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Chemistry and Biology of Prions†

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Ten years have passed since the term "prion" was introduced (Prusiner, 1982) and the prion protein discovered (Bolton et al., 1982; Prusiner et al., 1982a). Although the notion of prions was initially met with considerable skepticism, the steady accumulation of experimental data over the past decade has created a rather convincing edifice which argues that prions are unique among all infectious pathogens (Prusiner, 1982, 1991). While the human prion diseases once presented a rather confusing picture, the finding that prion diseases may be both inherited and transmissible has brought some clarity. The situation with the natural prion diseases of animals remains more problematic. Progress in understanding the human prion diseases has its roots in their transmission to animals (Masters et al., 1981a) and the discovery of the prion protein (PrP) (Bolton et al., 1982; Prusiner et al., 1982a) followed by the molecular cloning of the PrP gene (Chesebro et al., 1985; Oesch et al., 1985; Prusiner et al., 1984).

The prion diseases are a group of neurodegenerative disorders of animals and humans. These diseases are often transmissible to experimental animals by inoculation. Unlike other transmissible disorders, the prion diseases can also be caused by mutations in PrP, which is encoded by a chromosomal gene. Six diseases of animals and four of humans are caused by prions (Table I). Scrapie of sheep and goats is the prototypic prion disease. Mink encephalopathy, chronic wasting disease, bovine spongiform encephalopathy (BSE), feline spongiform encephalopathy, and exotic ungulate encephalopathy are all thought to occur after the consumption of prion-infected foodstuffs. Similarly, kuru of the New Guinea Fore people is thought to have resulted from the consumption of brains from dying relatives during ritualistic cannibalism (Alper, 1987; Gajdusek, 1977). Creutzfeldt-Jakob disease (CJD) occurs primarily as a sporadic disorder (Masters et al., 1981b), but iatrogenic CJD is thought to result from the accidental inoculation of patients with prions (Fradkin et al., 1991). Familial CJD, Gerstmann–Sträussler–Scheinker syndrome (GSS), and fatal familial insomnia (FFI) are all dominantly inherited prion diseases which have been shown to be caused by mutations in the PrP gene (Hsiao & Prusiner, 1990; Medori et al., 1992).

From studies of a once obscure disease of sheep, a new area of biological research is beginning to emerge. For many years, scrapie was considered an enigmatic disorder of sheep and goats, the etiology of which was unknown. By 1938, experimental transfer of scrapie from one sheep to another began to argue for an infectious etiology (Cuillé & Chelle, 1939). Meanwhile, observations that the genetic backgrounds of flocks profoundly influence their susceptibility to scrapie raised the possibility that scrapie might be an inherited disorder (Gordon, 1966). These opposing views sparked many controversial encounters (Dickinson et al., 1965; Parry, 1962) and foreshadowed a series of equally bitter arguments about the possible structure of the transmissible scrapie agent (Pattison, 1988).

As molecular biological and genetic analyses of both the human and animal prion diseases have advanced, the biochemistry of the prion protein continues to pose both methodological and conceptual problems. For example, transmissible prions are composed largely, if not entirely, of an abnormal isoform of cellular PrP designated PrP^{Sc} (Gabizon & Prusiner, 1990; Prusiner, 1991). Although PrP^{Sc} is synthesized from cellular PrP (PrP^{C}) by a posttranslational process (Basler et al., 1986; Borchelt et al., 1990, 1992; Caughey & Raymond, 1991), the precise nature of this protein transformation remains unknown. Whether the conversion of PrP^{C} to PrP^{Sc} involves an as yet unidentified chemical modification, perhaps labile under the conditions of analysis, or it only involves a conformational change (Stahl et al., 1992b) remains to be established.

Purification of Scrapie Infectivity. Many investigators attempted to purify the scrapie agent but with relatively little

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success. The slow, cumbersome, and tedious bioassays in sheep and later mice greatly limited the number of samples that could be analyzed. Since the ease of purifying any biologically active macromolecule is directly related to the rapidity of the assay, it is not surprising that little progress was made with sheep and goats where only very limited numbers of samples could be analyzed and incubation times exceeding 18 months were required (Pattison, 1988). Experimental transmission of scrapie to mice allowed many more samples to be analyzed, but one year was required to complete the measurement of scrapie infectivity by endpoint titration using 60 animals to evaluate one sample (Chandler, 1961).

The transmission of scrapie to Syrian hamsters of an inoculum previously passaged in rats produced disease in about 70 days (Marsh & Kimberlin, 1975). These shorter incubation times coupled with the development of standard curves relating the length of the incubation time to the size of the inoculated dose permitted much more rapid quantitation of specimens (Prusiner et al., 1980, 1982b). This methodological advance made possible the development of protocols for the significant enrichment of scrapie infectivity (Prusiner et al., 1982a, 1983).

Development of the Prion Concept. With partially purified fractions of scrapie agent from hamster brain, it became possible to demonstrate that those procedures which modify or hydrolyze proteins produce a diminution in scrapie infectivity (Prusiner, 1982; Prusiner et al., 1981). At the same time, tests done in search of a scrapie-specific nucleic acid were unable to demonstrate any dependence of infectivity on a polynucleotide (Prusiner, 1982) in agreement with earlier studies reporting the extreme resistance of infectivity to UV irradiation at 254 nm (Alper et al., 1967).

On the basis of these observations, it seemed likely that the infectious pathogen capable of transmitting scrapie was neither a virus nor a viroid. For this reason the term "prion" was introduced to embolden the concept that the scrapie agent was likely to be unique in its molecular structure (Prusiner, 1982). Indeed, considerable evidence accumulated over the past decade supports this hypothesis (Prusiner, 1991; Weissmann, 1991). Furthermore, the replication of prions and their mode of pathogenesis also appear to be unique and without precedent. After a decade of severe criticism and serious doubt, the prion concept is now enjoying considerable acceptance.

Discovery of the Prion Protein. Once it was established that scrapie prion infectivity depended upon protein (Prusiner et al., 1981), the search for a scrapie-specific protein intensified. While the insolubility of scrapie infectivity made purification problematic, we took advantage of this property along with its relative resistance to degradation by proteases to extend the degree of purification. Radioiodination of partially purified fractions revealed a protein unique to preparations from scrapie-infected brains (Bolton et al., 1982; Prusiner et al., 1982a). This protein was later named prion protein (PrP) with an apparent molecular mass of 27–30 kDa or PrP 27–30 (McKinley et al., 1983).

Subsequent studies showed that PrP 27–30 is derived from a larger protein of molecular mass 33–35 kDa designated PrP^{Sc} (Meyer et al., 1986; Oesch et al., 1985). At the same time, it was found that the brains of normal and scrapie-infected hamsters express similar levels of PrP mRNA and a protease-sensitive prion protein designated PrP^{C} (Oesch et al., 1985). The function of PrP^{C} is unknown although it has been suggested that a PrP-like molecule from chickens may have acetylcholine receptor inducing activity (ARIA) (Harris et al., 1991). Furthermore, PrP^{C} does not seem to be essential at least in young mice since disruption of the PrP gene has not caused any detectable abnormalities in the nervous, musculoskeletal, or lymphoreticular systems at 14 months of age (Büeler et al., 1992). These results argue that scrapie and the other prion diseases do not result from an inhibition of PrP^{C} function due to PrP^{Sc} but rather the accumulation of PrP^{Sc} interferes with some as yet undefined cellular process. Perhaps, the absence of PrP^{C} will result in abnormalities later in life as is the case for the p53 tumor suppressor protein where young animals lacking p53 are normal but neoplasms develop as they age (Donehower et al., 1992).

Table I: Prion Diseases[a]

disease	natural host
scrapie	sheep and goats
transmissible mink encephalopathy (TME)	mink
chronic wasting disease (CWD)	mule deer and elk
bovine spongiform encephalopathy (BSE)	cattle
feline spongiform encephalopathy (FSE)	cats
exotic ungulate encephalopathy (EUE)	nyala and greater kuru
kuru	humans—Fore
Creutzfeldt-Jakob disease (CJD)	humans
Gerstmann–Sträussler–Scheinker syndrome (GSS)	humans
fatal familial insomnia (FFI)	humans

[a] Alternative terminologies include slow virus infections, subacute transmissible spongiform encephalopathies, and unconventional slow virus diseases (Gajdusek, 1977).

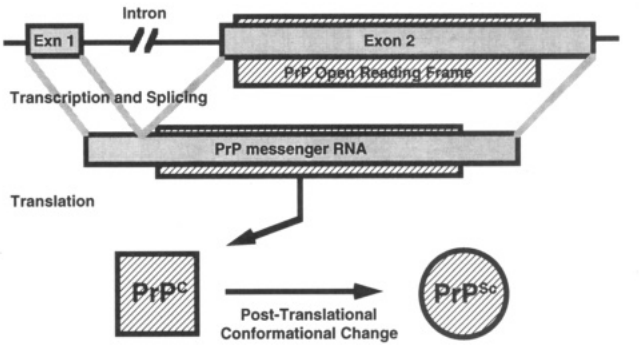

FIGURE 1: Structure and organization of the chromosomal prion protein gene. In all mammals examined, the entire open reading frame (ORF) is contained within a single exon. The 5′ untranslated region of the PrP mRNA is derived from either one or two additional exons. Only one PrP mRNA has been detected. PrP^{Sc} is thought to be derived from PrP^{C} by a posttranslational process. The amino acid sequence of PrP^{Sc} is identical to that predicted from the translated sequence of the DNA encoding the PrP gene, and no unique posttranslational chemical modifications have been identified that might distinguish PrP^{Sc} from PrP^{C}. Thus, it seems likely that PrP^{C} undergoes a conformational change as it is converted to PrP^{Sc}.

PrP Gene Structure and Organization. The entire open reading frame (ORF) of all known mammalian and avian PrP genes is contained within a single exon (Figure 1) (Basler et al., 1986; Gabriel et al., 1992; Hsiao et al., 1989; Westaway et al., 1987). This feature of the PrP gene eliminates the possibility that PrP^{Sc} arises from alternative RNA splicing (Basler et al., 1986; Westaway et al., 1987; Westaway et al., 1991); however, mechanisms such as RNA editing or protein splicing remain a possibility (Blum et al., 1990; Kane et al., 1990). 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 (Basler et al., 1986). The mouse (Mo) PrP gene is comprised of three exons with exon 3 analogous to exon 2 of the hamster (Westaway et al., 1991). 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 which may

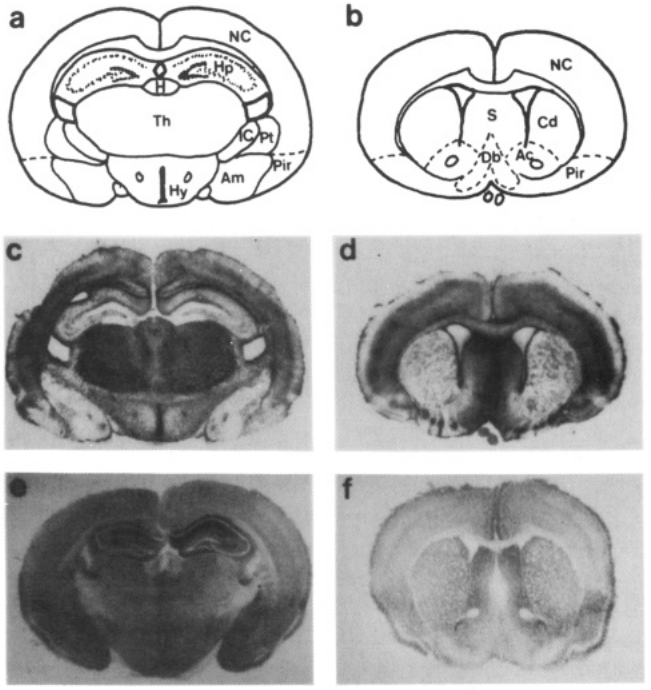

FIGURE 2: Histoblots of Syrian hamster brain immunostained for PrP^C or PrP^{Sc}. Coronal sections through the hippocampus–thalamus (a, c, and e) and the septum–caudate (b, d, and f). Brain sections of a Syrian hamster clinically ill after inoculation with Sc237 prions (c and d) and an uninfected control animal (e and f). Immunostaining for PrP^{Sc} is shown in panels c and d and for PrP^C in e and f. Abbreviations: Ac, nucleus accumbens; Am, amygdala; Cd, caudate nucleus; Db, diagonal band of Broca; H, habenula; Hp, hippocampus; Hy, hypothalamus; IC, internal capsule; NC, neocortex. Reproduced from Taraboulos et al. (1992a).

function as a canonical binding site for the transcription factor Sp1 (McKnight & Tjian, 1986).

Mapping PrP genes to the short arm of human chromosome 20 and the homologous region of Mo chromosome 2 argues for the existence of PrP genes prior to the speciation of mammals (Sparkes et al., 1986). Hybridization studies demonstrated <0.002 PrP gene sequences per ID_{50} unit in purified prion fractions, indicating that a gene encoding PrP^{Sc} is not a component of the infectious prion particle (Oesch et al., 1985). This is a major feature which distinguishes prions from viruses including those retroviruses that carry cellular oncogenes and from satellite viruses that derive their coat proteins from other viruses previously infecting plant cells.

Expression of the PrP Gene. Although PrP mRNA is constitutively expressed in the brains of adult animals (Chesebro et al., 1985; Oesch et al., 1985), it is highly regulated during development. In the septum, levels of PrP mRNA and choline acetyltransferase were found to increase in parallel during development (Mobley et al., 1988). In other brain regions, PrP gene expression occurred at an earlier age. In situ hybridization studies show that the highest levels of PrP mRNA are found in neurons (Kretzschmar et al., 1986).

PrP^C expression in brain was defined by standard immunohistochemistry (DeArmond et al., 1987) and by histoblotting (Figure 2) (Taraboulos et al., 1992a). 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 where scrapie infectivity was found to migrate in a pattern consistent with retrograde transport (Fraser & Dickinson, 1985; Jendroska et al., 1991; Kimberlin et al., 1983). While the rate of PrP^{Sc} synthesis appears to be a function of the level of PrP^C expression in transgenic (Tg) mice, the level to which PrP^{Sc} accumulates appears to be independent of PrP^C concentration (Prusiner et al., 1990).

Synthesis of PrP Isoforms. Metabolic labeling studies of scrapie-infected cultured cells have shown that PrP^C is synthesized and degraded rapidly while PrP^{Sc} is synthesized slowly by an as yet undefined posttranslational process (Figure 1) (Borchelt et al., 1990, 1992; Caughey et al., 1989; Caughey & Raymond, 1991). These observations are consistent with earlier findings showing that PrP^{Sc} accumulates in the brains of scrapie-infected animals while PrP mRNA levels remain unchanged (Oesch et al., 1985). Furthermore, the structure and organization of the PrP gene made it likely that PrP^{Sc} is formed during a posttranslational event (Basler et al., 1986).

Both PrP isoforms appear to transit through the Golgi apparatus where their Asn-linked oligosaccharides are modified and sialylated (Bolton et al., 1985; Endo et al., 1989; Haraguchi et al., 1989; Manuelidis et al., 1985; Rogers et al., 1990). PrP^C is presumably transported within secretory vesicles to the external cell surface where it is anchored by a glycosyl phosphatidylinositol (GPI) moiety (Figure 3) (Safar et al., 1990; Stahl et al., 1987, 1992a). In contrast, PrP^{Sc} accumulates primarily within cells where it is deposited in cytoplasmic vesicles, many of which appear to be secondary lysosomes (Borchelt et al., 1992; Caughey et al., 1991a; McKinley et al., 1991b; Taraboulos et al., 1990b, 1992b).

Whether PrP^C is the substrate for PrP^{Sc} formation or a restricted subset of PrP molecules are precursors for PrP^{Sc} remains to be established. Several experimental results argue that PrP molecules destined to become PrP^{Sc} exit to the cell surface as does PrP^C (Stahl et al., 1987) prior to their conversion into PrP^{Sc} (Borchelt et al., 1992; Caughey & Raymond, 1991; Taraboulos et al., 1992b). Interestingly, the GPI anchors of both PrP^C and PrP^{Sc}, which presumably feature in directing the subcellular trafficking of these molecules, are sialylated (Figure 3) (Stahl et al., 1992a). It is unknown whether sialylation of the GPI anchor participates in some aspect of PrP^{Sc} formation.

Although most of the difference in the mass of PrP 27–30 predicted from the amino acid sequence and that observed after posttranslational modification is due to complex-type oligosaccharides, these sugar chains are not required for PrP^{Sc} synthesis in scrapie-infected cultured cells on the basis of experiments with the Asn-linked glycosylation inhibitor tunicamycin and site-directed mutagenesis studies (Taraboulos et al., 1990a).

Cell-free translation studies have demonstrated two forms of PrP: a transmembrane form which spans the bilayer twice at the transmembrane (TM) and amphipathic helix domains and a secretory form (Lopez et al., 1990; Yost et al., 1990). The stop transfer effector (STE) domain controls the topogenesis of PrP. That PrP contains both a TM domain and a GPI anchor poses a topologic conundrum. It seems likely that membrane-dependent events feature in the synthesis of PrP^{Sc} especially since brefeldin A, which selectively destroys the Golgi stacks (Doms et al., 1989), prevents PrP^{Sc} synthesis

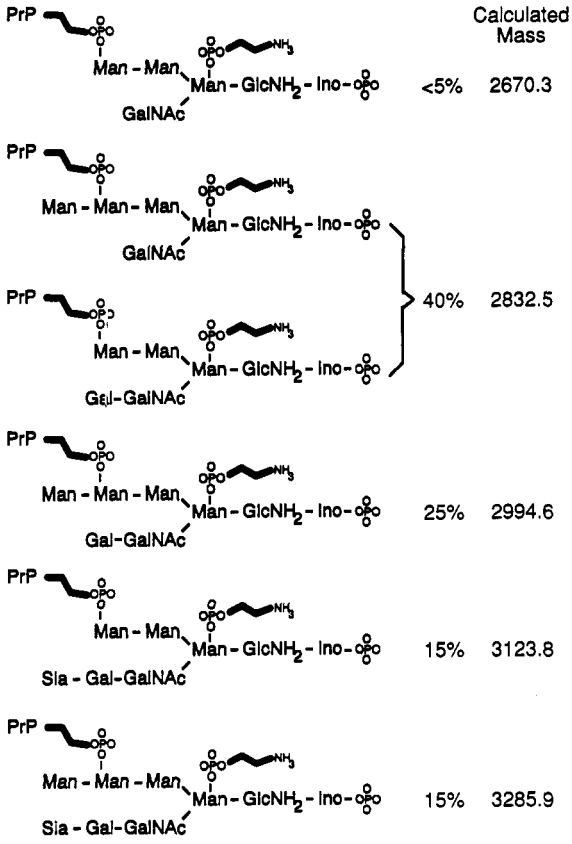

FIGURE 3: Glycoinositol phospholipid anchors of the prion protein. The proposed GPI anchor structures were determined for SHaPrP^{Sc} by mass spectrometry. The percentages indicate an estimate of the approximate relative abundance of each glycoform. The calculated masses are based on the average molecular weight of each element in the GPI and include the mass of the C-terminal PrP (K12) peptide which accounts for 1312.5 mass units of the total. Reproduced from Stahl et al. (1992).

in scrapie-infected cultured cells (Taraboulos et al., 1992b). For many years, the association of scrapie infectivity with membrane fractions has been appreciated (Gibbons & Hunter, 1967); indeed, hydrophobic interactions are thought to account for many of the physical properties displayed by infectious prion particles (Gabizon & Prusiner, 1990; Prusiner et al., 1980).

Genetic Linkage of PrP with Scrapie Incubation Times. Studies of PrP genes (*Prn-p*) in mice with short and long incubation times demonstrated genetic linkage between a *Prn-p* restriction fragment length polymorphism (RFLP) and a gene modulating incubation times (*Prn-i*) (Carlson et al., 1986). Other investigators have confirmed the genetic linkage, and one group has shown that the incubation time gene *Sinc* is also linked to PrP (Hunter et al., 1987; Race et al., 1990). *Sinc* was first described by Dickinson and colleagues over 20 years ago (Dickinson et al., 1968); whether the genes for PrP, *Prn-i*, and *Sinc* are all congruent remains to be established. The PrP sequences of NZW (*Prn-p*^{a}) and I/Ln (*Prn-p*^{b}) mice with short and long scrapie incubation times, respectively, differ at codons 108 (L → F) and 189 (T → V) (Westaway et al., 1987). While these amino acid substitutions argue for the congruency of *Prn-p* and *Prn-i*, experiments with *Prn-p*^{a} mice expressing *Prn-p*^{b} transgenes demonstrated a paradoxical shortening of incubation times (Westaway et al., 1991) instead of a prolongation as predicted from (*Prn-p*^{a} × *Prn-p*^{b}) F1 mice which exhibit long incubation times that are dominant (Carlson et al., 1986; Dickinson et al., 1968). Whether this paradoxical shortening of scrapie incubation times in Tg-(*Prn-p*^{b}) mice results from high levels of PrP^C-B expression remains to be established (Westaway et al., 1991).

Prion Diseases of Animals. An investigation into the etiology of scrapie followed the vaccination of sheep for looping ill virus with formalin-treated extracts of ovine lymphoid tissue unknowingly contaminated with scrapie prions (Gordon, 1946). Two years later, more than 1500 sheep developed scrapie from this vaccine. While the transmissibility of experimental scrapie became well established, the spread of natural scrapie within and among flocks of sheep remained puzzling. Parry argued that host genes were responsible for the development of scrapie in sheep. He was convinced that natural scrapie is a genetic disease which could be eradicated by proper breeding protocols (Parry, 1962, 1983). 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 (Dickinson et al., 1965). The incubation time gene for experimental scrapie in Cheviot sheep called *Sip* is said to be linked to a PrP gene RFLP (Hunter et al., 1989), a situation perhaps analogous to *Prn-i* and *Sinc* in mice. However, the null hypothesis of nonlinkage has yet to be tested, and this is important, especially in view of earlier studies which argue that susceptibility of sheep to scrapie is governed by a recessive gene (Parry, 1962, 1983). Although a polymorphism in the PrP ORF has been found at codon 171 (R → Q) in a Suffolk sheep (Goldman et al., 1990), no correlation with scrapie has been observed (D. Westaway and S. B. Prusiner, unpublished data).

Since the initial cases of BSE in 1985 (Wells et al., 1987), >70 000 cattle have died of bovine spongiform encephalopathy (BSE) in Great Britain (Dealler & Lacey, 1990; Wilesmith et al., 1992a,b). Neither the cause of BSE, often referred to as "mad cow disease", nor methods of controlling the spread of this disorder are known. Epidemiologic investigations suggest that prion-contaminated meat and bone meal derived from the offal of scrapie-infected sheep are the cause of BSE. Between 1978 and 1980, the majority of rendering plants in Great Britain stopped using hydrocarbon solvents for extraction of the rendered greaves prior to heating because of the expense and toxicity of the solvents. Presumably, the appearance of BSE ~5 years later resulted from the survival of sheep prions in meat and bone meal preparations. It seems likely that the solvent extraction methods which were widely employed before 1980 resulted in the delipidation of sheep prions, making them more susceptible to inactivation by subsequent heating steps used in the rendering process. The practice of feeding dietary protein supplements derived from rendered ruminant offal to cattle has been banned since 1988. Curiously, the majority of BSE cases have occurred in herds with a single affected animal within a herd; several cases of BSE in a single herd are infrequent (Wilesmith et al., 1992a,b). Whether the distribution of BSE cases within herds will change as the epidemic progresses and BSE will disappear with the cessation of feeding rendered meat and bone meal are uncertain.

Of particular importance to the BSE epidemic is kuru of humans, confined to the Fore region of New Guinea (Gajdusek, 1977; Gajdusek et al., 1966). Once the most common cause of death among women and children, kuru has almost disappeared with the cessation of ritualistic cannabalism (Alpers, 1987). These findings argue that kuru was transmitted orally as proposed for BSE. Of note are recent cases

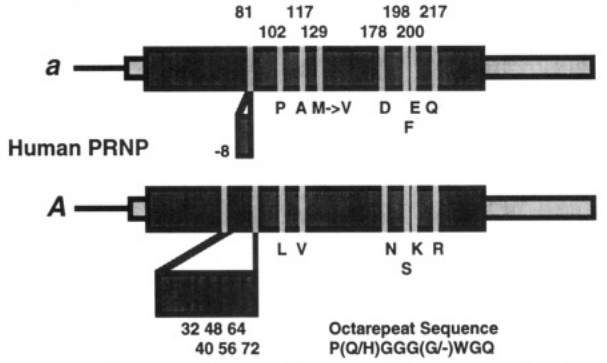

FIGURE 4: Human prion protein gene. The open reading frame (ORF) is denoted by the large gray rectangles and the exon by the smaller rectangles (diagonal lines). Codon numbers are indicated above the amino acid sequence. Human PrP wild-type polymorphisms are shown in the upper rectangle denoted "a", while mutations linked to or associated with prion diseases are depicted in the lower rectangle denoted "A". The wild-type human PrP gene contains five octarepeats [P(Q/H)GGG(G/–)WGQ] from codons 51 to 91. Deletion of a single octarepeat at codon 81 or 82 is not associated with prion disease. Whether such a deletion alters the phenotypic characteristics of a prion disease is unknown, but homozygosity for Met or Val at codon 129 appears to increase susceptibility to sporadic CJD. Octarepeat inserts of 32, 40, 48, 56, 64, and 72 amino acids at codons 67, 75, or 83 have been found and are either genetically linked to or associated with familial CJD. Point mutations at codons 102 (Pro → Leu), 117 (Ala → Val), and 198 (Phe → Ser) are found in patients with GSS. There are common polymorphisms at codons 117 (Ala → Ala) and 129 (Met → Val). Point mutations at codons 178 (Asp → Asn) and 200 (Glu → Lys) are found in patients with familial CJD. Point mutations at codons 198 (Phe → Ser) and 217 (Gln → Arg) are found in patients with GSS who have PrP amyloid plaques and neurofibrillary tangles.

of kuru which have occurred in people exposed to prions more than three decades ago.

Human Prion Diseases. The discovery of human prion diseases came from the recognition that the neuropathology of a cerebellar disorder of New Guinea natives was similar to that of scrapie. Spongiform degeneration in kuru prompted the suggestion that transmission studies in apes be performed (Hadlow, 1959). The success of those studies (Gajdusek et al., 1966) was followed by the transmission of CJD to apes (Gibbs et al., 1968) based on the earlier recognition that the neuropathological changes in kuru were similar to those found in CJD (Klatzo et al., 1959). In 1920, Creutzfeldt reported the case of a 23 year old woman who died of a neurodegenerative disease (Creutzfeldt, 1920), and the following year Jakob reported five cases (Jakob, 1921). Ironically, some investigators doubt that Creutzfeldt described the disease that now bears his name (Richardson, 1977).

In humans, genetics were first thought to have a role in CJD with the recognition that ~10% of cases are familial (Gajdusek, 1977; Masters et al., 1981b). Like sheep scrapie, the relative contributions of genetic and infectious etiologies in the human prion diseases remained puzzling. The discovery of the PrP gene raised the possibility that mutation might feature in the hereditary human prion diseases. A point mutation at codon 102 (P → L) was found and linked genetically to development of GSS with a LOD score exceeding 3 (Figure 4) (Hsiao et al., 1989). This mutation which may be due to the deamination of a methylated CpG in a germline PrP gene resulted in the substitution of a T for C. The codon 102 mutation has been identified in ten families in nine different countries including the family in which GSS was first described (Kretzschmar et al., 1991).

An insert of 144 bp at codon 53 containing six octarepeats has been seen in patients with CJD from four families all residing in southern England (Figure 4) (Poulter et al., 1992). This mutation must have arisen through a complex series of events since the human PrP gene contains only five octarepeats, indicating that a single recombination event could not have created the insert. Genealogic investigations have shown that all four families are related, arguing for a single founder born more than two centuries ago (Poulter et al., 1992). The LOD score for this extended pedigree exceeds 11. Studies from several laboratories have demonstrated that insertions of four, five, six, seven, eight, or nine octarepeats (in addition to the normal five) segregate with inherited CJD, whereas deletion of one octarepeat has been identified without the neurologic disease (Goldfarb et al., 1991a; Owen et al., 1992; Vnencak-Jones & Phillips, 1992).

For many years, 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 (Kahana et al., 1974). Recent studies have shown that some Libyan and Tunisian Jews in families with CJD have a PrP gene point mutation at codon 200 resulting in a E → K substitution (Goldfarb et al., 1990; Hsiao et al., 1991a). One patient was homozygous for the mutation, but her clinical presentation was similar to that of heterozygotes (Hsiao et al., 1991a) arguing that familial prion diseases are true autosomal dominant disorders like Huntington's disease (Wexler et al., 1987). The codon 200 mutation has also been found in Slovaks originating from Orava in North Central Czechoslovakia (Goldfarb et al., 1990), in a cluster of familial cases in Chile (Goldfarb et al., 1991b), and in a large German family living in the United States (Bertoni et al., 1992). Some investigators have argued that the codon 200 mutation originated in a Sephardic Jew whose descendants migrated from Spain and Portugal at the time of the inquisition (Goldfarb et al., 1991b). It is more likely that the codon 200 mutation has arisen independently multiple times by the deamidation of a methylated CpG as described above the codon 102 mutation (Hsiao et al., 1989, 1991a). In support of this hypothesis are historical records of Libyan and Tunisian Jews indicating that they are descended from Jews living on the island of Jerba who first settled there around 500 BC and not from Sephardim (Udovitch & Valensi, 1984).

Many families with CJD have been found to have point mutations at codon 178 (Brown et al., 1992; Fink et al., 1991; Goldfarb et al., 1991c). In these patients as well as those with the codon 200 mutation, PrP amyloid plaques are rare; the neuropathologic changes generally consist of widespread spongiform degeneration. Recently, a new prion disease which presents with insomnia has been described in three Italian families with the codon 178 mutation (Medori et al., 1992). The neuropathology in these patients with fatal familial insomnia is restricted to selected nuclei of the thalamus. It is unclear whether all patients with the codon 178 mutation or only a subset present with sleep disturbances.

Other point mutations at codons 117, 198, and 217 also segregate with inherited prion diseases. Patients with a dementing or telencephalic form of GSS have a mutation at codon 117 (Doh-ura et al., 1989; Hsiao et al., 1991b). These patients as well as some in other families were once thought to have familial Alzheimer's disease but are now known to have prion diseases on the basis of PrP immunostaining of amyloid plaques and PrP gene mutations (Ghetti et al., 1989; Giaccone et al., 1990; Nochlin et al., 1989). Patients with the codon 198 mutation have numerous neurofibrillary tangles that stain with antibodies to τ and have amyloid plaques (Ghetti et al., 1989) that are composed largely of a PrP fragment

extending from residue 58 to 150 (Tagliavini et al., 1991). A genetic linkage study of this family produced a LOD score exceeding 6 (Dlouhy et al., 1992; Hsiao et al., 1992). The neuropathology of two patients of Swedish ancestry with the codon 217 mutation (Hsiao et al., 1992; Ikeda et al., 1991) was similar to that of patients with the codon 198 mutation.

At PrP codon 129, an amino acid (Met/Val) polymorphism (Figure 4) has been identified (Owen et al., 1990). Patients with CJD following treatment with human pituitary growth hormone (Buchanan et al., 1991; Fradkin et al., 1991) or gonadotrophin have a preponderance of the Val allele (Collinge et al., 1991) compared to the general population. Sporadic CJD patients were found to be homozygous for the Met or Val allele at codon 129 but were rarely heterozygous (Palmer et al., 1991). This finding has been interpreted as being consistent with the hypothesis that PrP^{C}/PrP^{Sc} complexes feature in the replication of prions (Prusiner et al., 1990).

Attempts To Demonstrate de Novo Synthesis of Prions in Tg Mice Expressing GSS Mutant MoPrP. When the codon 102 point mutation was introduced into a MoPrP transgene, spontaneous CNS degeneration occurred, characterized by clinical signs indistinguishable from experimental murine scrapie and neuropathology consisting of widespread spongiform morphology and astrocytic gliosis (Hsiao et al., 1990). By inference, these results argue that PrP mutations genetically linked to GSS and familial CJD are the causes of these diseases. It is unclear whether low levels of protease-resistant PrP in the brains of Tg mice with the GSS mutation are PrP^{Sc} or residual PrP^{C}. Undetectable or low levels of PrP^{Sc} in the brains of these Tg mice are consistent with the results of transmission experiments that suggest low titers of infectious prions. Brain extracts from two Tg(GSSMoPrP) mice that developed spontaneous neurodegeneration transmitted CNS degeneration to inoculated recipients and show de novo synthesis of prions as evidenced by serial passage (K. Hsiao, D. Groth, D. Foster, M. Torchia, M. Scott, and S. B. Prusiner, manuscript in preparation). If these observations can be supported by additional data and the possibility of contamination eliminated, then it can be argued that these findings are in accord with the results of many other experimental studies which contend that prions are devoid of a foreign nucleic acid (Kellings et al., 1992; Neary et al., 1991; Prusiner, 1991).

One view of the PrP gene mutations has been that they render individuals susceptible to a common "virus" (Aiken & Marsh, 1990; Chesebro, 1992; Kimberlin, 1990). In this scenario, the putative scrapie virus is thought to persist within a worldwide reservoir of humans, animals, or insects without causing detectable illness. Yet one in 10^6 individuals develops sporadic CJD and dies from a lethal "infection", while ~100% of people with PrP point mutations or inserts appear to eventually develop neurologic dysfunction. That germline mutations found in the PrP genes of patients and at-risk individuals are the cause of familial prion diseases is supported by experiments with Tg(GSSMoPrP) mice described above (Hsiao & Prusiner, 1990). The Tg mouse studies also argue that sporadic CJD might arise from the spontaneous conversion of PrP^{C} to PrP^{CJD} due to either a somatic mutation of the PrP gene or rare event involving modification of wild-type PrP^{C} (Prusiner, 1991).

Species Barriers in the Transmission of Prion Diseases. Passage of prions between species is a stochastic process characterized by prolonged incubation times (Pattison & Jones, 1967). Prions synthesized de novo reflect the sequence of the host PrP gene and not that of the PrP^{Sc} molecules in the inoculum (Bockman et al., 1987). On subsequent passage in an homologous host, the incubation time shortens to that recorded for all subsequent passages, and it becomes a nonstochastic process. 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 beef.

To test the hypothesis that differences in PrP gene sequences might be responsible for the species barrier, Tg mice expressing SHaPrP were constructed (Prusiner et al., 1990; Scott et al., 1989). The PrP genes of Syrian hamsters and mice encode proteins differing at 16 positions. Inoculation of four lines of Tg(SHaPrP) mice with SHa prions demonstrated abrogation of the species barrier resulting in abbreviated incubation times due to a nonstochastic process (Figure 5A) (Prusiner et al., 1990; Scott et al., 1989). The length of the incubation time after inoculation with SHa prions was inversely proportional to the level of $SHaPrP^{C}$ in the brains of Tg(SHaPrP) mice (Figure 5A,B) (Prusiner et al., 1990). $SHaPrP^{Sc}$ levels in the brains of clinically ill mice were similar in all four Tg(SHaPrP) lines inoculated with SHa prions. 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 (Figure 5C). Conversely, inoculation of Tg(SHaPrP) mice with SHa prions led to only the synthesis of SHa prions (Figure 5D). Thus, the de novo synthesis of prions is species specific and reflects the genetic origin of the inoculated prions. Similarly, the neuropathology of Tg(SHaPrP) mice is determined by the genetic origin of prion inoculum. Mo prions injected into Tg(SHaPrP) mice produced a neuropathology characteristic of mice with scrapie. A moderate degree of vacuolation in both the gray and white matter was found, while amyloid plaques were rarely detected. Inoculation of Tg(SHaPrP) mice with SHa prions produced intense vacuolation of the gray matter, sparing of the white matter and numerous SHaPrP amyloid plaques characteristic of Syrian hamsters with scrapie.

Prion Replication. Although the search for a scrapie-specific nucleic acid continues to be unrewarding, some investigators steadfastly cling to the notion that this putative polynucleotide drives prion replication. If prions are found to contain a scrapie-specific nucleic acid, then such a molecule would be expected to direct scrapie agent replication using a strategy similar to that employed by viruses. In absence of any chemical or physical evidence for a scrapie-specific polynucleotide (Aiken & Marsh, 1990; Kellings et al., 1992; Prusiner, 1991), it seems reasonable to consider some alternative mechanisms that might feature in prion biosynthesis.

The multiplication of prion infectivity is an exponential process in which the posttranslational conversion of PrP^{C} or a precursor to PrP^{Sc} appears to be obligatory (Borchelt et al., 1990). In the simplest model, one PrP^{Sc} molecule combines with one PrP^{C} molecule to form a heterodimeric intermediate which is transformed into two molecules of PrP^{Sc}. In the next cycle, each of the two PrP^{Sc} molecules combines with a PrP^{C} molecule giving rise to four PrP^{Sc} molecules. In the third cycle, each of the four PrP^{Sc} molecules combines with a PrP^{C} molecule giving rise to eight PrP^{Sc} molecules, thus creating an exponential process. Assuming prion biosynthesis simply involves amplification of posttranslationally altered PrP molecules, we might expect Tg(SHaPrP) mice to produce both SHa and Mo prions after inoculation with either prion since these mice synthesize both SHa and $MoPrP^{C}$. Yet Tg(SHaPrP) mice produce only those prions present in the inoculum (Figure 5C,D). We have interpreted these results as indicating that the incoming PrP^{Sc} molecules interact with

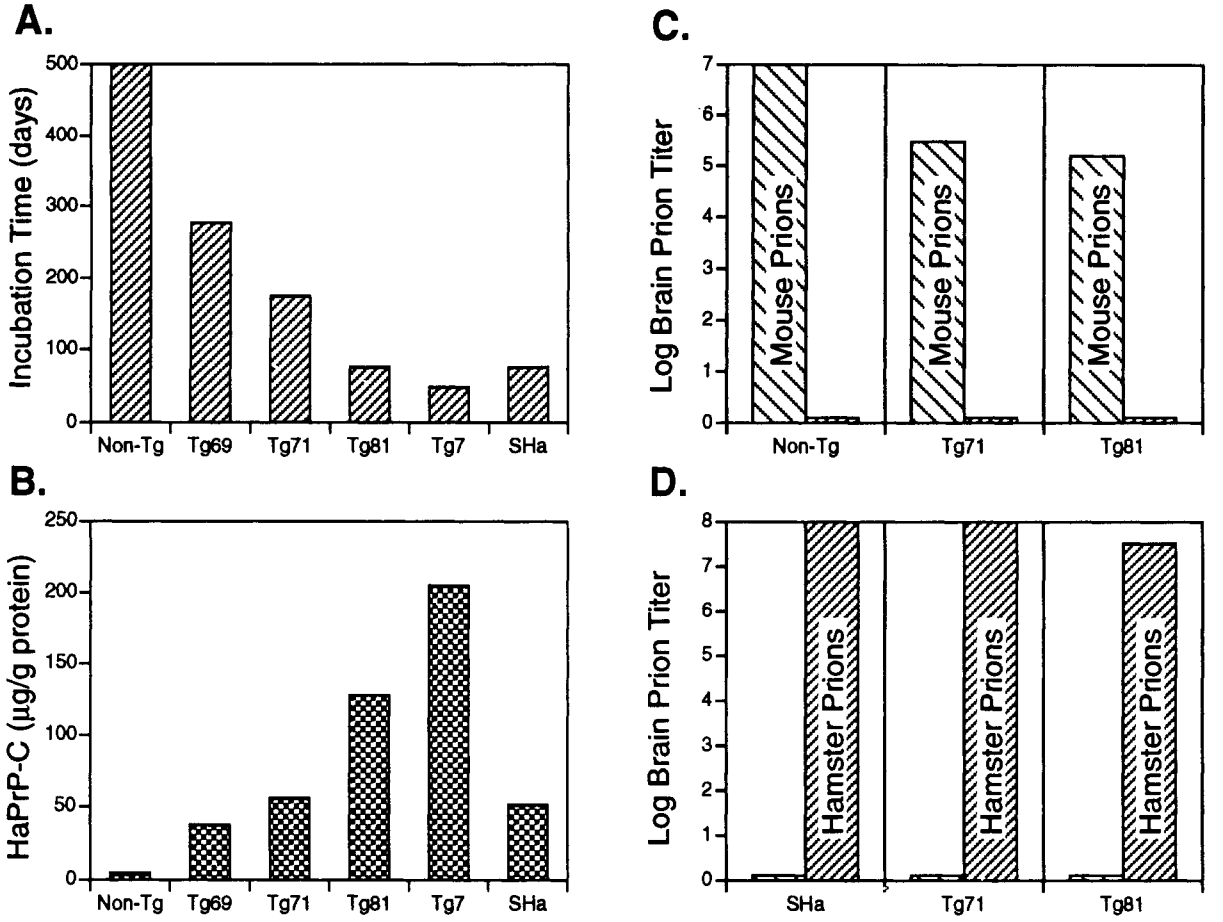

FIGURE 5: Transgenic mice expressing Syrian hamster (SHa) prion protein exhibit species-specific scrapie incubation times, infectious prion synthesis, and neuropathology (Prusiner et al., 1990). (A) Scrapie incubation times in nontransgenic mice (Non-Tg) and four lines of transgenic mice expressing SHaPrP and Syrian hamsters inoculated intracerebrally with $\sim 10^7$ ID_{50} units of Sc237 prions serially passaged in Syrian hamsters. The four lines of transgenic mice have different numbers of transgene copies: Tg69 and 71 mice have two to four copies of the SHaPrP transgene, whereas Tg81 have 30 to 50 and Tg7 mice have >60. Incubation times are number of days from inoculation to onset of neurologic dysfunction. (B) Brain $SHaPrP^C$ in transgenic mice and hamsters. $SHaPrP^C$ levels were quantitated by an enzyme-linked immunoassay. (C) Prion titers in brains of clinically ill animals after inoculation with Mo prions. Brain extracts from Non-Tg, Tg71, and Tg81 mice were bioassayed for prions in mice (left) and hamsters (right). (D) Prion titers in brains of clinically ill animals after inoculation with SHa prions. Brain extracts from Syrian hamsters as well as Tg71 and Tg81 mice were bioassayed for prions in mice (left) and hamsters (right).

the homologous PrP^C substrate to replicate more of the same prions (Prusiner et al., 1990).

Additional evidence in support of the proposed model for prion replication comes from Tg(Mo/SHaPrP) mice expressing chimeric Mo/$SHaPrP^C$ (M. Scott, D. Groth, M. Torchia, D. Foster, and S. B. Prusiner, manuscript in preparation). The chimeric Mo/SHaPrP gene was constructed by substituting the SHaPrP sequence for MoPrP from codon 94 to 188; within this domain, there are five amino acid substitutions which distinguish Mo from SHaPrP. When inoculated with either Mo or SHa prions, these Tg(Mo/SHaPrP) mice develop scrapie after ~ 140 days. The chimeric Tg mice produce Mo/$SHaPrP^{Sc}$ and Mo/SHa prions after inoculation with SHa prions and probably Mo prions as well. Evidence for chimeric Mo/SHa prions comes from the development of scrapie in Tg(Mo/SHaPrP) mice at ~ 70 days after inoculation with brain extracts from Tg(Mo/SHaPrP) mice containing the chimeric prions.

In the absence of any candidate posttranslational chemical modifications (Stahl et al., 1992b) that differentiate PrP^C from PrP^{Sc}, we are forced to consider the possibility that conformation distinguishes these isoforms. By comparing the amino acid sequences of 11 mammalian and one avian prion proteins, structural analyses predicted four α-helical regions (J.-M. Gabriel, F. Cohen, R. A. Fletterick, and S. B. Prusiner, manuscript in preparation; Cohen et al., 1986). Peptides corresponding to these regions of the SHaPrP were synthesized and, contrary to predictions, three of the four spontaneously formed amyloids as shown by electron microscopy and Congo red staining (Gasset et al., 1992b). By infrared spectroscopy, these amyloid peptides were found to exhibit a secondary structure comprised largely of β-sheets. The first of the predicted helices is the 14-residue peptide corresponding to SHaPrP codons 109–122; this peptide and the overlapping 15-residue sequence 113–127 both form amyloid. The most highly amyloidogenic peptide is the sequence AGAAAAGA corresponding to PrP codons 113–120. This peptide is in a region of PrP that is conserved across all known species. Two other predicted α-helices corresponding to SHaPrP codons 178–191 and 202–218 form amyloids and exhibit considerable β-sheet structure when synthesized as peptides. These findings suggest the possibility that the conversion of PrP^C to PrP^{Sc} involves the transition of one or more putative PrP α-helices into β-sheets. Infrared spectroscopy of PrP 27–30 has shown a high β-sheet content (Caughey et al., 1991b) which decreased when PrP 27–30 was denatured and scrapie infectivity diminished concomitantly (Gasset et al., 1992a).

These structural investigations of synthetic PrP peptides and the correlations between PrP 27–30 secondary structure and scrapie infectivity offer a structural model for the conversion of PrP^C to PrP^{Sc} as well as the replication of infectious prion particles involving a transition from α-helices

to β-sheets in PrP. Whether any of these synthetic PrP peptides can induce brain degeneration, PrP^{Sc} formation or prion infectivity is currently being investigated. If additional data can be obtained to support the hypothesis set forth here, then it may be useful to examine other degenerative diseases with respect to proteins undergoing similar structural changes.

In humans carrying point mutations or inserts in their PrP genes, mutant PrP^{C} molecules might spontaneously convert into PrP^{Sc}. While the initial stochastic event may be inefficient, once it happens the process becomes autocatalytic. The proposed mechanism is consistent with individuals harboring germline mutations who do not develop CNS dysfunction for decades and with studies on Tg(GSSMoPrP) mice that spontaneously develop CNS degeneration (Hsiao et al., 1990). Whether all GSS and familial CJD cases contain infectious prions or some represent inborn errors of PrP metabolism in which neither PrP^{Sc} nor prion infectivity accumulates is unknown. If the latter is found, then presumably mutant PrP^{C} molecules alone can produce CNS degeneration.

Some investigators have suggested that scrapie agent multiplication proceeds through a crystallization process involving PrP amyloid formation (Gajdusek, 1988, 1990; Gajdusek & Gibbs, 1990). Against this hypothesis is the absence or rarity of amyloid plaques in many prion diseases as well as the inability to identify any amyloid-like polymers in cultured cells chronically synthesizing prions (McKinley et al., 1991a; Prusiner et al., 1990). Purified infectious preparations isolated from scrapie-infected hamster brains exist as amorphous aggregates; only if PrP^{Sc} is exposed to detergents and limited proteolysis, does it then polymerize into prion rods exhibiting the ultrastructural and tinctorial features of amyloid (McKinley et al., 1991a). Furthermore, dispersion of prion rods into detergent–lipid–protein complexes results in a 10–100-fold increase in scrapie titer, and no rods could be identified in these fractions by electron microscopy (Gabizon et al., 1987).

Prion Diversity. There is good evidence for multiple "strains" or distinct isolates of prions as defined by specific incubation times, distribution of vacuolar lesions, and patterns of PrP^{Sc} accumulation (Bruce et al., 1989; Carp & Callahan, 1991; Dickinson et al., 1968; Fraser & Dickinson, 1973; Hecker et al., 1992). The mechanism by which isolate-specific information is carried by prions remains enigmatic; indeed, explaining the molecular basis of prion diversity seems to be a formidable challenge. For many years, some investigators argued that scrapie is caused by a virus-like particle which contains a scrapie-specific nucleic acid that encodes the information expressed by each isolate (Bruce & Dickinson, 1987). To date, no such polynucleotide has been identified by a wide variety of techniques including measurements of the nucleic acids in purified preparations. An alternative hypothesis has been suggested where PrP^{Sc} alone is capable of transmitting disease but the characteristics of PrP^{Sc} might be modified by a cellular RNA (Weissmann, 1991). This accessory cellular RNA or "coprion" is postulated to induce its own synthesis upon transmission from one host to another.

Two additional hypotheses not involving a nucleic acid have been offered to explain distinct prion isolates: a non-nucleic acid second component might create prion diversity or posttranslational modification of PrP^{Sc} might be responsible for the different properties of distinct prion isolates (Prusiner, 1991). Whether the PrP^{Sc} modification is chemical or conformational remains to be established, but no candidate chemical modifications have been identified. Structural studies of the GPI anchors of two SHa isolates have failed to reveal any differences; interestingly, about 40% of the anchor glycans have sialic acid residues (Figure 3) (Stahl et al., 1992a). A portion of the PrP^{C} GPI anchors also have sialic acid residues; PrP is the first protein found to have sialic acid residues attached to GPI anchors.

Although the structures of Asn-linked CHOs have been analyzed for PrP^{Sc} of one isolate (Endo, 1989), no data are available for PrP^{Sc} of other isolates of PrP^{C}. The great diversity of Asn-linked CHOs makes them candidates for isolate-specific information, but there is no precedent for Asn-linked CHOs instructing the synthesis of more of the same Asn-linked CHOs. In recent studies, we found that distinct isolates produce different, reproducible patterns of PrP^{Sc} accumulation (Hecker et al., 1992). These findings have given rise to the hypothesis that PrP^{Sc} synthesis occurs in particular sets of cells for a given prion isolate. Specific Asn-linked CHOs bound to PrP^{Sc} might function to target a distinct isolate to a particular set of cells where the same Asn-linked CHOs would be coupled to PrP^{C} prior to its conversion to PrP^{Sc}. Even though this hypothesis is attractive, it must be noted that PrP^{Sc} synthesis in scrapie-infected cells occurs in the presence of tunicamycin, which inhibits Asn-linked glycosylation, and with PrP molecules mutated at the Asn-linked glycosylation consensus sites (Taraboulos et al., 1990b). Whether SHa scrapie prions can be synthesized in Tg mice expressing SHaPrP with mutated Asn-linked glycosylation consensus sites and the properties exhibited by distinct isolates is currently under investigation.

Various isolates of scrapie prions (Bruce & Dickinson, 1987; Dickinson & Fraser, 1979; Dickinson & Outram, 1988; Kimberlin et al., 1987) could also be accommodated by multiple conformers that act as templates for the folding of de novo synthesized PrP^{Sc} molecules during prion "replication" (Hecker et al., 1992; Prusiner, 1991). Although this proposal is rather unorthodox, it is consistent with observations generated from Tg(SHaPrP)Mo studies contending that PrP^{Sc} in the inoculum binds to homologous PrP^{C} to form a replication intermediate (Prusiner et al., 1990). Whether foldases, chaperonins, or other types of molecules feature in the conversion of the PrP^{C} to PrP^{Sc} is unknown. The molecular mass of a PrP^{Sc} dimer or trimer is consistent with the ionizing radiation target size of 55 000 ± 9000 daltons as determined for infectious prion particles independent of their polymeric form (Bellinger-Kawahara et al., 1988). Of note, two different isolates from mink dying of transmissible mink encephalopathy exhibit different sensitivities of PrP^{Sc} to proteolytic digestion, supporting the suggestion that isolate specific information might be carried by PrP^{Sc} (Bessen & Marsh, 1992).

An Overview of Prion Structure and Replication. Although many experimental studies reviewed above argue persuasively that prions are devoid of nucleic acid, the complete structure of the prion particle remains to be established. Whether the enigma of prion diversity will eventually be shown to depend upon an as yet undetected scrapie-specific nucleic acid or it arises through an unorthodox mechanism such as Asn-linked glycosylation of PrP^{Sc} is uncertain.

Consider the remote possibility that prions do contain an as yet undetected polynucleotide; then presumably prion replication would involve a virus-like strategy. The putative scrapie-specific nucleic acid would act as a template for its own synthesis using cellular polymerases. By an as yet undefined mechanism, the putative scrapie-specific nucleic acid would stimulate the conversion of PrP^{C} to PrP^{Sc}. While this putative scrapie-specific nucleic acid would provide a plausible explanation for prion diversity, it would require that the nucleotide sequence be able to discriminate between

SHaPrP and MoPrP in Tg(SHaPrP) mice. In addition, the putative scrapie-specific nucleic acid would have to be ubiquitous in order to explain how sporadic CJD occurs with an incidence of $1/10^6$ (Brown et al., 1987; Masters et al., 1978) all over the planet while virtually all people carrying PrP gene mutations develop prion disease.

A more likely scenario is that prions do not contain a scrapie-specific nucleic acid; rather, they are composed entirely of PrP^{Sc} molecules. If this is case, then the species barrier for prion transmission and the results with Tg(SHaPrP) and Tg-(Mo/SHaPrP) mice, as well as infectious prions in the brains of patients with inherited prion diseases, can be more readily explained. If prions are composed entirely of PrP^{Sc}, then replication must involve the interaction of nascent PrP^{C} or a precursor with PrP^{Sc} (Prusiner, 1991). Although there are no physical data at this time demonstrating the existence of PrP^{C}/PrP^{Sc} replication intermediates, it is difficult to explain the results obtained with Tg(SHaPrP) and Tg(Mo/SHaPrP) mice in studies of prion synthesis. Moreover, other studies have shown that patients homologous for the Met/Val polymorphism at codon 129 are predisposed to sporadic CJD while those with heterozygous alleles at codon 129 are relatively protected (Palmer et al., 1991). These findings have been interpreted as being consistent with the hypothesis that prion replication is most efficient when the primary structures of PrP^{C} and PrP^{Sc} are the same.

The formal possibility remains that prions contain a second component which is not a nucleic acid. A small polypeptide, a polysaccharide, a lipid-glycan, or a phospholipid/sterol complex are all possibilities, but there is no evidence for any of these molecules as prion components at this time.

Conclusions and Prospective. The study of prions has taken several unexpected directions over the past few years. The discovery that prion diseases in humans are uniquely both genetic and infectious has greatly strengthened and extended the prion concept. To date, 12 different mutations in the human PrP gene all resulting in nonconservative substitutions have been found to be either linked genetically to or segregate with the inherited prion diseases. Yet, the transmissible prion particle is composed largely, if not entirely, of an abnormal isoform of the prion protein (PrP) designated PrP^{Sc} (Prusiner, 1991). These findings argue that prion diseases should be considered pseudoinfections since the particles transmitting disease appear to be devoid of a foreign nucleic acid and thus differ from all known microorganisms as well as viruses and viroids. Because much information especially about scrapie of rodents has been derived using experimental protocols adapted from virology, we continue to use terms such as infection, incubation period, transmissibility, and endpoint titration in studies of prion diseases.

Several major features distinguish prions from viruses. First, prions can exist in multiple molecular forms while viruses exhibit a distinct ultrastructural morphology. Second, prions are nonimmunogenic in contrast to viruses, which almost always provoke a vigorous immune response. Third, there is no evidence for an essential nucleic acid, foreign or host-derived, within the infectious prion particle while viruses have genomes that provide the template for progeny virions. Fourth, the only known protein component of the prion is PrP^{Sc}, which is encoded by a chromosomal gene in contrast to viruses where viral genomes encode most or all of the proteins found in mature virions.

Although relatively little is known about the replication of prions, Tg mice expressing foreign or mutant PrP genes now permit virtually all facets of prion diseases to be studied and have created a framework for future investigations. Furthermore, the structure and organization of the PrP gene suggested that PrP^{Sc} is derived from PrP^{C} or a precursor by a posttranslational process. Studies with scrapie-infected cultured cells have provided much evidence that the conversion of PrP^{C} to PrP^{Sc} is a posttranslational process that probably occurs in the endocytic pathway. The molecular basis of the PrP^{Sc} synthetic process remains to be elucidated, but extensive protein chemical studies suggest that this process is likely to involve a conformational change.

It seems likely that the principles learned from the study of prion diseases will be applicable to elucidating the causes of more common neurodegenerative diseases. Such disorders include Alzheimer's disease, amyotrophic lateral sclerosis, and Parkinson's disease. 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. If PrP^{C} can be diminished in humans without deleterious effects as is the case for Prn-$p^{0/0}$ mice (Büeler et al., 1992), then reducing the level of PrP mRNA with antisense oligonucleotides might prove an effective therapeutic maneuver in delaying the onset of CNS symptoms and signs.

The study of prion biology and diseases seems to be a new and emerging area of biomedical investigation. While prion biology has its roots in virology, neurology, and neuropathology, its relationships to the disciplines of molecular and cell biology as well as protein chemistry have become evident only recently. Certainly, the possibility that learning how prions multiply and cause disease, thus opening up new vistas in biochemistry and genetics, seems likely.

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