MINIREVIEW

A Critical Review of the Nature of the Spongiform Encephalopathy Agent: Protein Theory Versus Virus Theory[1]

HARASH NARANG[2]

Ken Bell International, Newcastle Upon Tyne NE2 3DH, United Kingdom

All spongiform encephalopathies (SEs) result in brain disorders brought about by a slow virus. Since the origin of bovine SE (BSE), the infectious nature of the disease has been firmly established. Tubulofilamentous particles/scrapie termed nemavirus (NVP) and scrapie-associated fibrils (SAF) are ultrastructural markers, whereas protease-resistant protein (PrP^{sc}) is a protein marker. The PrP molecules aggregate to form SAF. Each NVP consists of three layers: an outer protein coat, an intermediate ssDNA layer, and inner PrP/SAF. Therefore, ssDNA and PrP/SAF are physically associated with each other. The existence of at least 20 stable strains of SEs implies that a nucleic acid molecule serves as the information molecule. Animals inoculated with PrP^{sc} do not develop the clinical disease, however, ssDNA purified from scrapie-hamster brains by alkaline gel electrophoresis mixed with binding proteins before inoculation developed the clinical disease. It appears that an "accessory protein" coded by the ssDNA of the NVP interacts with normal PrP^{c} molecules, resulting in their conversion to PrP^{sc}/SAF. The pathogenesis process in the infected animal, with increasing incubation periods, reveals that larger amounts of normal PrP molecules are modified to form SAF. This interferes with the normal supply of PrP to cell membranes, which become disrupted and eventually fragment, resulting in the vacuoles typical of those found in the SEs. Critical review of scientific literature has demonstrated that the agent contains a DNA genome. [Exp Biol Med Vol. 227(1):4–19, 2002]

Key words: Creutzfeldt-Jakob disease; bovine spongiform encephalopathy; Kuru; nemavirus; protease-resistant protein (PrP); scrapie; scrapie-associated fibrils

This study was funded by Ken Bell International.

[1] This manuscript is an update of a previously published minireview entitled, "Origin and Implications of Bovine Spongiform Encephalopathy" (Proc Soc Exp Biol Med 211:306–322, 1996).

[2] To whom requests for reprints should be addressed at Ken Bell International, 22-40 Brentwood Avenue, Newcastle Upon Tyne NE2 3DH, United Kingdom. E-mail: Harash@compuserve.com

1535-3702/02/2271-0004$15.00

The spontaneous outbreak of bovine spongiform encephalopathy (BSE) in cattle in the 1980s was followed by transmission of the same strain of the agent to zoo animals and humans via contaminated food (1–3). Like scrapie in sheep, we also have witnessed BSE being transmitted by maternal transmission within cattle. In the past, we also witnessed Kuru in the Fore Tribe in New Guinea caused by cannibalistic practice (4–5). The BSE strain of the agent has unique properties and infects a wide range of hosts, causing degenerative disease of the central nervous system (CNS) (1–3). Because of the strikingly similar histopathological vacuolar lesions in the CNS, they have been grouped together as transmissible SEs (TSEs) (1–3). These include scrapie, Kuru, Creutzfeldt-Jakob disease (CJD), Gerstmann-Sträussler-Scheinker syndrome (GSSS), transmissible mink encephalopathy (TME), and BSE (1–5). The review of the scientific literature has firmly established the transmissible and infectious nature of the TSE agent (1–3).

Nature of the Agent

Even with the development of biological techniques in the mid-20th century, the nature of the agent responsible for these diseases has remained elusive and has baffled scientists. Much of the information about the nature of the TSE agent has come via indirect evidence from transmission and sterilization studies. It has been concluded that there is a common agent, and like conventional viruses, it has many strains (6–13). In the past, most of our knowledge of the properties of slow viruses has come from the study of the scrapie agent, while comparative research undertaken using the BSE strain produced contradictory results, which has extensively enhanced our understanding about the nature of the agent.

As would be understood from the terminology, TSE

agents replicate slowly with long asymptomatic incubation periods of months, years, or several decades in some cases as discussed previously (1, 2, 14, 15). Chronological studies have revealed that the replication of the agent is slow in the sense that clinical symptoms develop after a long incubation period. However, the infective titer gradually rises up to 10^{10} to 10^{12}/g of the brain tissue, 4–6 logarithmic units more than any other commonly known viruses (16). Once the animals are infected, there is a long asymptomatic period of incubation that appears to be related to the dose, the route of infection, the strain of the TSE agent, and the age of the host at the time of infection. Pathological damage is only seen in the CNS, and the disease is subacute, progressive, and always fatal (1–5, 15–18).

A number of hypotheses on the nature of the TSE agent have been proposed and discussed before (1–3, 15, 19). Several hypotheses seriously addressed the possibility that the replicating agent was a piece of cell membrane, a complex glycolipid, or protein (20, 21). Depending upon which hypothesis an author favors, references are selected to support a chosen hypothesis and completely ignore references that do not fit a particular way of thinking. In this paper, two major hypotheses, protein versus virus and mutation in the PrP gene, are discussed in detail. At times, hypotheses are being misquoted and stressed as facts. Since the public and the media have joined in the debate, the issue has become very complicated. Therefore, it is important to distinguish between a hypothesis and a fact. As hypotheses are based on knowledge of the general properties of slow viruses, it is important to re-analyze those properties used to support this hypothesis.

General Properties of the TSE Agent

Even before BSE appeared, like many other common viruses, more than 20 distinct strains of the TSEs had been isolated, each with different biological properties such as incubation period and distribution of the lesions. However, the importance of strains had not been appreciated (1–3, 6, 13, 22). Numerous experimental results have revealed that the BSE strain of the agent in cattle has some unique properties with the shortest incubation period (23). In the past, this strain had existed as a minor strain in sheep, causing ataxic, trembling symptoms, and it has been termed Type II scrapie (1–3). The BSE strain is the single most virulent strain and is capable of infecting a new host by an oral route in animals such as cats, mink, cows, and humans (1–3). In all animal species, the clinical disease is revealed with leading signs expressed as ataxia and balancing. Compared with the BSE strain of the agent, other common scrapie strains show up in animals exhibiting itchiness and loss of wool; in humans, the leading sign is dementia (24). It is obvious from the range of host species infected that the strain of the agent has an upperhand over clinical signs and incubation period, rather than the host's genetic makeup.

Unlike many common viruses, the TSE agent infects a range of hosts and it has no species barrier effect. This property is not unique to the TSE agent. Foot and mouth and rabies viruses infect with equal potency across a wide range of animal species. Supporters of the protein-only hypothesis site some of the unique physical and chemical properties of the TSE agent that would destroy common viruses. The ability of the TSE agent to remain remarkably stable over a wide range of physical and chemical conditions is an interesting yet worrying feature of the agent (4, 5, 15, 19, 25). The stability of common viruses is dependent on their protein coat. However, the TSE agent uses host protein to protect and infect a new host cell. In particular, its resistance to nucleases, irradiation with ultraviolet light, hydrolysis, the degree of its physiochemical stability, and that a remarkable amount of the infective dose can often survive when embedded within soil-containing pots that were buried in a garden for 3 years (26) and to heat of 132°C for 30 min have been discussed before (1–3, 19). The stability of a slow virus, where an infective dose can often survive embedded within soil for 3 years is not unique, as cowpox and smallpox are also known to survive open, harsh conditions. Many of these properties such as the resistance to nucleases, irradiation with ultraviolet light, or hydrolysis are not quite as unusual as previously thought; *Escherichia coli* and *Clostridium botulinum* developed resistance in a single strain after repeated irradiation.

It is obvious that heat treatment does destroy infectivity. In one study (27), a scrapie-infected hamster brain homogenate, a 1:10 dilution (w/v) made in saline, and an aliquot before inoculation were left overnight at 121°C. Of eight hamsters inoculated with this material, one developed scrapie after 285 days. Hamsters inoculated with the untreated infected material at dilutions of 1000 developed scrapie after 90 days, whereas the incubation period increased to 175 days with a million-fold dilution (19, 27). Similar studies have shown that the infectivity titer of the infectious agent decreased by two or three $\log_{10}$ following treatment at 80°–100°C for 30 min (27). However, the majority of infectivity can be abolished by autoclaving, and almost all by 1 N sodium hydroxide (over 55°C) and hypochlorite treatment (27).

Also, an important difference observed between strains was the susceptibility to UV irradiation. The susceptibility of the drowsy strain was reduced by 99% by levels of germicidal UV irradiation that had no effect on hyper strains of TME agent (1–3). Furthermore, the chemical, which survives harsh conditions even in fossils, is DNA and not protein. It appears that the interpretations and references used to support the protein-only hypothesis have been selective.

Evidence for and against a Nucleic Acid

The integrity and infectivity of all conventional viruses normally depend on a protein coat, and thus, treatment with proteases reduces the infectivity (28). This property is not unique to the TSE agent. Evidence for the concept that the protein is the agent has come largely from the fact that the TSE agent has been found to resist inactivation by harsh

procedures that specifically hydrolyse or modify nucleic acids, a feature that argues that SEs are devoid of polynucleotides, as discussed previously (19). It was demonstrated that after nuclease digestion, up to 4 kb of polyadenylated DNA sequences have been detected in CJD infectious fractions (29). UV and ionizing radiation inactivation studies have not concluded the absence of a nucleic acid; rather, they suggested that DNA must be small (25). It is a well-established practice in molecular biology laboratories to purify nucleic acids from cells and plasmid by boiling the sample and treating it with NaOH, SDS, phenol, and chloroform. DNA remains biologically active after these procedures (30). In the polymerase chain reaction, DNA is heated several times to over 90°C. Molecular cloning hybridization techniques involve harsh chemical treatments, and during all this processing, RNA and DNA remain biologically active. Differential susceptibility of DNA and RNA to cleavage by metal ions has shown that polyribonucleotides are readily degraded by heating in the presence of various metal ions, while negligible damage occurs to DNA with or without zinc (31, 32). Furthermore, DNA has been amplified from fossils and tissues that have been fixed with formalin and blocked for over 40 years (33), while no protein can withstand such drastic treatment.

Immune Response

The TSE agent, in contrast with other known conventional viral infections of humans and animals, does not initiate a virus-associated antibody immune or inflammatory response (34). So far, it has not been possible to develop a method to detect antibody (13) or neutralize the antigen with serum from the infected host. Because of these properties, this group of slow viruses stand out as being entirely different from all other known viruses, and the apparent lack of specific antigenicity make it an unconventional replicating agent (5, 15, 27, 35). Again, this is not unique to the TSE agent. The AIDS virus neither produces an inflammatory response nor can the antibody neutralize the virus. We can summarize from the general properties that the TSE agent contains a genome and is a virus.

Morphologically, two structures have consistently been seen by electron microscopy (EM) in all cases of TSEs in both experimental and natural diseases. The first are scrapie-associated fibril (SAF) seen in SE-infected brain fractions by negative contrast staining techniques (Fig. 1) (19, 36, 37). The second are tubulofilamentous virus-like particles termed nemavirus particles (NVP) seen in thin sections in synaptic terminals and axons (Fig. 2) (16, 38–45). Nemavirus/SAF are used as ultrastructural markers (16, 19, 36, 37, 46, 47), whereas protease-resistant protein (PrP^{SC}), termed prion, is a protein marker (28, 48–52).

SAF/Prion/Mutation in the Prion Gene Hypothesis

Since SAF were consistently observed in all TSEs, including CJD and BSE, it has been postulated that SAF are

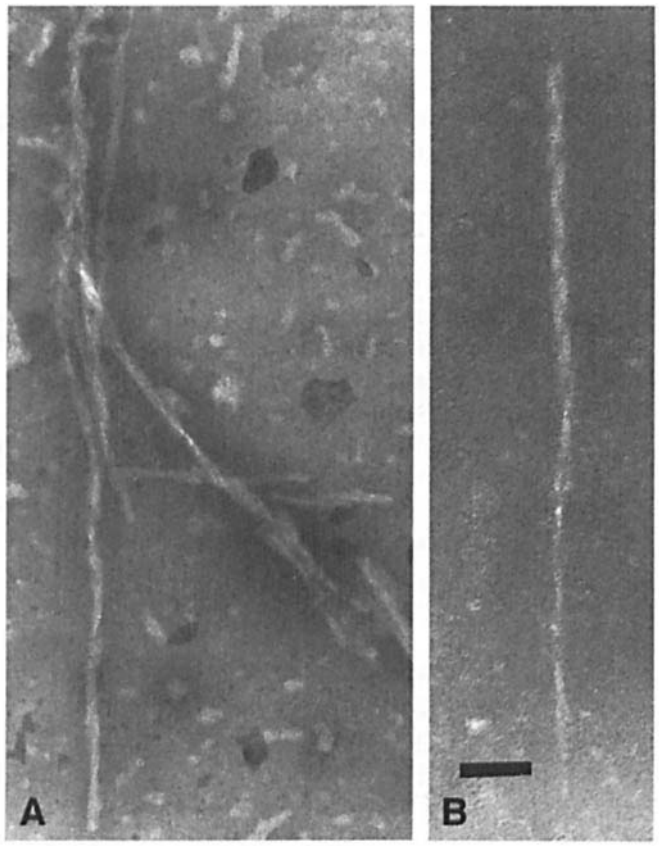

Figure 1. Electron micrograph of negatively stained impression grid from brain of a terminally ill hamster. (A) The preparation was exposed to 2% SDS solution. Note fluffy masses of adherent material in several places on the surface of SAF. (B) The preparation was first exposed to collagenase for 30 min and then 30 min to DNase. Note the fuzzy material within the twisted internal filament in the upper part of the structure and SAF below. Bar = 100 nm.

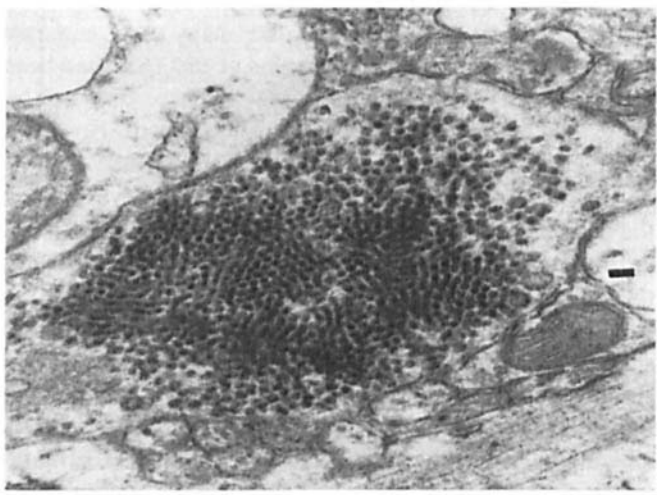

Figure 2. Section from scrapie mouse brain cortex showing a nerve terminal. Note the group of nemavirus particles. Bar = 100 nm.

candidates for the scrapie, Kuru, and CJD agents or that SAF represent component(s) of the agent (36, 37, 53–56). Biochemical analysis of highly purified SAF preparations were shown to consist of a single, major polypeptide of 27–30 kDa, a protease-resistant protein (PrP^{SC}) (28, 48–52)

that is derived from a normal host precursor sialoglycoprotein (PrP^c) (54). It has been demonstrated that PrP^c molecules aggregate to form "prion rods"/SAF (56, 57). The biochemical and immunological evidence so far presented has demonstrated that the SAF and PrP^{SC} cross-react antigenically and are the same structures (19, 49–51, 55, 58). Prusiner (59–61) considered that the individual prion is the agent and suggested that protease-resistant protein, PrP^{SC}, is its sole component. To understand its role in the disease process, it is important to review the nature and origin of the protease-resistant protein.

The Protease-Resistant Protein

It has been accepted that the PrP 33- to 35-kDa precursor protein is coded by a normal cellular gene and not by a putative nucleic acid carried within the prion (62). The complete translated precursor protein of 254 amino acids has been sequenced from a number of mammalian species. The protein is expressed to an equal amount, giving rise to the same primary translation product in normal and scrapie-infected brain (54, 63). Furthermore, tests revealed that the primary structure of 254 amino acids of the naturally occurring PrP33-35 precursor protein in a normal host before inoculation displays no amino acid differences compared to post-inoculated clinically sick animals with the scrapie agent (19, 64). However, when normal tissue is extracted with proteolytic enzyme, most, if not all, of the normal precursor protein is completely degraded, whereas when infected brain tissue is similarly extracted, variable quantities of converted, 27- to 30-kDa protease-resistant protein are detected (28, 48–51). To differentiate between the two types of protein, terms for normal PrP33-35 kDa (PrP^c) and scrapie PrP27-30 kDa (PrP^{SC} or PrP) have been commonly used (19, 53, 59, 60). What pathological role, if any, PrP^{SC} has in the disease process remains unknown; however, demonstration of a protease-resistant protein in a sample is used as a diagnostic tool for the disease. Logically, it cannot be an index of infectivity, although that assumption is often carelessly made.

PrP^c: a Housekeeping Protein

The additional findings of multiple transcription initiation sites and promoter region, which are rich in guanine and cytosine residues, just upstream from the short 5′ exon are reminiscent of many ubiquitous 'housekeeping genes.' The protein is predominantly found on the surface of neurons attached by a glycoinositol phospholipid anchor (65, 67). The function of normal PrP33-35 is unknown. However, several observations have suggested that it might be involved in receptor sites for the scrapie agent (1, 19). Key questions are: How are the normal isoforms of the precursor protein converted into the protease-resistant protein? What is the conversion mechanism of PrP^c to protease-resistant protein/SAF? Why does it occur only after the host has been infected?

PrP Post-Translation Modification Process

The precise relationship between normal PrP^c and scrapie PrP^{SC} has been difficult to establish because of the uncertainty whether *in vitro* analysis of brain extracts accurately reflects the *in vivo* situation. For a very long time, it has been considered that "an abnormal form of cellular" protein PrP^{SC} is the only major and necessary component of the TSE agents. The original proposal was that the interaction of PrP^{SC} with the normal PrP^c causes the post-translational modification in the normal isoform of the protein. Until recently (68), this has been the main theme of Prusiner's hypothesis (57, 59–61). Several ingenious hypotheses discussed previously (1, 19) have been devised in which a protein might accumulate by virtue of being involved in the regulation of its own synthesis (69–72), but they fall short of comprehensive explanations. To answer some of these questions, it was suggested that a point mutation in the PrP gene may cause the disease.

Role of Mutations in the PrP Gene

Based on basic structure, PrP amino acid peptide sequence, the backbone has been divided into eight domains, A through H (64). A comparative backbone amino acid sequence of precursor proteins from different animal species shows the same gross structure. However, it is important to stress that there are minor nucleotide differences, some of which are seen within the coding region in different host species and also different strains of the same species (65, 73), as discussed previously in detail (1, 19, 64). Like different animal species, in some human cases, minor nucleotide differences were also observed in the backbone of amino acid sequence. In humans, these differences in backbone amino acid sequence are termed point mutations. Once it was realized that prion protein is host derived, molecular genetics entered on the scene and this has attracted a great deal of research interest.

In some familial GSSS cases, point mutations were observed in the PrP gene, and it was suggested that these mutations may cause the disease (74–76). In general, but without documentation, sporadic cases of CJD are also considered to be caused by a spontaneous mutation in the PrP gene. In his evidence to the BSE Inquiry (www.bse.org.uk), Professor Roger Morris suggested that a spontaneous mutation in a somatic cell of the prion protein gene in a single cow led to a sporadic case of BSE, while feeding of this animal to other animals was the cause of the eventual epidemic. Professor Morris offered no evidence to the BSE Inquiry to support his theory. Further, Dr. Jim Hope's response to the BSE Inquiry suggested that mutation of the prion gene in a somatic or germ cells not of cattle but of sheep resulted in the generation of a novel scrapie strain. Feeding of this sheep to other animals caused the eventual BSE epidemic. It is important to stress that Dr. Hope is suggesting not one but two mutations, one in the PrP gene of the germ cell of sheep and another in the genome of the

agent. However, no point mutations have been observed in cattle or sheep PrP genes. Furthermore, transmission studies suggest that the TSE strains of the agent are very stable (1–3). Questions have to be answered: Does a mutation in the PrP gene create PrP^{SC} or clinical disease? Does infection create a *de novo* mutation in the PrP gene? It is, therefore, important to consider, in detail, the role of PrP and mutation in the PrP gene.

A search began for mutations in the corresponding human gene among some of the affected families. Some of the familial GSSS cases have a point mutation in the gene that codes for the precursor protein of 254 amino acids; over 25 mutations have been described. The first was found in codon 129 and subsequently in codons 102, 117, 178, and 200. An increasing number of exceptions are being found in the PrP gene mutations even in a single family with a particular PrP mutation (74). It is presumed that the amino acid changes that result from these mutations make the protein more susceptible to the conformational changes associated with the protease resistance. It is also presumed that mutations within the PrP gene can cause TSE if they are involved in a critical region.

These mutations have been seen in a number of first degree relatives of affected patients who are still healthy into their 60s and even middle 70s (77). According to Brown (77), in spite of great temptations of molecular genetics, the heyday of mutation hunters has probably come and gone. Sporadic CJD cases, which form the majority, do not have these mutations. The agent of these so-called unconventional slow virus diseases neither spontaneously creates a *de novo* mutation in the PrP gene nor copies any mutation in the PrP after being infected from one host to another, from the familial case or any other source of infection. There is no experimental evidence to support these presumptions. However, when genetically well-characterized mice are injected with different strains of the agent, the same mice group develop disease with different incubation periods with a different strain of the agent. Therefore, it is obvious that the host PrP gene has no control over the incubation periods.

Evidence from Transmission Studies

Since tissues from the familial patients inoculated into laboratory animals transmitted the disease, an explanation is needed as to how a genetic disease can be infectious with a predictable incubation period. Transmission of experimentally sheep- goat-, or pig-passaged BSE give a similar pattern of the incubation periods and pathology, demonstrating that the BSE agent is unchanged when passaged through a range of species. The "donor" species, irrespective of their PrP genetic make up, has little influence on the disease characteristics (6, 19, 78). In experimental transmission, the replication of the infectious agent starts only after inoculation or feeding of a new host species: monkeys, chimpanzees, pigs, or mink; all of these species have a different genetic makeup of the PrP (amino acid sequence) as compared to the original host, which may be human, cow, or sheep. The affected host animals would develop the clinical disease, while their PrP33-35 protein is modified into PrP27-30 as discussed previously (19, 24). None of the point mutations from the donor host animals are copied in the PrP gene or its product, PrP27-30; however, the TSE agent replicates, maintaining its specific strain characteristic (19, 24). It is a well-established fact that the strain of the TSE agent "breeds true," retaining its original properties during the passage from one animal species to another (19). These findings clearly demonstrate that these strain differences cannot be contained within the host-coded PrP molecules that do not "breed true" and have been discussed in a previous review (19, 24).

Since the protein model relies exclusively on a normal host-encoded protein, the hypothesis must first explain why the disease does not occur in everyone; it must then explain how it can be transmissible. Four major lines of indirect evidence have been developed in favor of the protein-only hypothesis. With PrP, scrapie infectivity copurify (19, 28, 52, 57, 79–81); some studies have suggested that no specific nucleic acid can be detected in highly purified PrP preparations (82–85); resistance of the scrapie agent to harsh procedures (48); and genetic evidence points to a possible intimate linkage between Prn-p and the gene that is considered to control the incubation period of the disease (86, 87). It is assumed that the prion must violate part of the central dogma of molecular biology and that genetic information is transferred indirectly from nucleic acid to proteins (60–62, 88).

The mechanism by which these mutations in the PrP gene lead to altered susceptibility or incubation periods has not been defined. However, it has been proposed that the human polymorphisms at amino acids 129 and 219 are associated with altered incubation time or susceptibility (89). As discussed previously (19, 24), Balter (90), in summarizing the main points relating to the protein only hypothesis at the Diagnosis of Prion Diseases Meeting in Germany, concluded "there was much new knowledge on display, but still no consensus on whether this mysterious disease agent is acting alone." Many researchers consider that the species can be overcome by introducing an appropriate PrP gene into transgenic mice (89). Although the above mutation may alter the susceptibility of an animal following exposure to an infectious agent, Prusiner's group (88) suggested that a number of point mutations and insertions in the human PrP gene apparently lead to spontaneous genetic disease. Prusiner's (74) group generated transgenic mice using multiple copies of mutant PrP gene referred to as P102L in humans and P101L in its mouse version, which had earlier been thought to cause GSSS in humans. The mutant mice, who expressed high levels of the mutant PrP, appeared to have spontaneously developed GSSS-like neurological symptoms (74), and this inherent instability makes the PrP^{c} protein more likely to convert to and accumulate as PrP^{SC}. At the time, many researchers thought that the protein-only

hypothesis had clinched victory. The hypothesis is based on negative and indirect evidence. Later, it appeared that in this degenerative brain disease of the transgenic mice, the pathology was different, no protease-resistant protein was detected, and it did not seem to be infective (91).

Manson *et al.* (92) repeated some of the Prusiner experiments using a new technique called double replacement gene targeting by removing the normal PrP mouse gene and then replacing it with a marker gene with a PrP gene containing the GSSS mutation. These transgenic homozygous mice on each duplicate chromosome harbor two copies of this mutation (101LL) in their PrP gene. Although the mice expressed high PrP levels, they did not spontaneously develop transmissible spongiform encephalopathy disease during their life span of about 900 days. Unlike the Prusiner study, Manson *et al.* (92) found that irrespective of genetic make up, mice had to be inoculated with brain extracts from a GSS source.

However, the incubation period following inoculation of the agent was dramatically reduced in mice homozygous for this mutation (101LL) and averaged 288 days compared with over 500 days in wild-type mice. Furthermore, when inoculated with brain extracts as a second pass from a GSS source mice (101LL) that had been culled at the terminal stage of clinical disease (254 days) with no apparent PrP deposition, disease was transmitted to all three groups of mice genotypes (101PP, 226 days; 101PL, 201 days; and 101LL, 148 days). Significantly, a reduction of incubation periods was seen in the second passage both in the wild-type (101PP) mice from over 500 days to 226 days compared with transgenic mice 101LL from 288 days to 148 days. On the contrary, 101LL transgenic mice were infected with ME7 strain of the scrapie had a significantly prolonged incubation period (338 days) compared with wild-type mice (161 days). These observations suggest that the species barrier had not been completely overcome the transgenic mice and, therefore, that PrP gene itself with or without a GSSS mutation is not the TSE agent or controls the incubation period. Similarly, familial as well as sporadic varieties of CJD could be transmitted to nontransgenic experimental animals, confirming the infectious nature of the disease (93).

Another point stressed by Balter (90), where there appears to be some misunderstanding, is that the genetic susceptibility to the infectious agent is well established in sheep. Based on their response to the scrapie agent challenge, Cheviot sheep at the Neuropathogenesis Unit (Edinburgh, UK) are divided into "positive" and "negative" lines (94). When sheep were inoculated with the scrapie strain of the agent, there was some evidence of intimate linkage between the susceptibility and incubation period with Prn-p, the gene (78, 86, 94, 95). In a wide range of sheep breeds, a single sheep gene called *Sip,* with alleles sA and pA, was considered to exert precise control over the timing of the appearance of symptoms. However, comparative experiments have revealed that the BSE strain controls the susceptibility and incubation period both in "positive" and "negative" lines of sheep rather than the host Prn-p gene compared with the scrapie strain. From the comparative evidence, it could be concluded that the PrP gene does not control the susceptibility or incubation period (94, 95) and, therefore, the significance of mutation would be negligible.

The crucial points, however, concern infectivity. Neither the gel-purified PrP protein nor the *in vitro*-translated and -modified synthetic protein have proven to be capable of transmitting the disease when inoculated into susceptible hosts. Infectivity is separable from the protein itself (96), and PrP is not an essential component of the infectious agent (97–99), and this has been discussed in detail in a previous review (1, 2, 19). Infectivity has been demonstrated in the absence of prion rods (96). SAF/PrP can be demonstrated in brains of infected mice and hamsters, but not in their spleen, which has a similar titer of infectivity (98). It has been demonstrated that treatment of hamsters inoculated with the 263K strain of the scrapie agent with amphotericin B can retard both clinical symptoms and the appearance of PrP in the brain without affecting the replication of the agent (99).

Additionally, in a recent study involving transmission of the BSE agent to mice, it was found that although all of the mice injected with homogenate from BSE-infected cattle brain exhibited neurological symptoms and neuronal death, more than 55% of them had no vacuoles or detectable PrP^{SC} (100). Next, when brains of PrP^{SC-} mice were used to inoculate a second series of mice, most developed neurological signs, but a few presented the PrP^{SC-} pattern again. In the third pass, use of brains of PrP^{SC-} mice from the second pass transmitted the classic form, and almost all of the PrP^{SC-} negative pattern except in one single mouse had disappeared. Therefore, it is clear that the BSE agent was replicating in the new host without benefit of PrP^{SC}. As the agent became adapted to the new host during serial passage, PrP^{SC} and vacuoles appeared, whereas in the first passage, more than 55% of the mice had no detectable PrP^{SC}. The study demonstrates that PrP^{SC} may be involved in the species adaptation, but it is not the agent itself. The result of adaptation suggests that the transmissible agent has some relationship with the PrP^{SC}. Therefore, it is obvious that PrP^{SC} is not the agent or part of the agent. The PrP post-translation modification process is a pathological process and PrP^{SC} is a by-product of the disease process during the development of the clinical disease and needs to be examined in some detail.

Identification of PrP^{SC} in the brain and other tissues or body fluids (1, 22, 24) is often taken as a definitive marker of the TSE disease. In many TSEs, a close correlation may exist between the level of infectivity and level of PrP^{SC}. However, there are a number of examples in which extremely low levels of disease-associated PrP (91) or no PrP^{SC} has been demonstrated despite presence of a rapid TSE transmission (100).

The Lasmézas *et al.* (100) study has further implications, particularly in relation to transmission studies from one species to another. Fraser *et al.* (101) used RIII mice to bioassay non-neuronal tissues from BSE-affected cattle, although mice died with incubation periods ranging from 222 to over 861 days. As histopathological examination revealed no vacuoles, the authors concluded that the tissue samples tested did not contain the agent. There were no details given concerning histopathology and how many brains from each group were examined. Samples of tissues included lymph node, semen, and fat (Table I). It is obvious from the data given in Table I for each type of tissue sample that the pattern in the incubation periods observed for the different tissue samples tested strongly suggests transmission with the TSE agent. Lasmézas *et al.* (100) were to use Fraser *et al.* (101) criteria, where negative pathology and PrP^{SC-} pattern was observed, 55% of mice could have been classified as negative transmission with the TSE agent. It is more than possible that negative pathology and PrP^{SC-} pattern are the likely outcomes when transmitting the disease from non-neuronal tissues and therefore would be misdiagnosed. It is also a well-established fact that in some hosts infected with a low dose of the TSE agent, both clinical disease and vacuoles may not appear in the life span of the host. The disease remains subclinical. Under these circumstances, it is common practice to make a second and third pass with negative brains (78, 100). However, it appears that no attempt was made by Fraser *et al.* (101) to inoculate a second series of mice with brains of mice. This revealed no spongiform changes that would have conclusively demonstrated presence or absence of the agent in the non-neuronal tissues. It is quite possible that the PrP^{SC} negative pattern property is unique in transmission of the BSE agent.

In primary transmission from one species to another, the TSE agent affects most of the new host species animals inoculated. However, in some cases, the clinical disease develops only after a prolonged incubation period. This species barrier may disappear on one further passage in the new host, and the incubation period is usually shortened. It has been shown that in transgenic mice the species barrier (the incubation period shortening) for transmission of the TSEs agent can be abrogated by expression of a specific host gene.

To answer some of these questions relating to trans-

Table I. RIII Mice Were Injected I.C. with 0.02 ml as 10-fold Homogenate from Samples of Tissues from Affected Cows

Tissue	Sample number	Number of histological examinations/number pending and still alive	Survival, days of cases with "negative" histology (mean ± SD)	Number of mice surviving >650 days
Lymph node	1	16/0	222–650 (a) (404 ± 138)	0
	2	14/0	277–650 (472 ± 128)	0
	3	7/14	230–498	0
Semen	1	21/0	447–861 (606 ± 132)	0
	2	7/14	216–497	5
Testis	1	5/13	209–463	5
Placental cotyledon	1	17/0	(378–768) (587 ± 145)	0
Buffy coat	1	16/0	204–916 (641 ± 211)	0
Bone marrow	1	17/0	300–793 (554 ± 138)	0
Skeletal muscle	1	23/0	268–870 (511 ± 137)	0
	2	14/0	334–888 (562 ± 162)	0
	3	5/20	221, 356, 402 405, 414	15
Fat	1	4/19	382, 407, 412, 543	6
Kidney	1	3/19	343, 393, 457	10
Oesophagus	1	6/14	245, 370, 393, 421, 430, 434	4
Distal ileum(c)	1	2/1	335, 448	0
Udder	1	8/17	263–531	2
Sciatic nerve	1	8/16	248–418	10
	2	6/19	211, 294, 392, 426, 461, 613	2
Cauda equina	1	4/20	268, 313, 422, 532	6

Note. Twenty-four mice used per sample. Intercurrent illness necessitating the sacrificing of mice before 200 days postinoculation, excluded.

mission and incubation period, a number of transgenic mouse lines have been produced by insertion of a nonhost gene (hamster or human), some with foreign normal PrP, and some with the PrP gene with mutation (102, 103). Since it is known that the SEs agent can be transmitted easily to a wide variety of nontransgenic hosts, it is important to understand that the phenomenon of the species barrier can only be examined in the light of reductions or increases in the incubation period from first to subsequent passages.

The role of transgenic mice in the presence of foreign PrP gene, nor nontransgenic mice, developed SEs or any other clinical neurological disorder and has been discussed in detail in the previous review (19). On the contrary, when both transgenic and nontransgenic mice were inoculated with a known dose of the scrapie agent, only a few of the inoculated animals developed SE. The most important finding was the comparison of transgenic and nontransgenic mice studies, which revealed that irrespective of PrP gene makeup, there is still the need for an infectious agent. These studies suggest the need of an unknown replicating putative agent that causes the disease. However, some differences may exist in the incubation period in terms of days in some of these experiments. These studies further stress that genetic differences (with or without additional PrP gene or with a point mutation) do not protect the animals from developing the clinical disease in their life span. Furthermore, using the same strain of the agent, the incubation period can vary in different strains of nontransgenic mice where no genetic alterations were carried out. These findings suggest that a normal PrP gene or gene inserted from another animal is not the agent, but may alter the susceptibility by creating receptor sites on the cell surface, thus removing the species barrier.

It has been suggested that the PrP gene is identical or closely linked to genetic loci *Sinc*/Prn-p (in mice) and *Sip*/Prn-p (in sheep) gene that control the incubation period (86). Mice have been experimentally produced in which the PrP gene has been deleted (104, 105). By further breeding, homozygous mice have been generated for disrupted *Sinc*/Prn-p gene (Prn-$p^{o/o}$ mice). According to Prusiner (60), PrP^c does not seem to be essential for normal development, at least in young mice, since disruption of the PrP gene did not cause any detectable abnormalities in the nervous, musculoskeletal, or lymphoreticular system up to 9 months (104, 105). Prusiner (60) emphasized this point that PrP gene disrupted mice remained well up to 9 months, although it has been suggested that perhaps the absence of PrP^c will result in abnormalities later in life. However, after 4 years, this information has not been updated (60).

The results of these studies suggest that somehow the scrapie agent has gained entry into the brain cells and PrP molecules on the surface of cells that act as receptor sites. To understand these points further, transgenic mice expressing chimeric PrP genes derived from the Syrian hamster (SHa) and the mouse (Mo) PrP gene were constructed (103). Transgenic mice MHM2 PrP, who produced 2- to 4-fold more PrP^c per microgram of total brain protein than normal hamsters, were resistant to the hamster strain of scrapie, whereas those who produced normal amounts of PrP^c were susceptible (102). Some lines of animals developed the disease with 306 to 448 days incubation period, compared with 134 days in MH2M PrP line. It is also strange that in some of these studies, transgenic mice expressing chimeric human PrP have been shown to express 4- to 8-fold higher levels of normal human PrP^c. Yet upon inoculation with human strain of the agent, they failed to develop the disease more frequently compared with transgenic mice expressing low levels of normal human PrP^c (68). It is evident from transgenic mice studies that despite human PrP being expressed 4- to 8-fold higher in levels compared with normal human PrP^c levels, these transgenic mice with higher levels failed to develop PrP^{SC} containing human or human-mice hybrid PrP^{SC} (68). These studies suggested that expression ratios of PrP^c present in the tissues also do not appear to control the final PrP^{SC} product.

Following a number of experiments in transgenic mice, Prusiner's group (68) has concluded that another "X" protein, a macromolecular chaperone other than PrP^{SC}, is required in the post-translational process, as previously described by Narang (64). This mechanism suggests that the PrP^{SC} does not act as the infectious agent nor is it required for modification of PrP^c into PrP^{SC}, thus implying that the introduction of PrP^{SC} itself into a host does not elicit the pathological process as suggested previously. These findings suggest post-translation conversion of PrP^C to PrP^{SC}, with the help of an accessory protein that is being formed in the near vicinity of precursor nonpathogenic PrP^c. Since PrP^c is host derived and will always be present in host cells, as the modification has been due to interaction of the accessory "X" protein, the post-translation conversion of PrP^c to PrP^{SC} is more likely to be a secondary process involving a fusion protein (1, 19, 64, 106).

It is obvious from the Lasmézas *et al.* (100) study that PrP^{SC} is not the agent or part of the agent, and Prusiner's group acknowledged from their own experiments that "X" protein is essential for the post-translation process. The "X" protein would be coded by a "X" DNA of the TSE agent (Fig. 3). Balter (90) quotes Charles Weissmann, "One conclusion you may not draw is that (Manson's experiments, 92) has disapproved Prusiner's contention that this mutation can cause a prion disease." Neurologist John Collinge also agrees, saying that although "Manson's work (92) is intriguing, it should not be the take off point to launch a search for a virus or other microbe." The evidence against protein or point mutation being the agent or part of the agent is pretty overwhelming. On the contrary, there is strong experimental evidence in support of a virus being the agent. The protein-only hypothesis has no direct evidence that links PrP^{SC} being the agent. On the contrary, most of the new evidence supports a virus hypothesis.

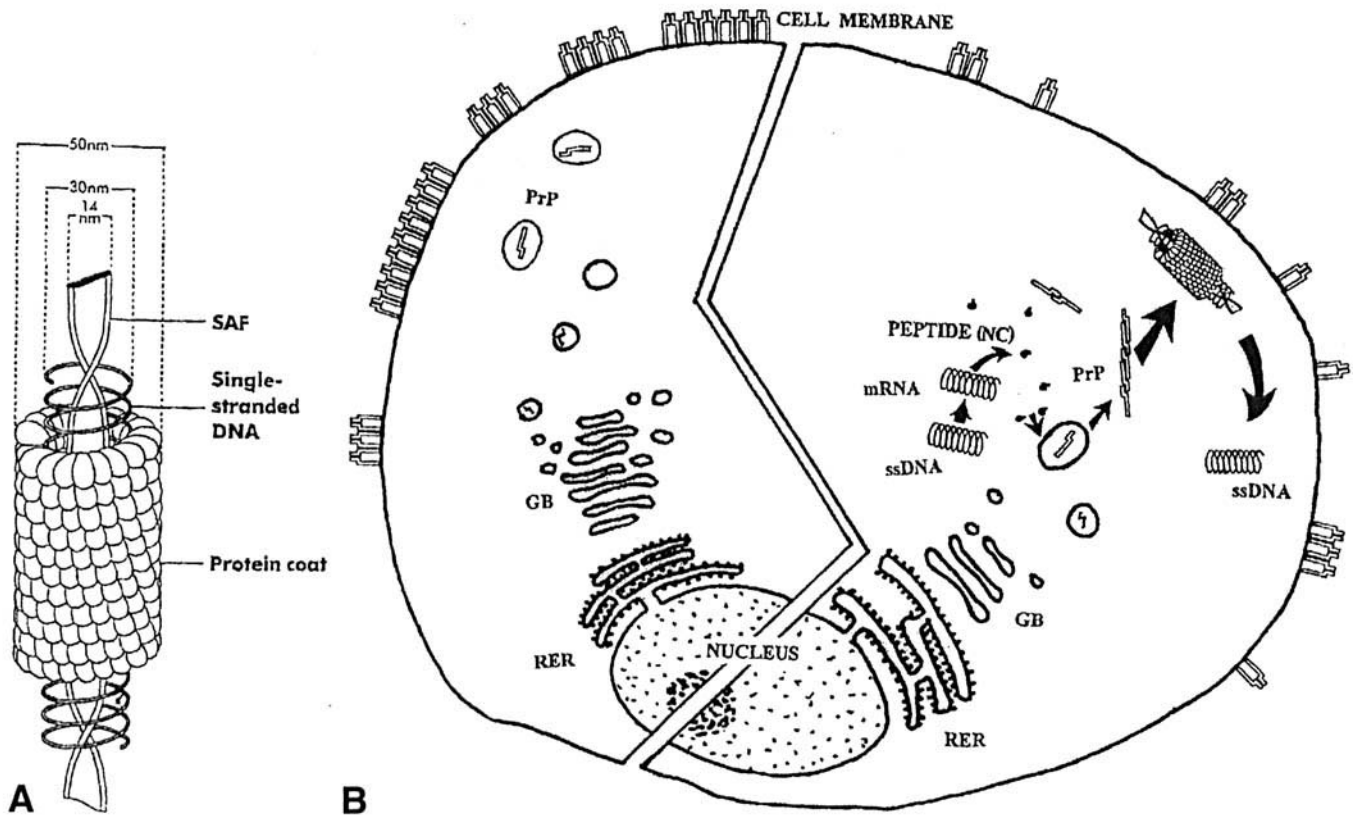

Figure 3. (A) A model of nemavirus, ssDNA coiled around the SAF as a spring protected by an outer protein coat. (B) A schematic representation of a cell showing endoplasmic reticulum (ER), Golgi body (G) showing synthesis of PrP 33-35, and *Nemo corrupta* (NC) is coded by ssDNA. When molecules of PrP33-35 make contact with the NC molecules, the later interacts either as an enzyme or has very similar receptor site as cell membrane. They act against each other and join head-to-head and tail-to-tail position to form a protease-resistant protein, morphologically seen as SAF. The ssDNA is wrapped around to form nemavirus. The part of the cell membrane that receives a continuous supply of PrP33-35 (left side) and the opposite side (right) because of trapping of PrP molecules a slow disruption in the infected cell causes gaps and weakening of cell membrane. Weak cell membranes reach a breakage point, vacuolation follows, and clinical symptoms become evident.

Scrapie Strain Variations

Only after the appearance of BSE have the strain differences become more obvious and their importance realized. Different strains of the TSE agent behave differently in the same strain of animal. Also, same strains of the agent behave differently in different strains of animal species. The mechanism of "breeding true" the agent must be explained by understanding how a protein-only molecule holds genetic information.

There appears to be a surprisingly high efficiency of transmission of BSE to a so-called "negative" (resistant) line of sheep selected for differences in susceptibility to scrapie. Furthermore, with the BSE agent, no significant or clear difference in efficiency of infection or incubation periods between the routes or lines by either i.c. and or oral routes was observed as seen in scrapie sheep "positive" (susceptible) and "negative" (resistant) lines (101, 107). Like scrapie in sheep, the resistant line of sheep failed to identify any species barrier resistance with the BSE strain. Similar transmission experiments in mink also revealed a 100% efficiency of the agent of bovine origin both by i.c. and oral routes. By comparison, 15% of mink developed SE with the scrapie sheep strain by i.c. route, whereas none developed clinical disease when fed scrapie-infected tissues (108). Thus, allelic complexity in the sheep PrP gene, which was considered to control the susceptibility and incubation periods and was used as one of the strongest points in evidence for support of the protein-only hypothesis group, also failed to identify any genotype resistance to the BSE agent (101, 107). It appears that the strain differences of the agent have an upper hand in controlling susceptibility and the incubation period rather than the PrP gene.

These studies revealed that the agent can be propagated in animals homozygous or heterozygous for the PrP gene, although the incubation periods may vary, thus suggesting that an interaction of host and a nonhost nucleic acid is an essential component of the agent. The protein model must also account for a number of typical viral features seen under various experimental conditions: 1) agent strain differences seen in the same strain of host inoculated with different pathogenic isolates; 2) strain competition: the ability of one strain of the agent to inhibit replication of a different strain inoculated later into the same animals; 3) host strain differences, either in susceptibility to disease or

in different disease characteristics in different host strains; 4) species barrier effect: resistance to disease transmission among different species; 5) adaptation: gradual change on early serial passage in a new host species; and 6) mutation: unpredictable, sudden change on passage in a single host strain.

The PrP precursor protein is identical in the same strain of animals. Since differences are seen in the pathological and incubation periods when inoculated by different strains of the agent, these differences can only be influenced by the accessory "chaperone" protein and, therefore, have to be different in different strains of the agent. To fulfil this condition, both PrP and the accessory protein cannot be host derived. Since PrP is host derived, the accessory protein has to be foreign and has to be coded by a foreign DNA.

Prusiner's group (68) is now in agreement with the suggestion that was previously put forward by Narang (64) that another protein, a macromolecular "chaperone" other than PrP^{SC}, is required in one-to-one ratio that interacts with the normal PrP^{c} during the post-translational process. Before the modification can begin, a macromolecule chaperone is required in the pathogenesis of the disease. To code for another protein macromolecule or for this process to occur, another DNA gene other than PrP gene is required (64). Explanation of the transmissible and mutable nature of the agent led to the hypothesis that the agent replicates with the help of a "conjectured normal" host nucleic acid serving as coprion that is present in a normal cell (62). This cannot explain the existence of the phenomenon analogous to "interference." It also fails to show how normal host nucleic acid in the same strain of mice produces different incubation times and distribution with different genetically stable strains of scrapie (6–13, 109). All transmission experiments with the SE agent suggest that PrP sequence is not copied in a new host species, rather, that the host's own PrP is used to form SAF, the protease-resistant protein. If the "conjectured normal" host nucleic acid is serving as coprion, it must have some function and be active during some developmental stage in the normal host, while PrP^{c} is always present in the host's cell, and potentially it follows that most of the animals should develop SE without inoculation. Furthermore, the agent of SE replicates and increases in titer, whereas the host brain cells do not divide and, therefore, the "conjectured normal" host nucleic acid gene would not replicate. To say this "conjectured normal" host nucleic acid is activated only by the entry of PrP^{SC} into a new host cannot be true for a number of reasons.

So far, we know that the disease has always been experimentally established in all animal species that have been inoculated or fed with the infective agent while maintaining the strain characteristics. Once the animals are infected, there is a period of incubation, at the end of which the fatal disease has always declared itself. This finding suggests that the gene coding for the "chaperone" protein (1, 19, 110, 111), which recently has been termed *Nemo corrupta* (Nemo was the name of the captain of a submarine who was everywhere but no one could find him; and corrupta is formed from "corrupts true function"), must be part of the TSE agent. In some studies it has been demonstrated that this macromolecule is coded by the ssDNA of the nemavirus (1, 19, 64, 106, 110). Further evidence that the DNA coding for the "chaperone" protein *N. corrupta* must be nonhost comes from the fact that different strains behave differently in the same host.

A further requirement for a satisfactory molecular model of a virus for the TSEs was that they were consistent with the pathogenesis and pathology of the disease. A lot of understanding of molecular biology can be obtained from the ultrastructural morphology of nemavirus, the structure of which explains production of "X chaperone" protein and post-translation modification of PrP into SAF the protease-resistant protein, PrP^{SC}.

As the PrP molecules are added into the chain, the morphological assembly of protease resistant SAF takes place while ssDNA wraps around SAF and, after acquiring a protein coat, forms nemavirus particles (1, 19, 111). As PrP molecules are diverted away from cell membranes to form SAF, there is a gradual weakening of cell membranes (Fig. 3). As the incubation period progresses, the process of replication of ssDNA and the accessory protein accelerates, and more and more of the PrP molecules are diverted to form SAF. The weak cell membranes reach breakage point; thus, vacuolation occurs. This suggests that there is some correlation between conversion, normal PrP 33- to 35-kDa precursor protein into protease-resistant protein and vacuolation. This correlation has became very obvious in a recent study involving transmission of the BSE agent to mice. More than 55% exhibited neurological symptoms and neuronal death but had no vacuoles or detectable PrP^{SC} (100). However, in a second series of mice inoculated with brains of PrP^{SC-}, most developed neurological signs and had vacuoles and PrP^{SC}. The BSE agent is not PrP and is virulent enough to replicate in a new host without PrP accumulation and production of vacuoles. As a result of this adaptation, the transmissible agent would be tightly associated with PrP.

Evidence from Ultrastructural Studies

Ultrastructurally, the pathological findings in BSE-affected cows resemble those in natural and experimental scrapie- and CJD-infected animals. The results of EM studies have revealed differences between Alzheimer's disease (AD), a nontransmissible neurological disease, and TSEs. In AD brains, neurofibrillary paired helical filaments (NPHFs) have been observed, the hallmarks of AD, which are not seen in CJD (111, 112). On the contrary, brains from cases of CJD, Kuru, BSE, and scrapie contain tubulofilamentous particles termed nemavirus (16, 38–45, 110) and are not seen in AD. The number and the density of the nemavirus increase during subsequent weeks until the particles are readily seen, which correlates with infective titers (16, 41–45), and the appearance of nemavirus in experimental CJD

and scrapie precedes the onset of clinical disease (16, 41, 42). The presence of these nemavirus in natural sheep scrapie, BSE, and CJD is not due to the blind passage of a carrier virus as may be the case in experimental animals. These particles are readily distinguishable from normal cellular or degraded normal cellular structures.

Nemavirus particles were not considered to be the infectious agent because they were thought to be absent from the brains of hamsters with experimental scrapie (41, 45, 113, 114) in which the highest reported concentrations of the agent occur (113, 114). However, the recent demonstration of these particles in hamsters infected with scrapie (40–43, 58) and the independent confirmation of these particles in scrapie-infected hamsters, CJD, BSE, and naturally infected sheep, suggest that these particles are viral in nature (39, 43). From the large number of independent studies, it now appears that nemavirus are specific to all TSEs. Difficulties caused by random sampling using EM may explain the failure to find these particles in the previous studies.

Using an "impression" negative staining technique and treatment of grids with different concentrations of SDS (without proteinase K) or with a combination of proteases and nuclease, it was possible to demonstrate that the nemavirus particles consist of an outer protein coat, a middle layer of ssDNA, and an innermost resistant SAF/PrP layer that stayed intact (Fig. 3) (59, 109, 111, 115–117). SAF/PrP are observed from 10 days postinoculation from right side inoculated sides of the brain, and from 18 days postinoculation from both right and left side. This would suggest that replication of the agent starts at the local site of inoculation (27).

Relationship of Nemavirus with PrP/SAF

Nemavirus particle morphologically has a very unusual three-layer structure not seen in other viruses. In contrast to the morphology of common viruses that have a two layer structure of nucleic acid protected by an outer protein coat, nemavirus has the ssDNA lying sandwiched between two layers of protein, the inner being SAF/ SAF/PrP (19, 27, 58, 115–117). What appears to be a strange coincidence is that SAF/PrP forms the central core of the nemavirus. The morphological relationship between the PrP, ssDNA, the outer protein coat, and NVP is obvious, whereas the involvement in the disease pathogenesis or its relationship to the agent remains to be explained. In all animal species, normal PrP 33- to 35-kDa precursor protein PrP^{c} is an essential housekeeping component normally found on the cell surface (65, 118). Conversion of PrP^{c} to PrP/SAF is a post-translational process. Recently, Prusiner's group (68), following a number of experiments on transgenic mice, concluded that a macromolecular chaperone other than PrP^{SC} is required in the post-translational process.

The nature of the outer protein coat is still undetermined, but the grid touch negative-staining technique suggests that the outer coat is very hydrophilic and may be a host-derived, ssDNA binding protein that may help in recombination and repair processes (58). It is possible that the outer protein coat with ssDNA is the infectious agent and, because of its close physical association with the PrP/SAF ssDNA of the agent, co-purifies with the SAF/PrP fractions in various amounts (19, 27, 40, 15–117) and is not visible without ruthenium red staining (40, 15–117). It has been demonstrated that after mild detergent treatment, SAF show differing sedimentation characteristics and titer changes (79, 80).

Characterization of ssDNA

It is possible to purify ssDNA corresponding to about 1.2 kb from homogenized brain tissue from scrapie-infected hamsters by phenol/chloroform extraction procedure and by alkaline gel electrophoresis (106, 119, 120). A sample of the DNA preparation was examined by EM, which revealed ssDNA of about 0.49×10^{6} daltons (Fig. 4) (121). Alkaline gel analysis of scrapie-infected hamster preparations revealed a specific unusual band of DNA. This band stained with ethidium bromide under denatured conditions, whereas ordinary nucleic acid did not (106, 119, 120). As anticipated from this unusual staining property, the sequence of ssDNA revealed a repeat uniform symmetrical multipalindromic repeat of $(TACGTA)_{n}$. As expected, point mutations have been seen in this sequence in some CJD cases (19). This very high base-pairing sequence property appears unique to this ssDNA band and may explain why it remained stained with ethidium bromide under denatured conditions. Because of the unusual multipalindromic nature under normal physiological conditions, ssDNA would base pair and coil in a very complicated fashion within the same molecule or with other molecules, forming a long chain (106). It is evident from this self-pairing property within the same molecule and in other molecules (Fig. 5) why most of the previous studies have failed to reveal a specific nucleic acid by sub-

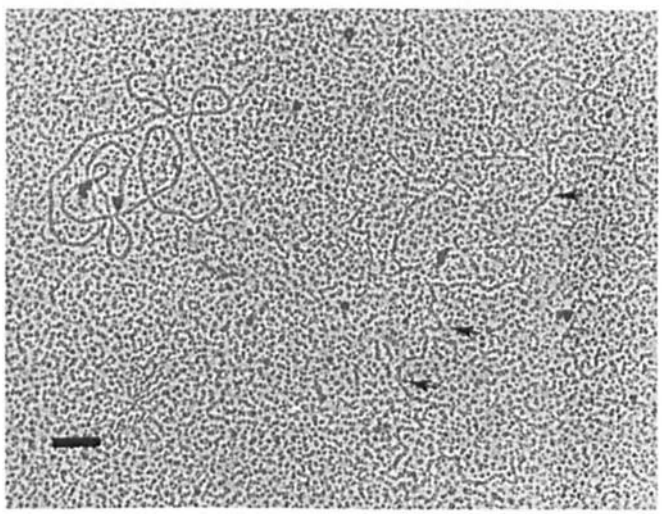

Figure 4. Purified DNA from scrapie-infected hamster brain spread over hypophase in a mixture of 40% formamide and 0.4 mg/ml cytochrome cat. The grids were fixed and stained by uranyl acetate in ethanol rotary shadowed with Pt/Pd. Note mitochondrial circular dsDNA and ssDNA (arrows). Bar = 100 nm.

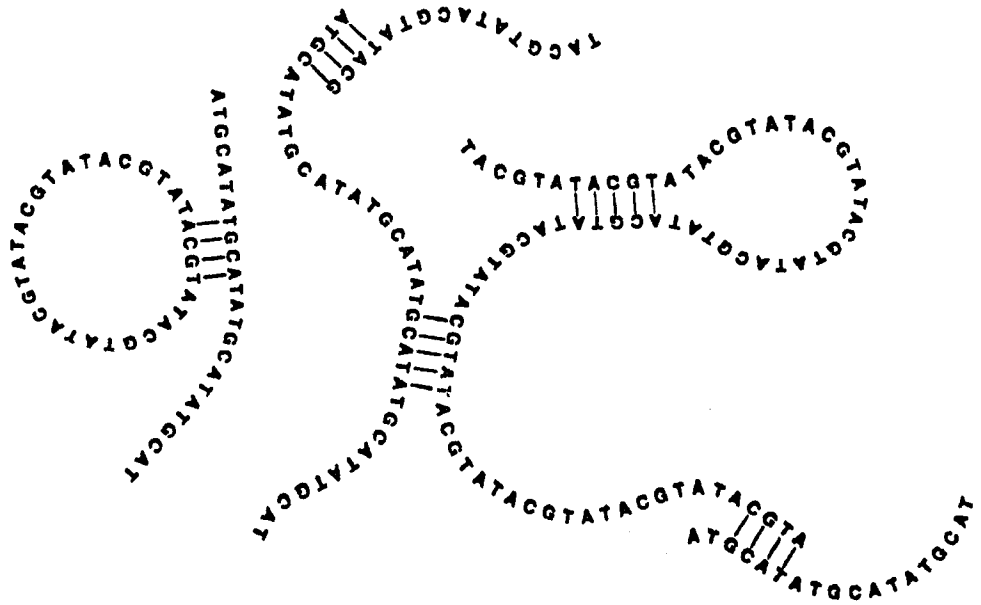

Figure 5. Because of the unusual multi-palindromic nature of the ssDNA, its molecule under normal physiological conditions base pair and coil in a very complicated fashion within the same or with other molecules. The terrible beauty of the ssDNA is that it has its own built-in survival kit, and it is so evolutionarily primitive that it can repair itself and multiply whenever and wherever the opportunity arises. All it needs is a living cell.

tractive hybridization methods (122–124). Furthermore, it is important to realize that when purifying ssDNA, because of its binding capacity to nitrocellulose, one must avoid the use of nitrocellulose centrifuge tubes or else anticipate a loss of large quantities of ssDNA.

From this palindromic sequence of the scrapie genome, one can see the reasons why many who have searched for a putative scrapie genome revealed no specific nucleic acid. The property of high base pairing, as demonstrated by alkaline gel electrophoresis, might have a direct bearing on the binding capacity of receptor protein molecules that might, in turn, affect the protection of the nucleic acid against methylation, UV, or DNase degradation (125).

Direct evidence that ssDNA wrapped around "nemavirus" is the genome of the scrapie agent comes from a recent study by injecting highly purified ssDNA mixed with binder protein. Lipofectin has been used successfully to transfect DNA (124) and RNA (127) into a variety of tissue culture cell type. Lipofectin reagent interacts spontaneously with DNA to form lipid-DNA complex (126). The fusion of the DNA complex with tissue cells results in the efficient uptake and expression of the DNA. The mixing of ssDNA with lipofectin, BP, and NHB homogenate must result in a DNA complex that helps transfect the hamster cells.

In four previous attempts, no infectivity was demonstrated in the phenol/chloroform-purified preparations of DNA by intracerebral inoculation into hamster brain (120). This demonstrated that DNA, like other conventional viral DNA, also requires a carrier protein for the uptake and its expression. In a recent study, Narang (120) used phenol/chloroform-purified nucleic acid, which was further subjected to alkaline gel electrophoresis, and a single band of ssDNA corresponding to about 1.2 kb visualized was cut from the gel. The gel-purified ssDNA was mixed before inoculation with: a) $MgCl_2$, b) lipofectin, c) ssDNA-binding protein, and d) normal brain homogenate. Hamsters in groups b, c, and d injected with a mixture of ssDNA developed the clinical disease, and the brain pathology revealed generalized vacuolation (Fig. 6), whereas animals injected with ssDNA mixed with $MgCl_2$ (group a) and the control group remained healthy. This is direct evidence that sup-

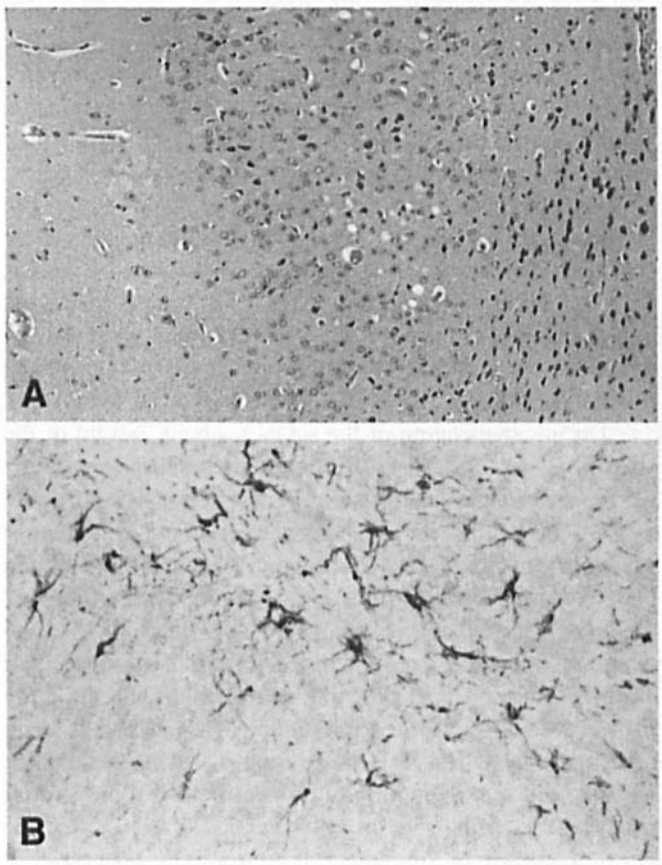

Figure 6. Section of the cortex from cases of hamster injected with ssDNA mixed with suspension of lipofectin showing (A) vacuolation and shrunken neurons. (B) Brain sections were stained with GFAP showing astrocytes. (A) Magnification ×130.

ports the hypothesis that the ssDNA is the genome of the scrapie agent.

The infectivity demonstrated in the 1.2-kb ssDNA preparation after alkaline electrophoresis is highly unlikely to be due to presence of Prpsc, because no Prpsc was demonstrated by the Western blot in the phenol/chloroform-purified preparations. It is also important to stress that proteins, if any are present in the DNA preparations during gel electrophoresis, would run in the opposite direction to DNA. Furthermore, treatment of the DNA preparations with 0.5 M NaOH at 65°C would eliminate infectivity, and animals inoculated with ssDNA suspended in $MgCl_2$ remained healthy. On the basis of incubation periods, clinical signs, and lesion profiles in hamsters, it is evident that the ssDNA, with the help of carrier protein, does transmit the disease, demonstrating that ssDNA is the genome of the agent and requires a carrier protein for cell infection.

There are three important known facts that have become obvious: 1) The natural transmission of the BSE agent to a large number of animal species has proved beyond doubt the transmissible nature of the agent, regardless of how small an infective dose. 2) Emergence of the BSE strain, highlights importance of different strains of the TSE agent, and 3) the agent is resistant to a variety of chemical and physical treatments (1, 2, 5, 6, 19, 35). The precision of the scrapie agent-host interactions has been one of the important factors that has provided the opportunity to establish reliable details about disease pathogenesis.

A search for a putative scrapie genome using a refocusing gel electrophoresis method also failed to produce a specific nucleic acid, but the authors (85) state that they cannot rule out the existence of a small nucleic acid or, alternatively, that the molecules exist as variable lengths. Important differences exist in the preparation of the nucleic acid in the present study as compared with previous studies (82–85). Since it was apparent from the "touch" technique that the ssDNA lies wrapped around SAF/PrP protected by a protein coat (18, 35, 36, 38), no detergents were used to disrupt the tissue.

During sequencing of the ssDNA, about 280 bp of the amyloid B-protein (APP)751 mRNA gene sequenced (27) suggested that the gene might be interlinked with the ssDNA. Post-transcription modification of the PrP or amyloid proteins might be achieved by interlinking of the ssDNA with the host PrP or amyloid gene. Interlinking of the ssDNA with the host PrP or APP gene might lead to the overexpression of these proteins. This would also explain a built-in mechanism of post-translation modification of the PrP and APP with plaque formation in TSEs in the late stage of incubation period. The integration of ssDNA with host DNA would also explain familial inheritance of the natural scrapie in sheep, GSSS, and familial CJD cases.

Conclusion

It is a well-established fact that the strains of the TSE agent "breed true" with their own particular biological properties. A study of Icelandic sheep scrapie and a review of the European scientific literature has demonstrated the existence of two strains of scrapie in sheep: Type I, "itchy" and Type II, the ataxic "trembly" type. These clinical signs, trembling and ataxia, are similar in BSE, vCJD, Kuru, and sheep inoculated with brain tissues from cows. Since the clinical signs of Type II scrapie in sheep with trembling and ataxia are similar to those seen in BSE and vCJD, this suggests that Type II is the cause of BSE and vCJD.

For a number of years, the phenomenon of interference between the two strains of the agent has been known (106). Based on this phenomenon, I proposed that the Type I strain of scrapie acts as vaccine against BSE, Type II scrapie. Many people have eaten Type I strain unknown to them and, therefore, will have a natural protection. In the summer of 2000, at Birmingham, Dr. S. Prusiner stated that his colleague, Dr. Mike Scott, believes that sheep carry two strains of the agent: scrapie strain and the BSE strain (128). The agent has always been in sheep and probably always will be. Now Dr. Stanley Prusiner has openly admitted the phenomena of interference between the two strains of the agent. He believes that the scrapie strain is somewhat dominant, preventing the BSE strain from infecting cattle and people when both are present. In other words, the scrapie strain acts as a vaccine against the BSE strain. The phenomena of interference will only be effective if the agent causing the disease is a virus. Therefore it must be a virus.

The author is indebted to many colleagues, particularly the advice of Drs. Dmitry Goldgaber, Luisa Gregori, Angela Ryan, and Colin Leakey. The financial support from Ken Bell International is also greatly appreciated.

1. Narang HK. The Link: Creutzfeldt-Jakob Disease: Bovine Spongiform Encephalopathy. Scrapie from Sheep to Cow to Man. Newcastle, United Kingdom: HH Publishers, 1997.
2. Narang HK. Origin and implications of bovine spongiform encephalopathy. Proc Soc Exp Biol Med **211**:306–322, 1996.
3. Narang HK. Lingering doubts about spongiform encephalopathy and Creutzfeldt-Jakob disease. Proc Soc Exp Biol Med **226**:640–652, 2001.
4. Gajdusek DC. Unconventional viruses and origin and disappearance of Kuru. Science **197**:943–960, 1977.
5. Gajdusek DC. Unconventional viruses causing subacute spongiform encephalopathies. In: Field BN, Melnick L, Chanock R, Shope RF, Roizman B, Eds. Human Viral Infections. New York: Raven Press, pp1519–1557, 1985.
6. Bruce ME, Dickinson AG. Biological evidence that scrapie agent has an independent genome. J Gen Virol **68**:79–89, 1987.
7. Dickinson AG, Fraser H. Genetic control of the concentration of ME7 scrapie agent in mouse spleen. J Comp Path **79**:363–366, 1969.
8. Fraser H. Neuropathology of scrapie, the precision of the lesions and their diversity. In: Prusiner SB, Hadlow WJ, Eds. Slow Transmissible Disease of the Nervous System. New York: Academic Press, pp387–406, 1979.
9. Carp RI, Merz PA, Kascsak RJ, Merz G, Wisniewski HM. Biological properties of scrapie, an unconventional slow virus. Gen Virol **53**:596–606, 1985.
10. Kim YS, Carp RI, Callahan SM, Wisniewski HM. Clinical course of three strains in mice injected stereotaxically in different brain regions. J Gen Virol **68**:695–702, 1987.

11. Pattison IH. The relative susceptibility of sheep, goat and mice to two types of the goat scrapie agent. Res Vet Sci **7:**207–212, 1966.
12. Gibbs CJ Jr, Gajdusek DC, Amyx H. Strain variation in the viruses of Creutzfeldt-Jakob disease and kuru. In: Prusiner SB, Hadlow WJ, Eds. Slow Transmissible Disease of the Nervous System. New York: Academic Press, pp87–110, 1979.
13. Gibbs CJ Jr, Gajdusek DC, Morris JA. Viral characteristics of the scrapie agent in mice. NINDB Monograph No 2. Slow, Latent and Temperate Viral Infections, pp195–202, 1965.
14. Sigurdsson B. Rida, a chronic encephalitis of sheep. Br Vet J **110:**341–354, 1954.
15. Narang HK. Scrapie, an unconventional virus: the current views. Proc Soc Exp Biol Med **184:**375–388, 1987.
16. Narang HK. A chronological study of experimental scrapie in mice. Virus Res **9:**293–306, 1988.
17. Hadlow WJ, Kennedy RC, Race RE. Natural infection of Suffolk sheep with scrapie virus. J Infect Dis **146:**657–664, 1982.
18. Wells GAH, Hancock RD, Cooley WA, Richards MS, Higgins RH, Davis GP. Bovine spongiform encephalopathy: diagnostic significance of vacuolar changes in selected nuclei of the medulla oblongata. Vet Rec **125:**521–524, 1989.
19. Narang HK. The nature of the scrapie agent: the virus theory. Proc Soc Exp Biol Med **211:**208–224, 1996.
20. Gibson RA, Hunter GD. Nature of the scrapie agent. Nature **215:**1041–1043, 1967.
21. Cho HJ. Requirement of a protein component for scrapie infectivity. Intervirology **14:**213–216, 1980.
22. Narang HK. Death on the Menu: CJD Victims: Diagnosis and Care: Families Devastated by Mad Cow Disease Reveal Their Tragic Stories. Newcastle, United Kingdom: HH Publishers, 1997.
23. Narang HK. Bovine spongiform encephalopathy. Report and Proceedings of the Agricultural Committee Memorandum HMSO, House of Commons. Appendix **22:**237–240, 1990.
24. Narang HK. A critical review of atypical cerebellum type Creutzfeldt-Jakob disease: its relationship to "new variant" CJD and bovine spongiform encephalopathy. Proc Soc Exp Biol Med **226:**629–639, 2001.
25. Rohwer RG. Scrapie infectious agent is virus-like in size and susceptibility to inactivation. Nature **308:**658–662, 1984.
26. Brown P, Gajdusek DC. Survival of scrapie virus after 3 years' interment. Lancet **337:**269–270, 1991.
27. Narang HK. The structure and transmission of BSE and CJD. In: Collinge P, Ed. The BSE Business. Birmingham, United Kingdom: The Public Health Trust, pp30–44, 1995.
28. Bolton DC, McKinley MP, Prusiner SB. Identification of a protein that purifies with the scrapie prion. Science **218:**1309–1311, 1982.
29. Akowitz A, Sklaviadis T, Manuelidis EE, Manuelidis L. Nuclease-resistant polyadenylated RNAs of significant size are detected by PCR in highly purified Creutzfeldt-Jakob disease preparations. Microb Pathogen **9:**33–45, 1990.
30. Sambrook J, Fritsch EF, Maniatis T. Molecular Cloning: A Laboratory Manual. (2nd ed.). Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press, 1989.
31. Shin YA, Eichhorn GL. Interaction of metal ions with polynucleotides and related compounds. XI. The reversible unwinding and rewinding of deoxyribonucleic acid by zinc (II) ions through temperature manipulation. Biochemistry **7:**1026–1032, 1968.
32. Butzow JJ, Eichhorn GL. Different susceptibility of DNA and RNA to cleavage by metal ions. Nature **254:**358–359, 1975.
33. Shibata D, Martin WJ, Arnheim N. Analysis of DNA sequences in forty-year-old paraffin-embedded thin tissue sections: a bridge between molecular biology and classical histology. Cancer Res **48:**4564–4566, 1988.
34. Porter DD, Porter HG, Cox NA. Failure to demonstrate a humoral immune response to scrapie in mice. J Immunol **111:**1407–1410, 1973.
35. Gajdusek DC. Subacute spongiform encephalopathies: transmissible cerebral amyloidoses caused by unconventional viruses. In Virology, Field BN, Knipe DM, Chanock RM, Hirsch MS, Melnick LJ, Monath TP, Roizman B. Eds. New York: Raven Press, pp2289–2324, 1990.
36. Merz PA, Somerville RA, Wisniewski HM, Iqbal K. Abnormal fibril from scrapie infected brains. Acta Neuropathol **54:**63–74, 1981.
37. Merz PA, Rohwer RG, Kascsak R, Wisniewski HM, Somerville RA, Gibbs CJ Jr, Gajdusek DC. Infectious-specific particles from the unconventional slow virus diseases. Science **225:**437–440, 1984.
38. Narang HK, Shenton B, Giorgi PP, Field EJ. Scrapie agent and neurons. Nature **24:**106–107, 1972.
39. Narang HK. An electron microscopic study of natural scrapie sheep brain: further observations on virus-like particles and paramyxovirus like tubules. Acta Neuropathol **28:**317–329, 1974.
40. Narang HK, Asher DM, Pomeroy KL, Gajdusek DC. Abnormal tubulovesicular particles in brains of hamsters with scrapie. Proc Soc Exp Biol Med **184:**504–509, 1987.
41. Liberski PP, Yanagihara R, Gibbs CJ Jr, Gajdusek DC. Tubulovesicular structures in experimental spongiform encephalopathy Creutzfeldt-Jakob disease and scrapie. Intervirology **29:**115–119, 1988.
42. Liberski PP, Yanagihara R, Gibbs CJ Jr, Gajdusek DC. Appearance of tubulovesicular structures in experimental Creutzfeldt-Jakob disease and scrapie proceeds the onset of clinical disease. Acta Neuropathol **79:**349–354:1990.
43. Liberski PP. Ultrastructural neuropathological features of bovine spongiform encephalopathy. J Am Vet Med Assoc **196:**1682, 1990.
44. Gibson PH, Doughty LA. An electron microscopic study of inclusion bodies in synaptic terminals of scrapie-infected animals. Acta Neuropathol **77:**420–425, 1989.
45. Baringer JR, Prusiner SB, Wong JS. Scrapie associated particles in post-synaptic processes: further ultrastructural studies. J Neuropathol Exp Neural **40:**281–288, 1981.
46. Stack MJ, Aldrich AM, Kitching AD, Scott AC. Comparative study of electron microscopical techniques for the detection of scrapie-associated fibrils. Res Vet Sci **59:**247–254, 1995.
47. Narang HK, Perry RH. Diagnosis of Creutzfeldt-Jakob disease by electron microscopy. Lancet **335:**663–664, 1990.
48. Prusiner SB. Novel proteinaceous infectious particles cause scrapie. Science **216:**136–144, 1982.
49. Prusiner SB, Bolton DC, Groth DF, Bowman KA, Cochran SP, McKinley MP. Further identification and characterisation of scrapie prions. Biochemistry **21:**6942–6950, 1982.
50. Bendheim PE, Berry RA, DeArmond SJ, Stites DP, Prusiner SB. Antibodies to a scrapie prion protein. Nature **310:**418–421, 1984.
51. Bode L, Pocchiari M, Gelderblom H, Diringer H. Characterisation of antisera against scrapie-associated fibrils (SAF) from affected hamster and cross-reactivity with SAF from scrapie-affected mice and from patients with Creutzfeldt-Jakob disease. J Gen Virol **66:**2471–2478, 1985.
52. McKinley MP, Bolton DC, Prusiner SB. A protease-resistant protein in a structural component of scrapie prion. Cell **35:**57–62, 1983.
53. Merz PA, Rubenstein R, Carp R, Kascsak R. Evidence to implicate SAF as form of infectious agent. In: Symposium sur les Virus Non-Conventionnels du Système Nerveux Central (2nd ed.). Paris, 1988.
54. Oesch B, Westaway D, Walchli M, McKinley MP, Kent SBH, Aebersold R, Barry RA, Tenpst P, Teplow DB, Hood LE, Prusiner SB, Weissmann C. A cellular gene encodes scrapie PrP 27-30 protein. Cell **40:**735–746, 1985.
55. Diringer H, Rahn HC, Bode L. Antibodies to the protein of scrapie-associated fibrils. Lancet **2:**345, 1984.
56. DeArmond SJ, McKinley MP, Barry RA, Braunfeld MB, McColloch JR, Prusiner, SB. Identification of prion amyloid filaments in scrapie-infected brains. Cell **41:**221–235, 1985.
57. Prusiner SB, McKinley M, Bowman KA, Bolton DC, Bendheim PE, Groth DF, Glenner GG. Scrapie prions aggregate to form amyloid-like birefringent rods. Cell **35:**349–358, 1983.
58. Narang HK, Asher DM, Gajdusek DC. Tubulofilaments in negatively stained scrapie-infected brains: relationship to scrapie-associated fibrils. Proc Natl Acad Sci U S A **84:**7730–7734, 1987.
59. Prusiner SB. Molecular Biology of prion diseases. Science **252:** 1515–1522, 1991.
60. Prusiner SB. Transgenic mice in prion diseases. Curr Top Micro Immunol **206:**275–304, 1996.
61. Prusiner SB. Prions. Sci Am **251:**50–59, 1984.
62. Weissmann C. Unified theory of prion propagation. Nature **352:**679–683, 1991.
63. Chesebro B, Race R, Wehrly K, Nishio J, Bloom M, Lechner D, Bergstrom S, Robbins K, Maye, L, Keith JM, Garon C, Haase A. Identification of scrapie prion protein-specific mRNA in scrapie-infected and uninfected brains. Nature **315:**331–333, 1985.

64. Narang HK. Relationship of protease-resistant protein, scrapie-associated fibrils and tubulofilamentous particles to the agent of spongiform encephalopathies. Res Virol **143:**381–386, 1992.
65. Brown P. The molecular biology of the transmissible spongiform encephalopathies (scrapie, kuru and Creutzfeldt-Jakob disease). Dis Neurosci **5:**57–69, 1988.
66. Hay B, Barry RA, Lieberburg I, Prusiner SB, Lingappa VR. Biogenesis and transmembrane orientation of the cellular isoform of the scrapie prion protein. Mol Cell Biol **7:**914–920, 1987.
67. Caughey B, Raymond GJ. The scrapie-associated from of PrP is made from a cell surface precursor that is both protease- and phospholipase-sensitive. J Biol Chem **266:**18217–18223, 1991.
68. Telling GC, Scott M, Mastrianni J, Gabizon R, Torchia RM, Cohen F, DeArmond SJ, Prusiner SB. Prion propagation in mice expressing human and chimeric .PrP transgenes implicates the interaction of cellular PrP with another protein. Cell **83:**79–90, 1995.
69. Bolton DC, Bendheim PE. A modified host protein model of scrapie. In Bock G, Marsh J, Eds. Ciba Foundation for Symposium 135. Chichester, United Kingdom: J. Wiley & Sons, pp164–181, 1988.
70. Wills PR. Induced frameshifting mechanism of replication for an information-carrying scrapie prion. Microb Pathogen **6:**235–249, 1988.
71. Carp RI, YE X, Kascsak RJ, Rubenstein R. The nature of the scrapie agent: biological characteristics of scrapie in different scrapie strain-host combinations. Ann NY Acad Sci **724:**221–234, 1994.
72. Come JH, Fraser PE, Lansbury PT. A kinetic model for amyloid formation in the prion diseases: importance of seeding. Proc Natl Acad Sci U S A **90:**5959–5963, 1993.
73. Basler K, Oesch B, Scott M, Westaway D, Walchli M, Groth DF, McKinley MP, Prusiner SB, Weissmann C. Scrapie and cellular PrP isoforms are encoded by the same chromosomal gene. Cell **46:**417–428, 1986.
74. Hsiao KK, Baker HF, Crow TJ, Poulter M, Owen F, Terwilliger JD, Westaway D, Ott J, Prusiner SB. Linkage of prion protein missense variant to Gerstmann-Sträussler syndrome. Nature **338:**342–345, 1989.
75. Owen F, Lofthouse R, Crow TJ, Baker HF, Poulter M, Collinge J, Risby D, Ridley RM, Hsiao K, Prusiner SB. Insertion in prion protein gene in familial Creutzfeldt-Jakob disease. Lancet **i:**51–52, 1989.
76. Collinge J, Harding AE, Owen F, Poulter M Lofthouse R, Boughey AM, Shah T, Crow TJ. Diagnosis of Gerstmann-Sträussler-syndrome in familial dementia with prion protein gene analysis. Lancet **2:**15–17, 1989.
77. Brown P. The "Brave New World" of transmissible spongiform encephalopathy (infectious cerebral amyloidosis). Mol Neurobiol **8:**79–87, 1994.
78. Bruce ME, Chree A, McConnell I, Foster J, Pearson G, Fraser H. Agent strain variation in BSE and scrapie. In: Bradley R, B Marchant, Eds. EEC Meeting, 1993: Proceedings of the Consultation BSE Scientific Vet Committee Commission European Communities. Brussels: pp189–201, 1994.
79. Somerville RA, Carp RI. Altered scrapie infectivity estimates by titration and incubation period in the presence of detergents. J Gen Virol **64:**2045–2050, 1983.
80. Somerville RA, Merz PA, Carp RI. Partial copurification of scrapie-associated fibrils and scrapie infectivity. Intervirology **25:**48–55, 1986.
81. Diringer H, Hilmert H, Simon D, Werner E, Ehlers B. Toward purification of the scrapie agent. Eur J Biochem **134:**555–560, 1983.
82. Gabizon R, McKinley MP, Prusiner SB. Purified prion protein and scrapie infectivity copartition into liposomes. Proc Natl Acad Sci U S A **84:**4017–4021, 1987.
83. Bellinger-Kawahara C, Diener TO, McKinley MP, Groth DF, Smith DR, Prusiner SB. Purified scrapie prion resist inactivation by procedures that hydrolyze, modify, or shear nucleic acids. Virology **160:**271–274, 1987.
84. Oesch B, Groth DF, Prusiner SB, Weissmann C. Search for a nucleic acid: a progress report. In: Bock G, Marsh J, Eds. Novel Infectious Agents and the Central Nervous System, Ciba Foundation Symposium. New York: John Wiley & Sons, pp209–223, 1988.
85. Meyer N, Rosenbaum V, Schmidt B, Gilles K, Mirenda C, Groth D, Prusiner SB, Riesner D. Search for a putative scrapie genome in purified prion fractions revealed a paucity of nucleic acids. J Gen Virol **72:**37–49, 1991.
86. Carlson GA, Kingsbury DT, Goodman PA, Coleman S, Marshall ST, DeArmond, SJ, Westaway D, Prusiner SB. Linkage of prion protein and scrapie incubation time genes. Cell **46:**503–511, 1986.
87. Hunter N, Hope J, McConnell I, Dickinson AG. Linkage of the scrapie-associated fibril protein (PrP) gene and Sinc using congenic mice and restriction fragment length polymorphism analysis. J Gen Virol **68:**2711–2716, 1987.
88. Prusiner SB. Biology and genetics of prion diseases. Annu Rev Microbiol **48:**655–686, 1994.
89. Palmer M, Dryden A, Hughes J, Collinge J. Homozygous prion protein genotype predisposes to sporadic Creutzfeldt-Jakob disease. Nature **352:**340–342, 1991.
90. Balter M. Prions: a lone killer or a vital accomplice? Science **286:**660–662, 1999.
91. Chesebro B. PrP and the scrapie agent. Nature **356:**560, 1992.
92. Manson J, Jamieson E, Baybutt H, Tuzi NL, Barron R, McConnell I, Somerville R, Ironside J, Will R, Sy MS, Melton DW, Hope J, Bostock C. A single amino acid alteration (101L) introduced into murine PrP dramatically alters incubation time of transmissible spongiform encephalopathy. EMBO J **18:**6855–6864, 1999.
93. Ferber RA, Wiesenfeld SL, Roos RP, Bobowick AR, Gibbs CJ Jr, Gajdusek DC. Familial Creutzfeldt-Jakob disease: transmission of the familial disease to primates. In: Subirana A, Espadaler JM, Burrows EH, Eds. Genetic and Transmissible Dementias. Amsterdam: Excerpta Med, pp358–380, 1974.
94. Hunter N, Goldmann W, Benson G, Foster JD, Hope J. PrP genotype studies. In: Bradley R, Marchant BM, Eds. EEC Meeting, 1993: Proceedings of the Consultation BSE Scientific Vet Committee Commission European Communities. Brussels: pp125–139, 1994.
95. Dickinson AG, Meikle VH, Fraser H. Identification of a gene which controls the incubation period of some strains of scrapie in mice. J Comp Path **78:**293–299, 1968.
96. Meyer RK, McKinley MP, Bowman KA, Braunfeld M, Berry RA, Prusiner SB. Separation and properties of cellular and scrapie prion proteins. Proc Natl Acad Sci U S A **83:**2310–2314, 1986.
97. Sklaviadis TK, Manuelidis L, Manuelidis EE. Physical properties of the Creutzfeldt-Jakob disease agent. J Virol **63:**1212–1222, 1989.
98. Czub M, Braig HR, Blode H, Diringer H. The major protein of SAF is absent from spleen and thus not an essential part of the scrapie agent. Arch Virol **91:**383–386, 1986.
99. Xi YG, Ingrosso L, Ladogana A, Masullo C, Pocchiari M. Amphotericin B treatment dissociates in vivo replication of the scrapie agent from PrP accumulation. Nature **356:**598–601, 1992.
100. Lasmézas CI, Deslys J-P, Robain O, Jaegly A, Beringue V, Peyrin J-M, Fournier J-G, Hauw J-J, Rossier J, Dormont D. Transmission of the BSE agent to mice in the absence of detectable abnormal prion protein. Science **275:**402–405, 1997.
101. Fraser H, Foster JD. Transmission to mice, sheep and goats and bioassay of bovine tissues. In: Bradley R, Marchant BM, Eds. EEC Meeting, 1993: Proceedings of the Consultation BSE Scientific Vet Committee Commission European Communities. Brussels: pp145–159, 1994.
102. Scott M, Foster D, Mirenda C, Serban D, Coufal F, Wälchli M, Torchia M, Groth D, Carlson G, DeArmond SJ, Westaway D, Prusiner SB. Transgenic mice expressing hamster prion protein produce species-specific scrapie infectivity and amyloid plaques. Cell **59:**847–857, 1989.
103. Scott M, Groth D, Foster D, Torchia M, Yang S-L, DeArmond SJ, Prusiner SB. Propagation of prions with artificial properties in transgenic mice expressing chimeric PrP gene. Cell **73:**979–988, 1993.
104. Büeler H, Fischer M, Lang Y, Bluethmann H, Lipp HP, DeArmond SJ, Prusiner SB, Aguet M, Weissmann C. Normal development and behaviour of mice lacking the neuronal cell-surface PrP protein. Nature **356:**577–582, 1992.
105. Büeler H, Aguzzi A, Sailer A, Greiner R-A, Autenried P, Aguet M, Weissmann C. Mice devoid of PrP are resistant to scrapie. Cell **73:**1339–1347, 1993.
106. Narang HK. Molecular cloning of single-stranded DNA purified from scrapie-infected hamster brain. Res Virol **144:**375–387, 1993.
107. Foster JD, Hope J, McConnell I, Bruce ME, Fraser H. Transmission of bovine spongiform encephalopathy to sheep, goats and mice. Ann NY Acad Sci **724:**300–303, 1994.
108. Robinson MM. Transmissible encephalopathy research in the United States. In: Bradley R, Marchant BM, Eds. EEC Meeting, 1993: Pro-

ceedings of the Consultation BSE Scientific Vet Committee Commission European Communities. Brussels: pp261–270, 1994.
109. Dickinson AG, Fraser H. An assessment of the genetics of scrapie in sheep and mice. In: Prusiner SB, Hadlow WJ, Eds. Slow Transmissible Disease of the Nervous System. New York: Academic Press, pp367–385, 1979.
110. Narang HK. The molecular pathogenesis of the tubulofilamentous particles: evidence of a homologous single-stranded DNA in scrapie, Creutzfeldt-Jakob disease and bovine spongiform encephalopathy. In: Bradley R, Marchant BM, Eds. EEC Meeting, 1993: Proceedings of the Consultation BSE Scientific Vet Committee Commission European Communities. Brussels: pp385–419, 1994.
111. Narang HK. Scrapie-associated tubulofilamentous particles in human Creutzfeldt-Jakob disease. Res Virol **143:**387–395, 1992.
112. Narang HK. High-resolution electron microscopic analysis of the amyloid fibril in Alzheimer's disease. J Neuropathol Exp Neurol **39:**621–631, 1980.
113. Carp RI, Merz PA, Kascsak RJ, Merz G, Wisniewski HM. Biological properties of scrapie, an unconventional slow virus. Gen Virol **53:**596–606, 1985.
114. Marsh R, Kimberlin RH. Comparison of scrapie and transmissible mink encephalopathy in hamsters: clinical signs, pathology and pathogenesis. J Infect Dis **131:**104–110, 1975.
115. Narang HK. Evidence of ssDNA in tubulofilamentous particles: their relationship to scrapie-associated fibrils. Intervirology **32:**185–192, 1991.
116. Narang HK. Scrapie-associated tubulofilamentous particles in scrapie hamsters. Intervirology **34:**105–111, 1992.
117. Narang HK, Asher DM, Gajdusek DC. Evidence that DNA is present in abnormal tubulofilamentous structures found in scrapie. Proc Natl Acad Sci U S A **85:**3575–3579, 1988.
118. Blalock JE, Smith EM. Hydropathic anti-complementary of amino acids based on the genetic code. Biochem Biophys Res Commun **121:**203–207, 1984.
119. Narang HK. Evidence that homologous ssDNA is present in scrapie, Creutzfeldt-Jakob disease, and bovine spongiform encephalopathy: slow virus of the central nervous system. Ann NY Acad Sci **724:**314–326, 1994.
120. Narang HK. Evidence that single-stranded DNA wrapped around the tubulofilamentous particles "Nemavirus" is the genome of the scrapie agent. Res Virol **149:**375–382, 1998.
121. Narang HK. Detection of single-stranded DNA in scrapie-infected brain by electron microscopy. J Mol Biol **216:**469–473, 1990.
122. Wietgrefe S, Zupancic M, Haase A, Chesebro B, Race R, Frey W, Rustan T, Freedman RL. Cloning of a gene whose expression is increased in scrapie and in senile plaques in human brains. Science **230:**1177–1181, 1985.
123. Diedrich J, Wietgrefe S, Zupancic M, Staskus K, Retzel E, Haase AT, Race R. The molecular pathogenesis of astrogliosis in scrapie and Alzheimer's disease. Microb Pathogen **2:**435–442, 1987.
124. Duguid JR, Rohwer RG, Seed B. Isolation of cDNAs of scrapie-modulated RNAs by subtractive hybridisation of a cDNA library. Proc Natl Acad Sci U S A **85:**5738–5742, 1988.
125. Lewin B. Genes (3rd ed.). New York: John Wiley & Sons, 1988.
126. Felgner PL, Gadek TR, Holm M, Roman R, Chan HW, Wenz M, Northrop JP, Ringold GM, Danielsen M. Lipofectin: a highly efficient lipid-mediated DNA transfection procedure. Proc Natl Acad Sci U S A **84:**7413–7417, 1987.
127. Malone RW, Felgner PL, Verma IM. Cationic liposome-mediated RNA transfection. Proc Natl Acad Sci U S A **86:**6077–6081, 1989.
128. Coghlan A. Reservior sheep. New Sci **2249:**20, 2000.