

## Molecular Genetics of Transmissible Spongiform Encephalopathies\*

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Transmissible spongiform encephalopathies are degenerative disorders of the central nervous system that occur naturally in man and in a variety of other animals. They include scrapie of sheep and goats, bovine spongiform encephalopathy (BSE)<sup>1</sup> in cattle, and several human diseases such as Creutzfeldt-Jakob disease (CJD), Gerstmann-Sträussler-Scheinker syndrome (GSS), and fatal familial insomnia (FFI). Since its first recognition in 1986, BSE reached epidemic proportions in the UK (1), and there is substantial evidence that a new variant of CJD may be because of consumption of BSE-contaminated products (2).

The unusual properties of the transmissible agent, or prion, early on suggested that it might be devoid of nucleic acid (3). Currently, the most widely accepted proposal is the "protein only" hypothesis, first outlined in general terms by Griffith (4) and enunciated in its updated and detailed form by Prusiner (5, 6). It proposes that the prion is identical with a conformational isoform of PrP<sup>C</sup> (5), a normal host protein (7-9) found predominantly on the outer surface of neurons. Introduction of the abnormal conformer into the organism would result in the conversion of PrP<sup>C</sup> into a likeness of itself (Fig. 1).

During the course of prion disease, a largely protease-resistant, aggregated form of PrP, designated PrP<sup>Sc</sup>, accumulates mainly in brain (7, 10). Prusiner and co-workers (11) proposed that PrP<sup>Sc</sup> is the main or only constituent of the prion. Because no chemical differences were found between PrP<sup>C</sup> and PrP<sup>Sc</sup>, the two species are believed to differ in their conformation. The three-dimensional structure of PrP<sup>C</sup> has been elucidated (12) but not that of PrP<sup>Sc</sup>; however, the  $\beta$ -sheet content of PrP<sup>Sc</sup> was found to be high whereas that of PrP<sup>C</sup> is low (13). Because the ratio of infectious units to PrP<sup>Sc</sup> molecules is only about 1:100,000 (14), the structure of the PrP molecule actually associated with infectivity cannot be definitively inferred. For this reason the PrP species responsible for infectivity is presently better designated as PrP\* (15). The conclusion that some form of PrP is the essential (perhaps only) constituent of the infectious agent is based on compelling biochemical and genetic evidence (5, 16).

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<sup>1</sup> The abbreviations used are: BSE, bovine spongiform encephalopathy; CJD, Creutzfeldt-Jakob disease; GSS, Gerstmann-Sträussler-Scheinker syndrome; FFI, fatal familial insomnia; CNS, central nervous system.

## Familial Spongiform Encephalopathies Are Associated with Mutations in the PrP Gene

Although most cases of human prion disease are sporadic, about 10% are familial and linked to one of a number of mutations in the *PrP* gene (Fig. 2) (for reviews see Refs. 17 and 18). It is believed that these mutations allow spontaneous conversion of PrP<sup>C</sup> into PrP<sup>Sc</sup> with a frequency sufficient to cause disease within the lifetime of the individual (5, 19). Sporadic CJD could be attributed to rare instances of spontaneous conversion of PrP<sup>C</sup> into PrP<sup>Sc</sup> or rare somatic mutations in the *Prnp* gene. In both cases the initial conversion would be followed by autocatalytic propagation. Mice overexpressing a murine *PrP* transgene with a mutation corresponding to the human GSS mutation Pro-102  $\rightarrow$  Leu spontaneously contract a lethal scrapie-like disease, and it has been reported that this disease can be transmitted to mice expressing the same mutant transgene at lower levels, which do not lead to spontaneous disease (20, 21).

## Mice Devoid of PrP<sup>C</sup> Are Resistant to Scrapie

The "protein only" hypothesis predicts that in the absence of PrP<sup>C</sup> mice should be resistant to scrapie and fail to multiply the infectious agent. Mice devoid of PrP (*Prnp*<sup>0/0</sup>) were generated by homologous recombination and found to be essentially normal (22). When challenged with mouse prions, mice devoid of PrP were completely protected against scrapie disease, and prions failed to accumulate in spleen and brain, in contrast to wild-type mice (23, 24). Introduction of murine *Prnp* transgenes into *Prnp*<sup>0/0</sup> mice resulted in several lines with varying expression levels of PrP<sup>C</sup>, which were susceptible to mouse prions; the higher the PrP<sup>C</sup> content of the brain, the shorter the incubation times (25).

The demonstration that disruption of the *PrP* gene confers resistance to scrapie and that reintroduction of a PrP-encoding transgene restores susceptibility paved the way to reverse genetics of PrP, that is the introduction of deletions or mutations into the *Prnp* gene and determination of the capacity of the modified gene to confer susceptibility to scrapie to a PrP knockout mouse. Transgenes encoding PrP with deletions extending to codon 93, but not to 106, restored susceptibility to scrapie (25).<sup>2</sup>

## Transport of Prions from the Periphery to the CNS

Acquired forms of prion diseases are mostly transmitted through oral uptake of prions or peripheral administration, raising the question as to how the agent finds its way to the CNS.

*Prnp*<sup>+/+</sup> neuroectodermal tissue introduced into the brain of newly born *Prnp*<sup>0/0</sup> mice develops into differentiated *Prnp*<sup>+/+</sup> nervous tissue. Intracerebral inoculation of such engrafted mice leads to typical scrapie pathology in the graft but not in the surrounding *Prnp*<sup>0/0</sup> tissue (26). However, because intraperitoneal inoculation of these mice does not lead to pathology in the *Prnp*<sup>+/+</sup> graft, transport of prions from the periphery to the CNS is seen to require interposed PrP-bearing tissue (27). It has also been shown that *Prnp*<sup>+/+</sup> immunodeficient mice lacking mature B cells fail to transport prions from the periphery to the CNS, suggesting a role of the lymphoreticular system in transport (28). It is believed that the follicular dendritic cells,

<sup>2</sup> D. Shmerling and E. Flechsig, unpublished results.

which depend on B cells for their maintenance in a mature state, are the site of prion replication in the spleen (29, 30) and that transport to the CNS is, in addition, dependent on the peripheral nervous system (31).

### The Species Barrier Is Abolished by Introducing the PrP Transgene of the Prion Donor into the Recipient

Prions are transmitted from one species to another much less efficiently, if at all, than within the same species and only after prolonged incubation times. In the case of prion transmission from hamsters to mice, this so-called species barrier was overcome by introducing hamster *Prnp* transgenes into recipient wild-type mice (32, 33). Importantly, the properties of the prions produced in these transgenic mice corresponded to the prion species used for inoculation (32), that is infection with hamster prions led to production of hamster prions, but infection with mouse prions gave rise to mouse prions. Within the framework of the “protein only” hypothesis this means that

hamster PrP<sup>C</sup> but not murine PrP<sup>C</sup> (which differs from the former by 10 amino acids) is a suitable substrate for conversion to hamster PrP<sup>Sc</sup> by hamster prions and vice versa. Interestingly, susceptibility of the mouse to prions from other species, such as hamster, mouse, or man, is increased when the *PrP* transgenes are introduced into a PrP knockout mouse, suggesting that the resident murine gene inhibits the propagation of the alien prions (23).

### The Puzzle of Prion Strains

Dickinson and colleagues (34, 35) showed that many distinct strains of scrapie prions can be derived from sheep isolates. These strains differ by their incubation times in various inbred mouse lines and by the lesion patterns they occasion in the affected brains. Interestingly, different strains can be propagated in one inbred mouse strain (homozygous with regard to its *PrP* gene) (35). Within the framework of the protein only hypothesis this is at first blush puzzling, because it means that one and the same polypeptide chain is able to mediate different strain phenotypes. The *conformational hypothesis* proposes that each strain is associated with a different conformation of PrP<sup>Sc</sup> (or PrP\*) and that each of these can convert the PrP<sup>C</sup> of its host into a likeness of itself (19). Marsh and colleagues (36) showed that the PrP<sup>Sc</sup> species associated with two hamster-adapted scrapie strains, HY and DY, are cleaved to products of different length by proteinase K; the different susceptibility to protease is readily explained by different conformations. Similar findings were made with other prion strains propagated in the mouse (37, 38). Moreover, PrP<sup>Sc</sup> of certain strains differ in the ratio of the diglycosylated to the monoglycosylated form (39).

Is it possible that a dozen different strains of prions can differ in regard to the conformation of the PrP<sup>Sc</sup> they are associated with and that all these conformations can “breed true”? It has recently been claimed that PrP<sup>Sc</sup> molecules of as many as eight different strains could be differentiated by virtue of their relative affinity for a monoclonal antibody directed against an epitope that is fully available in PrP<sup>C</sup> but partially occluded in PrP<sup>Sc</sup>. In addition, some strains differed in their susceptibility to denaturation by guanidinium chloride, adding further credibility to the conformational hypothesis of strain specificity (40).

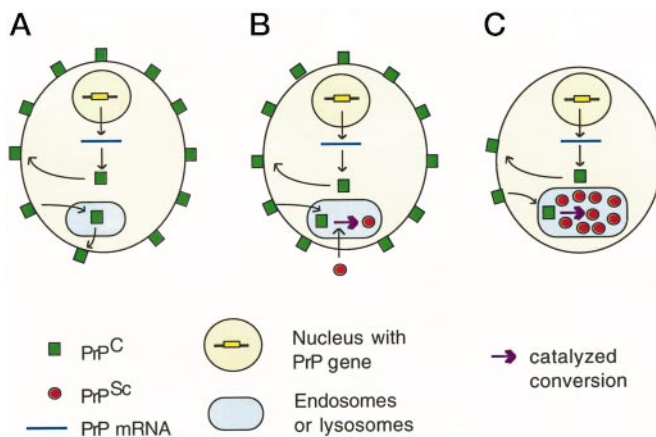


FIG. 1. The “protein only” model. A, in the normal cell PrP<sup>C</sup> is synthesized, transported to the cell surface and recycled. B, the protein only model assumes that the prion is identical with PrP<sup>Sc</sup> (or PrP\*). Exogenous prions cause the conversion of the normal cellular protein PrP<sup>C</sup> into PrP<sup>Sc</sup>, either at the cell surface or after internalization. C, PrP<sup>Sc</sup> accumulates intracellularly in late endosomes or lysosomes, and the cell surface is depleted of PrP<sup>C</sup>. PrP<sup>Sc</sup> is also released into the extracellular space.

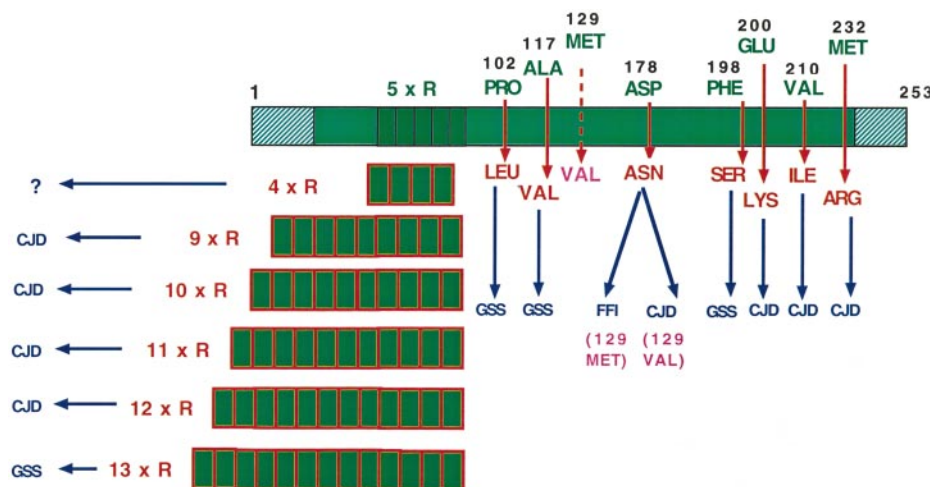
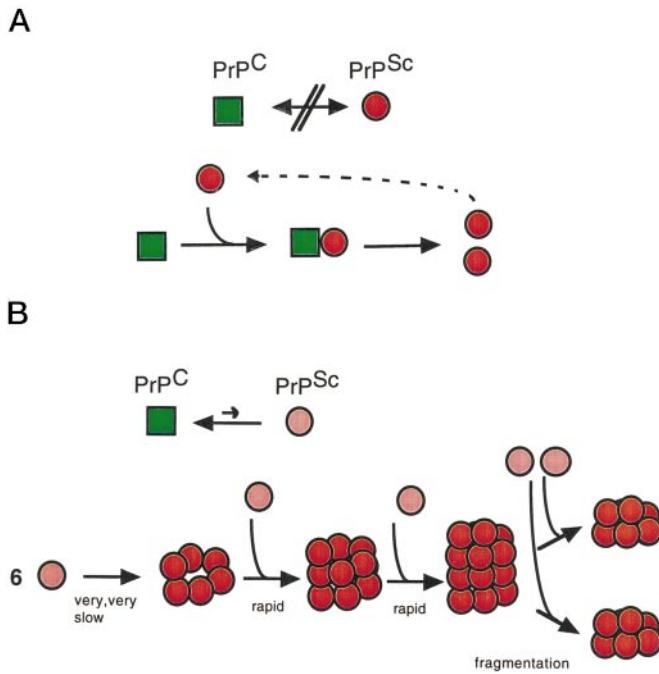


FIG. 2. Mutations of the *PrP* gene are associated with prion diseases. The top rectangle represents the coding region of the human *PrP* gene; the hatched green areas indicate the amino- and carboxyl-terminal signal sequences, and the five dark green boxes (R) are the “octa repeats,” sequences of 8 amino acids. The red arrows indicate amino acid replacements in individual mutant *PrP* genes. The diseases associated with these mutations are given in blue (CJD, GSS, FFI). The Met-129 → Val replacement represents a polymorphism, which in itself is not associated with disease; however, it modifies the effects of the Asp-178 → Asn mutation, such that when the latter is combined with Met-129 it is associated with FFI and when combined with Val-129 it is associated with CJD. Amplification of the number of octa repeats has been found in cases of familial CJD and GSS.



**FIG. 3. Models for the conformational conversion of PrP<sup>C</sup> to PrP<sup>Sc</sup>.** *A*, the “refolding” model. The conformational change is kinetically controlled, a high activation energy barrier preventing spontaneous conversion at detectable rates. Interaction with exogenously introduced PrP<sup>Sc</sup> causes PrP<sup>C</sup> to undergo an induced conformational change to yield PrP<sup>Sc</sup>. This reaction may involve extensive unfolding and refolding of the protein to explain the postulated high energy barrier and could be dependent on an enzyme or chaperone. In the case of certain mutations in PrP<sup>C</sup> (see Fig. 2), spontaneous conversion to PrP<sup>Sc</sup> may occur as a rare event, explaining why familial CJD or GSS arises spontaneously, albeit late in life. Sporadic CJD may come about when an extremely rare event (occurring in one among a million individuals per year) leads to spontaneous conversion of PrP<sup>C</sup> to PrP<sup>Sc</sup> and gives rise to a conversion cascade (5, 32). *B*, the “seeding” model. PrP<sup>C</sup> and PrP<sup>Sc</sup> (or a PrP<sup>Sc</sup>-like molecule) are in equilibrium strongly favoring PrP<sup>C</sup>. PrP<sup>Sc</sup> is only stabilized when it adds onto a crystal-like seed or aggregate of PrP<sup>Sc</sup>. Seed formation is a rare event; once a seed is present, monomer addition can ensue at a rapid rate (43, 44). To explain exponential conversion rates, it must be assumed that the aggregates are continuously fragmented to present increasing surface for accretion.

### The Conversion Reaction

Conversion of PrP<sup>C</sup> to PrP<sup>Sc</sup> in scrapie-infected cells is a late post-translational process, occurring after PrP<sup>C</sup> has reached its normal extracellular location or thereafter (41). Why is spontaneous formation of PrP<sup>Sc</sup> an extremely rare event and how does scrapie infection promote conversion? The “refolding model” (Fig. 3A) proposes that conversion requires that PrP<sup>C</sup> be unfolded to some extent and refolded under the influence of a PrP<sup>Sc</sup> molecule (19), a process that would have to overcome a high activation energy barrier and might require a chaperone and an energy source. The “nucleation model” (Fig. 3B) proposes that PrP<sup>C</sup> is in equilibrium with PrP<sup>Sc</sup> (or a precursor thereof) and that PrP<sup>Sc</sup> is only stabilized when it adds onto a crystal-like seed or aggregate of PrP<sup>Sc</sup>. If a stable aggregate needs to consist minimally of a substantial number of PrP<sup>Sc</sup> molecules, then its spontaneous formation would be a very rare event. However, once a seed is present, monomer addition could ensue at a rapid rate (42–44). Trapping of PrP by essentially irreversible aggregation would drive the bulk conversion process. The proposed process is akin to the assembly of (protease-sensitive) flagellin to (protease-resistant) flagellar filaments (45). Interestingly, the same flagellin molecule can assemble into two types of flagella, depending on the provenance of the seed (46), thereby providing an analogy for conformationally determined prion strain specificity.

Caughey and colleagues (47) have shown that incubation of <sup>35</sup>S-labeled PrP<sup>C</sup> with PrP<sup>Sc</sup> yields a radioactive product that resembled PrP<sup>Sc</sup> with regard to its protease resistance. The conversion reaction exhibited the “species specificity” observed *in vivo*; thus PrP<sup>C</sup> from mouse was readily converted to protease resistance by murine PrP<sup>Sc</sup> but poorly by bovine PrP<sup>Sc</sup> and vice versa (48). Moreover, even strain specificity has been demonstrated in this type of reaction, in that labeled PrP<sup>C</sup> incubated with PrP<sup>Sc</sup> from the DY or the HY hamster transmissible mink encephalopathy strains described above yielded labeled PrP<sup>Sc</sup> with the properties typical for the input PrP<sup>Sc</sup> (49). Despite efforts by several groups, it has not been possible to demonstrate net synthesis of infectivity over the background given by the PrP<sup>Sc</sup> preparation.

### Implications and Outlook

Although each individual piece of evidence described above could be explained in several ways, the conjunction of data strongly supports the proposal that the prion is composed partly or entirely of a PrP-derived molecule (PrP\* or PrP<sup>Sc</sup>) and that protein-encoding nucleic acid is not an essential component. Probably the closest one could come to irrefutable proof for the protein only hypothesis would be the demonstration that biosynthetic, pure PrP<sup>C</sup> can be converted not only into a protease-resistant form but to infectious scrapie agent under defined conditions *in vitro*.

Many questions still need to be elucidated, in particular the mechanism and the requirements for the conversion reaction, the transport of the prion from periphery to CNS, and the mechanism of pathogenesis, to name but a few. From a practical side, early diagnosis of prion disease and treatments aimed at arresting or reversing the disease in humans are important goals.

Finally one may raise the question whether prion-like agents cause other diseases or appear in non-vertebrate organisms. Although several human diseases accompanied by amyloid formation are known, none of them have been reproducibly transmitted. Interestingly, two yeast phenotypes have been ascribed to “heritable protein conversion,” namely the [URE3] and the [PSI] systems (50), and have opened new perspectives for the elucidation of this phenomenon.

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