rapidly or frequently than nonTg controls (197).

The successful breaking of the species barrier between humans and mice has its origins in a set of studies with Tg mice expressing chimeric PrP genes derived from SHa and Mo PrP genes (181). One SHa/MoPrP gene, designated MH2MPrP, contains five amino acid substitutions encoded by SHaPrP, while another construct designated MHM2PrP has two substitutions. Tg(MH2M) mice were susceptible to both SHa or Mo prions, whereas three lines expressing MHM2PrP were resistant to SHa prions (180). The brains of Tg(MH2M) mice dying of scrapie contained chimeric PrP^{Sc} and prions with an artificial host range favoring propagation in mice that express the corresponding chimeric PrP but were also transmissible, albeit at reduced efficiency, to nonTg mice and hamsters. These findings provided additional genetic evidence for homophilic interactions between PrP^{Sc} in the inoculum and PrP^{C} synthesized by the host.

With the recognition that Tg(HuPrP) mice were not suitable recipients for the transmission of Hu prions, we constructed Tg(MHu2M) mice analogous to the Tg(MH2M) mice described above. HuPrP differs from Mo PrP at 28 of 254 positions (108), whereas chimeric MHu2MPrP differs at nine residues. The mice expressing the MHu2M transgene are susceptible to human prions and exhibit abbreviated incubation times of $\sim$200 days (197). In these initial studies, the chimeric MHu2M transgene encoded a Met at codon 129 and all of the patients were homozygous for Met at this residue.

From Tg(SHaPrP) mouse studies, prion propagation is thought to involve the formation of a complex between PrP^{Sc} and the homotypic substrate PrP^{C} (166). Propagation of prions may require the participation of other proteins, such as chaperones, which might be involved in catalyzing the conformational changes that feature in the formation of PrP^{Sc} (146). Notably, efficient transmission of HuCJD prions to Tg(HuPrP)/$Prnp^{0/0}$ mice was obtained when the endogenous MoPrP gene was inactivated, suggesting that $MoPrP^{C}$ competes with $HuPrP^{C}$ for binding to a cellular component (197). In contrast, the sensitivity of Tg(MHu2M) mice to HuCJD prions was not affected by the expression of $MoPrP^{C}$. One explanation for the difference in susceptibility of Tg(MHu2M) and Tg(HuPrP) mice to Hu prions in mice may be that mouse chaperones catalyzing the refolding of PrP^{C} into PrP^{Sc} can readily interact with the $MHu2MPrP^{C}/HuPrP^{CJD}$ complex but not with $HuPrP^{C}/HuPrP^{CJD}$. The identification of protein X is an important avenue of research since isolation of this protein or complex of proteins would presumably facilitate studies of PrP^{Sc} formation. To date, attempts to isolate specific proteins that bind to PrP have been disappointing (138, 218a). Whether identification of protein X will require isolation of a ternary complex composed of PrP^{C}, PrP^{Sc}, and protein X remains to be determined.

To accomplish the conversion of PrP^{C} into PrP^{Sc}, participation of one or more molecular chaperones may be required, as discussed above for protein X. Scrapie-infected cells in culture display marked differences in the induction of heat-shock proteins (193, 194), and Hsp70 mRNA has been reported to increase in scrapie of mice (99). By two-hybrid analysis in yeast, PrP has been shown to interact with Bcl-2 and Hsp60 (51, 109). Although these studies are suggestive, no direct identification of a molecular chaperone involved in prion formation in mammalian cells has been accomplished, to date.

SPECTRUM OF HUMAN PRION DISEASES

The human prion diseases present as infectious, genetic, and sporadic disorders (155). This unprecedented spectrum of disease presentations demanded a new pathogenic mechanism. Knowledge of prions provides such a conceptual framework within which this spectrum of disease presentations can be accommodated.

Human prion disease should be considered in any patient who develops a progressive subacute or chronic decline in cognitive or motor function. Typically adults between 40 and 80 years of age, patients often exhibit clinical features helpful in providing a premorbid diagnosis of prion disease, particularly sporadic CJD (83, 173). The young age of 17 people who have died of variant (v) CJD in Britain and France has raised the possibility that these individuals were infected with bovine prions that contaminated beef products (26, 38, 215). Ninety-one young adults have also been diagnosed with iatrogenic CJD 4 to 30 years after receiving human growth hormone (HGH) or gonadotropin derived from cadaveric pituitaries (11, 103). The longest incubation periods (20–30 years) are similar to those associated with more recent cases of kuru (62, 102, 160).

Sporadic forms of prion disease comprise most cases of CJD and possibly some cases of GSS (126). In these patients, mutations of the PrP gene are not found. How prions arise in patients with sporadic forms is unknown; hypotheses include horizontal transmission from humans or animals (60), somatic mutation of the PrP gene ORF, and spontaneous conversion of PrP^{C} into PrP^{Sc} (86, 155). Numerous attempts to establish an infectious link between sporadic CJD and a preexisting prion disease in animals or humans have been unrewarding (37, 122).

Inherited Prion Diseases

The recognition that 10% of cases are familial led to the suspicion that genetics might play a role in this disease (125, 131, 190). Like sheep scrapie, the relative contributions of genetic and infectious etiologies in the human prion diseases

remained puzzling. The discovery of the PrP gene and its linkage to scrapie incubation times in mice (21) raised the possibility that mutation might feature in the hereditary human prion diseases. A mutation in the ORF or protein-coding region of the PrP gene has been found in all reported kindreds with inherited human prion disease (Figure 1*B*).

Gerstmann-Sträussler-Scheinker Disease and Genetic Linkage

The discovery that GSS, which was known to be a familial disease, could be transmitted to apes and monkeys was first reported when many still thought of scrapie, CJD, and related disorders as being caused by viruses (125). Only the discovery of a proline (P)→leucine (L) mutation at codon 102 of the human PrP gene that was genetically linked to GSS permitted the unprecedented conclusion that prion disease can have both genetic and infectious etiologies (85, 155). In that study, the codon 102 mutation was linked to development of GSS with a logarithm of the odds (LOD) score exceeding 3, demonstrating a tight association between the altered genotype and the disease phenotype (Figure 1*B*). This mutation may be caused by the deamination of a methylated CpG in a germline PrP gene resulting in the substitution of a thymine (T) for cytosine (C). This mutation has been found in many families in numerous countries, including the original GSS family (50, 74, 105).

fCJD Caused by Octarepeat Inserts

An insert of 144 bp containing six octarepeats at codon 53 was described in patients with CJD from four families residing in southern England (142, 154). Genealogic investigations have shown that all four families are related, arguing for a single founder born more than two centuries ago. The LOD score for this extended pedigree exceeds 11. Studies from several laboratories have demonstrated that two, four, five, six, seven, eight, or nine octarepeats in addition to the normal five are found in individuals with inherited CJD (69, 142); whereas deletion of one octarepeat has been identified without the neurologic disease (113, 203).

fCJD in Libyan Jews

The unusually high incidence of CJD among Israeli Jews of Libyan origin was thought to be due to the consumption of lightly cooked sheep brain or eyeballs (97). Molecular genetic investigations have shown that some Libyan and Tunisian Jews in families with CJD have a PrP gene point mutation at codon 200 resulting in a Glu→Lys substitution (Figure 1*B*) (72, 86). The E200K mutation has been genetically linked to the mutation with a LOD score exceeding 3 (57) and has been found in Slovaks originating from Orava in North Central

Slovakia (72), in a cluster of familial cases in Chile (70), and in a large German family living in the United States (6).

Most patients are heterozygous for the mutation and thus, express both mutant and wtPrP^{C}. In the brains of patients who die of fCJD (E200K), the mutant PrP^{Sc} is both insoluble and protease resistant, whereas much of wtPrP differs from both PrP^{C} and PrP^{Sc} in that it is insoluble but readily digested by proteases. Whether this form of PrP is an intermediate in the conversion of PrP^{C} into PrP^{Sc} remains to be established (58).

Penetrance of fCJD

Studies of carriers with the codon 200 mutation using life table analyses have concluded that prion disease caused by this mutation exhibits complete penetrance (25, 187). In other words, if the carriers live long enough, they will all eventually develop prion disease. Some investigators have argued that the inherited prion diseases are not fully penetrant, and thus an environmental factor such as the ubiquitous "scrapie virus" is required for illness to be manifest, but as reviewed above no evidence for a viral pathogen has been found in prion disease (66, 70).

Fatal Familial Insomnia

Studies of inherited human prion diseases demonstrate how changing a single polymorphic residue alters the clinical and neuropathologic phenotype. Mutation of the PrP gene at codon 178 resulting in the substitution of Asn for Asp with a Met encoded at the polymorphic codon 129 results in a prion disease called fatal familial insomnia (FFI) (Figure 1*B*) (73, 130). In this disease, adults generally over age 50 present with a progressive sleep disorder and die within about a year of onset (121). In their brains, deposition of PrP^{Sc} is confined largely within the anteroventral and the dorsal medial nuclei of the thalamus. In contrast, the same D178N mutation with a Val encoded at position 129 produces fCJD in which the patients present with dementia and widespread deposition of PrP^{Sc} is found postmortem (71). The first family to be recognized with CJD was recently found to carry the D178N mutation (106, 131, 190).

The discovery that FFI is an inherited prion disease clearly widens the clinical spectrum of these disorders and raises the possibility that many other degenerative diseases of unknown etiology may be caused by prions (130). The codon 178 mutation has been linked to the development of prion disease with a LOD score exceeding 5 (153). More than 30 families worldwide with FFI have been recorded (63).

When the PrP^{Sc} molecules produced in the two prion diseases with the D178N mutations were examined by Western immunoblotting after limited digestion with proteinase K followed by deglycosylation with PNGase F, the

PrP^{Sc}(D178N, M129) of FFI exhibited an M_r of 19K and that of fCJD(D178N, V129) an M_r of 21 kDa (135). This difference in molecular size was shown to be due to different sites of proteolytic cleavage at the NH_2 termini of the two PrP^{Sc} molecules. This differential proteolytic cleavage was interpreted as reflecting different tertiary structures.

Human PrP Gene Polymorphisms

At PrP codon 129, an amino acid polymorphism for the M→V has been identified (Figure 1*B*) (141). This polymorphism appears able to influence prion disease expression not only in inherited forms, but also in iatrogenic and sporadic forms of prion disease. A second polymorphism resulting in an amino acid substitution at codon 219 (E→K) has been reported in the Japanese population, in which the K allele occurs with a frequency of about 12% (101). A third polymorphism is the deletion of a single octarepeat (24 bp) that has been found in 2.5% of the population (24, 113, 203). In another study of over 700 individuals, deletion of this single octarepeat was found in 1.0% of the population (145).

Studies of Caucasian patients with sCJD have shown that most are homozygous for Met or Val at codon 129 (144). This contrasts with the general population, in which frequencies for the codon 129 polymorphism in Caucasians are 12% V/V, 37% M/M, and 51% M/V (33). In contrast, the frequency of the Val allele in the Japanese population is much lower (49, 133), and heterozygosity at codon 129 (M/V) is more frequent (18%) in CJD patients than the general population, where the polymorphism frequencies are 0% V/V, 92% M/M, and 8% M/V (192).

While no specific mutations have been identified in the PrP gene of patients with sporadic CJD (67), homozygosity at codon 129 in sCJD (144) is consistent with the results of Tg mouse studies. The finding that homozygosity at codon 129 predisposes to sCJD supports a model of prion production that favors PrP interactions between homologous proteins, as appears to occur in Tg mice expressing SHaPrP inoculated with either hamster prions or mouse prions (156, 166, 179), as well as Tg mice expressing a chimeric SHa / Mo PrP transgene inoculated with “artificial” prions (180).

DE NOVO GENERATION OF PRIONS

The codon 102 point mutation found in GSS patients was introduced into the MoPrP gene and Tg(MoPrP-P101L) mice were created expressing high levels of the mutant transgene product. Five lines of Tg(MoPrP-P101L) mice developed CNS degeneration indistinguishable from experimental murine scrapie, with neuropathology consisting of widespread spongiform morphology and

astrocytic gliosis and PrP amyloid plaques (88, 195). Brain extracts prepared from spontaneously ill Tg(MoPrP-P101L) mice transmitted CNS degeneration to Tg196 mice (88, 195). The Tg196 mice express low levels of the mutant transgene MoPrP-P101L but do not develop spontaneous disease. These studies as well as transmission of prions from patients who died of GSS to apes and monkeys (125) and Tg(MHu2M-P102L) mice (198) argue persuasively that prions are generated de novo by mutations in PrP. In contrast to species-specific variations in PrP, 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 (89, 167, 219).

The initial attempt to detect de novo production of prions in uninoculated Tg(MoPrP-P101L) mice used nonTg CD-1 mice, Syrian hamsters, and the Tg196 mice (88). None of the CD-1 mice developed disease whereas many of the Syrian hamsters did. We chose Syrian hamsters not as negative controls but because these animals have relatively short incubation times and thus are sensitive hosts for prion formation. Just how the presence of a Leu at codon 102 might alter binding of mutant PrP^{Sc} to $wtPrP^{C}$ was unknown, but its place in the central region of PrP suggested that the effect might be large, as it proved to be. Not unexpectedly, Tg196 mice expressing the mutant transgene proved to be the best hosts. It is noteworthy that extracts from the brains of patients who died with GSS (P102L) have transmitted disease to Tg(MHu2M-P102L) mice but not to Tg(MHu2M) mice (198). In contrast, human prions from patients with sCJD, fCJD(E200K) and FFI have all transmitted disease to Tg(MHu2M) (196, 198).

Why mutations of the PrP gene that produce seemingly unstable PrP^{C} molecules require many decades in humans to be manifest as CNS dysfunction is unknown. In Tg(MoPrP-P101L) mice, the level of expression of the mutant transgene is inversely related to the age of disease onset. In addition the presence of the wtMoPrP gene slows the onset of disease and diminishes the severity of the neuropathological changes.

Artificial PrP Gene Mutations Generating Prions de Novo

The conversion of α-helices into β-sheets appears to be a fundamental step in formation of PrP^{Sc}. Previous studies with synthetic peptides have suggested that a region of PrP, encompassing residues 108–121, may form into a β-sheet in the pathogenic isoform, PrP^{Sc} (65). This region appears to be required for formation of PrP^{Sc}, since deletion of residues 108–121 prevented formation of PrP^{Sc} in scrapie-infected N2a cells, whereas deletion of other regions of PrP preceding or following this segment allowed formation of protease-resistant PrP^{Sc} (136). To test a prediction of this hypothesis, alanine residues within the region 113–118 sequence were substituted with potentially β-sheet promoting amino acids,

and a variety of computational secondary structure prediction methods were applied. It was found that increasing substitution of Ala→Val led to a high probability of β-sheet in this region, whereas the wild-type sequence always gave a high probability of forming an α-helix, using a number of secondary structure computational methods (182). One particular mutant, with Ala→Val substitutions at 113, 115, and 118, was selected for further study, and when the purified mutant and wild-type proteins expressed at high level in *Escherichia coli* were denatured in 6M GdnHCl and refolded by rapid dilution in aqueous buffer at physiological pH, the mutant protein displayed a marked transition to β-sheet, whereas the wild-type protein was α-helical (182).

To test whether formation of β-sheet conformation caused neurodegeneration, the Ala→Val mutated ORF was inserted into the transgene expression vector, cosSHa.Tet, for expression in Tg mice (181). Following microinjection, severe difficulties in identifying mice with high copy numbers of the transgene were initially experienced. Upon further analysis, it was recognized that many animals were showing neurological signs and dying within a period of two to four weeks after birth, prior to weaning and screening for the presence of the transgene. To date, every animal identified with a transgene copy number equal to the established Tg(SHaPrP)81 line (179) succumbed to disease within two months after birth. These studies strongly support the hypothesis that the region PrP(108–122) adopts a β-sheet conformation during formation of PrP^{Sc} (90, 146). Confirming that prions are produced de novo, one brain from a founder animal sick at four weeks of age was found to transmit disease to Syrian hamsters at 120 days: 15 of 16 animals inoculated developed disease, and the brains of the afflicted hamsters contained high concentrations of protease-resistant $SHaPrP^{Sc}$ and displayed extensive spongiform degeneration and astrocytic gliosis. Although several other founder animals have not yet transmitted disease, this same founder and another have also transmitted disease to Tg(MH2M)229/ $Prnp^{0/0}$ mice at 90-120 days following inoculation (182).

STRAINS OF PRIONS

Scrapied goats with two different syndromes, one in which the goats became hyperactive and the other in which they became drowsy, raised the possibility that strains of prions might exist (152). Subsequent studies with mice documented the existence of multiple strains in mice through careful measurements of incubation times and the distribution of vacuoles in the CNS (46, 55). Two different groups of prion strains were identified using two strains of mice, C57BL and VM: One group typified by Me7 prions exhibited short incubation times in C57BL mice and long ones in VM mice. The other group of strains including 22A and 87V showed antithetical behavior with respect to the length

of the incubation time. Particularly puzzling was the finding that long incubation times were a dominant trait for either group of prion strains, as evidenced by studies in F1(C57BL × VM) mice.

Molecular genetic studies later showed genetic linkage of incubation times to the PrP gene, using NZW and Iln mice (21) that later proved to be analogous in the C57BL and VM mice (94). The PrPs of NZW and Iln mice differ at residues 108 and 189 and are termed PrP-A and PrP-B, respectively (207). Using transgenic mice expressing different levels of PrP^{C}-A and PrP^{C}-B, it was shown than long incubation times in F1 mice were not a dominant trait but, rather, they were due to a gene dosage effect (20).

Selective Neuronal Targeting

With the development of a new procedure for in situ detection of PrP^{Sc}, designated histoblotting (191), it became possible to localize and quantify PrP^{Sc} as well as to determine whether "strains" produce different, reproducible patterns of PrP^{Sc} accumulation. The patterns of PrP^{Sc} accumulation were found to be different for each prion strain if the genotype of the host was held constant (42, 81). This finding was in accord with earlier studies showing that spongiform degeneration is strain specific (55) since PrP^{Sc} accumulation precedes vacuolation. Because a single prion strain produced many different patterns when inoculated into mice expressing various PrP transgenes, we concluded that the pattern of PrP^{Sc} deposition is a manifestation of the particular strain but not related to its propagation (SJ DeArmond & SB Prusiner, in preparation). The results of studies of three prion strains prepared from three brain regions and spleens of inbred mice support this contention (22).

While studies with both mice and Syrian hamsters established that each strain has a specific signature as defined by a specific pattern of PrP^{Sc} accumulation in the brain (20, 42, 81), comparisons must be done on an isogenic background (88, 180). When a single strain is inoculated into mice expressing different PrP genes, variations in the patterns of PrP^{Sc} accumulation were found to be equally as great as those seen between two strains (SJ DeArmond & SB Prusiner, in preparation). Based upon the initial studies performed in animals of a single genotype, we suggested that PrP^{Sc} synthesis occurs in specific populations of cells for a given distinct prion isolate (81, 155).

Isolation of New Strains

Further studies implicating PrP in the phenomenon of prion strains comes from studies on the transmission of strains from one species to another. Such studies were especially revealing when mice expressing chimeric SHa / MoPrP transgenes were used. Evidence was obtained showing that new strains pathogenic for Syrian hamsters could be obtained from prion strains that had been previously cloned by limiting dilution in mice (45) (M Scott & SB Prusiner,

in preparation). Both the generation and propagation of prion strains seem to be results of interactions between PrP^{Sc} and PrP^{C}. Additionally, strains once thought to be distinct that were isolated from different breeds of scrapied sheep were shown to have indistinguishable properties. Such findings argue for the convergence of some strains and raise the issue of the limits of prion diversity.

While other comparisons of prion strains had not revealed any biochemical or physical differences in PrP^{Sc} (81), a difference in PrP^{Sc} related to strains was found when two prion strains were isolated from mink with transmissible encephalopathy (9). One strain (HY) produced hyperactivity in Syrian hamsters and the other (DY) was manifest as a drowsy syndrome like the scrapie strains first seen in goats (9, 10). PrP^{Sc} produced by the DY prions showed diminished resistance to proteinase K digestion and truncation of the NH_2 terminus compared to HY and many other strains (8), providing evidence for the hypothesis that different strains might represent different conformers of PrP^{Sc} (156). This altered sensitivity to protease displayed by the DY strain in vivo was demonstrated in vitro when partially denatured, radiolabeled PrP^{C} was bound to PrP^{Sc} (7). Notably, it was not possible to demonstrate the de novo synthesis of infectious prions using this system.

Enciphering Diversity Through Protein Conformation

Although the notion that PrP^{Sc} tertiary structure might encipher the information for each strain is consistent with all of the foregoing experimental data, such a hypothesis enjoyed little enthusiasm. Only with the transmission of two different inherited human prion diseases to mice expressing chimeric Hu/ Mo PrP transgenes has persuasive evidence emerged supporting this concept. In fatal familial insomnia (FFI), the protease-resistant fragment of PrP^{Sc} after deglycosylation has an M_r of 19K, whereas that from other inherited and sporadic prion diseases is 21K (Figure 2) (135, 147). 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-kDa PrP^{Sc}, whereas fCJD (E200K) and sCJD produced the 21-kDa PrP^{Sc} in the Tg mice (196). These findings argue 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.

The Interplay Between the Species and Strains of Prions

The recent advances described above in our understanding of the role of the primary and tertiary structures of PrP in the transmission of disease have given new insights into the pathogenesis of the prion diseases. The amino acid sequence of PrP encodes the species of the prion (Table 1) (179, 198) and the prion derives

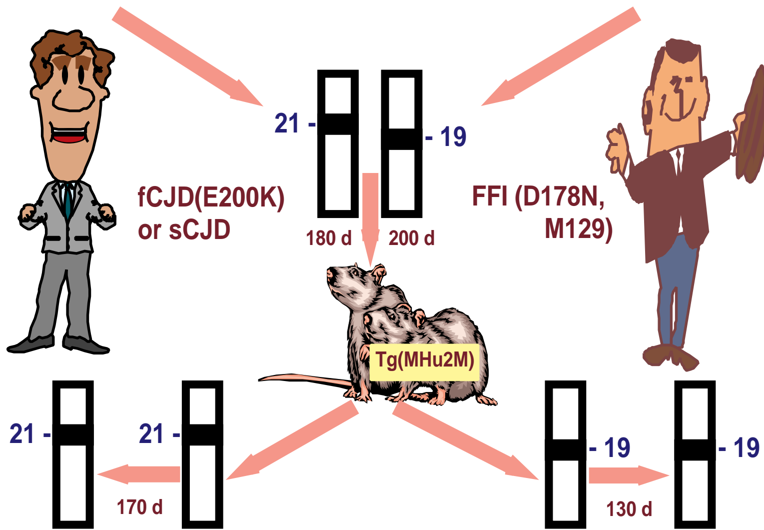

Figure 2 Evidence for prion diversity being enciphered within the conformation of PrP^{Sc}. Mutant prions template specific conformations onto PrP^{Sc} formed in Tg(MHu2M)$Prnp^{0/0}$ mice. Western blot analysis of PrP^{Sc} from the brains of patients who died of fCJD(E200K) or FFI(D178N, M129) showed PrP molecules of 21 or 19 kDa, respectively (196). Homogenates were digested with proteinase K, denatured, and digested with PNGase F prior to SDS-PAGE followed by immunoblotting with α-PrP 3F4 mAbs. Aliquots of brain homogenates from patients were inoculated intracerebrally into Tg(MHu2M)$Prnp^{0/0}$ mice, and the mice were sacrificed about 200 days later when they developed signs of CNS dysfunction. Western blot analysis of PrP^{Sc} from the brains of Tg(MHu2M) mice showed MHu2M PrP^{Sc} molecules of 21 or 19 kDa. If the mice were inoculated with homogenates prepared from the brains of the patients who died of FFI(D178N; M129), then after proteinase K and PNGase F digestions, MHu2M PrP^{Sc} migrated with an M_r of 19 kDa. In contrast, if the mice were inoculated with fCJD(E200K) prions, then MHu2M PrP^{Sc} migrated at 21 kDa. On the second serial passage in Tg(MHu2M)$Prnp^{0/0}$ mice, aliquots of brain homogenates from first passage Tg(MHu2M)$Prnp^{0/0}$ mice were inoculated intracerebrally into Tg(MHu2M)$Prnp^{0/0}$ mice, and the mice were sacrificed when they developed signs of CNS dysfunction. For those mice receiving an inoculum that began with a fCJD(E200K) patient, the incubation time was ~170 days. For mice receiving an inoculum that began with an FFI patient, the incubation time was ~130 days on second passage. Western blot analysis of PrP^{Sc} from the brains of Tg(MHu2M) mice showed MHu2M PrP^{Sc} molecules of 21 or 19 kDa. If the mice were initially inoculated with homogenates prepared from the brains of the patients who died of FFI(D178N, M129), then after proteinase K and PNGase F digestions, MHu2M PrP^{Sc} migrated with an M_r of 19 kDa. In contrast, if the mice were inoculated with fCJD(E200K) prions, then MHu2M PrP^{Sc} migrated at 21 kDa.

Table 1 Influence of prion species and strain on transmission from Syrian hamsters to hamsters and mice*

Inoculum	Recipient	Sc237	139H
		Incubation time [days ± SEM] (n/n_o)	
SHa→SHa	SHa	77 ± 1 (48/48)	167 ± 1 (94/94)
SHa→SHa	Non-Tg mice	>700 (0/9)	499 ± 15 (11/11)
SHa→SHa	Tg(SHaPrP)81 mice	75 ± 2 (22/22)	110 ± 2 (19/19)

*The *species* of prion is *encoded* by the primary structure of PrP^{Sc} and the *strain* of prion is *enciphered* by the tertiary structure of PrP^{Sc}. We recognize that the primary structure as well as posttranslational chemical modifications determine the tertiary structure of PrP^{C} but we argue that the conformation of PrP^{C} is modified by PrP^{Sc} as it is refolded into a nascent molecule of PrP^{Sc} (196).

its PrP^{Sc} sequence from the last mammal in which it was passaged. While the primary structure of PrP is likely to be the most important or even sole determinant of the tertiary structure of PrP^{C}, existing PrP^{Sc} seems to function as a template in determining the tertiary structure of nascent PrP^{Sc} molecules as they are formed from PrP^{C} (32, 156). In turn, prion diversity may be enciphered in the conformation of PrP^{Sc} and prion strains may represent different conformers of PrP^{Sc} (10, 196)

While the foregoing scenario seems to be unprecedented in biology, considerable experimental data now support these concepts. However, it is not yet known whether multiple conformers of PrP^{C} also exist that serve as precursors for selective conversion into different PrP^{Sc}. In this light, it is useful to consider another phenomenon that is not yet understood: the selective targeting of neuronal populations in the CNS of the host mammal. Recent data suggest that variations in glycosylation of the Asn-linked sugar chains may influence the rate at which a particular PrP^{C} molecule is converted into PrP^{Sc} (SJ DeArmond & SB Prusiner, in preparation). Since Asn-linked oligosaccharides are known to modify the conformation of some proteins (137, 140), it seemed reasonable to assume that variations in complex CHOs might alter the size of the energy barrier that must be traversed during formation of PrP^{Sc}. If this is the case, then regional variations in CHO structure in the CNS could account for selective targeting, i.e. formation of PrP^{Sc} in particular areas of the brain. Such a mechanism could also explain the variations in the ratio of the various PrP^{Sc} glycoforms observed by some investigators (35). But such a mechanism, while accounting for specific patterns of PrP^{Sc} distribution, does not seem to influence to any measurable degree the properties of the resulting PrP^{Sc} molecule. In fact, molecular modeling and NMR structural studies may provide an explanation for such phenomena, since the Asn-linked CHOs appear to be on the opposite face

of PrP from where PrP^{C} and PrP^{Sc} are expected to interact during the formation of nascent PrP^{Sc} (89, 90, 167, 219).

ANIMAL PRION DISEASE

Sheep and Cattle PrP Gene Polymorphisms

Parry argued that host genes were responsible for the development of scrapie in sheep. He was convinced that natural scrapie is a genetic disease that could be eradicated by proper breeding protocols (148, 149). He considered its transmission by inoculation of importance primarily for laboratory studies and communicable infection of little consequence in nature. Other investigators viewed natural scrapie as an infectious disease and argued that host genetics only modulate susceptibility to an endemic infectious agent (47).

In sheep, polymorphisms at codons 136, 154, and 171 of the PrP gene that produce amino acid substitutions have been studied with respect to the occurrence of scrapie in sheep (Figure 1*B*) (31, 76, 77, 112). Studies of natural scrapie in the USA have shown that ~85% of the afflicted sheep are of the Suffolk breed. Only those Suffolk sheep homozygous for Gln (Q) at codon 171 were found with scrapie, although healthy controls with QQ, QR, and RR genotypes were also found (91, 95, 209). These results argue that susceptibility in Suffolk sheep is governed by the PrP codon 171 polymorphism. In Cheviot sheep, the PrP codon 171 polymorphism has a profound influence on susceptibility to scrapie as in Suffolks and codon 136 seems to play a less pronounced role (75, 92).

In contrast to sheep, different breeds of cattle have no specific PrP polymorphisms. The only polymorphism recorded in cattle is a variation in the number of octarepeats: Most cattle, like humans, have five octarepeats, but some have six (78, 159). Humans with seven octarepeats develop fCJD (68), but six octarepeats do not seem to be overrepresented in BSE (78, 93, 159).

Bovine Spongiform Encephalopathy

Beginning in 1986, an epidemic of a previously unknown prion disease named bovine spongiform encephalopathy (BSE) or "mad cow" disease appeared in cattle in Great Britain (204), in which protease-resistant PrP was found in brains of ill cattle (84, 159). It has been proposed that BSE represents a massive common source epidemic caused by meat and bone meal (MBM) fed primarily to dairy cows (213). The MBM was prepared from the offal of sheep, cattle, pigs and chickens as a high-protein nutritional supplement. In the late 1970s, the solvent extraction method used in the rendering of offal began to be abandoned, resulting in an MBM with a much higher fat content (214). It is now thought that this change in the rendering process allowed scrapie prions from sheep to

survive rendering and to pass into cattle. Alternatively, bovine prions that had caused clinical CNS dysfunction at such a low level as not to be recognized survived the rendering process and were passed back to cattle through the MBM. Recent statistics argue that the epidemic is now disappearing as a result of the 1988 food ban (2a).

Brain extracts from BSE cattle have transmitted disease to mice, cattle, sheep, pigs, and mink after intracerebral inoculation (56, 170). Transmissions to mice and sheep suggest that cattle preferentially propagate a single "strain" of prions (16). Of particular significance is the transmission of BSE to the marmoset after intracerebral inoculation and more recently, to the macaque (3, 115). In addition, a young adult monkey born and reared in Britain has died of a neurodegenerative disorder thought to be a prion disease in a zoo in Montpellier, France (14). Whether this monkey's illness is due to bovine prions in contaminated foodstuffs or is a case of sporadic prion disease remains to be established.

Have Bovine Prions Been Transmitted to Humans?

The recent cases of vCJD in 3 teenagers, 13 young adults, and 1 older adult in the UK and France raise the possibility of transmission of BSE to humans (26, 215). The average age of the initial patients was 27 years, much younger than any other group of people who have died of CJD except for those who received pituitary HGH. Not only does age set these teenagers and young adults apart from other individuals who died of prion disease but so does the neuropathology. The deposition of PrP in the brains of these patients is extreme, and numerous multinucleated PrP amyloid plaques surrounded by intense spongiform degeneration have been observed. These neuropathologic changes seem to be unlike any observed in other forms of prion disease. Why such cases should be largely confined to young people is unclear. Whether the young CNS is more vulnerable to invasion by bovine prions or the dietary habits of these young individuals exposed them to a greater dose of bovine prions is unknown.

Attempts to transmit bovine prions to Tg(HuPrP)Prnp$^{0/0}$ mice have been negative for more than 500 days (34). These same mice routinely develop illness approximately 200 days after inoculation with Hu prions (198). The interpretation of such studies must be guarded in the absence of positive controls in Tg mice with a bovine PrP gene. In our own studies of Tg(MBov2M)Prnp$^{0/0}$ mice expressing a chimeric Bov/Mo PrP transgene, no transmission of disease has been seen more than 400 days after inoculation (GC Telling & SB Prusiner, unpublished data). Since the Tg(MBov2M)Prnp$^{0/0}$ chimera may not be capable of propagating bovine prions, we have also inoculated Tg(BovPrP)Prnp$^{0/0}$ mice with BSE, and these mice have not developed disease at >200 days following inoculation (M Scott & SB Prusiner, unpublished data). Notably, Tg(HuPrP)Prnp$^{0/0}$ mice are susceptible to Hu CJD prions (104, 198).

Development of an efficient murine model for BSE may require the creation of novel gene sequences that do not exist in nature.

Epidemiological studies over nearly three decades have failed to establish convincing evidence for transmission of sheep prions to humans (37, 122). Of interest is the high incidence of CJD among Libyan Jews that was initially attributed to the consumption of lightly cooked sheep brain (97); however, subsequent studies showed that this geographical cluster of CJD is due to the E200K mutation (Figure 1*B*) (66, 86).

YEAST PRIONS

Although prions were originally defined in the context of an infectious pathogen (157), it is now becoming widely accepted that prions are elements that impart and propagate variability through multiple conformers of a normal cellular protein. Such a mechanism must surely not be restricted to a single class of transmissible pathogens. Indeed, it is likely that this original definition will need to be extended to encompass other situations where a similar mechanism of information transfer occurs. As we describe, two notable prion-like determinants, [URE3] and [PSI], have already been reported in yeast.

The [URE3] Determinant

In considering what properties a yeast prion-like determinant would possess, Wickner and colleagues (212) have proposed a series of useful criteria: (*a*) they would behave as non-Mendelian genetic elements; (*b*) the associated phenotype would be reversible; (*c*) a maintenance gene encoding the normal protein would manifest as a related, Mendelian genetic element; (*d*) overproduction of the maintenance gene product would increase the generation of the non-Mendelian element; and (*e*) defective, interfering replicons would not be evident. Two non-Mendelian genetic determinants that fulfill these criteria, [URE3] and [PSI], were first described over 25 years ago. The ure2 and [URE3] mutations were isolated by their ability to utilize ureidosuccinate in the medium, thereby overcoming a defect in uracil biosynthesis caused by mutations in aspartate transcarbamylase (Ura2p) (110). Whereas the behavior of the ure2 mutations was entirely consistent with a normal chromosomal locus, when [URE3] strains were mated with wild-type strains, an irregular segregation pattern was observed (1, 110). Subsequently, it was shown by cytoduction that [URE3] could be transferred in the absence of nuclear fusion, confirming their non-Mendelian nature, and that [URE3] could be cured by growth of cells on rich medium containing 5 mM Guanidine HCl (GdnHCl) (212). Significantly, the cured strains could then be used to generate further [URE3] mutants, arguing strongly against the participation of a nucleic acid genome, and thus satisfying two of the

aforementioned criteria expected of a prion-like determinant. Notably, the ure2 mutations are recessive and display the same phenotype as [URE3] mutants, and a series of genetic arguments showed clearly that the URE2 chromosomal gene is necessary for propagation of the [URE3] phenotype (211). However, since the phenotype of ure2 mutants is the same as that observed in the presence of [URE3], it seems most likely that URE2 encodes the normal, active form of the protein. Conversion to the abnormal, inactive form leads to the [URE3] state (211). Following the introduction of the URE2 gene on a high copy plasmid, an increase of 50-100 fold was observed in the frequency with which [URE3] mutants were obtained (211). This is entirely as expected for a prion mechanism, since the stochastic event that gives rise to the abnormal conformer will increase in frequency in cells in which the normal 'precursor' is overproduced. A similar mechanism may lead to spontaneous prion disease in transgenic mice overexpressing PrP (206).

The [PSI] Determinant

Another non-Mendelian genetic element in yeast called the [PSI] factor exaggerates the effect of a weak chromosomal ochre suppressor, SUQ5 (39). Subsequent studies showed that the action of [PSI] was more general, affecting other weak ochre suppressors (15) and that strong ochre suppressors become lethal in the presence of [PSI] (39), probably due to an intolerably low frequency of correct translational termination. [PSI] also affects the efficiency of suppression of UGA and UAG codons by the aminoglycoside antibiotics (143).

Many lines of evidence suggest that [PSI] is an abnormal, prion-like conformer of the Sup35 protein (Sup35p) (for reviews see 118, 201, 212). Like [URE3], [PSI] can be cured by growth on 5mM GdnHCl (202), as well as on hyperosmotic media (185). Other characteristics of [PSI] mirror those of [URE3]: The [PSI] phenotype is the same as that of the omnipotent suppressor mutations sup35 and sup45 (80), and overproduction of Sup35p leads to a 100-fold increase in the frequency of occurrence of [PSI] (27). In addition to these similarities to [URE3], the influence of [PSI] upon protein synthesis in vitro provides further evidence for a prion-like mode of propagation (200). When the efficiency of translational readthrough by extracts of yeast cells in the presence of added suppressor tRNAs in vitro was assessed, it was found that substantial readthrough occurred only when the extracts were prepared using strains that contained [PSI] (200).

Yeast Prion Domains

[PSI] and [URE3] share another important characteristic. In both cases the "functional" determinants have been mapped to the COOH-terminal region of the protein, distinct from the "prion" domain, which comprises the NH_2-terminal

65 and 114 residues of Ure2p (124) and Sup35p (44, 48, 199), respectively. Although neither of the prion domains display any sequence identity to each other or to PrP, the NH_2-terminal regions of Sup35p and mammalian PrPs both contain short repeated sequence elements: PQGGYQQYN in Sup35p and PHGGGWGQ in PrP (201).

Dependence of Yeast Prions on Molecular Chaperones

The intrinsic power of the yeast genetic system has, however, provided striking evidence for the involvement of chaperones in propagation of yeast [PSI] "prions." A genetic screen for factors that suppress the [PSI] phenotype resulted in the isolation of a single suppressor plasmid, which was found to contain the chaperone Hsp104 (28). Furthermore, propagation of [PSI] was eliminated by either overproduction or absence of Hsp104, and treatment of cells with guanidine or UV light led to induction of Hsp104p (28, 150). The significance of Hsp104 is unclear, since, to date, there are no published data to indicate that [URE3] utilizes Hsp104.

Differences Between the Yeast and Mammalian Prions

Although the preceding arguments provide an intriguing case for the existence of prion-like elements in yeast, it is essential to state that no biochemical data exist showing that the basis for the yeast "prion" phenomenon is a change in the conformation of a protein. Perhaps most importantly, the putative "prion" state is proposed to be functionally inert in the case of both [PSI] and [URE3], and produces the same phenotype as inactivation of the maintenance gene. In contrast, prion diseases in mammals cannot be explained simply by the loss of function of PrP, since ablation of the PrP gene had no detectable deleterious effect (18). Furthermore, the existence of distinct prion "strains" described elsewhere in this review argues that PrP^{Sc} may be both conformationally and functionally diverse.

SOME CONCLUDING REMARKS ON PRION GENETICS

Although the study of prions has taken several unexpected directions over the past three decades, a rather complete and fascinating story of prion biology is emerging. While learning the details of the structures of the prion proteins and deciphering the mechanism of PrP^{C} transformation into PrP^{Sc} will be important, the broader principles of prion biology have become reasonably clear. While some investigators prefer to view the composition of the infectious prion particle as unresolved, such a perspective denies an enlarging body of data, none of which refutes the prion concept.

The discovery that prion diseases in humans are uniquely both genetic and infectious greatly strengthened and extended the prion concept. To date, 20 different mutations in the human PrP gene all resulting in nonconservative substitutions have been found either to be linked genetically to or to segregate with the inherited prion diseases (Figure 1*B*). Yet the transmissible prion particle is composed largely, if not exclusively, of an abnormal isoform of the prion protein designated PrP^{Sc} (156).

Understanding how PrP^{C} unfolds and refolds into PrP^{Sc} will be of paramount importance in transferring advances in the prion diseases to studies of other degenerative illnesses. The mechanism by which PrP^{Sc} is formed must involve a templating process where existing PrP^{Sc} directs the refolding of PrP^{C} into a nascent PrP^{Sc} with the same conformation. Undoubtedly, molecular chaperones of some type participate in a process that seems confined to caveolae-like domains of the cell.

Studies of prion-like proteins in yeast may prove particularly helpful in dissecting some of the events that feature in PrP^{Sc} formation (211). Conversion to the prion-like state in yeast requires the molecular chaperone Hsp104; however, no homolog of Hsp104 has been found in mammals (28, 150). Other examples of acquired inheritance in lower organisms may also occur through a prion-like mechanism (43, 111).

As our understanding of prion propagation increases, it should be possible to design effective therapeutics. Since people at risk for inherited prion diseases can now be identified decades before neurologic dysfunction is evident, the development of an effective therapy is imperative. Moreover, the possible transmission of bovine prions to humans in Britain and France presents considerable concern as to the number of people who might succumb to prion disease in the future (38). Although we have no way of predicting the number of individuals who may develop neurologic dysfunction from bovine prions in the future, seeking an effective means of therapy now seems most prudent. From our current knowledge, the conversion of PrP^{C} into PrP^{Sc} seems to be the most attractive target (32). Either stabilizing the structure of PrP^{C} by binding a drug or interfering with the action of protein X, which presumably functions as a molecular chaperone, seem reasonable strategies for the rational design of a pharmacotherapeutic.

Whether therapies designed to prevent the conversion of PrP^{C} into PrP^{Sc} will be effective in the more common neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, and amyotrophic lateral sclerosis (ALS) is unknown. Alternatively, developing a therapy for the prion diseases might provide a blueprint for designing somewhat different drugs for these disorders. Like the inherited prion diseases, an important subset of Alzheimer's

disease and ALS is caused by mutations that result in nonconservative amino acid substitutions in proteins expressed in the CNS.

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