MINIREVIEW

The Nature of the Scrapie Agent: The Virus Theory[1] (44009)

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Abstract. **All spongiform encephalopathies (SEs) are slow virus-transmissible infectious disorders of the brain. Tubulofilamentous particles/scrapie-associated fibrils (SAF) are ultrastructural markers, while protease-resistant protein (PrP) is a protein marker. The PrP molecules aggregate to form SAF, which occurs as an internal part of the tubulofilamentous particle termed nemavirus (NVP). Each NVP consists of three layers: (i) an outer protein coat, (ii) an intermediate ssDNA layer, and (iii) inner PrP/SAF. A chronological study of scrapie-infected hamster brain revealed that NVP and SAF are seen 10 days postinoculation from the inoculated right side of the brain and from 18 days postinoculation from both sides of the brain. The existence of at least 20 stable strains of SEs implies that a nucleic acid molecule serves as the information molecule. This is incompatible with the hypothesis that PrP by itself or a specific point mutation is the agent. It appears that an "accessory protein" coded by the ssDNA of the nemavirus interacts with normal PrP^{c} molecules, resulting in their conversion to PrP^{sc}/SAF. The pathogenesis process in infected animals 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.**
[P.S.E.B.M. 1996, Vol 212]

Over the years, a remarkable series of discoveries have been made: (i) scrapie disease of sheep was found to be transmissible to mice (1); (ii) kuru, an epidemic neurodegenerative disorder, shared pathological similarities with scrapie (2); (iii) kuru was experimentally transmitted to primates; (iv) the neuropathological similarity of scrapie with Creutzfeldt-Jakob disease (CJD) and bovine spongiform encephalopathy (BSE) was established; and (v) subsequently CJD and BSE were in turn successfully transmitted to susceptible host species. Because of the strikingly similar histopathological vacuolar degenerative lesion of the central nervous system (CNS), scrapie, CJD, kuru, Gerstmann-Sträussler-Scheinker syndrome (GSSS), and some other animal diseases have been grouped together as spongiform encephalopathies (SEs) (3). The considerable amount of experimental work that has already been conducted on scrapie, kuru, and CJD is being applied to BSE. Based on different clinical characteristics and lesions distribution in the brain, different strains of scrapie agent have been identified. The different strains consistently have either a long or a short incubation period. Since the emergence of BSE, numerous experimental results have revealed that the BSE agent is a novel major new

[1] This article is Part II of an update of a previously published minireview entitled, "Scrapie, an Unconventional Virus: The Current Views" Proc Soc Exp Biol Med **184**:375–388, 1987. (Part I of the update appears in Proc Soc Exp Biol Med **211**:306–322, 1996.)

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This work was supported by Ken Bell International.

0037-9727/96/2123-0208$10.50/0

strain of pathogen (3). It is naturally transmitted with food from one species to another, jumping species barriers, and within a few years has infected domestic cats and 16 exotic zoo animals (3).

Nature and Properties of the Scrapie Agent

In the laboratory, most studies have been directed at understanding the virus-like pathogenesis of disease and characterizing the nature of the infectious agent. The agent appears to multiply by replicating very slowly with a very long asymptomatic incubation period of months or even years and a protracted clinical course. Because of the long incubation period, the SEs disorders were first described as slow virus infections (4). According to the definition, the SEs agent replicate slowly; however, the titer reaches up to 10^{10}–10^{12}/g of brain tissue. This titer is 4–6 logs more than any other commonly known viruses. The incubation period in slow viruses does not vary within very wide limits in the same animal species and appears to be related to the dose, the route of infection, the strain of scrapie agent, and the age of the host at the time of inoculation. The replication is slow in the sense that clinical symptoms develop after a long incubation period. Otherwise, diseases follow a course that is just as regular as the course in acute infections.

The precision of scrapie agent-host interactions has been one of the important factors that has provided the opportunity to establish reliable details about disease pathogenesis. After many years of research, despite much work, the nature of the agent responsible for these diseases still remains controversial. There are two important known facts: (i) the agent is transmissible, and (ii) it is resistant to a variety of chemical and physical treatments. The scrapie agent remained viable after scrapie-infected hamster brain homogenate was mixed with soil and packed into perforated petri dishes which were then embedded within soil-containing pots buried in a garden in the Washington, DC, area for 3 years (5). The biological properties of the scrapie agent—in particular, its resistance to nucleases, irradiation with ultraviolet light, and hydrolysis, the degree of its physiochemical stability, and the fact that a remarkable amount of the infective dose can often survive a heat of 132°C for a half an hour—have been discussed before (6, 7). However, the majority of infectivity can be abolished by autoclaving, and almost all by 1 *N* sodium hydroxide (over 55°C) and hypochlorite treatment (8). Successive determination of the size of the scrapie agent have given progressively smaller estimates (9). The agent will pass through a gradocol membrane of about 100 nm, but not one of 20 nm, in pore size, demonstrating the virus-like properties of the agent (6, 10). These atypical infectious diseases contrast with other known conventional viral diseases of humans and animals in that they do not initiate a virus-associated antibody immune or inflammatory response (11). From its unusual properties, it appears that the SEs agent is very different from all known viruses of animals and plants, and also viroids (7). Many researchers have been much embarrassed by the agent's stubborn refusal to reveal itself in any morphologically visible form and therefore have pursued unorthodox hypotheses. Several papers seriously addressed the possibility that the replicating agent was a piece of cell membrane, a complex glycolipid or protein (12, 13). To distinguish and separate the slow virus group from the rest of the other infectious microorganisms, the term "unconventional viruses" is used (6). A number of hypotheses on the nature of the SEs agent have been proposed and discussed before (7). In this paper, two major hypotheses are discussed in detail.

Prion/scrapie–associated Fibril Hypothesis

Abnormal fibrils termed "scrapie-associated fibrils" (SAF) were first identified by electron microscopy (EM) in extracts of scrapie-infected brains (14) and thereafter have been consistently observed in other SEs (15, 16), including in CJD and BSE (17, 18). Originally, SAF were the only abnormal structures seen by EM (19) and have been found only in scrapie and related diseases caused by unconventional viruses (14–16). SAF are not seen in non-neurological diseases or in a variety of other human and animal diseases exhibiting similar histopathological and ultrastructural features. Further, the fact that the occurrence and number of SAF have been shown to parallel the increase in infectivity (15, 16, 20), with a high degree of co-purification between SAF and infectivity in relation to protein concentration (19, 21), has made them excellent candidates for the scrapie, kuru, or CJD agent, or a form of infectious agent (6, 14–16, 19, 22) or a component(s) of the agent (16). It has been demonstrated that after mild detergent treatment, SAF show differing sedimentation characteristics and titer changes (23). The physiochemical properties and infectivity have been determined only on crude, or partially purified extracts of SAF fractions which may have contained up to 10% impurities. The apparent infectivity of fibrillary material may be due to coincidental co-purification of the agent (24). Biochemical analysis of highly purified SAF preparations were shown to consist of a single, major polypeptide of 27–30 kDa (PrP^{sc}), a protease-resistant protein (PrP) (25), which is derived from the full-length (33–35 kDa; PrP^{c}) normal host precursor sialoglycoprotein (26). The biochemical and immunological evidence so far has demonstrated that the SAF and PrP cross-react antigenically (21, 25, 27) and are the same structures (7, 19, 21, 30, 31).

It has been suggested that the scrapie agent is

composed exclusively of the host-coded PrP, without a scrapie-specific nucleic acid (32), and to distinguish it from both viruses and viroids, a term "prion" for the scrapie agent was proposed (32). This implies that the introduction of PrP^{sc} into a host both elicits the pathological processes and gives rise to more PrP^{sc} than the amount used for inoculation. How could this transformation be triggered? Is it a direct or indirect action? There is, however, no direct evidence whatsoever that PrP^{sc} arises from PrP^{c} by proteolytic cleavage. The most important discovery in this work was that the protein was not encoded by a foreign DNA; therefore, one has to conclude that host-encoded, nonpathogenic PrP^{c} precursor is converted into the modified, infectious form as a consequence of the introduction of PrP^{sc} into a new host. One might envisage PrP^{sc} initiating a cascade of events, which would lead to the conversion of PrP^{c} to PrP^{sc} form. The protein found in normal host tissue PrP^{c} differs from PrP^{sc} in its physical properties, and its susceptibility to proteinase K (PK), probably due to post-translational modifications (26, 33). Post-translational modification process and formation of SAF does not appear to be a reversible process. Models that involve the protein-only hypothesis are in direct conflict with the existence of distinct scrapie strains.

It has been demonstrated that PrP molecules aggregate to form "prion rods"/SAF and plaques (29, 34). A number of studies (35–39) have demonstrated that PrP27–30 molecules do not aggregate to form "prion rods" during purification steps as suggested previously (29, 34), but exist as morphological structure seen as SAF. In certain parts of infected brains, SAF accumulate to form plaques. If PrP^{sc} acts as the infectious agent, by converting PrP^{c} into PrP^{sc}, this conversion process is not replication by itself. PrP^{sc} molecules aggregate to form SAF, protease-resistant protein, and if this linking is a continuous process, how does the titer increase? An explanation is also needed for how PrP^{sc} molecules, once they have assembled as SAF, break up to form monomers in a new host. These findings suggest that post-translation conversion of PrP^{c} to PrP^{sc}, protease-resistant protein, is more likely to be a secondary process involving a fusion protein (39).

Immunohistochemical studies have demonstrated that plaques can be classified into two types according to the kind: (i) β-protein–positive amyloid plaques seen both in Alzheimer's disease and CJD, and (ii) PrP–positive plaques present in CJD, kuru, and other transmissible encephalopathies (40–42). It is known that amyloid β-protein is derived from amyloid precursor protein (APP), coded by a normal gene mapped to Chromosome 21 (43), and on the homologous murine Chromosome 16 (44). The function of the amyloid β-protein remains unknown.

PrP33–35 kDa precursor protein is coded by a normal gene assigned to mouse Chromosome 2 and in humans on the short arm of Chromosome 20 (45–48). The PrP gene coding for the complete translated precursor protein of 254 amino acids protein has been sequenced from a number of mammalian species (Fig. 1) (33, 49, 50). Identification of the PrP gene in numerous vertebrates, with the additional findings of multiple transcription initiation sites, and promoter regions rich in guanine and cytosine, just upstream from the short 5′ exon, are reminiscent of many ubiquitous "housekeeping genes." In all animal species, normal PrP contains a hydrophobic sequence at the N terminus (51, 52) and is predominantly found on the surface of neurons attached by a glycoinositol phospholipid anchor (52). There is only a single PrP gene, expressed to an equal extent in normal and scrapie-infected animals, giving rise to the same the primary translation product in normal and scrapie-infected brain (26, 45). What is the role of the normal host protein? Is it a major component of the infectious agent?

Role of Mutations in the PrP Gene

Surprisingly, the protein isolated from normal individuals does not differ in any biochemical or immunological feature from the protein isolated from affected individuals (33). Once it was realized that the PrP was host derived, molecular genetics entered on the scene and a search began for mutations in the corresponding human gene, among affected families. Some of the familial GSSS cases have mutations in the gene that codes for the precursor protein of 254 amino acids. It has been suggested that these point mutations observed in the PrP gene may cause the disease. The first was found in codon 129, and the subsequent in codons 102, 117, 178, and 200. There are reports of other mutations in codons 51 and 118 (53, 54). In fact, no affected family is known to exist that does not carry one or another mutation, and new mutations are being discovered every year (55). According to Brown (56), in spite of the great temptations of molecular genetics the heyday of mutation hunters has probably come and gone. It is unlikely that the next dozen mutations are, at the least, predisposing. Sporadic CJD cases, which form the majority, do not have these mutations.

Currently, these mutations have been classified into four groups according to the disease phenotype. Those in Group I express heterogenous clinical and pathological type (5, 58, 59). In phenotype that are consistent with GSSS, the mutations occur at codons 102, 117, and 198 (59, 60). The most common occur at codons 102, 178, and 200. The mutation at codon 102 segregates with the ataxic form and the encephalic form is linked to mutation at codon 117; while neurofibrillary tangles have been linked with the mutation at codon 198 (60). Similar clinical illnesses have been de-

-22
Met Ala Asn Leu Ser Tyr Trp Leu Leu Ala Leu Phe Val Ala Met Trp Thr Asp Val Gly Leu Cys
1

A Lys Lys Arg Pro Lys (Pro Gly Gly)^1 Trp Asn Thr Gly Gly Ser Arg Tyr Pro Gly Gln Gly Ser
(Pro Gly Gly)^1 (Asn Arg Tyr Pro)^2 (Pro Gly Gly)^1 Gly Gly Thr

B (Trp Gly Gln Pro His Gly Gly Gly)^3 (Trp Gly Gln Pro His Gly Gly Gly)^3
(Trp Gly Gln Pro His Gly Gly Gly)^3 (Trp Gly Gln Pro His Gly Gly Gly) ^{3}
Trp Gly Gln Gly Gly Gly Thr

C His Asn Gln Trp Asn Lys Pro Ser Lys Pro Lys
Thr Asn Met Lys His Met (Ala Gly Ala)^4 Ala Ala (Ala Gly Ala)^4

D (Val Val Gly)^5 Gly Leu Gly Gly Tyr Met Leu Gly Ser Ala
Met Ser Arg Pro Met Met His Phe Gly Asn Asp Tyr Gly Asp Arg

E (Tyr Tyr Arg)^6 Glu Asn Met (Asn Arg Tyr Pro)^2 Asn Gln Val
(Tyr Tyr Arg)^6 Pro Val Asp Gln Tyr Asn Asn Gln
Asn Asn Phe Val His Asp Cys Val Asn

F Ile Thr Ile Lys Gln His Thr Val Thr Thr Thr Thr
Lys Gly Glu Asn Phe Thr Gly Thr Asp Ile Lsy Ile Met Gly Arg

G (Val Val Gly)^5 Gln Met Cys Thr Thr Gln Tyr Gln Lys Gly Ser Gln
Ala Tyr Tyr Asp Gly Arg Arg Ser---Ser Ala Val

H Leu Phe Ser Ser Pro Pro Val Ile Leu Leu Ile Ser Phe Leu Ile Phe Leu Met Val Gly

22 34 39 25 28 33 27 26 20

Figure 1. Amino acid sequence of PrP precursor protein with schematic representation from hamster. The segments of amino acid are moved without breaking the order to show similarities (no.) or underlining in Domain A–H. Numbers shown underneath are amino acids in each domain.

scribed among individuals with different mutations, and different clinical illnesses among individuals with the same mutation, even within the same family (61). The fatal familial insomnia cases have been linked to mutation at codon 178 (62).

Does infection create a *de novo* mutation in the PrP gene? Apart from variable point mutations seen in different families cases of GSSS, these mutations have been seen in a number of first degree relatives of affected patients who are healthy into their 60s and even mid 70s (5). 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. This finding does not support the idea of GSSS's being a simple genetic disease. Since PrP is involved in the pathogenesis process (39), these mutations in the PrP gene in some familial patients may be important for two reasons: (i) the mutation reflects an increased susceptibility, or (ii) the mutation has occurred in patients subsequent to infection.

Certainly the primary structure of the full-length PrP33–35 form the scrapie precursor protein displays no amino acid change compared with the naturally occurring form (33, 63). So far, no structural difference has been found between the normal noninfectious and the scrapie-infectious form of PrP. The agents of these so-called unconventional slow virus diseases neither spontaneously create a mutation in the PrP gene, nor do they copy any mutation in the PrP after being infected from familial case or any other source.

Another corollary of this paradigm, proven in a number of transmission studies, is that the replication of the infectious agent occurs after inoculation of a new host species, which has a different genetic makeup of the PrP (amino acid sequence) than does the original host. Monkeys or chimpanzees inoculated with human-infected tissues have no mutation in the PrP gene similar to those in human brain material infected with CJD, kuru, or BSE. CJD from patients with the 102, 117, 178, and 200 codon mutations has been transmitted to monkeys and chimpanzees. These inoculated monkeys and chimpanzees develop a scrapie-like disorder, and their PrP33–35 protein is modified into PrP27–30, containing PrP not of humans or cow (donor) but of monkey or chimpanzee (host) (Fig. 2). None of the point mutations are copied in the PrP gene or its product in the new host. The clinical infectious disease develops, and the scrapie agent replicates maintaining its specific strain characteristic. The inoculated host animals do not carry any of these point mutations, nor does the PrP made in these experimentally infected hosts contain these point mutations as recognized in CJD patients (64). Furthermore, when BSE tissue containing the agent and bovine PrP27–30 is inoculated into sheep, the affected host sheep modifies PrP33–35^{sh} into PrP27–30^{sh}. Similar re-

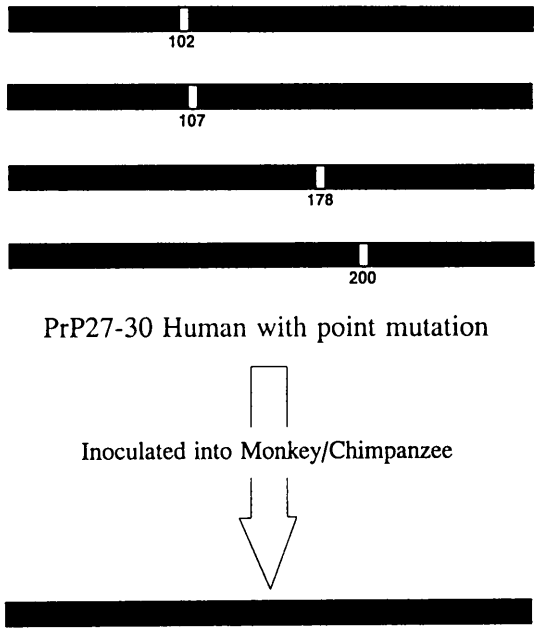


Figure 2. Human brain extract from four different amino acid point mutations in the PrP gene were used to inoculate monkeys and chimpanzees. Irrespective of the point mutation, infection results in *de novo* generation of monkey/chimpanzee PrP27–30 from the normal host precursor PrP33–35 protein.

sults are obtained by inoculating BSE tissue containing $PrP27–30^{Bo}$ into mice, pigs, and goats, where host PrP33–35 modifies into respective host PrP27–30 (Fig. 3). The strain of the agent "breeds true," retaining its original properties during the passage from one animal species to another (3). In both human and animal transmission studies, the fact that, on passage, PrP^{sc} converts to a new host species, PrP27–30 amino acid sequences, clearly demonstrates that these strain differences cannot be contained within the host-coded PrP molecules, since PrP27–30 does not "breed true" (Fig. 3).

Originally it was suggested that transgenic mice expressing a PrP gene with a specific 102 Pro to Leu point mutation, seen in GSSS, developed a degenerative brain disease, but later it appeared that the pathology is different (65). This degenerative brain disease in the transgenic mice expressing a PrP gene was also different in that there was no demonstratable protease-resistant protein and it did not seem to be infective (66).

Three transgenic mouse lines were produced by inserting hamster PrP gene into the mouse genome (67). The founder mice F1 were then mated to yield first-generation transgenic mice, and the first-generation mice were in turn mated, which provided a second generation. Neither transgenic in the presence of foreign PrP gene nor nontransgenic mice developed SEs or any other clinical neurological disorder. However, when these animals were inoculated with a known dose of the scrapie agent they developed SE, the incubation period in one experiment being 75 days and in the other 179 days. Nontransgenic mice showed resistance to hamster scrapie agent, with an incubation period extending over 400 days (67). An incubation of 75 days is similar to that seen in hamster inoculated with hamster scrapie. In the same study, the incubation time was observed following inoculation with a known dose of the mouse scrapie agent. All transgenic mice developed SE with an incubation period of 156–174 days, while with nontransgenic mice the incubation period was much shorter, at 134–151 days. The long incubation period of the second and third experiment have not been explained.

A comparative scrapie infection susceptibility study in chimeras with Human Down's syndrome, and control group mice, revealed that the symptoms of scrapie in the chimeras appeared at 137.8 ± 4.3 days, 17 days earlier than in the control group, while the time between inoculation and death was reduced by 30 days (68). Contrary to the situation in transgenic mice, in chimeras with Human Down's syndrome mice neither PrP nor the genes known to control the scrapie incubation period can be implicated. The likely possi-

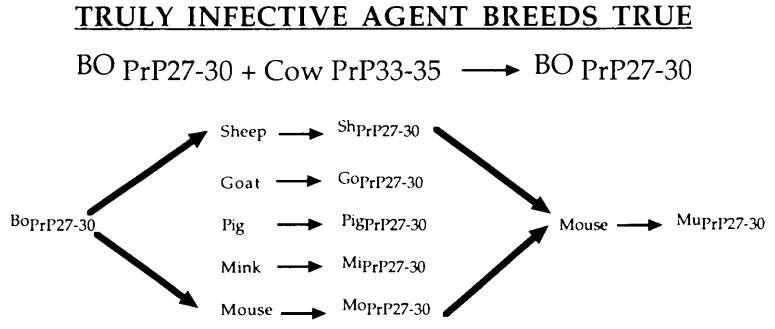


Figure 3. BSE brain extract used to inoculate sheep, goat, pig, mink, and mouse, and then passaged into mice. Respective of the source of infection, normal host precursor protein PrP33–35 modifies into respective host PrP27–30.

Figure shows On Interspecies Transmission PrP27-30 does not breed true

bility is that, in the chimeras with Human Down's syndrome, mouse neurons are developmentally immature relative to those in the control group and PrP, an essential housekeeping component, is diverted away from the neuron cell membranes, which would shorten the incubation period. These studies of species-specific scrapie infectivity do not rule out the possibility of the presence of an unknown putative agent present in the PrP preparations. The comparison of these incubation periods between transgenic and non-transgenic mice demonstrates that, irrespective of PrP gene makeup, there is still a need for an infectious agent (67).

PrP Null Mice

Mice homozygous for disrupted Prn-p gene (Prn-$p^{o/o}$ mice), although no PrP is detectable in the brains of these animals, develop without detectable physical or behavioral defects and reproduce normally (69). It thus became possible to study the response of Prn-$p^{o/o}$ mice to inoculation with the scrapie agent. In a comparative study using mice carrying a single Prn-p allele (Prn-$p^{o/+}$ mice) and Prn-$p^{o/o}$ mice reconstituted with Syrian hamster PrP genes, only one Prn-$p^{o/o}$ mouse developed the disease after inoculation with heat-inactivated (80°C for 20 min) Chandler-derived mouse scrapie agent, while the rest remained alive and free of symptoms for up to 13 months. Surprisingly, however, the unheated samples inoculated in Prn-$p^{o/o}$ mice caused the typical scrapie symptoms and death in all six animals 204 ± 14 days postinoculation (70). Since all six Prn-$p^{o/o}$ mice from the unheated inoculum developed the disease, it would appear that somehow the scrapie agent had gained entry into the brain cells. It is known that a considerable drop of titer occurs following incubation at 80°–100°C for 30 min (71). The mice inoculated with heat-inactivated inoculum remained free of symptoms at 13 months postinoculation, the reason for which may be that there was a considerable drop of the infective titer because of heat treatment. However, it may have been due to presence of a co-factor in the unheated inoculum, which contain crude protein particles that helped the absorption of the scrapie agent into cells. Heterogygous Prn-$p^{o/+}$ mice showed enhanced resistance to scrapie, and, after introduction of Syrian hamster PrP transgenes, Prn-$p^{o/o}$ mice became highly susceptible (incubation time: 56 days) to the hamster-derived scrapie agent but showed a long incubation period (>271 days) for mouse scrapie agent (70).

It has been shown that in transgenic mice the "species barrier" for transmission of the SEs agent can be abrogated by expression of the host gene. However, overall results of the transmission studies are variable and disappointing. Transgenic mice expressing chimeric PrP genes derived from Syrian hamster (SHa) and mouse (Mo) PrP gene were constructed (72), one with two substitutions designated MHM2 PrP (at position 108 Leu to Met and position 111 Val to Met), and the second containing five amino acid substitution designated MH2M PrP (additional three substitutions were lle to Met at position 138, a Tyr to Asn at position 154, and Ser to Asn at position 169) (72). When transgenic mice expressing these chimeric PrP molecules were challenged with Syrian or mouse-derived strain of the scrapie agent, it was found that MH2M PrP mice were susceptible to both hamster and mouse scrapie agent. Transgenic MHM2 PrP mice that 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, while those that produced normal amount of PrP^c were susceptible (67). Some lines developed the disease with a 306- to 448-day incubation period, compared with 134 days in MH2M PrP line. Making a simple interpretation of these results becomes more complicated.

Furthermore, 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 than transgenic mice expressing low levels of normal human PrP^c (73). These experiments—combining transgenic and Prn-$p^{o/o}$ mice, "knock-out" animals with a range of further crossed and back-crossed mouse strains and gene copy numbers—have become almost as difficult to interpret as those of classical scrapie agent strains, which has made the story even more complicated. These experiments, without evidence, imply that PrP is the agent, while it is evident that the infectious agent is something other than PrP^{sc}.

However, these experiments do strongly suggest that the PrP molecules on the cell surface may act as receptor sites for the agent and absence of such sites would prevent entry of the scrapie agent into the cell, thus making the animals resistant to infection (74). This hypothesis can only be resolved by further experimentation, particularly using the BSE agent, where the incubation period is an interaction of host and strain of the scrapie agent and not just influenced by the genetic makeup of the host PrP gene (3). Since transgenic mice produced in the above experiment contain PrP gene from hamster, as well as their own, they are susceptible to both mouse- and hamster-derived scrapie agent. These facts demonstrate that the disease is not a genetic disorder, but the evidence presented so far favors the view that SEs are infectious diseases involving a nonconventional scrapie nucleic acid genome. These findings suggest that a normal PrP by itself is not the agent.

There remains the requirement of an explanation for the presence of multiple stable strains of the

scrapie agent. The mechanism of propagation of the agent must be established for a protein-only information molecule. Several explanations of scrapie strains in the context of the PrP theory have been put forward, but, as further knowledge about the biochemistry and molecular biology of the PrP has become available, most theories have been abandoned. The current theory is that PrP can assume various tertiary structures and that these different structures can imprint upon the normal cellular infecting PrP molecule. Two approaches have been used to address the possible relationship between PrP tertiary structure and the dual requirement of the scrapie agent: replication and maintenance of the strain identity. The first approach involved serial passage of material derived from different brain areas, and the second was to determine the putative role of the tertiary structure of PrP to the treatment with PK that will denature the protein followed by analysis of the scrapie strain characteristic (75). Their preliminary results revealed that procedures which render the PrP preparations derived from scrapie-infected mouse and hamster brain PK sensitive do not affect the level of their scrapie strain-specified characteristics (75). A number of scrapie strains can be differentiated in the same strain of animals. To sustain true strain characteristics, there must be a nucleic acid which serves as informational molecules. The remarkable diversity and stability of scrapie strains demonstrated in a large number of studies make explaining genetically stable strains with the "prion" theory, with or without tertiary structure, very problematic.

Essential Backbone of Prusiner's Hypothesis

Prusiner (31) considered that the individual prion is the agent and is too small to contain a nucleic acid. The prion hypothesis has been based on two points: (i) no demonstratable nucleic acid in PrP preparation and (ii) resistance of the scrapie agent to harsh procedures (76). There are two major lines of indirect evidence in favor of the protein-only hypothesis. First, PrP and scrapie infectivity co-purifies by several procedures (19, 25, 32, 34), and no specific nucleic acid has been detected in highly purified PrP preparations (77–79). Second, genetic evidence points to an intimate linkage between the PrP disease and Prn-p, the gene that is considered to control incubation period (80, 81). There are two assumptions: either the PrP must bind to a specific host DNA sequence and act as a depressor, or the prion must violate part of the central dogma of molecular biology, that genetic information is transferred indirectly from nucleic acid to proteins (31, 32, 82). Many of the arguments used have been based on assumption that a nucleic acid of SEs is unprotected and free of a protein coat, as observed in viroids.

There are striking differences among the mammalian PrP gene sequences, and a great deal of allelic complexity in both PrP coding regions and in its flanking regions in the sheep (83–85). Reproducible differences in the incubation periods from different strains in different hosts' species with different genetic makeup indicate a genetic basis for the differences (75, 86–88) and that in mice one *Sinc* gene, and in sheep *Sip* gene, exerts a major influence on the incubation period of experimental scrapie (89). It has been suggested that the PrP gene is either closely linked or identical to genetic locus *Sinc* and *Sip* gene, which controls the incubation period (83). Later the *Sinc* gene and *Sip* gene have been described as Prn-i (80). Based on their response to the scrapie challenge, sheep *Sip* genotype are divided into "positive" (susceptible) and "negative" (resistant) lines (83). Comparative experimental transmission studies using brain extracts, collected from natural scrapie sheep and BSE, to mice and sheep of different *Sinc* and *Sic* genotypes revealed unexpectedly different patterns in incubation periods between transmission of BSE and sheep scrapie to mice and sheep (3). Both the "positive" (susceptible) and the "negative" (resistant) lines of sheep developed the clinical disease through both ic and oral transmission of the agent of BSE (3). These comparative studies clearly demonstrate that Prn-i–synonymous *Sinc*/*Sip* gene plays no role in the outcome of susceptibility, and does not affect the incubation period with the BSE agent. The incubation period in BSE is preferentially being influenced by the strain of BSE agent, which has a major influence on the outcome. A similar feature has been observed with the SEs agent isolated from domestic cats, tiger, and some exotic species of ruminants in zoos. However, different strains of the scrapie agent in different host species have different incubation periods, which exemplifies the influence of host and agent interaction.

Evidence for and Against a Nucleic Acid

Treatment with proteases reduces infectivity (82), which clearly demonstrated that, apart from any other classes of macromolecules, protein is an essential part of the scrapie agent (7). This property is not unique to the scrapie agent, because all conventional viruses normally depend on a protein coat for their integrity and infectivity. However, such negative results are always vulnerable to criticism. Evidence for the prion concept has come largely from the fact that the scrapie agent has been found to resist inactivation by harsh procedures that specifically hydrolyze or modify nucleic acids, a feature that argues that SEs are devoid of polynucleotides. It was demonstrated that after nuclease digestion up to 4-kb polyadenylated DNA sequences have been detected in CJD infectious fractions (90). Consideration also must be given to the fact that the nucleic acid of the scrapie genome, apart from

being small, might be very well protected like the *lac* gene, where binding of the protein to DNA is related to the dyad symmetry. Ultraviolet and ionizing radiation inactivation studies have not concluded the absence of a nucleic acid but, rather, have suggested that it must be small (91). It is unwise to use evidence of the failure of ultraviolet light inactivation of the scrapie agent as proof of the absence of nucleic acid, since a similar resistance spectrum to ultraviolet action can be demonstrated in a number of other microorganisms (92).

It has been well established that nucleic acids are resistant and remain biologically active after harsh chemical treatments, as observed in the routine preparation of plasmid DNA. Boiled and treated with NaOH, SDS, phenol, and chloroform, DNA is still found it to be biologically active (93). In the polymerase chain reaction, DNA is heated to over 90°C several times. Furthermore, DNA has been amplified after tissue has been fixed with formalin and blocked for over 40 years (94), while proteins cannot withstand such drastic treatment.

Differential Cleavage Susceptibility of dsDNA, ssDNA, and RNA to Zn^{2+} ion

Unwinding and rewinding of double-helical DNA by heating and cooling has been observed in the presence of copper (II) ions and zinc ions (95). 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 (95, 96). The presence of a heterogeneous population nucleic acid molecules <80 to 240 nt has been demonstrated in preparations of PrP that underwent extensive hydrolysis treatment with Zn^{2+} ions and nuclease (97). Thus, it appear that the PrP preparations extensively treated with Zn^{2+} ions used as inoculum contain enough nucleic acid to account for the infectivity (97).

Photochemical inactivation of viruses by psoralen derivatives depends on a number of factors, properties of viral nucleic acid, genome size, and interaction from psoralen cross-links, which in turn depends on existence of single-or double-strandedness and secondary structure "fold backs" in a nucleic acid (98). It is very obvious from the electron micrograph of DNA fragments that inactivation of double-stranded DNA is far more rapid than that of single-stranded DNA or RNA virus, by psoralen derivatives (98). In this study (98), many of the small closed circular molecules of single-stranded DNA from fd bacteriophage, included for the purposes of length calibration, appeared to have remained intact even after 30-min psoralen derivatives treatment (98). Most animal and plant viruses, as well as bacteriophage, are inactivated by hydroxylamine (99, 100), except for the paramyxoviruses (RNA), which are resistant to this action. Thus, the properties of an RNA and ssDNA virus would appear to be very different from that of dsDNA. Some additional evidence that no nucleic acid is present is the relative resistance to photochemical inactivation after treatment with the psoralen and resistance to treatment with 0.5 *M* hydroxylamine (101).

Evidence that the Scrapie Agent Contains Nucleic Acid

Host range of the scrapie agent. *Inoculation of non-natural hosts.* In the 1960s, a new chapter in the history of scrapie was started when Chandler reported the successful experimental transmission of the scrapie agent to mice inoculated with the brain suspension from naturally infected sheep (1). These experiments beyond doubt demonstrated the infectious nature of the disease. Subsequently, the disease was established in rats, in hamsters, and in over 20 other animal species, suggesting a very wide range of host susceptibility (6). Primary transmission from any species infected with SEs to a different new host species might affect only a minority of the inoculated animals and 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. Furthermore, transmission experiments involving both parenteral and intracerebral infections show the diversity of animal species that can be infected with scrapie agent, either directly or indirectly. One can conclude that almost every mammalian species can be affected by the scrapie agent to produce SEs, and clinically the disease appears in a wide range of host species, while the host genetic makeup may influence the length of the incubation period. The time scale of the disease in different species is characteristic: the incubation period usually bears a relationship to the average life expectancy of the species.

Strain Variations

There are a number of genetic markers of scrapie strain differences, and these are manifested in different organs and in different host functions, including behavioral, metabolic, and immunological (75). Scrapie strain differences have been described in several species (mice, hamsters, and mink), in natural scrapie in sheep and goats (75, 102–104), and in tissue culture systems (75). A phenomenon analogous to "interference" has been demonstrated by the inoculation of mice with a long-incubation (600 days+) strain of the scrapie agent followed a few weeks later by a short incubation (150 days) strain. Inoculation first with the "long" strain inhibits replication of the "shorter" strain, and vice versa. In experimental serial propagation, the scrapie strains breed true with a precise copy-

ing process to ensure stability of strains of the agent within a fixed host (103–106).

Unified Theory of Prion Propagation

Recently the transmissible and mutable nature of the agent led to hypothesis that the agent replicates, with the help of a conjectured normal host nucleic acid serving as co-prion (107). The hypothesis that the agent only contains a protein (32) or that the agent replicates with the help of a conjectured normal host nucleic acid (107), serving as co-prion, not only cannot explain 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 (103, 104). All transmission experiments with the SE agent suggest that PrP sequence is not copied in a new host species, but the host's own PrP is used to form SAF, the protease-resistant protein (Fig. 3). So far, there is no direct evidence to support that PrP is an infectious protein, and its role in pathogenesis remains unclear.

Evidence that PrP is not Essential for Infectivity

A number of studies have suggested that SAF/PrP is the infective agent or that infectivity is associated with it (15). However, a number of other studies have suggested that infectivity can be separated from both SAF and protein itself, and that PrP is not an essential component of the infectious agent (108). Infectivity has been demonstrated in the absence of prion rods (109). SAF/PrP can be demonstrated in brains of infected mice and hamsters but not in their spleen, which has a similar titer of infectivity (110). 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 (111). No effect was observed in mice or hamsters infected with two other scrapie strains. Furthermore, in some patients with "prion" disease PrP^{sc} is barely detectable or undetectable (61, 62, 112). The evidence supporting the concept that the scrapie agent is composed solely of protein is far from conclusive (35–39, 105), and Prusiner (31, 32) himself considers that the scrapie agent might contain some nucleic acid as well as protein.

Since its discovery (14, 27, 28) SAF/PrP's critical role in pathogenesis of the disease has remained to be resolved. However, once the protein was identified, our imagination, based on assumptions, has run as far as our experimental techniques will carry us. It is time to put behind us the question of the participation of a nucleic acid and stop pretending that it has simply not been identified. Working from basic principle of science, we can always start again in another direction. Looking back some years from the molecular viewpoint and approaching the problem through pathological observations may lead to the postulate that SAF/PrP is not the agent, but involved in the pathogenesis.

Tubulofilamentous Particles

Tubulofilamentous virus-like particles (Fig. 4, A and B) were originally described in scrapie mice and have been observed in hamsters infected with scrapie (113) in natural scrapie sheep, human CJD, and BSE (113–116). With the recent independent confirmation of these particles in scrapie-infected hamsters (117) and natural scrapie sheep, human CJD, and BSE (118–120), it now appears that the tubulofilamentous particles are specific to all the SEs (117–121). These structures have been demonstrated by negative staining in scrapie-infected hamsters at 20–31 days, which precedes the appearance of other neurological changes (35), and in mice, about half way through the incubation period, before vacuolation is apparent in the thalamus (122). The number and the density of the tubulofilamentous particles increase during subsequent weeks until the particles are readily seen, which correlates with infective titers (118, 122). It has also been established that the appearance of tubulofilamentous particles in experimental CJD and scrapie precedes the onset of clinical disease (118). The presence of these tubulofilamentous particles in natural CJD, scrapie sheep, and BSE is unlikely to be due to the blind passage of a carrier virus, as may be the case in experimental animals. Consequently these particles can no longer be dismissed as incidental to the disease process. These particles are readily distinguishable from normal cellular structures.

Using an "impression" negative staining technique, it has been demonstrated that the tubulofilamentous particles are more complex than previously expected (Fig. 4, A and B) and that SAF form the core of these particles (18, 35–37). By treating impression grids with a combination of proteases and nuclease, it was possible to demonstrate that the tubulofilamentous particles consist of an outer protein coat and a middle layer of ssDNA, while an innermost resistant SAF/PrP layer stayed intact (Fig. 4, C and D) (36–38). In some publications, tubulofilamentous particles have been referred to as tubulovesicular structures (118, 120, 122). To differentiate tubulofilamentous particles from SAF/PrP the term nemavirus (NVP; nema = filamentous) was introduced to describe tubulofilamentous particles (18, 39). The definitive diagnosis of SEs can be made by the "touch" impression technique, where both SAFs and NVP can be demonstrated from unfixed brain tissues within an hour. This method has

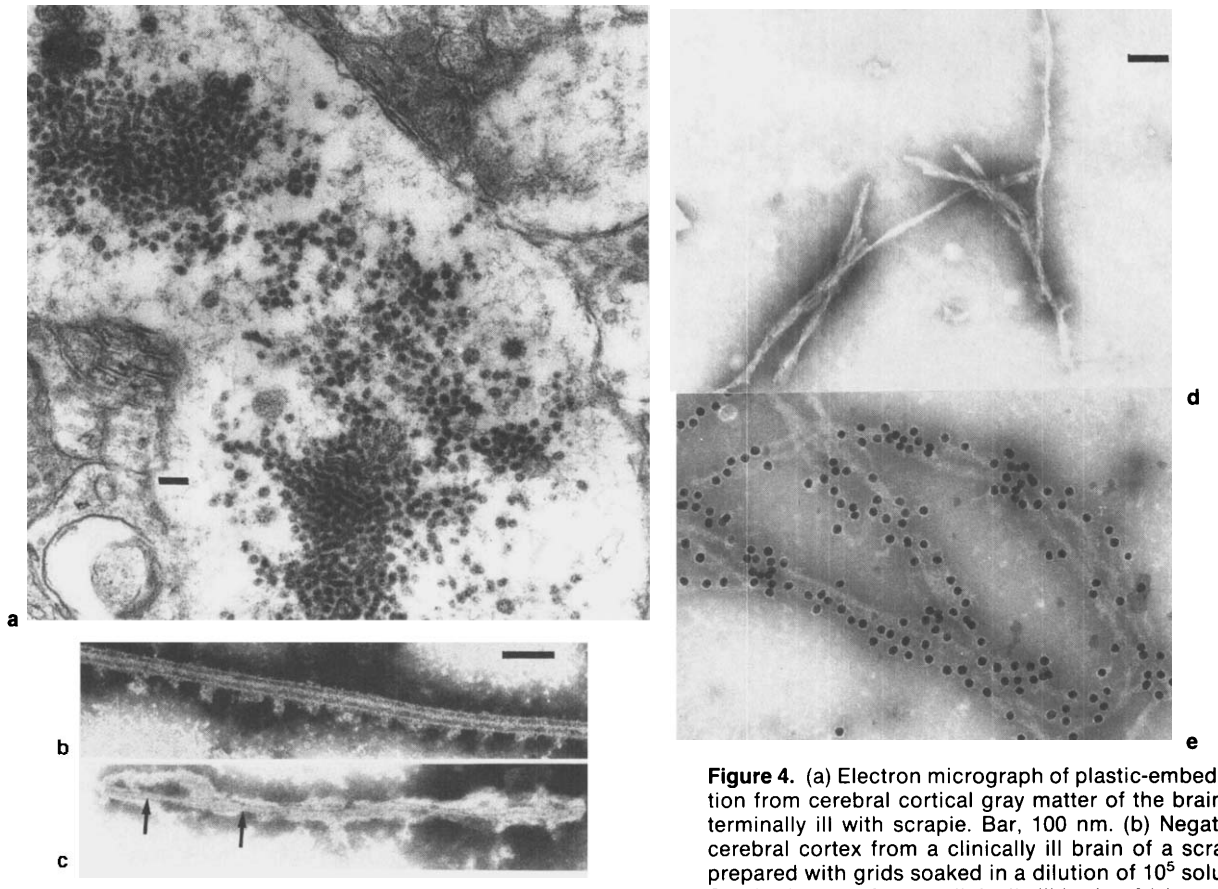


Figure 4. (a) Electron micrograph of plastic-embedded thin section from cerebral cortical gray matter of the brain of a mouse terminally ill with scrapie. Bar, 100 nm. (b) Negatively stained cerebral cortex from a clinically ill brain of a scrapie hamster prepared with grids soaked in a dilution of 10^5 solution of SDS. Cerebral cortex from a clinically ill brain of (c) a scrapie mouse prepared with grid soaked in 10^5 dilution of SDS and (d) a scrapie hamster soaked in 1% solution of SDS and (e) stained with an immunogold labeling technique. Note typical SAF. Grid bar, 100 nm.

been successfully applied using biopsy and autopsy brain tissue for the diagnosis of human CJD (3, 10, 17, 18).

Furthermore, treatment of grids with different concentrations of SDS (without proteinase K) have confirmed that the NVP contain a twisted filament core, SAF. Only after disruption of the outer coat of the NVP was it possible to stain SAF by both anti-SAF and anti-PrP, which forms the integral part of the NVP (37, 38). No SAF or SAF-like structures were seen in normal animals by this or any other method. These studies demonstrated that SAF/PrP is not an artifact of protease treatment of proteins at the purification step (18, 35–37). As NVP and SAF were observed from 10 days postinoculation from the right inoculated side of the brain, and from 18 days postinoculation from both right and left sides, this would suggest that replication of the agent starts at the local site of inoculation (10). This view is supported by the fact that the length of the NVP and SAF appeared to increase with incubation period, as was evident from the twists observed in the SAF. The appearance of NVP in the infected brains from 10 days postinoculation and their replication are consistent with the gradual increase of infective titer in the brain tissue. The formation of NVP and SAF as early as 10 days postinoculation is not consistent with the proposal that aggregation of PrP involves a nucleation event analogous to the seeding of a crystal (123).

Relationship of Tubulofilamentous Particles to SAF/PrP

It is a strange coincidence that SAF/PrP, once considered to be the agent or part of the agent, forms the core of the abnormal NVP, as seen in preparations from scrapie-infected hamster, mice, and human CJD brains (18, 35, 37). In contrast to the morphology of common viruses, which have a two-layer structure—a nucleic acid protected by an outer protein coat, the tubulofilamentous particle has a novel three-layer structure with the ssDNA lying sandwiched between two layers of protein, the inner being the protease-resistant protein (Fig. 4B). The nature of the outer protein coat still remains to be determined but appears

to be very hydrophilic (35, 36). It is possible that the outer protein coat with ssDNA is the infectious agent which co-purifies with the SAF/PrP fractions in various amounts as fluffy fragments.

Purification of Nucleic Acid

Nucleic acid purified from enriched preparations of both nemavirus and mitochondria with an infective titer of $10^{8.5}$/brain, and further subjected to alkaline gel electrophoresis, showed a striking difference in the mitochondria DNA (mtDNA) from scrapie-infected and uninfected brains (124, 125). In scrapie-infected brain tissue, mtDNA was present in multimers of high molecular weight as well as in normal monomers that did not differ in size or amount from monomeric mtDNA of uninfected control tissue. Since removal of mitochondrial outer membranes resulted in no detectable loss of titer, a mitochondrial association with scrapie infectivity was considered (124, 125). The mtDNA dimers and higher multimeric forms of mtDNA have been previously reported, particularly in established mammalian cell lines, such as HeLa (126) and mouse fibroblast L cells (127), as well as in certain tumor cells (128). Complex forms of mtDNA generally appear in very small amounts in normal cells, but under conditions of stress. The apparent disappearance of the slower migrating mtDNA band after incubation of the preparation of scrapie-infected tissue with mung bean nuclease suggested association of specific a single-stranded DNA with the monomeric mtDNA (124).

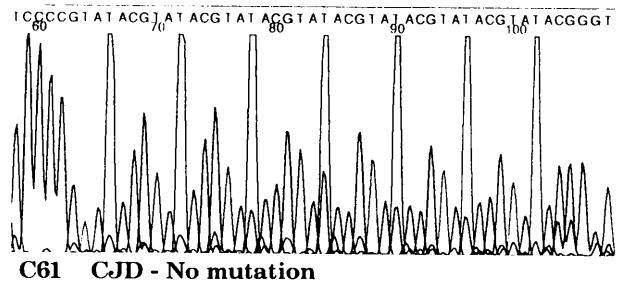
A single-stranded DNA (ssDNA) of 1.2 kb of about 0.49×10^6 Daltons has been visualized by electron microscopy in DNA prepared from scrapie-infected brain. Therefore, the argument that no nucleic acid has been demonstrated in scrapie-infected tissue is not true (129). It is interesting to note that the scrapie genome size estimation—obtained by comparing chemical and heat inactivation rate constant of the scrapie agent directly with the inactivation rate constants of bacteriophages of known nucleic acid size—was of the order of 0.75×10^6 for single-stranded and 1.6×10^6 for double-stranded virus (91).

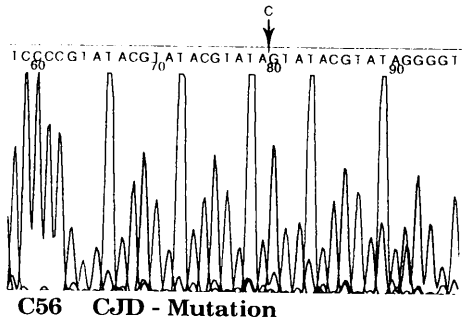
Further analysis of nucleic acid purified both from infected and from the equivalent fraction of uninfected brain, run on an alkaline agarose gel under alkaline conditions, revealed an unusual DNA band of about 1.2 kb which stained with ethidium bromide only from scrapie-infected brain (130). Ethidium bromide was only added to the resolving gel (first it was added by mistake in the alkaline gel). Staining of the ssDNA band with ethidium bromide under denaturing conditions suggested an unusual nature of the ssDNA band, which might have a very high base pairing property. ssDNA from scrapie-infected hamster brains and CJD- and BSE-infected brains has been cloned and partially sequenced, revealing an unusual palindromic six base $(TACGTA)_n$ repeat (130). An unusual palindromic six-base $(TACGTA)_{10}$ repeat sequence was obtained in BSE-infected brains. In three out of 10 CJD cases, both C and T were missing from one of the palindrome reading frame (Fig. 5). These changes in the sequence could account for the scrapie agent strain variations. These results demonstrate that each nemavirus ssDNA molecule consists of multiple copies of $(TACGTA)_n$ with intermediate segments (yet to be sequenced) spaced along the length of the ssDNA. Palindromic sequence features are common to many recognition sites for regulator proteins. Because of their unusual multipalindromic nature, under normal physiological conditions ssDNA molecules would base pair and coil in a very complicated fashion. Thus, in purified nucleic acid preparations the molecules of ssDNA may base pair within the same molecule or with other molecules forming a long chain (130). It is difficult to imagine how this nucleic acid exists *in vivo*, but once it has been disrupted the molecules would form a heterogeneous population. This was further evident from an experiment where NaOH-treated DNA was diluted to lower the concentration of NaOH to 100 m*M* NaOH and also from DNA samples treated with NaOH and formamide, and then run in alkaline conditions, in which the DNA remained stained, though with reduced intensity.

It is evident from this self-pairing property within the same molecule and with other molecules why most of the previous studies have failed to reveal a specific nucleic acid by subtractive hybridization methods, such as subtractive hybridization of cDNA libraries (131–133) or cloning residual DNA and RNA molecules present in highly purified infectious fractions. Neither of these approaches have identified a scrapie-specific nucleic acid (77).

A search for a putative scrapie genome using a refocusing gel electrophoresis method also failed to produce a specific nucleic acid, but the authors (78) 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 compared with previous studies (131–135). Since it was apparent from the "touch" technique that the ssDNA lies wrapped round SAF/PrP protected by a protein coat (18, 35, 36, 38), no detergents were used to disrupt the tissue.

The ssDNA molecule found is small. In a gel, it does not run as a single band. By the nature of single-stranded nucleic acid molecules, there is no melting point. From the structure of the ssDNA, one can see the reasons why many searches for a putative scrapie genome revealed no specific nucleic acid. The prop-





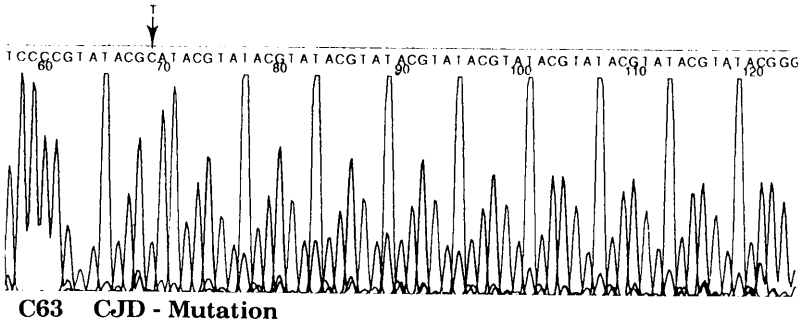


Figure 5. Sequence of three CJD clones showing mutation in the reading frame (arrows).

erty of such high base pairing as demonstrated by alkaline gel electrophoresis might have a direct bearing on the binding capacity of receptor protein molecules, which might in turn affect the protection of the nucleic acid against methylation, or ultraviolet or DNase degradation (136).

The morphological relationship between the PrP, ssDNA, the outer protein coat, and NVP is obvious, while the involvement in the disease pathogenesis, or its relationship to the agent remains to be determined. In all animal species, normal PrP 33–35-kDa precursor protein PrP^{c} is an essential housekeeping component, normally found on the cell surface (Fig. 6) (59). Conversion of PrP^{c} to protease-resistant protein/SAF is a post-translational process. 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 SEs agent. The interaction of PrP^{sc} with the normal PrP^{c} brings the post-translational modification in the normal isoform of the protein. This has been the main theme of Prusiner's hypothesis. Recently, Prusiner's group (73), 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 proposed chaperone interacts with the cellular PrP and modifies it into the protease-resistant protein PrP^{sc}. This is the first independent confirmation that "another" macromolecule than PrP^{sc} is required in the pathogenesis. There are two requirement for this process to occur. The first, to code for another protein macromolecule a DNA is required. Second, since, all hosts have PrP gene, to code for another protein macromolecule, DNA has to be nonhost. In previous studies, it had been demonstrated that this macromolecules is coded by the ssDNA of the nemavirus (10, 39).

It has been suggested (39) that in the infected cell the ssDNA wrapped around SAF/PrP codes for a nonhost peptide, an "accessory" protein which has an enzyme "double action" property, to cleave and cross-link (bind) precursor PrP into a chain. This brings a modification and fusion of precursor protein 33–35-kDa, possibly by a similar mechanism as in Willebrand factor (vWF) peptide, a plasma glycoprotein which exists as a single type homomultimeric units (137). The "double action" "accessory" protein

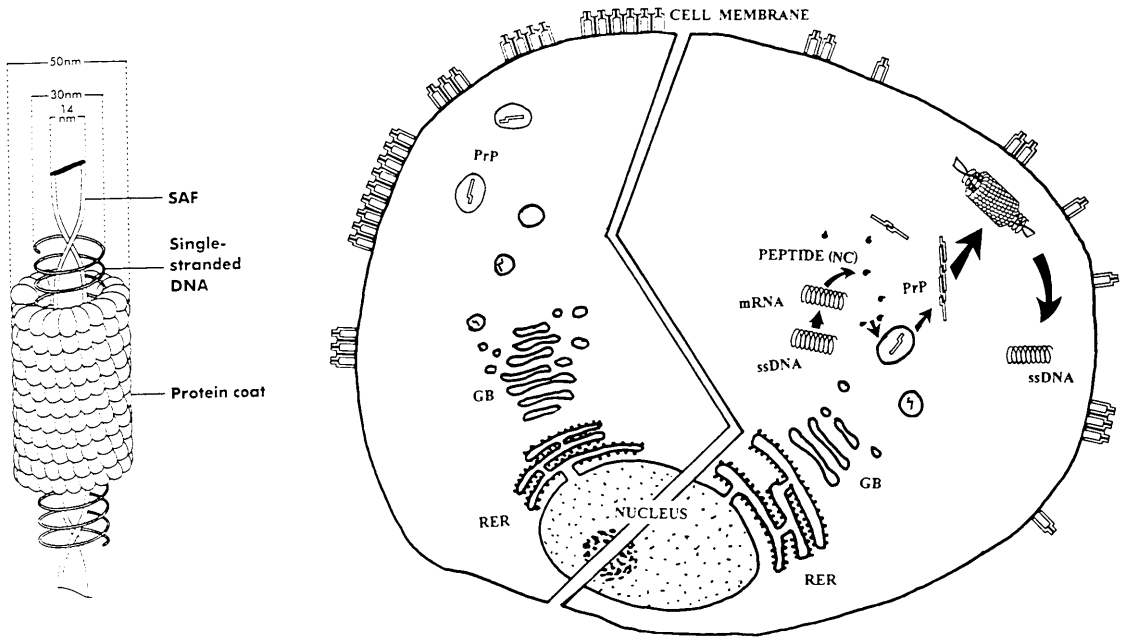


Figure 6. (a) A model of nemavirus, ssDNA coiled around the SAF as a spring protected by an outer protein coat. (b) A schematic representation of a cell showing endoplasmic reticulum (RER) Golgi body (GB) showing synthesis of PrP33–35, and *Nemo corrupta* (NC) is coded by ssDNA. When molecules of PrP33–35 make contact with the NC molecules, the latter interacts either as an enzyme or through having very similar receptor site to that of 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.

may have a very similar receptor site for PrP as on the cell membrane where PrP33–35 would lose the leader segment; thus, it will not reach its target site on the cell membrane (Fig. 6). After cleavage of the leader segment, the pro-peptide segment is converted into an active protease. The active enzyme peptide segments generated act against each other. This phenomenon has been well illustrated by chymotrypsinogen, which is converted by cleavage of a single peptide bond into active enzyme fragment called π-chymotrypsin then "self-acts" against other π-chymotrypsin molecules. This cleavage would explain the reduction in the size of precursor protein from 33–35 kDa to 27–30 kDa scrapie PrP (PrP^{sc}). Because of the "double action," it would be appropriate to designate the term *Nemo corrupta* for the "accessory" protein. It is also possible that this newly formed chain of PrP and peptide is not recognized by the host as nonprotein or, more likely, that protein, being protease resistant, cannot be degraded by macrophages, and therefore no antibodies are produced.

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 the tubulofilamentous particles. As PrP molecules are diverted away from cell membranes to form SAF, this results in a gradual weakening of cell membranes (Fig. 6). As the incubation period progresses, the process of replication of ssDNA and the accessory protein accelerates, more and more of the PrP molecules are diverted to form SAF, and the weak cell membranes reach breakage point. Vacuolation occurs and the clinical symptoms become evident after a long incubation period. In some hosts infected with the a low dose of SE agent, the breaking point of cell membranes may not be achieved in the life span of the host and thus the disease would remain subclinical. It is concluded that PrP, a normal protein, plays a role in the pathogenesis of SE and is closely associated with the ssDNA, which is securely anchored to SAF/PrP. The genomic information is held in this ssDNA; mutations in the ssDNA would explain the strains variation. The role of SAF/PrP is that it is being used as a convenient protein for holding and protection. This explains the true breeding of the strain of scrapie while the PrP changes in a new host.

During sequencing of the ssDNA, about 280 bp of the amyloid β-protein $(APP)_{751}$ mRNA gene sequenced (139) 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 in-

cubation period. The integration of ssDNA with host DNA would also explain familial inheritance of the natural scrapie in sheep, GSSS, and familial CJD cases.

I am indebted to many colleagues for advice on techniques, particularly, Drs. Anthony Sampson and Robert Ng, and to Miss Lisa Dowie for secretarial assistance.

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