

# Prions

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**ABSTRACT** Prions are unprecedented infectious pathogens that cause a group of invariably fatal neurodegenerative diseases by an entirely novel mechanism. Prion diseases may present as genetic, infectious, or sporadic disorders, all of which involve modification of the prion protein (PrP). Bovine spongiform encephalopathy (BSE), scrapie of sheep, and Creutzfeldt–Jakob disease (CJD) of humans are among the most notable prion diseases. Prions are transmissible particles that are devoid of nucleic acid and seem to be composed exclusively of a modified protein (PrP<sup>Sc</sup>). The normal, cellular PrP (PrP<sup>C</sup>) is converted into PrP<sup>Sc</sup> through a posttranslational process during which it acquires a high  $\beta$ -sheet content. The species of a particular prion is encoded by the sequence of the chromosomal PrP gene of the mammals in which it last replicated. In contrast to pathogens carrying a nucleic acid genome, prions appear to encipher strain-specific properties in the tertiary structure of PrP<sup>Sc</sup>. Transgenic studies argue that PrP<sup>Sc</sup> acts as a template upon which PrP<sup>C</sup> is refolded into a nascent PrP<sup>Sc</sup> molecule through a process facilitated by another protein. Miniprions generated in transgenic mice expressing PrP, in which nearly half of the residues were deleted, exhibit unique biological properties and should facilitate structural studies of PrP<sup>Sc</sup>. While knowledge about prions has profound implications for studies of the structural plasticity of proteins, investigations of prion diseases suggest that new strategies for the prevention and treatment of these disorders may also find application in the more common degenerative diseases.

The torturous path of the scientific investigation that led to an understanding of familial Creutzfeldt–Jakob disease (CJD) chronicles a remarkable scientific odyssey. By 1930, the high incidence of familial (f) CJD in some families was known (1, 2). Almost 60 years were to pass before the significance of this finding could be appreciated (3–5). CJD remained a curious, rare neurodegenerative disease of unknown etiology throughout this period of three score years (6). Only with transmission of disease to apes after inoculation of brain extracts prepared from patients who died of CJD did the story begin to unravel (7).

Once CJD was shown to be an infectious disease, relatively little attention was paid to the familial form of the disease since most cases were not found in families. It is interesting to speculate how the course of scientific investigation might have proceeded had transmission studies not been performed until after the molecular genetic lesion had been identified. Had that sequence of events transpired, then the prion concept, which readily explains how a single disease can have a genetic or infectious etiology, might have been greeted with much less skepticism (8).

Epidemiologic studies designed to identify the source of the CJD infection were unable to identify any predisposing risk factors, although some geographic clusters were found (9–12). Libyan Jews living in Israel developed CJD about 30 times

more frequently than other Israelis (13). This finding prompted some investigators to propose that the Libyan Jews had contracted CJD by eating lightly cooked brain from scrapie-infected sheep when they lived in Tripoli prior to emigration. Subsequently, the Libyan Jewish patients were all found to carry a mutation at codon 200 in their prion protein (PrP) gene (14–16).

My own interest in the subject began with a patient dying of CJD in the fall of 1972. At that time, I was beginning a residency in neurology and was most impressed by a disease process that could kill my patient in 2 months by destroying her brain while her body remained unaffected by this process. No febrile response, no leukocytosis or pleocytosis, no humoral immune response, and yet I was told that she was infected with a “slow virus.”

**Slow Viruses.** The term “slow virus” had been coined by Bjorn Sigurdsson in 1954 while he was working in Iceland on scrapie and visna of sheep (17). Five years later, William Hadlow had suggested that kuru, a disease of New Guinea highlanders, was similar to scrapie and thus, it, too, was caused by a slow virus (18). Seven more years were to pass before the transmissibility of kuru was established by passaging the disease to chimpanzees inoculated intracerebrally (19). Just as Hadlow had made the intellectual leap between scrapie and kuru, Igor Klatzo made a similar connection between kuru and CJD (20). In both instances, these neuropathologists were struck by the similarities in light microscopic pathology of the central nervous system (CNS) that kuru exhibited with scrapie or CJD. In 1968, the transmission of CJD to chimpanzees after intracerebral inoculation was reported (7).

In scrapie, kuru, CJD, and all of the other disorders now referred to as prion diseases (Table 1), spongiform degeneration and astrocytic gliosis is found upon microscopic examination of the CNS (Fig. 1) (21). The degree of spongiform

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Editor's Note: This article is an abbreviated version of Stanley B. Prusiner's Nobel Lecture, “Prions.” The 1997 Nobel Prize in Physiology or Medicine was awarded to Dr. Prusiner for his discovery of prions, an entirely new genre of disease-causing agents, and for elucidating the fascinating principles that underline their mode of action. The Nobel Foundation graciously has granted us permission to reprint this article. The Nobel Lectures provide examples of successful approaches to major scientific problems as well as authoritative reviews. However, in recent years, these lectures have rarely been read, perhaps because of the difficulty in obtaining the collections. By reprinting this lecture we hope to broaden their exposure.

Abbreviations: Bo, bovine; BSE, bovine spongiform encephalopathy; CJD, Creutzfeldt–Jakob disease; sCJD, sporadic CJD; fCJD, familial CJD; iCJD, iatrogenic CJD; vCJD, (new) variant CJD; CNS, central nervous system; CWD, chronic wasting disease; FFI, fatal familial insomnia; FSE, feline spongiform encephalopathy; FSI, fatal sporadic insomnia; GFAP, glial fibrillary acidic protein; GPI, glycosylphosphatidylinositol; GSS, Gerstmann–Sträussler–Scheinker disease; HGH, human growth hormone; Hu, human; MBM, meat and bone meal; Mo, mouse; r, recombinant; SHa, Syrian hamster; Tg, transgenic; TME, transmissible mink encephalopathy; wt, wild-type.

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<sup>‡</sup>Prions are defined as proteinaceous infectious particles that lack nucleic acid. PrP<sup>C</sup> is the cellular prion protein; PrP<sup>Sc</sup> is the pathologic isoform. NH<sub>2</sub>-terminal truncation during limited proteolysis of PrP<sup>Sc</sup> produces PrP 27–30.

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Table 1. The prion diseases

Disease	Host	Mechanism of pathogenesis
Kuru	Fore people	Infection through ritualistic cannibalism
iCJD	Humans	Infection from prion-contaminated HGH, dura mater grafts, etc.
vCJD	Humans	Infection from bovine prions?
fCJD	Humans	Germ-line mutations in PrP gene
GSS	Humans	Germ-line mutations in PrP gene
FFI	Humans	Germ-line mutation in PrP gene (D178N, M129)
sCJD	Humans	Somatic mutation or spontaneous conversion of PrP <sup>C</sup> into PrP <sup>Sc</sup> ?
FSI	Humans	Somatic mutation or spontaneous conversion of PrP <sup>C</sup> into PrP <sup>Sc</sup> ?
Scrapie	Sheep	Infection in genetically susceptible sheep
BSE	Cattle	Infection with prion-contaminated MBM
TME	Mink	Infection with prions from sheep or cattle
CWD	Mule deer, elk	Unknown
FSE	Cats	Infection with prion-contaminated bovine tissues or MBM
Exotic ungulate encephalopathy	Greater kudu, nyala, oryx	Infection with prion-contaminated MBM

iCJD, iatrogenic CJD; vCJD, variant CJD; fCJD, familial CJD; sCJD, sporadic CJD; GSS, Gerstmann-Sträussler-Sheinker disease; FFI, fatal familial insomnia; FSI, fatal sporadic insomnia; BSE, bovine spongiform encephalopathy; TME, transmissible mink encephalopathy; CWD, chronic wasting disease; FSE, feline spongiform encephalopathy; HGH, human growth hormone; MBM, meat and bone meal.

degeneration is quite variable, whereas the extent of reactive gliosis correlates with the degree of neuron loss (22).

**Prions: A Brief Overview.** Before proceeding with a detailed discussion of our current understanding of prions causing scrapie and CJD, I provide a brief overview of prion biology. Prions are unprecedented infectious pathogens that cause a group of invariably fatal neurodegenerative diseases mediated by an entirely novel mechanism. Prion diseases may present as genetic, infectious, or sporadic disorders, all of which involve modification of the prion protein (PrP), a constituent of normal mammalian cells (23). CJD generally presents as progressive dementia, whereas scrapie of sheep and bovine spongiform encephalopathy (BSE) are generally manifest as ataxic illnesses (Table 1) (24).

Prions are devoid of nucleic acid and seem to be composed exclusively of a modified isoform of PrP designated PrP<sup>Sc</sup>.<sup>‡</sup> The normal, cellular PrP, denoted PrP<sup>C</sup>, is converted into PrP<sup>Sc</sup> through a process whereby a portion of its  $\alpha$ -helical and coil structure is refolded into  $\beta$ -sheet (25). This structural transition is accompanied by profound changes in the physicochemical properties of the PrP. The amino acid sequence of PrP<sup>Sc</sup> corresponds to that encoded by the PrP gene of the mammalian host in which it last replicated. In contrast to pathogens with a nucleic acid genome that encode strain-specific properties in genes, prions encipher these properties in the tertiary structure of PrP<sup>Sc</sup> (26–28). Transgenic studies argue that PrP<sup>Sc</sup> acts as a template upon which PrP<sup>C</sup> is refolded into a nascent PrP<sup>Sc</sup> molecule through a process facilitated by another protein.

More than 20 mutations of the PrP gene are now known to cause the inherited human prion diseases, and significant genetic linkage has been established for five of these mutations (4, 16, 29–31). The prion concept readily explains how a disease can be manifest as a heritable as well as an infectious illness.

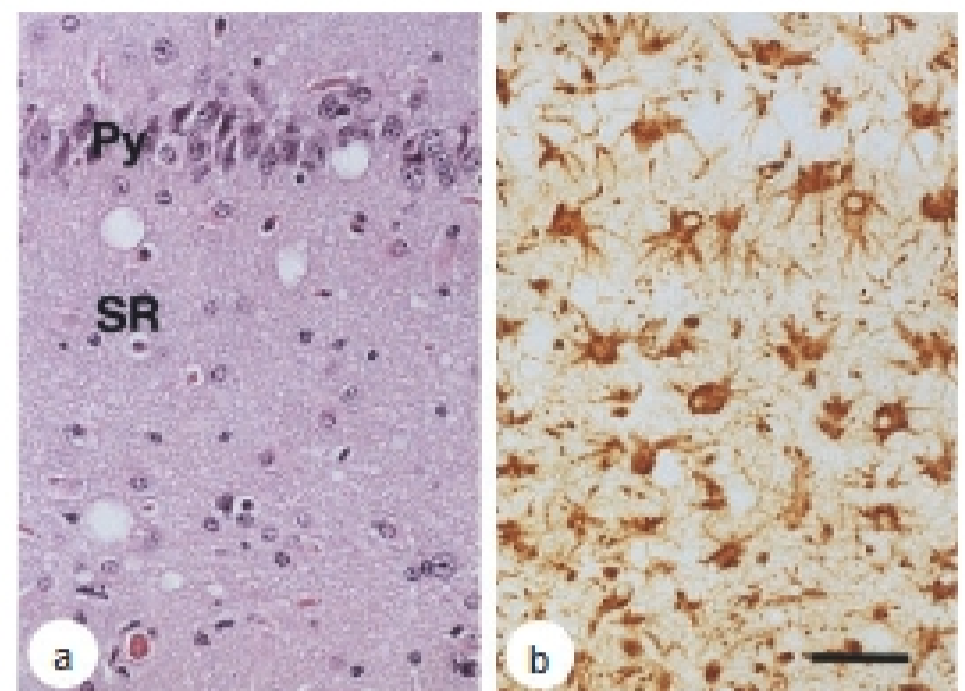


FIG. 1. Neuropathologic changes in Swiss mice after inoculation with RML scrapie prions. (a) Hematoxylin and eosin stain of a serial section of the hippocampus shows spongiform degeneration of the neuropil, with vacuoles 10–30  $\mu$ m in diameter. Brain tissue was immersion fixed in 10% buffered formalin solution after the animals had been sacrificed and was then embedded in paraffin. Py, pyramidal cell layer; SR, stratum radiatum. (b) Glial fibrillary acidic protein (GFAP) immunohistochemistry of a serial section of the hippocampus shows numerous reactive astrocytes. (Bar in b = 50  $\mu$ m and also applies to a.) Photomicrographs were prepared by Stephen J. DeArmond.

**Resistance of Scrapie Agent to Radiation.** My fascination with CJD quickly shifted to scrapie once I learned of the remarkable radiobiological data that Tikvah Alper and her colleagues had collected on the scrapie agent (32–34). The scrapie agent had been found to be extremely resistant to inactivation by UV and ionizing radiation, as was later shown for the CJD agent (35). It seemed to me that the most intriguing question was the chemical nature of the scrapie agent; Alper's data had evoked a torrent of hypotheses concerning its composition. Suggestions as to the nature of the scrapie agent ranged from small DNA viruses to membrane fragments to polysaccharides to proteins, the last of which eventually proved to be correct (36–42).

Scrapie of sheep and goats possesses a history no less fascinating than that of CJD. The resistance of the scrapie agent to inactivation by formalin and heat treatments (43), which were commonly used to produce vaccines against viral illnesses, suggested that the scrapie agent might be different from viruses, but it came at a time before the structure of viruses was understood. Later, this resistance was dismissed as an interesting observation but of little importance since some viruses can survive such treatments; indeed, this was not an unreasonable viewpoint. More than two decades were to pass before reports of the extreme resistance of the scrapie agent to inactivation by radiation again trumpeted the novelty of this infectious pathogen. Interestingly, British scientists had argued for many years about whether natural scrapie was a genetic or an infectious disease (44–46). Scrapie, like kuru and CJD, produced death of the host without any sign of an immune response to a "foreign infectious agent."

My initial studies focused on the sedimentation properties of scrapie infectivity in mouse spleens and brains. From these studies, I concluded that hydrophobic interactions were responsible for the nonideal physical behavior of the scrapie particle (47, 48). Indeed, the scrapie agent presented a biochemical nightmare: infectivity was spread from one end to the other of a sucrose gradient and from the void volume to fractions eluting at 5–10 times the included volume of chromatographic columns. Such results demanded new approaches and better assays (49).

**Bioassays.** As the number of hypotheses about the molecular nature of the scrapie agent began to exceed the number of

laboratories working on this problem, the need for new experimental approaches became evident. Much of the available data on the properties of the scrapie agent had been gathered on brain homogenates prepared from mice with clinical signs of scrapie. These mice had been inoculated 4–5 months earlier with scrapie agent that originated in sheep but had been passaged multiple times in mice. Once an experiment was completed on these homogenates, an additional 12 months was required to obtain the results of an endpoint titration in mice (50). Typically, 60 mice were required to determine the titer of a single sample. This slow, tedious, and expensive system discouraged systematic investigation.

Although the transmission of scrapie to mice had ushered in a new era of research, the 1.5- to 2-year intervals between designing experiments and obtaining results discouraged sequential studies. Infrequently, the results of one set of experiments were used as a foundation for the next and so on. Moreover, the large number of mice needed to measure the infectivity in a single sample prevented studies where many experiments were performed in parallel. These problems encouraged publication of inconclusive experimental results.

In 1972, when I became fascinated by the enigmatic nature of the scrapie agent, I thought that the most direct path to determining the molecular structure of the scrapie agent was purification. Fortunately, I did not appreciate the magnitude of that task, although I had considerable experience and training in the purification of enzymes (51). Although many studies had been performed to probe the physicochemical nature of the scrapie agent by using the mouse endpoint titration system, few systematic investigations had been performed on the fundamental characteristics of the infectious scrapie particle (42). In fact, 12 years after introduction of the mouse bioassay, there were few data on the sedimentation behavior of the scrapie particle. Since differential centrifugation is frequently a useful initial step in the purification of many macromolecules, some knowledge of the sedimentation properties of the scrapie agent under defined conditions seemed mandatory. To perform such studies, Swiss mice were inoculated intracerebrally with the Chandler isolate of scrapie prions and the mice were sacrificed about 30 and 150 days later, when the titers in their spleens and brains, respectively, were expected to be at maximal levels. The two tissues were homogenized, extracted with detergent, and centrifuged for increasing times and speeds (47, 52). The disappearance of scrapie infectivity was measured in supernatant fractions by endpoint titration, which required 1 year to score.

**Incubation time assays in hamsters.** In view of these daunting logistical problems, the identification of an inoculum that produced scrapie in the golden Syrian hamster (SHA) in ~70 days after intracerebral inoculation proved to be an important advance (53, 54) once an incubation time assay was developed (55, 56). In earlier studies, SHA had been inoculated with prions, but serial passage with short incubation times was not reported (57). Development of the incubation time bioassay reduced the time required to measure prions in samples with high titers by a factor of 5: only 70 days were required instead of the 360 days previously needed. Equally important, 4 animals could be used in place of the 60 that were required for endpoint titrations, making possible a large number of parallel experiments. With this bioassay, research on the nature of the scrapie agent was accelerated nearly 100-fold and the hamster with high prion titers in its brain became the experimental animal of choice for biochemical studies.

The incubation time assay enabled development of effective purification schemes for enriching fractions for scrapie infectivity. It provided a means to assess quantitatively those fractions that were enriched for infectivity and those that were not. Such studies led rather rapidly to the development of a protocol for separating scrapie infectivity from most proteins and nucleic acids. With a ~100-fold purification of infectivity

relative to protein, >98% of the proteins and polynucleotides were eliminated, permitting more reliable probing of the constituents of these enriched fractions.

**The Prion Concept.** As reproducible data began to accumulate indicating that scrapie infectivity could be reduced by procedures that hydrolyze or modify proteins but was resistant to procedures that alter nucleic acids, a family of hypotheses about the molecular architecture of the scrapie agent began to emerge (58). These data established, for the first time, that a particular macromolecule was required for infectivity and that this macromolecule was a protein. The experimental findings extended earlier observations on resistance of scrapie infectivity to UV irradiation at 250 nm (33) in that the four different procedures used to probe for a nucleic acid are based on physical principles that are independent of UV radiation damage.

Once the requirement of protein for infectivity was established, I thought that it was appropriate to give the infectious pathogen of scrapie a provisional name that would distinguish it from both viruses and viroids. After some contemplation, I suggested the term "prion," derived from **proteinaceous** and **infectious** (58). At that time, I defined prions as proteinaceous infectious particles that resist inactivation by procedures that modify nucleic acids. I never imagined the irate reaction of some scientists to the word "prion"—it was truly remarkable!

**Current definitions.** Perhaps the best current working definition of a prion is a proteinaceous infectious particle that lacks nucleic acid (28). Because a wealth of data supports the contention that scrapie prions are composed entirely of a protein that adopts an abnormal conformation, it is not unreasonable to define prions as infectious proteins (25, 27, 59, 60). But I hasten to add that we still cannot eliminate a small ligand bound to PrP<sup>Sc</sup> as an essential component of the infectious prion particle. Learning how to renature PrP<sup>Sc</sup> accompanied by restoration of prion infectivity or to generate prion infectivity *de novo* by using a synthetic polypeptide should help address this as-yet-unresolved issue (61). From a broader perspective, prions are elements that impart and propagate conformational variability.

Although PrP<sup>Sc</sup> is the only *known* component of the infectious prion particles, these unique pathogens share several phenotypic traits with other infectious entities such as viruses. Because some features of the diseases caused by prions and viruses are similar, some scientists have difficulty accepting the existence of prions despite a wealth of scientific data supporting this concept (62–67).

**Families of hypotheses.** Once the requirement for a protein was established, it was possible to revisit the long list of hypothetical structures that had been proposed for the scrapie agent and to eliminate carbohydrates, lipids, and nucleic acids as the infective elements within a scrapie agent devoid of protein (58). No longer could structures such as a viroid-like nucleic acid, a replicating polysaccharide, or a small polynucleotide surrounded by a carbohydrate be entertained as reasonable candidates to explain the puzzling properties of the scrapie agent (58, 68).

The family of hypotheses that remained after identifying a protein component was still large and required a continued consideration of all possibilities in which a protein was a critical element (49). The prion concept evolved from a family of hypotheses in which an infectious protein was only one of several possibilities. With the accumulation of experimental data on the molecular properties of the prion, it became possible to discard an increasing number of hypothetical structures. In prion research, as in many other areas of scientific investigation, a single hypothesis is all too often championed at the expense of a reasoned approach that requires entertaining a series of complex arguments until one or more can be discarded on the basis of experimental data (69).