SPONGIFORM ENCEPHALOPATHY: A PROBLEM OF PRIONS

Medical Grand Rounds
Parkland Memorial Hospital
September 27, 1990

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INTRODUCTION

The recent discovery of a new class of proteins is challenging our long-held concepts of degenerative brain disease, infectious disease, heredity, and even of life itself. The proteins are called prions and they have the amazing ability to infect animals and to replicate despite a total lack of any hereditary material in the form of DNA or RNA. The discovery ties together an economically troubling disease of Scottish sheep, an infectious disease of New Guinea cannibals, and a classic set of hereditary brain diseases with hyphenated German names. All of these diseases are grouped together under the heading subacute spongiform encephalopathies.

KURU, AN INFECTIOUS DISEASE OF CANNIBALS

The story begins in the remote highlands of Papua New Guinea with a tribe known as the Fore (Fig. 1). In the 1950's Australian physicians noted a high

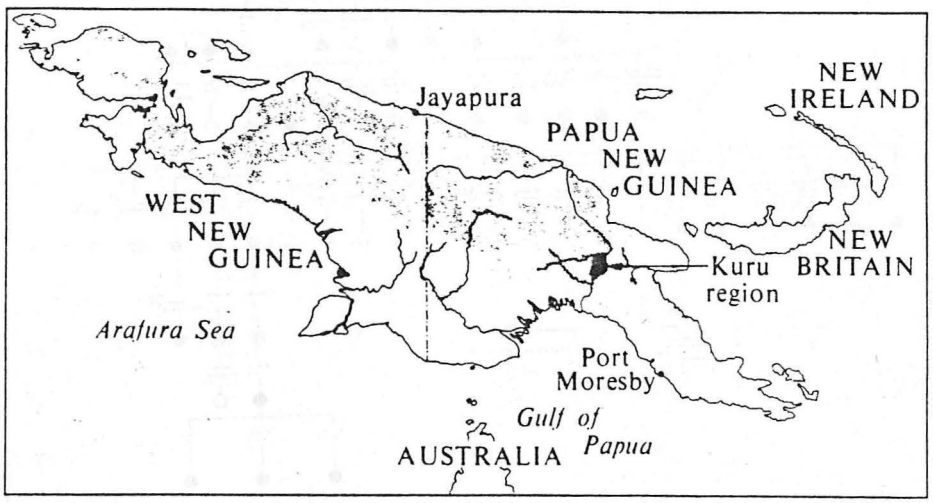

Fig. 1. The Kuru region of New Guinea.

incidence of a disease that the natives called kuru, which means shaking. Affected individuals develop cerebellar ataxia, progressive weakness and dementia, usually dying within months after onset. In 1959 a detailed description of kuru was published by Carleton Gadjusek, a young American physician (1). This article was read by a Scottish veterinarian named William J. Hadlow, who made the prescient suggestion that kuru was related to scrapie, a neurologic disease of sheep (2). The most striking similarity was in the pathology. In both diseases neurons become vacuolated, and large empty spaces

appear between the cells, giving the brain a spongy appearance. This is accompanied by an astrocytic gliosis and a variable deposition of amyloid. Even though scrapie appeared to be infectious there was a striking absence of any inflammatory or lymphocytic infiltrate. Scrapie had been transmitted experimentally from one sheep to another by intracerebral injection of brain homogenates from affected animals (2). Would kuru behave the same? To answer this question in 1965, Gadjusek injected brain extracts from kuru victims into the brains of chimpanzees and produced kuru (3). In turn, the brains from these infected chimpanzees produced kuru when injected into fresh chimpanzees. Fig. 2 shows the serial passage of kuru through seven generations of chimpanzees.

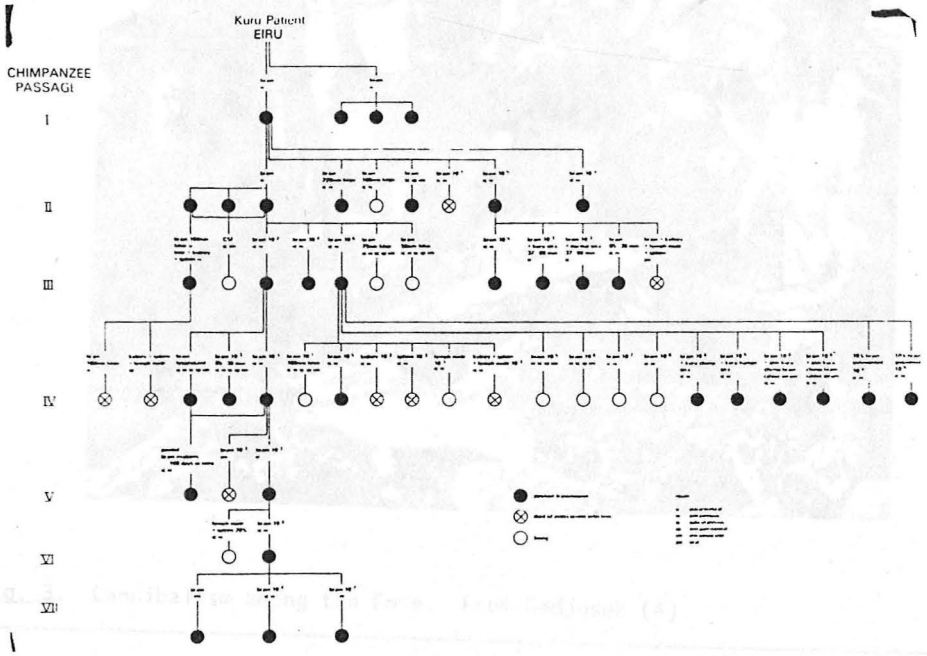

Fig. 2. Seven sequential passages of kuru agent from a single patient to multiple chimpanzees. From Gadjusek (4).

A striking feature of experimentally transmitted kuru and scrapie was the very long latent period. Recipient animals did not show symptoms until two years after the intracerebral injection. Once the symptoms appeared they were rapidly and relentlessly progressive. This led Gadjusek to suggest that kuru, like scrapie, is transmitted by a slow virus.

If kuru is an infectious disease how is it spread in nature? Gadjusek suggested that it was spread by cannibalism (4). Fore tribesmen ate the flesh of dead relatives, including those who died of kuru. This practice was most common among women and children, who were also the most frequently affected with the disease. Fig. 3 shows Fore villagers eating such a meal. The strongest

Fig. 3. Cannibalism among the Fore. From Gadjusek (4).

evidence in favor of the cannibalism hypothesis was Gadjusek's observation that the death rate from kuru declined precipitously after 1957 when cannibalism was halted (4) (Figs 4 and 5). The decline was steepest for 0-9 year old children, and next steepest for teenagers, which would be expected if the cessation of cannibalism were responsible. It is known that scrapie can be passed between sheep by the oral route, although the transmissibility by this route is 10' times less effective than it is by the intracerebral route. Kuru has also been transmitted orally to primates by feeding large amounts of brain extracts (5). However, spread by cannibalism need not have been oral. Women and children touched the flesh of the dead victims and smeared the tissue on their open wounds and abrasions (4). Thus, parenteral transmission was most likely.

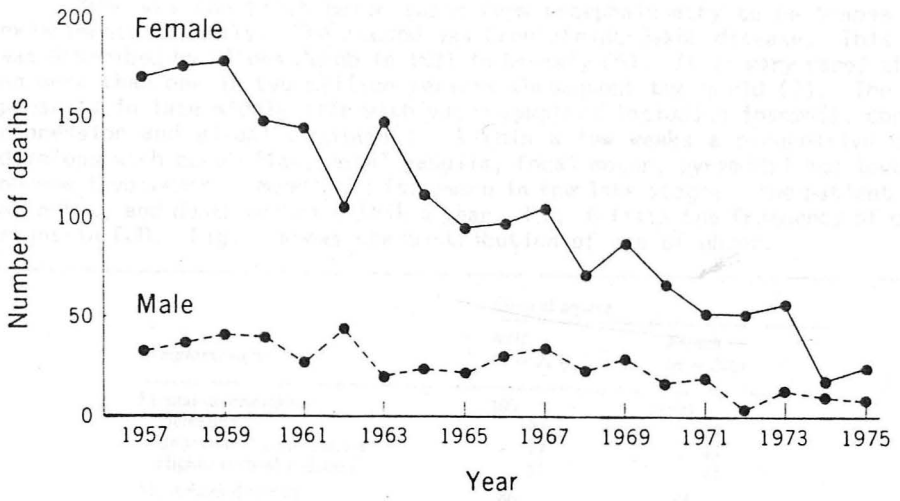

Fig. 4. Decline in number of deaths among Fore villagers following cessation of cannabilism in 1957 (4).

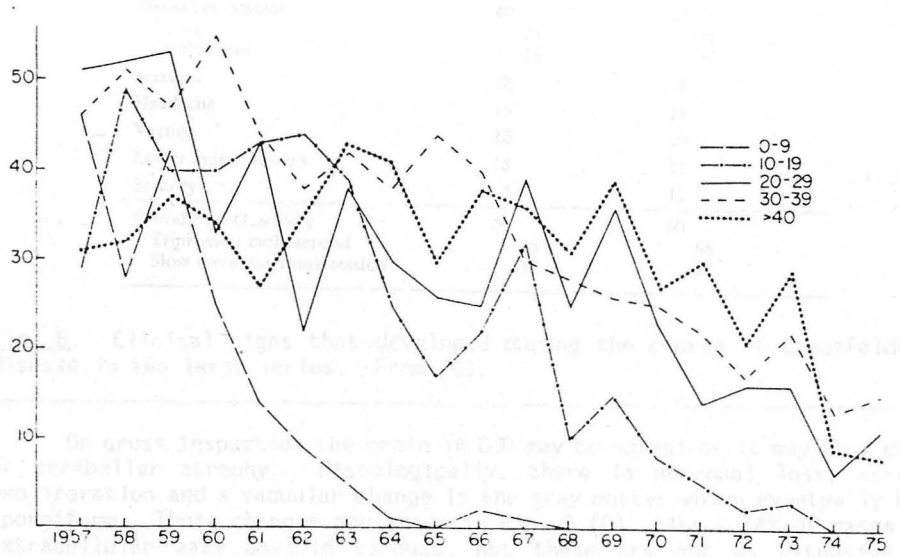

Fig. 5. Decline in deaths from kuru among Fore villagers each year since 1957 arranged by age at death (4).

CREUZFELDT-JAKOB DISEASE AND GERSTMANN-STRÄUSSLER SYNDROME: CIVILIZED VERSIONS OF KURU

Kuru was the first human spongiform encephalopathy to be transmitted to experimental animals. The second was Creutzfeldt-Jakob disease. This disease was described by Alfons Jakob in 1921 in Germany (6). It is very rare, affecting no more than one in two million persons throughout the world (7). The disease presents in late middle life with vague symptoms including insomnia, confusion, depression and visual complaints. Within a few weeks a progressive dementia develops with cerebellar, basal ganglia, focal motor, pyramidal and lower motor neuron involvement. Myoclonus is common in the late stages. The patient becomes akinetic, and death occurs within a year. Fig. 6 lists the frequency of clinical signs in CJD. Fig. 7 shows the distribution of age of onset.

Symptoms/signs	*Clinical course*	
	NIH (n = 223)	*French (n = 230)*
Mental deterioration	100	100
Dementia	100	96
Behavioural abnormalities	77	49
Higher cortical function	51	47
Movement disorder	86	91
Myoclonus	80	88
Other	37	26
Cerebellar	70	61
Pyramidal	67	43
Extra-pyramidal	57	67
Visual/oculomotor	40	42
Visual	23	31
Oculomotor	18	16
Seizures	18	8
Headache	16	14
Vertigo	16	10
Lower motor neuron	13	11
Sensory	5	11
Periodic EEG activity	59	80
Triphasic 1 cycle/second	46	56
Slow wave burst/suppression	14	32

Fig. 6. Clinical signs that developed during the course of Creuzfeldt-Jakob disease in two large series. From (6).

On gross inspection the brain in CJD may be normal or it may show cerebral or cerebellar atrophy. Histologically, there is neuronal loss, astrocytic proliferation and a vacuolar change in the gray matter which eventually becomes spongiform. These changes are shown in Fig. 8 (8). About 14% of cases have extracellular waxy amyloid plaques, but these are not as extensive as in Alzheimer's disease (9). About 15% of cases of CJD are familial and inherited as an autosomal dominant trait. There is a particularly high incidence of the genetic form in Libyan Jews. The remaining 85% are sporadic.

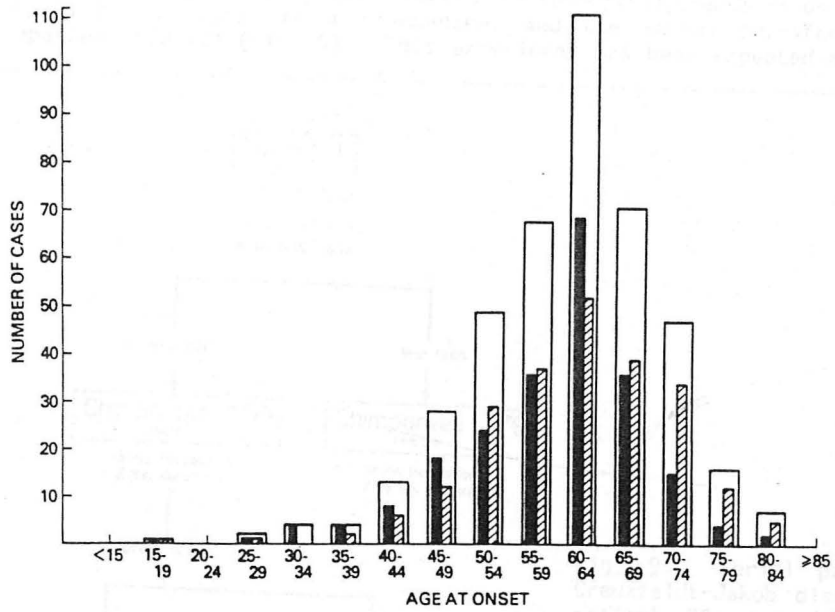

Fig. 7. Age at onset of Creutzfeldt-Jakob disease in NIH case series (solid bars), French case series (cross-hatched bars) and combined series (open bars). From (6).

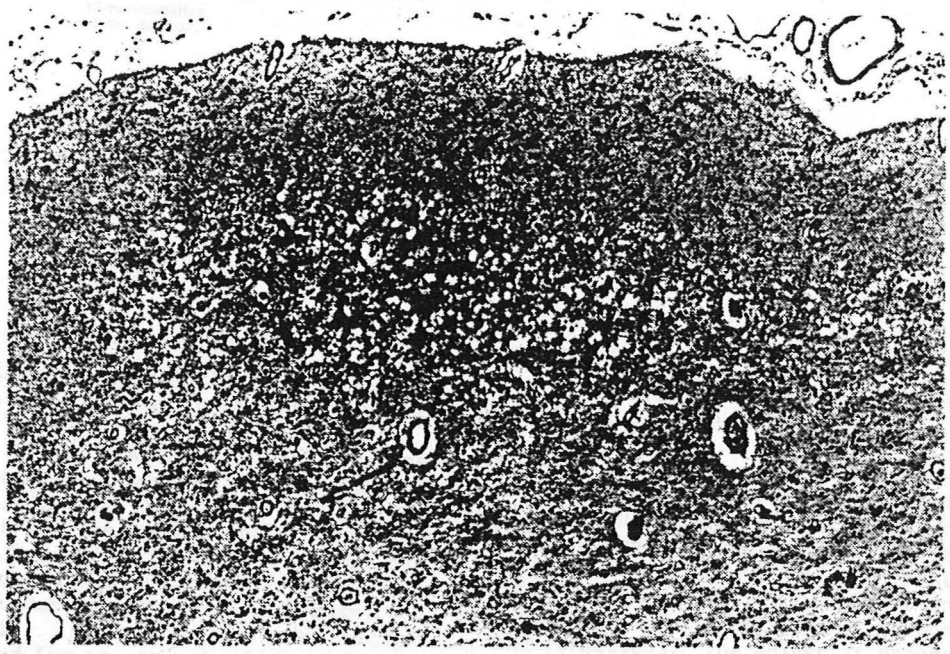

Fig. 8. Widespread spongiform change in frontal cortex of a patient with Creuzfeldt-Jakob disease (8).

In 1968 and 1969 Gadjusek reported the successful transmission of CJD from a human brain biopsy to a chimpanzee, and the serial transfer to other chimpanzees (10,11) (Fig. 9). This experiment has been repeated many times.

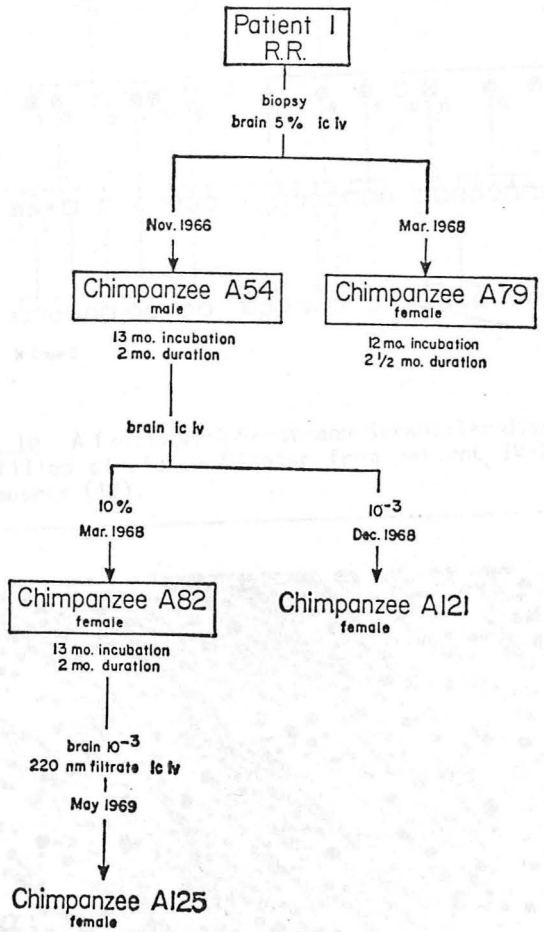

Fig. 9. Serial passage of Creuzfeldt-Jakob disease from patient RR. to chimpanzees. From Gadjusek (11).

Such transmissibility clearly qualifies CJD as a "slow virus" disease, and was a major factor in the awarding of the Nobel Prize to Gadjusek in 1976 (4).

As stated above, 15% of cases of CJD are genetic. There is also a closely related disease called Gerstmann-Sträussler syndrome that is entirely genetic (12). Fig. 10 shows a pedigree of a family with autosomal dominant Gerstmann-Sträussler syndrome (12). The major difference between Gerstmann-Sträussler and CJD is that it tends to occur earlier in life and to have a more protracted course. Also, there are many more amyloid plaques in the brain. Gerstmann-Sträussler disease was transmitted from a woman in this family to a marmoset (12). How can a disease be both genetic and infectious? The answer is prions, and for an account of their discovery we must first discuss scrapie.

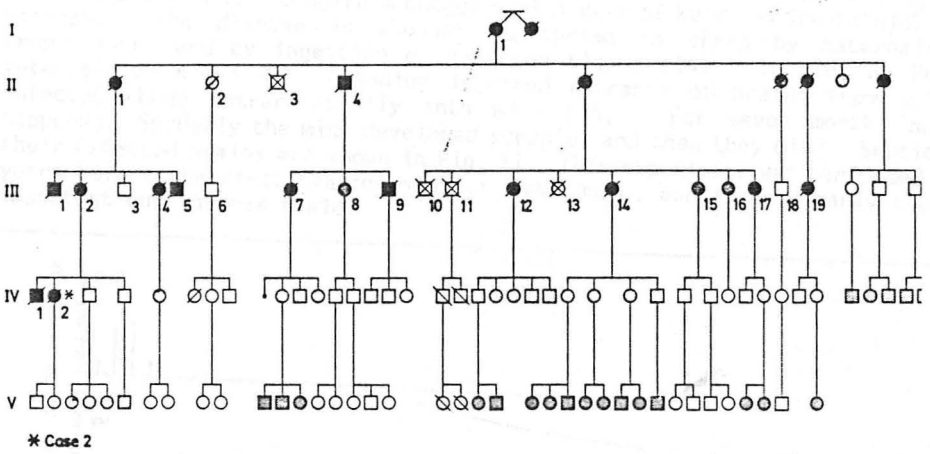

Fig. 10. A family with Gerstmann-Straussler disease. Affected individuals shown in filled circles. Disease from patient IV-2 (asterisk) was transmitted to marmosets (12).

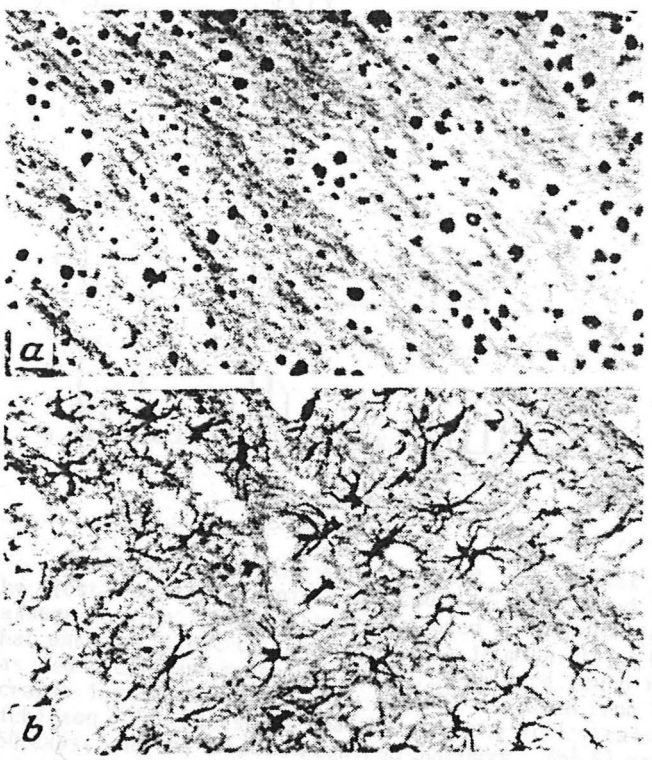

Fig. 11. Panel a (top): normal mouse brain. Panel b (bottom): corresponding area of brain in a mouse that was injected with a brain homogenate from a sheep with scrapie (15).

SCRAPIE, THE KEY TO IT ALL

Scrapie is a neurodegenerative disease of sheep and goats (2). The affected brain shows spongiform change reminiscent of kuru and Creutzfeldt-Jakob disease. The disease is thought to spread in sheep by maternal-fetal transmission and by ingestion of infected tissue (14). In 1961 an English veterinarian named R.L. Chandler injected extracts of brains from scrapie-infected sheep intracerebrally into mice (15). For seven months nothing happened. Suddenly the mice developed scrapie, and then they died. Sections of their affected brains are shown in Fig. 11. This experiment was performed four years before the first transmission of human kuru, and it is clearly the most important one in this field.

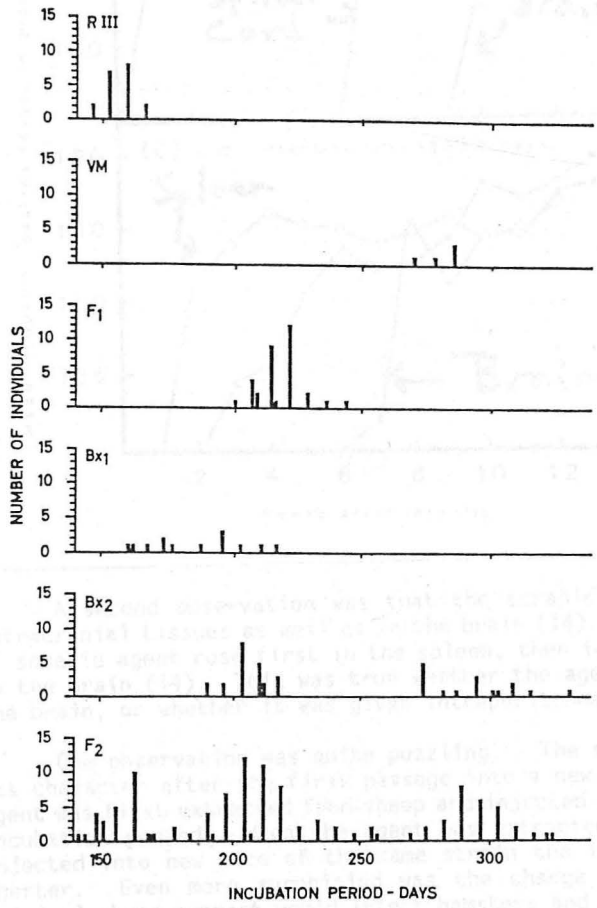

Fig. 12. Different incubation periods for mouse scrapie in different inbred mouse strains, and in various crosses (16).

The transfer of the scrapie agent to mice was a tremendous advance. For the first time the scrapie agent could be maintained in the laboratory by serial passage in mice. Methods were developed to titer the agent based on the dilution that was sufficient to produce disease in mice (14). Over the years a great deal was learned about scrapie. First, mice were found to vary genetically in the scrapie incubation time (16). Fig. 12 is taken from an important 1968 paper by Dickinson (16). It shows that mice of the RIII strain had incubation periods of 150 days, whereas VM mice required 300 days. The F1 generation was intermediate, and the F2 generation gave all three distributions. This is the expected behavior if the incubation period is controlled by a single gene with two alleles

that act in a co-dominant fashion. This finding was to have important utility in the evaluation of prions, as described below.

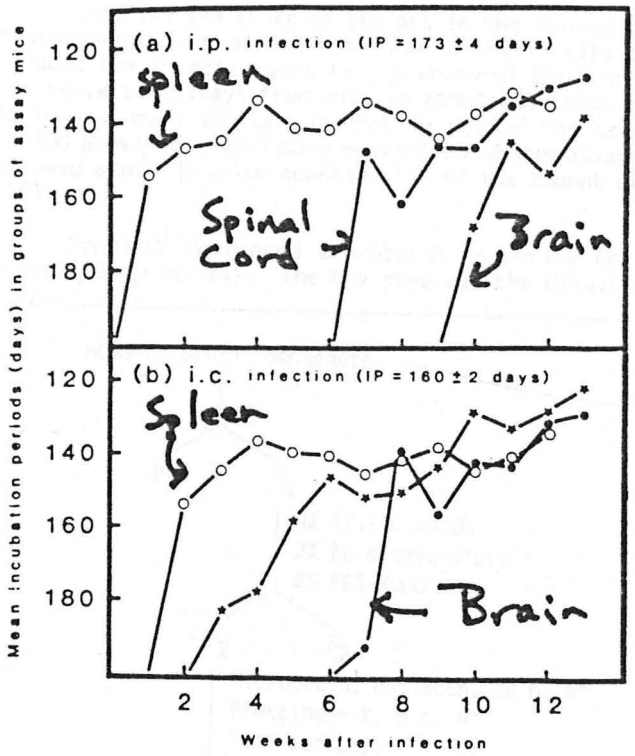

Fig. 13. Titer of scrapie agent in various tissues of mice after injection intraperitoneally (top) or intracerebrally (bottom) (14).

A second observation was that the scrapie agent could be recovered in extracranial tissues as well as in the brain (14). Fig. 13 shows that the titer of scrapie agent rose first in the spleen, then in the spinal cord, and finally in the brain (14). This was true whether the agent was injected directly into the brain, or whether it was given intraperitoneally.

One observation was quite puzzling. The scrapie agent seemed to change its character after the first passage into a new species (14). When a scrapie agent was first extracted from sheep and injected into mice there was a very long incubation period. When the agent was extracted from the affected mice, and injected into new mice of the same strain the incubation period was now much shorter. Even more surprising was the change in species specificity. The original sheep extract would infect hamsters and mice. However, once the agent was passaged in either of these species it would no longer infect the other species. Although mouse and hamster scrapie are both derived from sheep, each form of the agent is specific for the species in which it was first passaged. The scrapie agent must have undergone a profound change upon passage in animals. The nature of this change is now clear and will be discussed below.

Perhaps the most puzzling finding of all was that the scrapie agent did not seem to resemble any of the usual types of viruses. It did not seem to contain any nucleic acid (4). The agent was almost totally resistant to treatment with nucleic acid destroying enzymes, ultraviolet radiation and psoralen, all of which destroy viruses. On the other hand, it was sensitive to denaturing detergents

and other treatments that destroy proteins. Some people even suggested that the scrapie agent was not a virus, but rather some kind of infectious protein (4).

PRUSINER; PURVEYOR OF PRIONS

This was the state of the art in the late 1970's when Stanley Prusiner, an MD biochemist and neuroscientist at the University of California in San Francisco reduced the scrapie agent to a problem of biochemistry. Prusiner's first step was to switch assays from mice to hamsters, which had been shown by others (17) to have a much shorter incubation period for scrapie. The short incubation period provided a much more convenient 30-day assay for scrapie infectivity, and allowed a more precise quantitation of the amount of scrapie agent in any sample (18).

Prusiner developed a protocol to purify the scrapie agent from hamster brain (19) (Fig. 14). The key step was the digestion of the brain extract with

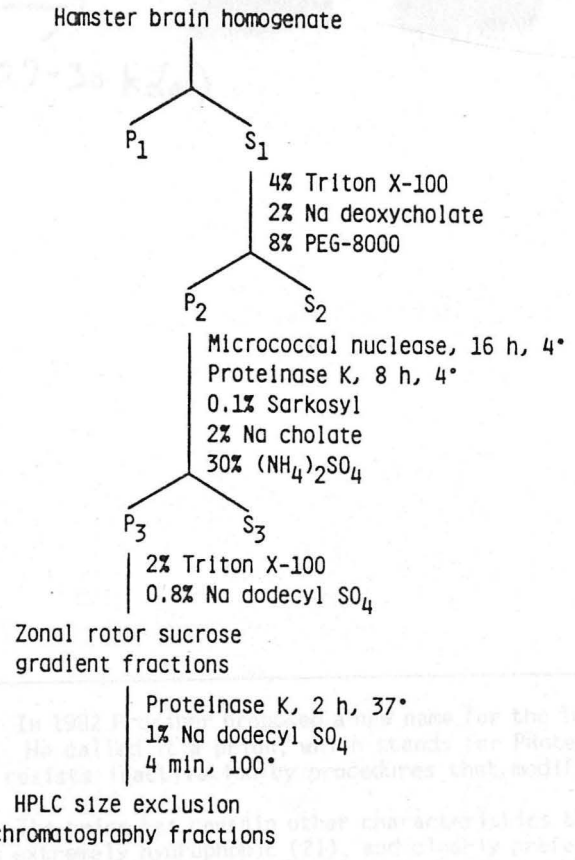

Fig. 14. Method for purification of infectious prions from brain of hamster infected with scrapie. S = supernatant; P = pellet (19).

proteinase K, an extremely powerful nonspecific protease. Nearly all of the proteins in the homogenate were destroyed, but scrapie infectivity was preserved. The purified protein showed a single major band on polyacrylamide gel electrophoresis (Fig. 15). It migrated with an apparent molecular weight of 27-30 kilodaltons. This protein, when injected into hamsters or mice, produced scrapie. There was no evidence for any associated nucleic acid, although it is impossible to rule this out with 100% certainty.

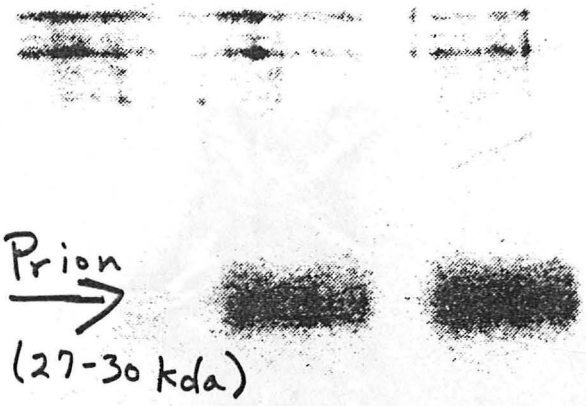

Fig. 15. Sodium dodecyl sulfate polyacrylamide gel electrophoresis of prion purified by high performance liquid chromatography. The top bands represent artifacts. The final preparation contains only one protein that migrates with a molecular weight of 27-30 kda (19).

In 1982 Prusiner proposed a new name for the infectious particle of scrapie (20). He called it a prion, which stands for PRoteinaceous INfectious particle that resists inactivation by procedures that modify nucleic acids.

The prion has certain other characteristics that are worth noting. First, it is extremely hydrophobic (21), and clearly prefers to be associated with cell membranes. This is attributable to the fact that it contains a covalently attached phospholipid anchor which is a phosphatidyl inositol glycan (21). Such anchors have been found on a variety of cell surface proteins. Second, the prion is a glycoprotein, containing carbohydrate in addition to amino acids (22). Third, it is infectious and resistant to proteases only when in its native form. If the protein is denatured with a strong detergent such as sodium dodecyl sulfate it no longer produces scrapie (22). It also becomes sensitive to protease digestion. Something in the 3-dimensional structure of the protein must allow it to produce scrapie, and at the same time makes it resistant to proteases.

A clue to the 3-dimensional structure came from microscopic study of the prion (23). Fig. 16 shows electron micrographs of a purified preparation of

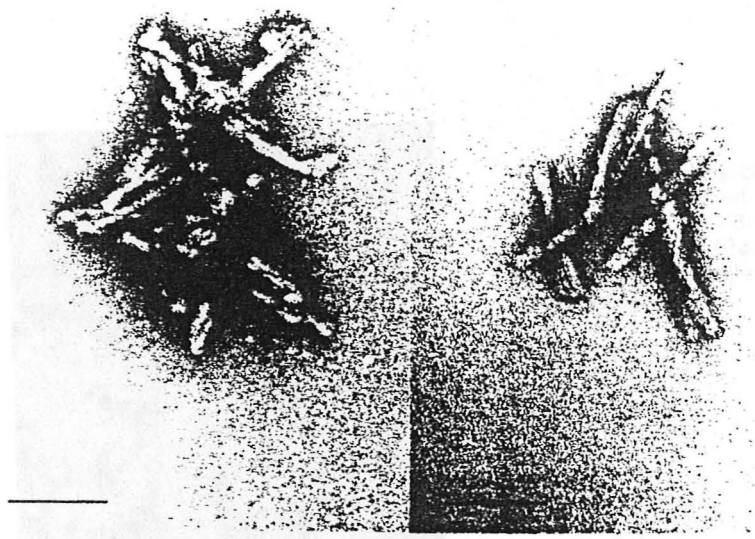

Fig. 16. Electron micrographs of prion rods that form when scrapie-infected hamster brains are extracted with detergents (23)

prions. The 27 kda proteins have lined up in linear arrays to form rods. Each rod contains as many as 1000 prion proteins. The rods stain with Congo red, and the stained rods show green birefringence (Fig. 17). All of these properties are those of amyloid proteins. It is clear from these studies that the prions form the amyloid deposits seen in the brains of animals or humans with spongiform encephalopathies. All proteins that form amyloid structures are resistant to proteases, and this explains the resistance of the scrapie agent to these enzymes.

Prusiner collaborated with Lee Hood at Cal Tech to obtained a partial amino acid sequence of the hamster prion protein (19). Then he collaborated with Charles Weissmann in Zurich to clone a cDNA complementary to the prion messenger RNA (24). The cDNA was cloned from a cDNA library prepared from the brain of a scrapie-infected hamster, and it was probed with an oligonucleotide corresponding to the protein sequence.

The cloning of the cDNA provided a big surprise (24). The prion was not a product of a viral genome. Rather, it was a normal hamster protein specified by a hamster gene. Fig. 18 shows Northern blots designed to detect the prion messenger RNA in hamster tissues (24). Panel A shows that the mRNA is present in uninfected control hamster brain. It does not increase after scrapie infection. Panel B shows that the same mRNA is present in heart and lung of uninfected hamsters. Panel C shows a series of "slot blots" that demonstrate the prion mRNA in brain, heart, lung, pancreas, spleen, testes and kidney of

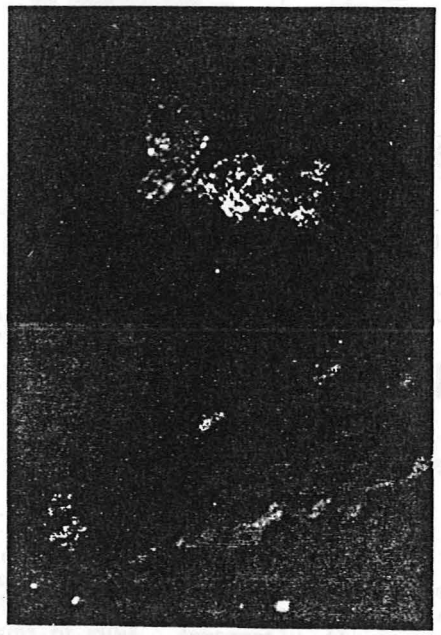

Fig. 17. Light micrographs of prion rods stained with Congo Red dye. Top: Bright field 500X. Middle:Polarized light 500X. Bottom: polarized light 200X. (23).

uninfected hamsters. In fact, the only tissue that lacks prion mRNA is the liver.

If the prion mRNA is present in uninfected hamsters, where is the prion protein? To answer this question Prusiner and Weissmann prepared an antibody against the prion protein, and performed immunoblots on detergent extracts of control and infected hamster brains (24) (Fig. 19). In both brains the antibody stained a protein of 33-35 kda. When the scrapie-infected extract was treated with proteinase K the size was reduced to 27-30 kda. When the normal extract was treated with proteinase K the scrapie protein was destroyed. A similar set of findings with hamster and mouse prions is shown in Fig. 20 (25).

These findings are staggering. They tell us that the prion protein is a normal membrane protein encoded by the host genome, and that something happens to the protein during scrapie infection that converts the protein into an alternate form. Fig. 21 compares the properties of the normal and altered forms of the prion (22). The alternate form of the protein forms amyloid, is resistant

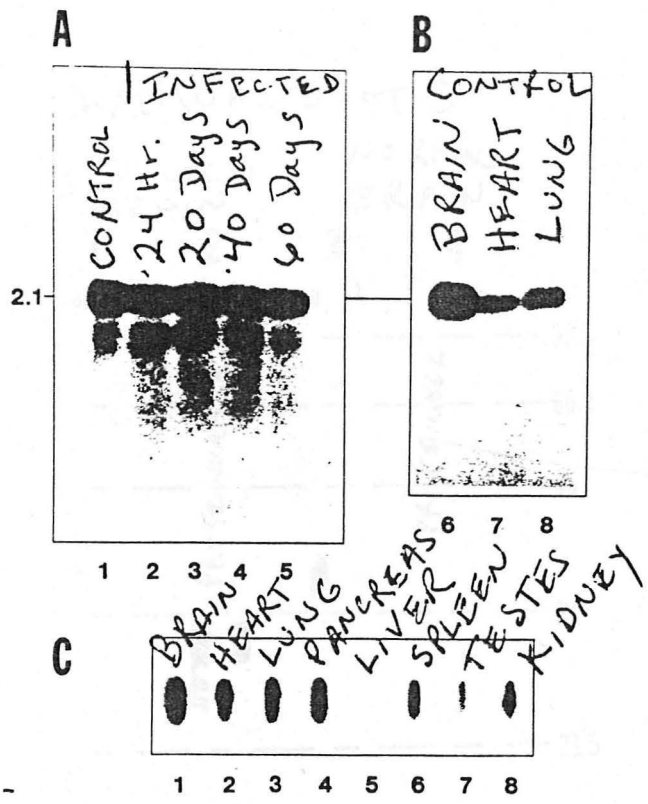

Fig. 18. A: Northern blot of mRNA extracted from brains of control and scrapie-infected hamsters at various times after infection. B: Northern blots of mRNA from three tissues of control (uninfected) hamsters. C: "Slot blots" of mRNA from various tissues of control, uninfected hamsters. All blots were probed with a cDNA encoding the hamster scrapie prion. (24)

to proteases, and has the ability to cause scrapie when injected into the brains of normal animals. It is important to note that the amount of the prion protein increases more than 10-fold in scrapie-infected brain, but there is no change in the amount of mRNA. Apparently, the scrapie change makes the protein resistant to proteases, and this allows it to accumulate in tissues. Fig. 22 is a model from Prusiner (22) that visually illustrates the differences between these two proteins.

These findings explain several of the most puzzling features of scrapie. First is the long latent period. When a few molecules of a modified prion enter the brain of a normal animal they modify the normal prion proteins that are already there. The modified prions are resistant to proteases and so they gradually accumulate, but this takes time.

The prion hypothesis also explains the alteration in species specificity that occurs with serial passage of the scrapie agent. When the sheep prion was injected into hamsters it caused the hamster prion to accumulate. When scrapie was passaged to the second hamster the hamster that was transmitted. Hamster prions do not infect mouse brains. Therefore, the scrapie agent had changed its specificity and would no longer infect the mouse.

What change could convert a normal cellular protein into a form that becomes toxic and resistant to proteases? There is no clue from the primary protein sequence, which is shown in Fig. 23 (27). The prion has a classic signal

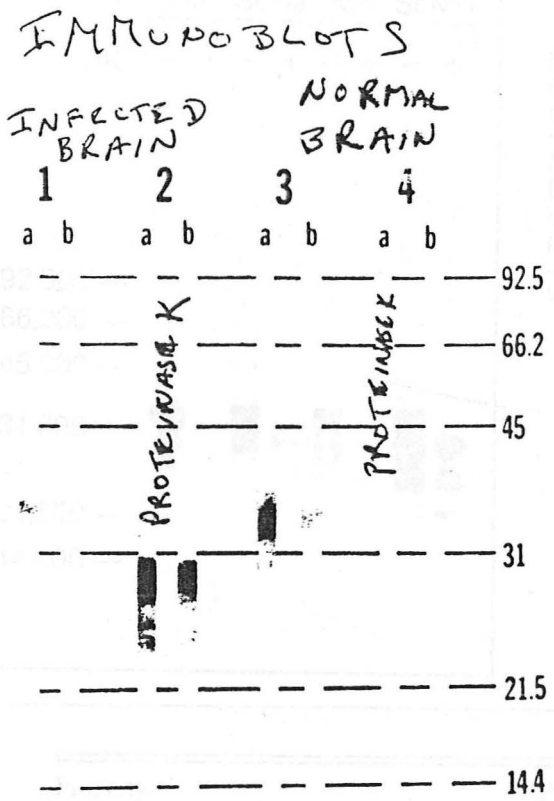

Fig. 19. Immunoblots of extracts of hamster brain subjected to polyacrylamide gel electrophoresis and incubated with a crude antiserum directed against hamster prion protein (lanes labeled a) or an affinity-purified immunoglobulin from the same antibody preparation (lanes b). Samples 1 and 2 are from a scrapie-infected hamster and samples 3 and 4 are from a control hamster. Samples 2 and 4 were treated with proteinase K prior to electrophoresis (24).

sequence, which is cleaved after synthesis. Then there are four (or five) repeats of an octamer beginning with tryptophane-glycine-glutamine (WGQ). Then there is an alanine-glycine rich region. At the C-terminus there is a hydrophobic region that serves as the initial membrane anchor and is cleaved off when the phosphatidyl inositol anchor is added. There are two sites for asparagine-linked glycosylation that occur in a loop region that is bounded by an intra-chain disulfide bond.

So far, the cellular and scrapie forms of the prion have been found to be identical (22). They migrate with the same molecular weight on SDS gels. They have the same amino acid sequence, and the same amino and carboxyl termini, suggesting that partial proteolysis does not occur. Both forms have carbohydrate, and it is not known whether the carbohydrate structure differs between the two forms. Since they migrate similarly on electrophoresis there is no gross change in the amount of carbohydrate.

Recall that the proteinase K fragment of the prion protein is infectious (22). The major site of cleavage is at amino acid 59 (27). Thus, the information that allows the scrapie prion to modify the host prion is contained between residue 59 and the carboxyl terminus at residue 232.

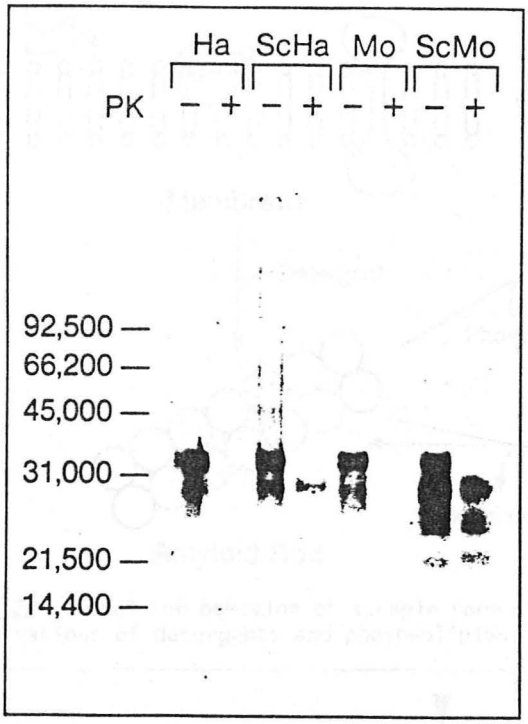

Fig. 20. Immunoblots of brain extracts from normal hamsters (Ha), scrapie-infected hamsters (ScHa), normal mice (Mo), and scrapie-infected mice (ScMo) probed with a polyclonal rabbit antibody against hamster prions. Extracts were either untreated, or digested with proteinase K as indicated. (25)

Property	PrP^C	PrP^{Sc}
Concentration in normal cells	$\sim 1\ \mu g\ g^{-1}$	–
Concentration in scrapie-infected cells	$\sim 1\ \mu g\ g^{-1}$	$\sim 10\ \mu g^{-1}$
Purifies with scrapie infectivity[b]	–	+
Released from membranes with PIPLC digestion	+	–
Polymerized into amyloid rods upon limited proteolysis and detergent extraction	–	+[c]
Resistant to proteinase K digestion	–	PrP 27–30

[a] +, yes; −, no.

[b] Two protocols were used: (*a*) detergent extraction, sedimentation, and protease digestion; (*b*) PrP 27–30 monoclonal antibody affinity chromatography.

[c] Indistinguishable from amyloid filaments forming plaques.

Fig. 21. Different properties of the prion protein in normal brain cells (PrP^c) and in scrapie-infected brain cells (PrP^{sc}) (22)

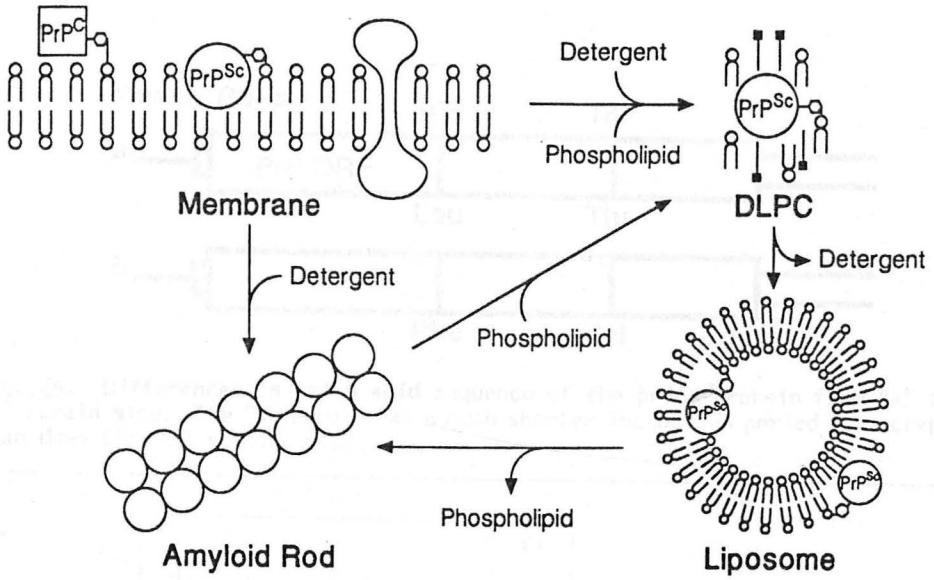

Fig. 22. Model for behavior of scrapie form of prion protein (PrP^{sc}) in various combinations of detergents and phospholipids. (22)

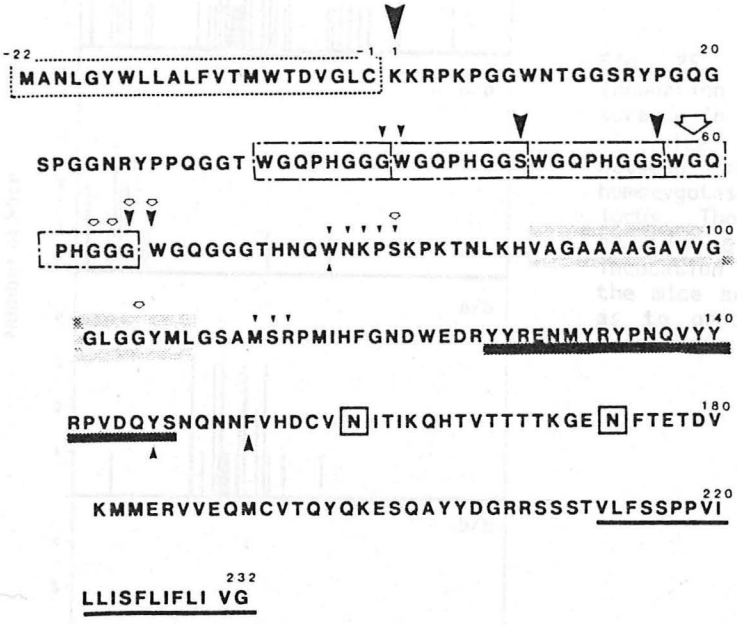

Fig. 23. Amino acid sequence of prion protein as deduced from cDNA sequence. Residues -22 to -1 are the signal sequence, which is removed during translation. The arrows indicate sites of proteolytic cleavage in vivo and in vitro (27)

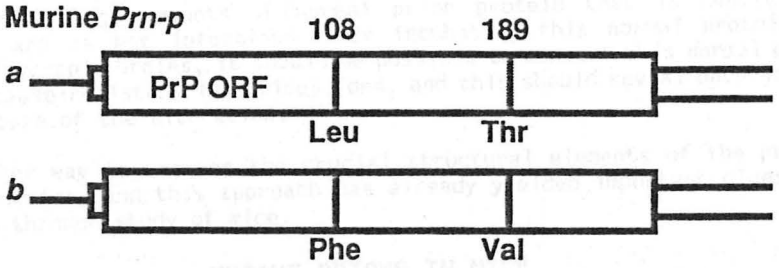

Fig. 24. Differences in amino acid sequence of the prion protein from "a" and "b" strain mice. The "a" strain has a much shorter incubation period for scrapie than does the "b" strain. (22).

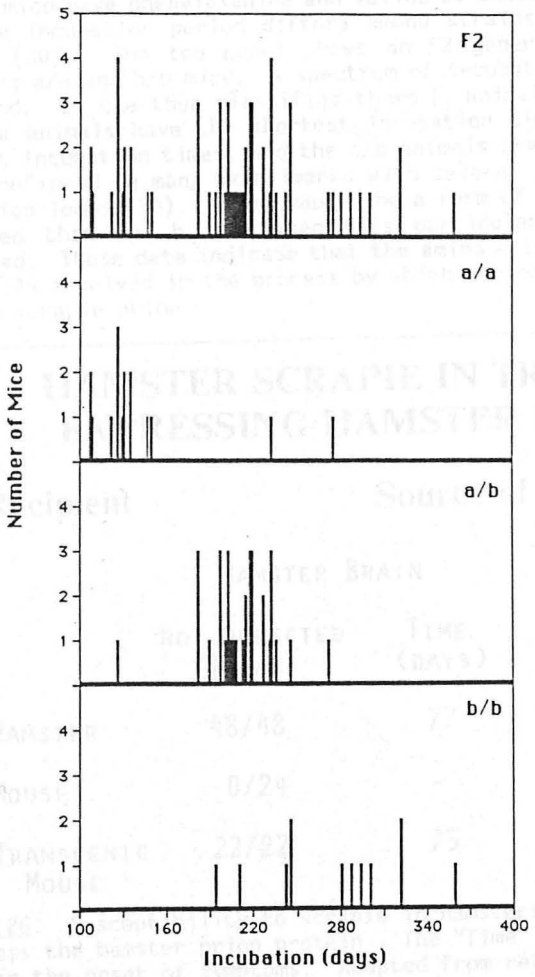

Fig. 25. Top panel: Incubation period for scrapie in F2 generation of mice from mating between a/a and b/b homozygotes at the prion locus. The bottom three panels show the incubation times when the mice are classified as to genotype at the prion locus. (30)

Despite these negative findings, there must be some structural explanation for the altered properties of the scrapie prion. So far only trace amounts of the normal cellular form of the prion have been purified, and the information about its structure is still sketchy. This will soon change. The normal prion gene has been introduced into cultured hamster cells by transfection (28). The cells produce large amounts of normal prion protein that is sensitive to proteases, and is not infectious. By incubating this normal protein with extracts of scrapie brains, it should be possible to convert this normal protein into a protease-resistant infectious form, and this should reveal once-and-for-all the nature of the alteration.

Another way to uncover the crucial structural elements of the prion is through genetics, and this approach has already yielded important clues. The first came through study of mice.

MUTANT PRIONS IN MICE

Recall that different strains of mice have different incubation periods for scrapie (16). We now know that the prion proteins in the short and long incubation strains have different sequences (22,29,30) (Fig. 24). The "a" strain mice have a leucine at position 108 and a threonine at position 189. The "b" strain mice have phenylalanine and valine at these two positions. Fig. 25 shows how the incubation period differs among strains with these two forms of the prions (30). The top panel shows an F2 generation which was derived from crossing a/a and b/b mice. A spectrum of incubation times from 100 to 350 days is found. If one then classifies these F_2 animals by genotype it is seen that the a/a animals have the shortest incubation times, the b/b animals have the longest incubation times, and the a/b animals are in between. This linkage has been confirmed in many experiments with several strains of mice that differ at the prion locus (30). Presumably the a form of the host prion is more readily modified than the b form when this particular strain of mouse scrapie is injected. These data indicate that the amino acids at positions 108 and 189 are crucially involved in the process by which the normal prion protein is modified by the scrapie prion.

HAMSTER SCRAPIE IN TRANSGENIC MICE EXPRESSING HAMSTER PRION PROTEIN

Recipient	Source of Scrapie Inoculum			
	Hamster Brain		Mouse Brain	
	No. Affected /Total	Time (days)	No. Affected /Total	Time (days)
Hamster	48/48	77	3/16	258-494
Mouse	0/24	-	17/21	126
Transgenic Mouse	22/22	75	0/21	-

Fig. 26. Susceptibility to scrapie in hamsters, mice and transgenic mice that express the hamster prion protein. The "Time" refers to the incubation period before the onset of symptoms. Adapted from reference 31.

PRIONS IN TRANSGENIC MICE

The crucial role of the host prion in scrapie has been demonstrated most dramatically by experiments in transgenic mice (31) (Fig. 26). Prusiner and co-workers injected the gene encoding hamster prions into mouse embryos. They then derived a strain of mice that produce the normal hamster prion protein as well as the mouse prion in the brain. These mice do not develop scrapie spontaneously because the hamster prion is the normal gene product. However, the transgenic mice now become sensitive to infection with the hamster scrapie agent. Whereas normal mice are strongly resistant to hamster scrapie, the transgenic mice are all sensitive. The incubation time is the same as in the usual hamster scrapie. This dramatic experiment confirms that the sensitivity of an animal to scrapie is dictated by the normal prion that it produces.

When the transgenic mice develop scrapie, the prion that they produce is a product of the hamster gene, and not the mouse gene. Fig. 27 shows that

SCRAPIE-INFECTED TRANSGENIC MICE PRODUCE HAMSTER SCRAPIE

Brain Sample Inoculated	Recipient: Hamster		Recipient: Mouse	
		Incubation Time		Incubation Time
	No.	(days)	No.	(days)
Hamster	48	77	-	-
Mouse	-	-	24	138
Hamster/Mouse Mixture	30	90	31	157
Transgenic Mouse with Scrapie	23	75	30	(>90)

Fig. 27. Extracts were prepared from the source indicated on the left, and were injected into normal hamsters or normal mice as indicated on the right. The number of animals that developed scrapie is indicated, as is the time in days to the onset of symptoms. Extracts from the transgenic mice with scrapie failed to produce symptoms in 30 mice up until the time the paper was written (>90 days). Adapted from reference 31.

extracts from the brains of scrapie-infected transgenic mice produce scrapie in hamsters, indicating that the hamster form of the prion is the one that is being modified. The brain extracts were much less potent in producing mouse scrapie. Although these results are still preliminary they suggest that the prion of hamster scrapie can only activate the normal hamster prion, and it cannot activate the mouse prion. This may explain the species specificity of scrapie.

PRIONS CAUSE CREUZFELDT-JAKOB DISEASE AND GERSTAMANN-STRÄUSSLER SYNDROME

All of the progress in scrapie is beginning to have an impact on our understanding of the human spongiform encephalopathies. Fig. 28 shows an

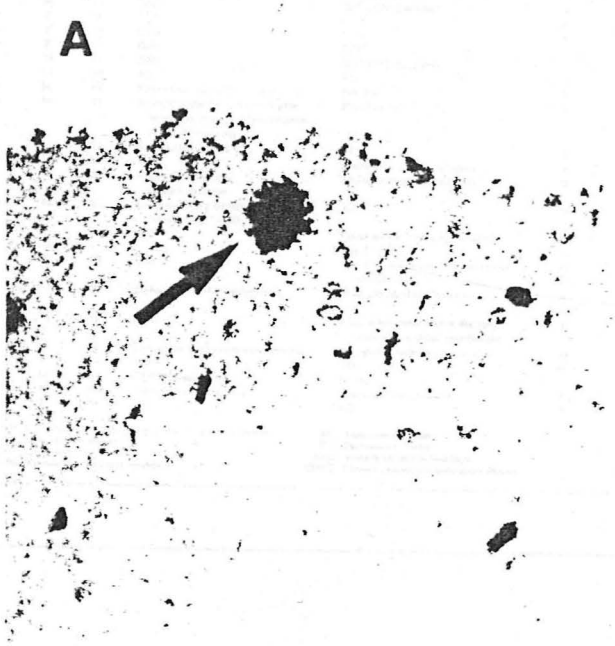

Fig. 28. An amyloid plaque in the cerebellum of a patient with Creutzfeldt-Jakob disease stained with a rabbit antiserum directed against the hamster prion protein and visualized with peroxidase. Amyloid plaques from patients with Alzheimer's disease were negative (not shown) (32).

immunoblot of an amyloid plaque from a CJD patient which shows staining with an antibody against hamster prions (32). Recently, Prusiner and co-workers have established an immunoassay for prions in extracts of brain that are treated with protease to destroy the normal prion protein. They have shown that this assay specifically detects patients with CJD and Gerstmann-Sträussler syndrome (33) (Fig. 29).

I have already discussed how Creutzfeldt-Jakob disease can occur in either sporadic or genetic forms. It can also occur in an infectious form that is iatrogenic. Fig. 30 shows a partial list of cases iatrogenic CJD (6). The first example occurred in a 55 year old woman who received a corneal transplant from a man who died from CJD (34). 18 months after the transplant the recipient developed CJD and she died shortly thereafter. Extracts of her brain were injected into chimpanzees, and they produced CJD (35). Other examples of infectious or iatrogenic CJD have now been widely recognized. The disease has occurred in neurosurgeons and in histology technicians who handled brains from CJD patients (36). It also occurred in several patients who received growth hormone that was isolated from pooled human pituitary glands (8). The upper

No.	Sex	Age	Clinical diagnosis	Pathology diagnosis	Nitrocellulose transfer PrP^{CJD}	Nitrocellulose transfer β-AP
1	M	57	CJD	CJD	+	0
2	M	61	CJD	No histology	+	0
3	F	68	CJD	CJD	+	Weak +
4	F	67	CJD	CJD	+	+
5	—	—	Familial CJD	CJD	+	0
6	M	57	CJD	No histology	+	0
7	M	54	CJD	CJD (PrP plaques)	+	0
8	F	70	CJD	CJD	+	0
9	F	78	CJD	CJD	+	Weak +
10	F	81	CJD	CJD	+	0
11	M	48	GSS	GSS (PrP plaques)	+	0
12	M	80	AD	AD	0	+
13	M	62	Pulmonary renal syndrome	Normal	0	+
14	M	78	Normal pressure hydrocephalus; dementia with gait disturbance; cortical blindness	Possible AD	0	+
15	M	79	AD; cardiac arrest	AD	0	+
16	M	70	CJD	Meningopolio encephalitis	0	0
17	F	63	Presenile dementia; seizure disorder	Multi-infarct dementia and AD	0	+
18	M	82	Long-standing dementia with myoclonus; ? CJD	AD	0	+
19	F	69	CJD	Focal spongiform degeneration	0	0
20	—	—		Shy-Drager	0	0
21	F	69	CJD	AD; PD; AV malformation upper cervical cord	0	Weak +
22	M	25	Refractory status epilepticus; sepsis	Multiple cerebral infarcts	0	0
23	M	49	ALL	ALL; adult respiratory distress syndrome; cranial irradiation	0	0
24	M	75	Dementia with movement disorder	PD; global ischemic necrosis	0	0
25	F	98	AD	AD	0	+
26	M	68	COPD; pneumonia	Normal	0	Weak +
27	M	59	Adenocarcinoma, lung	Small cortical infarcts	0	0
28	F	84	AD	AD	0	+

PrP^{CJD} Creutzfeldt-Jakob disease isoform of the prion protein.
β-AP Beta-amyloid peptide.
CJD Creutzfeldt-Jakob disease.
GSS Gerstmann-Sträussler syndrome.
AD Alzheimer's disease.
PD Parkinson's disease.
ALL Acute lymphocytic leukemia.
COPD Chronic obstructive pulmonary disease.

Fig. 29. Extracts of brains from patients with Creutzfeldt-Jakob disease react with an antibody to hamster prions after gel electrophoresis and blotting on nitrocellulose membranes. The extracts do not react with an antibody against the amyloid beta-protein that is found in Alzheimer's disease. (33)

Source of infection	*Route of inoculation*	*Incubation period*	*Clinical duration*
Corneal transplant	intraocular	18 months	8 months
Deep EEG electrodes	intracerebral	16 months	8 months
		20 months	23 months
Surgical instruments	intracerebral	18 months	2 months
		18 months	3 months
		19 months	3 months
		28 months	6 months
Dura mater graft	intracerebral	19 months	4 months
Human growth hormone	subcutaneous/ intramuscular	?–10 years	–[a]
		4–18 years	6 months
		6–13 years	19 months
		8–12 years	11 months
		12–15 years	14 months
		15–21 years	10 months
		19–23 years	3 months

Fig. 30. Iatrogenic cases of Creuzfeldt-Jakob disease as of 1987. More have been reported since then. (6).

panel of Fig. 31 shows that extracts from the brain of one of these patients contained fibrils that look just like the fibrils found in scrapie-infected hamster brains which are shown in the lower panel. Strikingly, the fibrils from

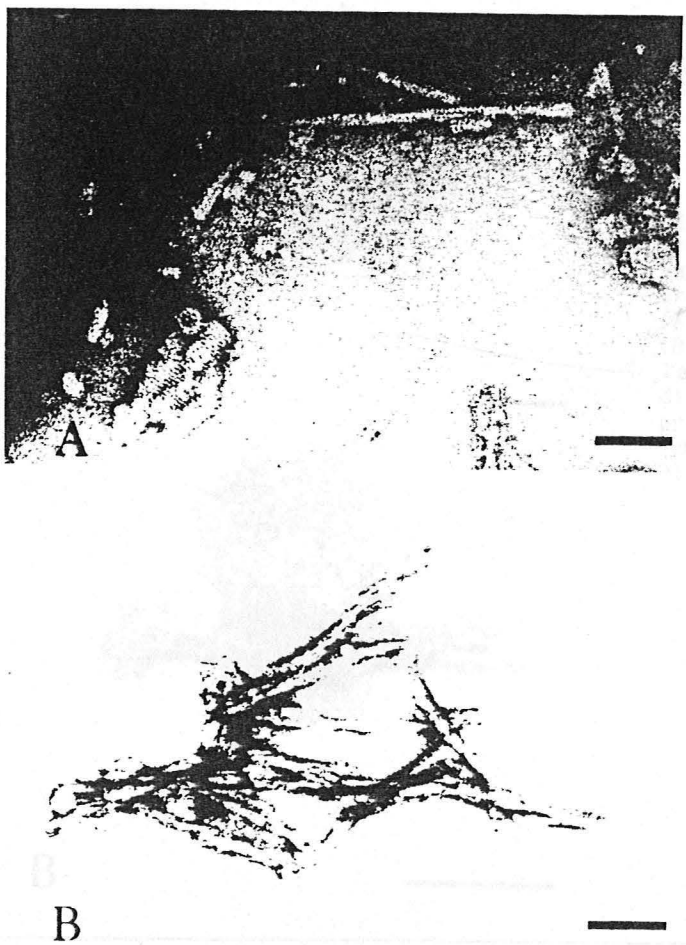

Fig. 31. Panel A: Scrapie fibrils in extracts of brain from a patient who developed Creutzfeldt-Jakob disease after human growth hormone administration. Panel B: For comparison is shown scrapie fibrils in extracts of brain from scrapie-infected hamster (8).

the patient's brain reacted positively with an antibody against the prion protein of hamster scrapie, as shown by the immunogold staining in the top panel of Fig. 32 (8). The bottom panel shows reactivity with authentic hamster prions for comparison.

These observations clearly demonstrate that human CJD can be infectious, and that infectivity is associated with the same prion protein that causes scrapie.

The link between CJD and scrapie is reinforced by recent genetic observations. Recall that 15% of CJD is inherited, and that Gerstmann-Sträussler syndrome is always inherited. The genetic forms of both of these diseases have recently been shown to be caused by mutations in the prion gene.

One mutation in CJD is an insertion of 144 basepairs in the gene (37,38). The insertion arises from a duplication of sequences that encode the repeats that occur near the amino terminus of the protein (Fig. 33). Whereas the normal

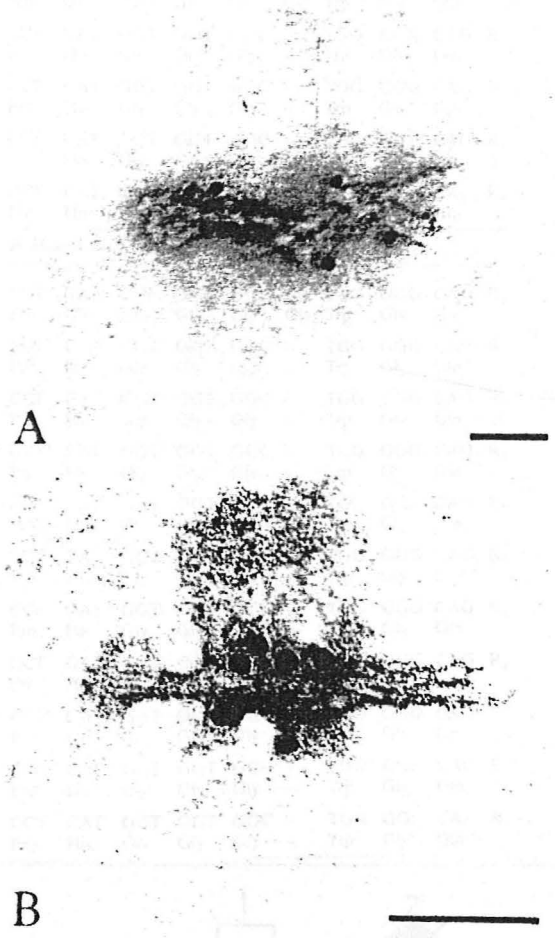

Fig. 32. Scrapie fibrils from brain of patient with iatrogenic Creuzfeldt-Jakob disease (A) and hamster brain (B) stained with gold-labeled antibody directed against hamster prion. Patient is the same as the one in Fig. 31. (8).

protein has 5 such repeats the mutant protein has 11 of them. Presumably, the additional 7 repeats modify the behavior of the protein so that it spontaneously forms a toxic structure. [Note that the sequence of the repeats in Fig. 32 differs from the sequence shown in Fig. 21 because these authors align the repeats differently.]

The 144 basepair insertion was originally reported to segregate with CJD in a family with the inherited disease (37). It was subsequently found in a family in which several members had died in their 30's and 40's of progressive dementia without a diagnosis of CJD (39). In another pedigree, shown in Fig. 34, the solid circles indicate women who developed progressive dementia in their early 20's. They had mild cerebral atrophy by CT scan, but only minimal cerebellum and motor signs (40). The women designated III-2 and III-4 were shown to have the insertion. Before their DNA was examined these individuals were considered to have early-onset Alzheimer's disease. After the DNA abnormality was discovered the diagnosis was changed to Gerstmann-Sträussler syndrome.

A. Wild-type allele

CCT	CAg	GGc	GGT	GGT	ggc	TGG	GGG	CAG	R_1
Pro	Gln	Gly	Gly	Gly	Gly	Trp	Gly	Gln	
CCT	CAT	GGT	GGT	GGC	–	TGG	GGG	CAG	R_2
Pro	His	Gly	Gly	Gly	–	Trp	Gly	Gln	
CCT	CAT	GGT	GGT	GGC	–	TGG	GGG	CAG	R_2
Pro	His	Gly	Gly	Gly	–	Trp	Gly	Gln	
CCc	CAT	GGT	GGT	GGC	–	TGG	GGa	CAG	R_3
Pro	His	Gly	Gly	Gly	–	Trp	Gly	Gln	
CCT	CAT	GGT	GGT	GGC	–	TGG	GGt	CAa	R_4
Pro	His	Gly	Gly	Gly	–	Trp	Gly	Gln	

B. Mutant allele

CCT	CAg	GGc	GGT	GGt	ggc	TGG	GGG	CAG	R_1
Pro	Gln	Gly	Gly	Gly	Gly	Trp	Gly	Gln	
CCT	CAT	GGT	GGT	GGC	–	TGG	GGG	CAG	R_2
Pro	His	Gly	Gly	Gly	–	Trp	Gly	Gln	
CCT	CAT	GGT	GGT	GGC	–	TGG	GGG	CAG	R_2
Pro	His	Gly	Gly	Gly	–	Trp	Gly	Gln	
CCT*	CAT	GGT	GGT	GGC	–	TGG	GGG	CAG	R_2
Pro	His	Gly	Gly	Gly	–	Trp	Gly	Gln	
CCc	CAT	GGT	GGT	GGC	–	TGG	GGa	CAG	R_3
Pro	His	Gly	Gly	Gly	–	Trp	Gly	Gln	
CCT	CAT	GGT	GGT	GGC	–	TGG	GGG	CAG	R_2
Pro	His	Gly	Gly	Gly	–	Trp	Gly	Gln	
CCc	CAT	GGT	GGT	GGC	–	TGG	GGG	CAG	R_3
Pro	His	Gly	Gly	Gly	–	Trp	Gly	Gln	
CCT	CAT	GGT	GGT	GGC	–	TGG	GGG	CAG	R_2
Pro	His	Gly	Gly	Gly	–	Trp	Gly	Gln	
CCT	CAT	GGT	GGT	GGC	–	TGG	GGG	CAG	R_2
Pro	His	Gly	Gly	Gly	–	Trp	Gly	Gln	
CCc	CAT	GGT	GGT	GGC	–	TGG	GGa	CAG	R_3
Pro	His	Gly	Gly	Gly	–	Trp	Gly	Gln	
CCT	CAT	GGT	GGT	GGC	–	TGG	GGt	CAa	R_4
Pro	His	Gly	Gly	Gly	–	Trp	Gly	Gln	

Fig. 33. Normal prion protein has 5 repeats (panel A), whereas prion from patient with hereditary Creuzfeldt-Jakob disease has 11 repeats owing to duplication of 144 base pairs (panel B). Numbers R1 to R4 at right refer to authors' speculation about evolutionary relationships among repeats (38)

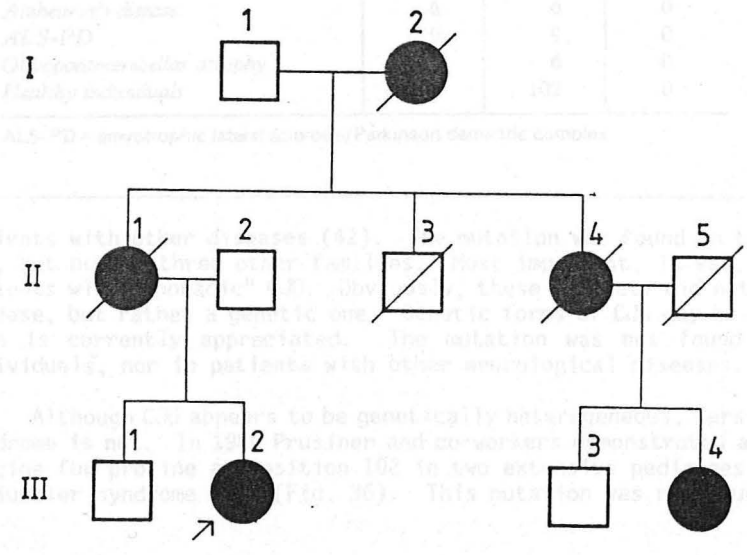

Fig. 34. A family originally considered to have early-onset Alzheimer's disease (see text). The two women in generation III had the 144 basepair insertion in the prion gene that was previously associated with Creuzfeldt-Jakob disease.

In medicine, when a new diagnostic test is developed it is customary to find that many cases of that disease were being mis-diagnosed or overlooked. That is certainly true with CJD and Gerstmann-Sträussler syndrome. Through the use of DNA probes for prions we will soon find many families with this disease who have previously been carrying some other diagnosis.

Even more remarkable, we will soon find that the histologic criteria for diagnosing spongiform encephalopathy are grossly inadequate. Collinge and co-workers recently reported another family with a diagnosis of familial early-onset Alzheimer's disease (39). Affected members were shown to have the 144 basepair insertion in the prion gene. At autopsy one of the patients' brains showed only mild neuronal loss. There was no vacuolization and none of the classic hallmarks of spongiform encephalopathy. Thus, not only the clinicians but also the pathologists have been missing these diseases. The spongiform change may occur quite late in the course and it will be missed if the patient dies early.

A second mutation in the prion gene has recently been described in patients with Creutzfeldt-Jakob disease (41). This is a mis-sense mutation that changes glutamic acid at position 200 to lysine. As shown in Fig. 35 Gadjusek and collaborators recently searched for this mutation in families with CJD, and in

SCREENING FOR THE PRIP CODON 200 MUTATION

Diagnosis	No tested	Genotype	
		Normal (Glu-Glu)	Mutated (Glu-Lys)
Familial CJD			
Family Ko	2	0	2
Family Ju	1	0	1
Family Ku	1	1	0
Family Si	1	1	0
Family Sh	1	1	0
Sporadic CJD	30	28	2
Iatrogenic CJD	4	4	0
Kuru	5	5	0
GSS	3	3	0
Alzheimer's disease	6	6	0
ALS-PD	9	9	0
Olivopontocerebellar atrophy	6	6	0
Healthy individuals	102	102	0

ALS-PD = amyotrophic lateral sclerosis/Parkinson dementia complex.

Fig. 35. Occurrence of the Glu^{200}-Lys mutation in two families with inherited CJD, and two patients who were previously considered to have the sporadic form (42)

patients with other diseases (42). The mutation was found in two families with CJD, but not in three other families. Most important, it was found in 2 of 30 patients with "sporadic" CJD. Obviously, these subjects did not have a sporadic disease, but rather a genetic one. Genetic forms of CJD may be much more common than is currently appreciated. The mutation was not found in 102 healthy individuals, nor in patients with other neurological diseases.

Although CJD appears to be genetically heterogeneous, Gerstmann-Sträussler syndrome is not. In 1989 Prusiner and co-workers demonstrated a substitution of leucine for proline at position 102 in two extensive pedigrees with Gerstmann-Sträussler syndrome (43) (Fig. 36). This mutation was not found in any

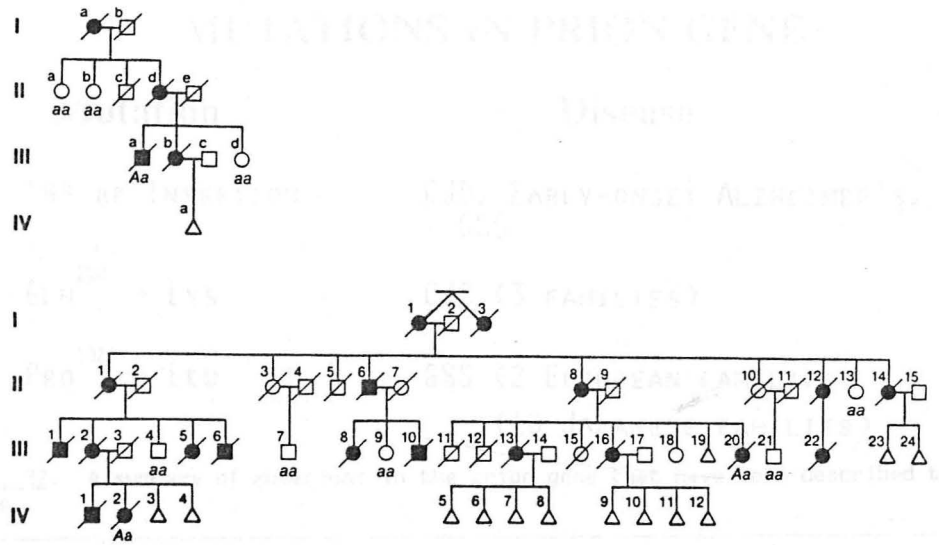

Fig. 36. Two families with ataxic Gerstmann-Straussler syndrome. "a" indicates a wild-type proline codon at position 102, and A indicates a mutant codon (leucine). Black symbols indicate affected family members, and slashes indicate death. The disease in patients III-20 and IV-2 has been transmitted by intracerebral injection into hamsters (43).

unaffected members of those families, nor was it found in 100 control subjects of the same ethnic background. Amazingly, the disease in one of these families could be transmitted to hamsters. Thus, we have the remarkable situation in which a genetically altered prion can convert the normal hamster prion into a form that causes disease! This is truly a new form of genetics as well as infection.

The proline to leucine change at position 200 was originally reported in two European families. The same mutation was found in all 11 Japanese Gerstmann-Sträussler patients who were tested (44). These came from 10 different unrelated families. These data suggest that this mutation cause the majority of cases of Gerstmann-Sträussler syndrome throughout the world. Whether the mutation occurred twice in evolution, or whether the Japanese and European patients have a common ancestor, is not known.

Fig. 37 summarizes all three known mutations in the prion gene in humans. The important point is that all three mutations lead to the production of a defective protein. None of them decreases expression. Clearly, these autosomal dominant diseases are caused by the production of an abnormal protein.

So far it is not possible to relate the amino acid changes to the propensity of these proteins to form prions. It is remarkable that these patients can live up to 40 years with the abnormal protein without showing any signs until they suddenly develop a rapidly progressive disease. This might suggest that another environmental factor is required in addition to the defective prion. Against this hypothesis is the uniform age of onset of symptoms. If an environmental agent, such as a viral infection, were required this should happen at various ages. It seems likely that the delayed onset of the genetic forms, like the infectious forms, is attributable to the slow

MUTATIONS IN PRION GENE

Mutation	Disease
144 BP INSERTION	CJD, EARLY-ONSET ALZHEIMER'S, GSS
$GLN^{200} \rightarrow LYS$	CJD (3 FAMILIES)
$PRO^{102} \rightarrow LEU$	GSS (2 EUROPEAN FAMILIES) (10 JAPANESE FAMILIES)

Fig. 37. A summary of mutations in the prion gene that have been described to date.

accumulation of the defective protein in the brain. Apparently a threshold must be reached, whereupon the disease progresses rapidly.

A crucial experiment will be to make a transgenic mouse who produces one of the mutant forms of the prion. If such a mouse spontaneously develops encephalopathy it should be possible to work out the natural history of the disease. Moreover, the cDNA encoding a mutant form should be transfected into cultured cells, and the extract should be assayed for its ability to produce scrapie in mice or hamsters. If this experiment works it should allow a dissection of the biochemical events underlying the pathogenicity of the mutant proteins.

BOVINE SPONGIFORM ENCEPHALOPATHY; A THREAT TO HUMANS?

In 1986 British scientists recognized the occurrence for the first time of a scrapie-like disease in cattle (45,46). The brains contain the classic scrapie fibrils (45). These fibrils react with antibodies against mouse scrapie. Moreover, the amino acid sequence of the protein was identical to that of the sheep scrapie prion. The cows are thought to have been infected through ingestion of protein-enriched meal that was made with extracts of sheep body parts. The disease is affecting more than 1000 cows per year in Britain.

These findings raise the troublesome question of whether this bovine scrapie agent might spread to man. Recall that infected animals harbor infectious prions in spleen and other body tissues - not in brain. So far there is no evidence of an increase in CJD in Britain or anywhere else, but this fact must be considered in light of the known latent period of this infection. The sheep products have now been eliminated from cattle feed, and it is hoped that the cattle disease will disappear.

AMYLOIDOGENIC PROTEINS AS NEUROTOXINS

Amyloid deposits occur in many different organs in more than a dozen diseases ranging from the brain in Alzheimer's disease to the pancreatic islets in Type II diabetes mellitus. Many physicians have been skeptical that these proteins actually damage tissues. Many hold that the amyloid proteins are simply

debris that accumulates after a tissue has been damaged by some other agent. The findings on spongiform encephalopathies, together with recent discoveries on the amyloid protein of Alzheimer's disease, should dispel these doubts forever. There is no longer any question that amyloid proteins can be primary neurotoxins in the brain and peripheral nervous systems.

The secret lies in genetics. The data on the genetic spongiform encephalopathies has been reviewed here. If an individual inherits a single nucleotide alteration at codon 102 of the prion gene this individual will develop Gerstmann-Sträussler syndrome before the fourth decade of life. The mutation somehow allows the prion to adopt an amyloid form, and this is sufficient to cause marked neuronal damage.

Last Spring a series of 4 articles in Science showed how a mis-sense mutation in another gene, the gene for the amyloid beta protein, produces hereditary cerebral amyloid angiopathy of the Dutch type (47-50). The amyloid beta-protein was originally discovered as the protein that produces amyloid plaques in Alzheimer's disease. Like the prion, the amyloid beta-protein normally does not form amyloid. However, in Alzheimer's disease patients the protein is modified so that it stains with Congo red and is resistant to proteases, the two classic hallmarks of amyloid. Most Alzheimer's patients do not have a structural mutation in the amyloid beta-protein gene: something else causes the protein to shift to an amyloidogenic form. However, in patients with hereditary cerebral amyloid angiopathy a mutation renders the protein spontaneously amyloidogenic and produces the disease without the need for any additional factors. This situation is formally analogous to the situation in Creuzfeldt-Jakob disease whereby some patients acquire an abnormality in the prion protein spontaneously, perhaps as a result of exposure to another prion protein, whereas others have a mutation.

So far, Alzheimer's disease has never been transmitted from humans to animals despite multiple attempts. Therefore, it is unlikely that the mechanism of the disease results from autocatalysis, as it does in Creuzfeldt-Jakob disease. Nevertheless, there must be some factor that alters the beta protein in this disease, and once this process begins the disease is inexorable.

How do amyloid proteins damage tissues, including the brain? The list of proteins that form toxic amyloid is large, ranging from plasma prealbumin in familial amyloid polyneuropathies to immunoglobulin light chains in so-called secondary amyloidosis. The only common feature is that they form amyloid. Somehow, the formation of amyloid, itself, must be toxic, but how?

It must be because these proteins are indigestible. The presence of an indigestible protein must somehow trigger a set of responses in a tissue that ends up being toxic. Perhaps the tissue increases its protease activity in a vain attempt to destroy the amyloid. Perhaps the tissue increases its oxidative defense mechanisms in an attempt to oxidize and thereby degrade the intruder. It seems likely to me that this defense against indigestibility is the real cause of these diseases.

I would like to make one additional point. The amyloid nature of Creutzfeldt-Jakob disease was not appreciated by the pathologists. Only 14% of patients have amyloid plaques, and yet the disease is clearly caused by the prion, an amyloidogenic protein. Obviously, the gross amyloid plaque is only the late stage of an amyloid disease. The plaque probably represents the failure of the defense mechanisms described above. Clearly, the indigestible amyloid proteins can cause disease long before they ever form plaques, and therefore we must greatly expand our notion of amyloid diseases to include those in which amyloid plaques have never been seen.

The way to diagnose amyloid diseases is to search for the hallmark feature: indigestibility. We should take a lesson from Prusiner. In puzzling diseases we should digest the organs with proteinase K, and examine whatever proteins survive. We should clone those proteins and then look for mutations in their genes. In my opinion such an approach would turn up a great many more amyloid diseases.

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