Prions

David W. Colby[1*] and Stanley B. Prusiner[1,2]

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

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

Correspondence: stanley@ind.ucsf.edu

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

PRION PROTEIN ISOFORMS

PrP^{Sc}, an alternative or abnormal isoform of PrP, stimulates the conversion of PrP^{C} into nascent PrP^{Sc}; in the brain, accumulation of PrP^{Sc} causes neurodegeneration. In Syrian hamsters, PrP^{C} and PrP^{Sc} are both 209-residue proteins with two glycosylation sites and a glycosylphosphatidyl inositol (GPI) anchor (Fig. 1). PrP is posttranslationally processed to remove a 22-amino-acid, amino-terminal signal peptide and a 23-amino-acid, carboxy-terminal peptide, which directs addition of the GPI anchor that tethers the protein to the cell membrane. No posttranslational modifications to the primary structure differentiate PrP^{C} from PrP^{Sc} (Stahl et al. 1993). Limited protease digestion of PrP^{Sc} often produces a smaller, protease-resistant molecule of approximately 142 amino acids, referred to as PrP 27–30 (Fig. 1). Under the same conditions, PrP^{C} and some forms of PrP^{Sc} are completely hydrolyzed. Although resistance to limited proteolysis has proved to be a convenient tool for detecting PrP^{Sc}, not all PrP^{Sc} molecules are resistant to protease digestion (Hsiao et al. 1994; Telling et al. 1996; Safar et al. 1998; Gambetti et al. 2008; Colby

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

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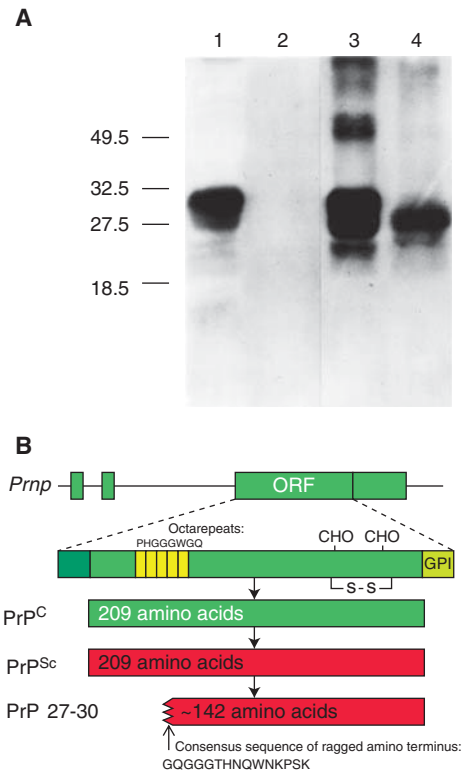

Figure 1. Prion protein isoforms. (*A*) Western immunoblot of brain homogenates from uninfected (lanes 1 and 2) and prion-infected (lanes 3 and 4) Syrian hamsters. Samples in lanes 2 and 4 were digested with 50 μg/μl proteinase K for 30 min at 37°C, completely hydrolyzing PrP^{C}. Proteinase digestion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

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

In the presence of detergent, PrP 27–30 polymerizes into amyloid (McKinley et al. 1991). The tendency of prions to form amyloids has also provided a useful means of prion detection (Colby et al. 2007); however, amyloid formation is a nonobligatory feature of prion disease (Wille et al. 2000). Prion rods formed by limited proteolysis and detergent extraction are indistinguishable from the filaments that aggregate to form PrP amyloid plaques in the CNS (DeArmond et al. 1985). Both the rods and the PrP amyloid filaments found in brain tissue show similar ultrastructural morphology and green-gold birefringence after staining with Congo red dye (Prusiner et al. 1983).

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

THE PrP GENE

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

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

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

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

HUMAN PRION DISEASES

Prion diseases occur as sporadic, genetic, and transmissible disease in humans (Table 1). Although infectious forms of prion disease are most well known to the general public, sporadic and heritable forms of the disease occur much more frequently in humans, with sporadic (s) CJD accounting for approximately 85% of cases. sCJD has no known cause although spontaneous misfolding of PrP^{C} into PrP^{Sc} is a leading hypothesis (Prusiner 1989; Hsiao et al. 1991a). Alternate hypotheses include somatic mutation of *PRNP*, undetected horizontal transmission (Gajdusek 1977), and infrequent amplification of low levels of PrP^{Sc} that are part of "normal" protein homeostasis. The brains of sCJD patients harbor infectious prions that are transmissible to experimental animals (Gibbs et al. 1968; Brown et al. 1994). In humans, virtually all forms of prion disease feature neuropathological changes including vacuolation (resulting in the spongiform appearance of brain tissue), astrocytic gliosis, and PrP deposition. The morphology of vacuoles and PrP deposits varies depending on the prion strain and host, as do the regions of the brain affected.

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

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

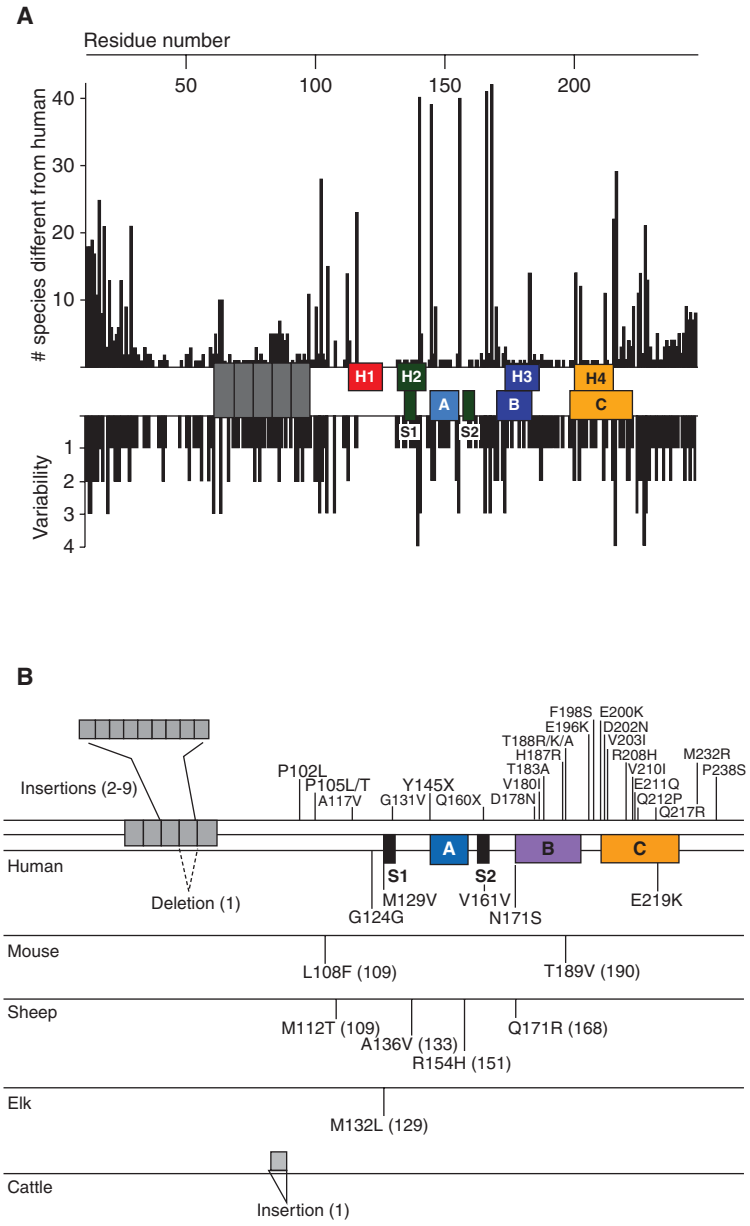

Figure 2. Variation of in the prion protein gene. (*A*) Species variations of the prion protein gene. The *x*-axis represents the human PrP sequence, with the five octarepeats and H1–H4 regions of the putative secondary structure shown, as well as the three α-helices A, B, and C and the two β-strands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

Table 1. Prion diseases in humans and animals.

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

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

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

Infectious forms of prion diseases include kuru, iatrogenic (i) CJD, and variant (v) CJD. Kuru in the highlands of New Guinea was transmitted by ritualistic cannibalism, as people in the region ate the brains of their dead relatives in an attempt to immortalize them (Glasse 1967; Alpers 1968; Gajdusek 1977). Iatrogenic transmissions include prion-tainted human growth hormone and gonadotropin, dura mater grafts, and transplants of corneas obtained from people who died of CJD (Koch et al. 1985; PHS 1997). In addition, CJD cases have been recorded after neurosurgical procedures in which ineffectively sterilized depth electrodes or instruments were used.

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

PRION DISEASES OF ANIMALS

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

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

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

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

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

TRANSGENIC MICE

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

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

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

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

PRION REPLICATION

Prion propagation requires conversion of PrP^{C} to PrP^{Sc}, thought to occur by a template-assisted process in which PrP^{Sc} acts as a template onto which PrP^{C} is refolded into the infectious conformation. The faithful replication of prion strains supports this theory. Evidence for this theory also comes from investigations of Tg mice expressing both SHaPrP and MoPrP, designated Tg(SHaPrP)*Prnp*$^{+/+}$ mice (Prusiner et al. 1990). When these mice were inoculated with prions originating from mice, $MoPrP^{C}$ was recruited and converted into $MoPrP^{Sc}$. Inoculation of these mice with hamster prions resulted in the conversion of $SHaPrP^{C}$ into $SHaPrP^{Sc}$. These findings indicate that molecules with the PrP sequence that is most well suited to adapt to the PrP^{Sc} template are selected for conversion.

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

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

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

BIOLUMINESCENCE IMAGING

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

PrP AMYLOID

As mentioned earlier, amyloid plaques are a nonobligatory feature of prion diseases. Approximately 10% of sCJD cases whereas 70% of kuru cases show amyloid plaques; all vCJD cases show amyloid plaques surrounded by a halo of spongiform degeneration—such structures are called florid plaques (Klatzo et al. 1959; Will et al. 1996). In Tg(SHaPrP)*Prnp*$^{+/+}$ mice expressing both MoPrP and SHaPrP, amyloid plaques were found when hamster prions replicated but not when mouse prions replicated (Prusiner et al. 1990). These experimental studies showed unequivocally that amyloid plaques need not accompany prion replication. In earlier studies, the 87V prion strain that produced numerous amyloid plaques was isolated from Cheviot sheep with scrapie and resulted in amyloid when passaged in *Prnp*$^{b/b}$ mice (Bruce et al. 1976; Jeffrey et al. 1994).

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

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

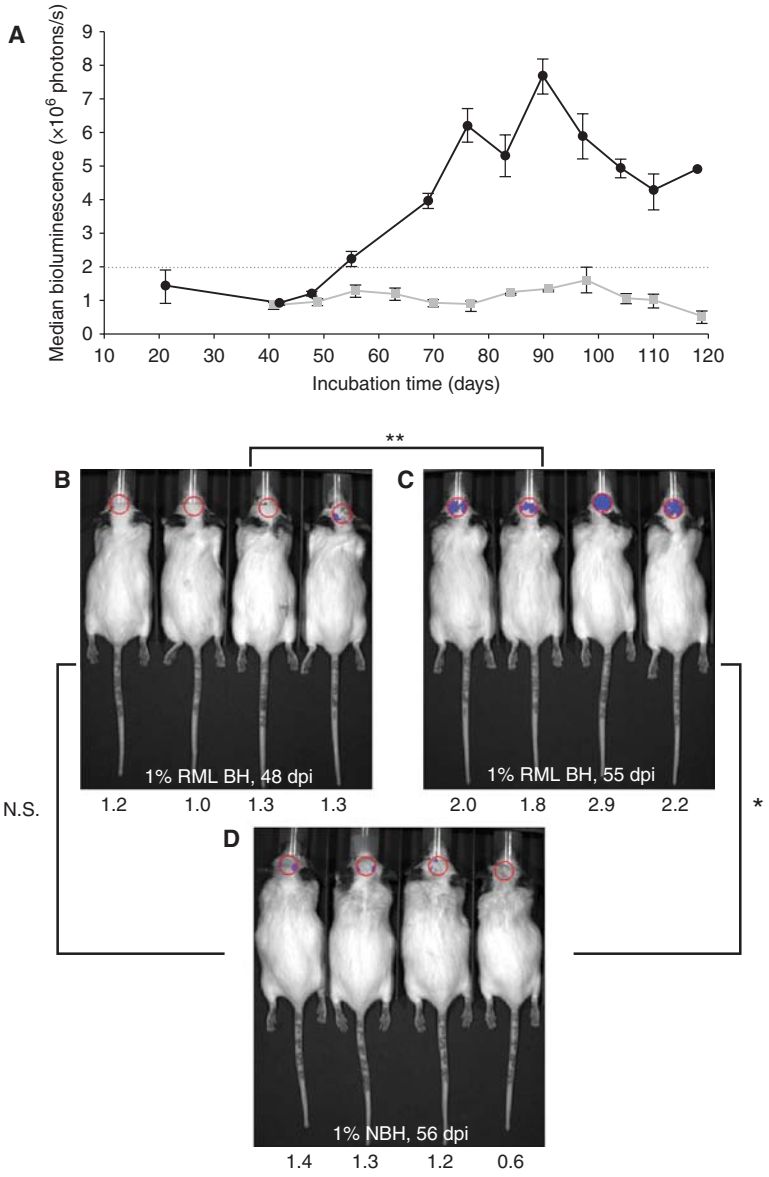

Figure 3. Bioluminescence in Tg(*Gfap*-luc) mice inoculated intracerebrally with RML prions ($n = 12$) indicated a reactive astrocytic gliosis. (*A*) Bioluminescence measured from the brains of prion-inoculated mice (black circles) began to increase at 55 d postinoculation (dpi). Bioluminescence in control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

CELL BIOLOGY OF PrP^{Sc} FORMATION

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

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

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

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

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

DE NOVO GENERATION OF PRIONS

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

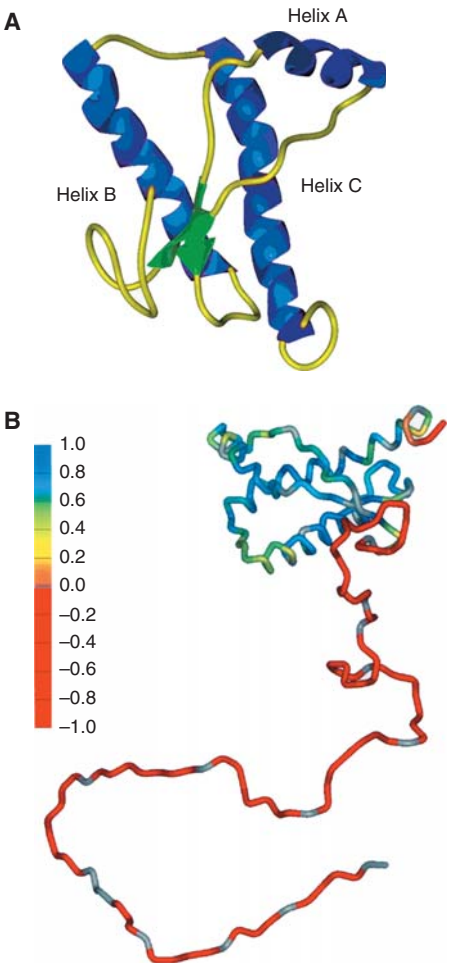

Figure 4. Structures of PrP^{C}. (*A*) NMR structure of Syrian hamster (SHa) recombinant (rec) PrP(90–231), which presumably resembles PrP^{C}. Blue, α-helices; yellow, loops; green, β-strands (James et al. 1997). (*B*) Schematic diagram showing degree of structure for entire PrP polypeptide chain based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

achieving this goal. In Tg(PrP,P101L) mice, an experimental model of human GSS, prion disease was transmitted from high-expressing Tg(PrP,P101L) mice to Tg mice expressing low levels of MoPrP(P101L), which are far less susceptible to spontaneous prion disease (Hsiao et al. 1990; Hsiao et al. 1994; Nazor et al. 2005). Similar transmissions were later accomplished with a synthetic, 55-residue peptide carrying the same P→L mutation and folded into a β-rich structure (Kaneko et al. 2000; Tremblay et al. 2004).

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

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

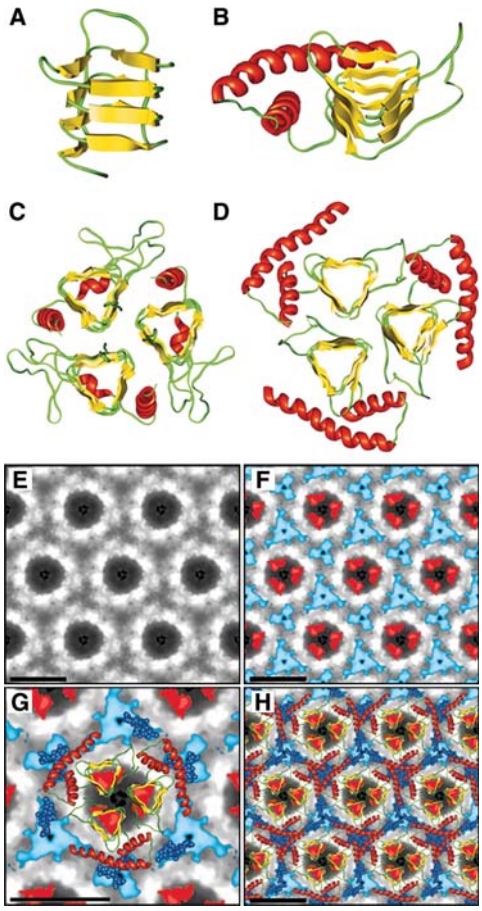

Figure 5. Structural models of PrP^{Sc}. (*A*) Residues 89–174 of PrP threaded into a left-handed β-helix based on UDP *N*-acetylglucosamine *O*-acyltransferase from *Escherichia coli* (PDB ID code 1LXA). (*B*) Model of the monomer of PrP 27–30 with the α-helical region (residues 177–227) as determined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

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

In other work, amyloid inoculation of Tg9949 mice overexpressing an amino-terminally truncated PrP resulted in novel, protease-sensitive, synthetic prions (Colby et al. 2010). Although these strains lacked protease resistance, they caused severe neuropathology and were serially transmissible both in Tg9949 mice and in Tg mice moderately overexpressing full-length PrP. Most, if not all, naturally occurring prions contain some fraction of PrP^{Sc} in a conformation that resists protease digestion (McKinley et al. 1983). This observation has led some researchers to equate protease resistance with prion infectivity and pathogenesis. However, many naturally occurring prion strains also contain PrP^{Sc} in a conformation that is sensitive to protease digestion (Safar et al. 1998). The novel, protease-sensitive, synthetic prion strains showed that $sPrP^{Sc}$ is both transmissible and pathogenic.

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

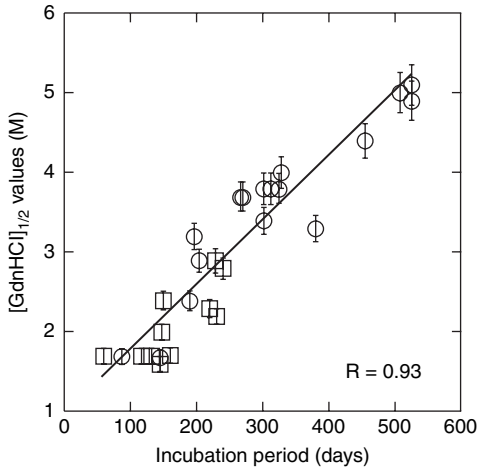

Figure 6. The conformational stability of prions is directly proportional to the length of the incubation time in mice. The $[GdnHCl]_{1/2}$ values for prions were plotted as a function of the incubation times. Synthetic prions (circles) in the brains of Tg9949, Tg4053, and non-Tg FVB mice were plotted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

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

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

PRION STRAINS

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

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

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

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

ENLARGING SPECTRUM OF PRION-LIKE DISEASES

The discovery that prions form amyloid prompted one of us to suggest that the common neurodegenerative diseases are also caused by prions (Prusiner 1984; Prusiner 2001) despite the inability to transmit such illnesses to monkeys and apes (Goudsmit et al. 1980). Brain extracts from either Alzheimer's patients or aged Tg mice expressing mutant APP injected into the brains of Tg mice expressing the amyloid precursor protein (APP) carrying the Swedish point mutation (Haass et al. 1995) accelerated the formation of Aβ amyloid plaques (Meyer-Luehmann et al. 2006; Eisele et al. 2009). Brain extracts from Tg mice expressing mutant tau injected into the brains of Tg mice expressing human wt tau produced aggregates of human tau (Clavaguera et al. 2009). Similar results were found for aggregated tau protein added to cultured cells, which induced the aggregation of nascent tau (Frost et al. 2009). These findings suggest that the tauopathies result from a prion-like process that induces hyperphosphorylation of tau followed by polymerization into filamentous aggregates. The production of hyperphosphorylated tau also appears to be stimulated by oligomers of the Aβ peptide, whereas amyloid fibrils comprised of Aβ are a much less efficient stimulus (Lambert et al. 1998). An expanded 44-mer polyglutamine repeat of a truncated huntingtin protein was found to stimulate aggregation of a "normal" 25 mer; this aggregated state could be maintained in cell culture over many generations, arguing for prion-like propagation of huntingtin aggregates (Ren et al. 2009). Patients suffering from Parkinson's disease who received fetal grafts of substantia nigral cells later showed aberrantly folded α-synuclein in Lewy bodies within the transplanted grafts, arguing that α-synuclein acted like a prion (Kordower et al. 2008; Li et al. 2008; Olanow and Prusiner 2009). Taken together, these findings argue that prion-like, self-propagating states feature in many different, if not all, neurodegenerative diseases.

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

Table 2. Some characteristics of mammalian prions.

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

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

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

TOWARD THERAPEUTICS FOR PRION DISEASES

Despite these advances in understanding prions and many of the neurodegenerative diseases, no treatment is currently available to halt the progression of any of these illnesses. Studies of prions in mice have elucidated several aspects of neurodegeneration that may prove useful in developing effective therapeutics. First, reduction of the precursor protein PrP^C prolongs the incubation time (Büeler et al. 1993; Prusiner et al. 1993; Safar et al. 2005). Second, slowing prion formation by inhibiting of the formation of nascent PrP^{Sc} prolongs the incubation time (Kawasaki et al. 2007). Third, reducing the availability of PrP^C in cells or mice where prion infection has already been established allows for existing prions to be cleared (Enari et al. 2001; Peretz et al. 2001b; Safar et al. 2005). Fourth, enhancing the clearance of PrP^{Sc} provides an alternative route of action for therapeutic intervention (Supattapone et al. 1999b; Supattapone et al. 2001).

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

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

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