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Current Review

Infectious and Genetic Manifestations of Prion Diseases

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Considerable data has accumulated during the past four decades indicating that many features of scrapie, kuru, Creutzfeldt-Jakob disease (CJD), and Gerstmann-Sträussler-Scheinker syndrome (GSS) are not typical of an infectious disease. Furthermore, the past 20 years have witnessed numerous reports of experimental observations arguing that the scrapie agent is a novel pathogen. To distinguish the scrapie and CJD agents from viroids and viruses, the term "prion" was introduced (Prusiner 1982).

Scrapie of sheep and goats is the prototypic prion disorder, because it was the first of the diseases mentioned above to be transmitted to laboratory rodents (Chandler 1961). Like kuru and CJD, scrapie causes death of the host without any sign of an immune response to a "foreign infectious agent." As described below, British scientists argued for many years about whether natural scrapie was a genetic or infectious disease (Dickinson *et al.* 1965; Parry 1983), and debate about scrapie was heightened in 1966 when Tikvah Alper and colleagues reported the extraordinary resistance of the scrapie agent to inactivation by ionizing and ultraviolet irradiation (Alper *et al.* 1966, 1967). Speculation about the composition of the scrapie agent increased during the following decade.

This review of the infectious and genetic manifestations of prion diseases begins with a brief summary of the structure and properties of prion particles. It is important to realize that, despite the apparent controversy arising from the diverse terminology used by different laboratories, much of this information has been confirmed and is now widely accepted (Diener 1987). Turning to the molecular genetics of prion diseases, we present evidence from recent studies with transgenic (Tg) mice expressing genes encoding foreign prion proteins (PrPs) that suggests that the molecular mechanism of prion replication differs fundamentally from those mechanisms employed by conventional viruses and viroids. Finally, we discuss the infectious, sporadic, and genetic manifestations of prion diseases using both natural and experimental scrapie and the recently described bovine spongiform encephalopathy as examples.

In 1959, Hadlow suggested that "it might be profitable, in view of the veterinary experience with scrapie, to examine the possibility of the experimental induction of kuru in a laboratory primate, for one might surmise that the pathogenic mechanisms involved in scrapie — however unusual they may be — are unlikely to be unique in the province of animal pathology" (Hadlow 1959). This prophetic statement was originally directed toward those interested in human or animal diseases; currently known prion diseases affect only vertebrates. Nevertheless, just as viroids were once considered to be unconventional viruses, we believe that the properties of these novel pathogens may provide new insight into a large number of plant diseases whose causal agents are still obscure.

Properties and structure of prions. Although both prions and viruses multiply, their properties, structures, and modes of replication seem to be fundamentally different. The nucleic acid genomes of conventional viruses encode most or all the proteins necessary for producing infectious viruses, thereby determining their biological and physical properties. In contrast, prions contain little or no nucleic acid. Scrapie prions do contain an abnormal isoform of the PrP, designated PrP^{Sc}, but the PrP is encoded by a chromosomal gene (Oesch *et al.* 1985). Unlike viruses, prions synthesized *de novo* possess PrP^{Sc} molecules reflecting the resident (or host) PrP gene but not necessarily the sequence of the PrP^{Sc} molecules found within the infecting prion (Bockman *et al.* 1987; Prusiner *et al.* 1990). Although viruses usually evoke an immune response during some phase of their infection, prions do not appear to do so (Oesch *et al.* 1985; Prusiner 1982, 1989).

That PrP is encoded by a cellular gene and not by a putative nucleic acid carried within the prion is a major feature which distinguishes prions from viruses. This discovery coupled with recent genetic studies showing linkage between a PrP missense variant and GSS demand that scrapie and CJD no longer be considered virological disorders. Although prion diseases resemble viral illnesses in some respects, the structure and cellular biological and genetic properties of prions clearly separate them from viruses. Whether prions are composed of only an abnormal isoform of the PrP or whether they contain some additional molecule is uncertain.

Many lines of evidence argue that PrP^{Sc} is the sole component of prions: 1) multiple forms of prions are infectious

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– membranes, rods, spheres, detergent-lipid-protein complexes (DLPC), and liposomes; 2) many attempts to show the dependence of scrapie infectivity on a nucleic acid have been unsuccessful; 3) the ionizing radiation target size of the prion is 55,000 Da; 4) PrP^{Sc} is encoded by a cellular gene; 5) mice with short and long incubation times have different PrP genes that encode distinct PrPs and produce prions with distinct properties; and 6) GSS is linked to a mutation in the PrP gene. The partitioning of infectivity in a wide variety of different forms argues for a single component but does not eliminate the possibility of a second macromolecule. Numerous attempts to inactivate scrapie prion infectivity by procedures that hydrolyze or modify nucleic acids have been consistently unsuccessful. Ultraviolet irradiation of membranes, rods, and DLPC suggests that if prions have an essential nucleic acid, then it will be <5 bases if single-stranded or 30–45 base pairs if double-stranded (Bellinger-Kawahara *et al.* 1987a; Gabizon *et al.* 1988a). Ionizing radiation studies give a target size too small to protect a large nucleic acid but do not rule out some other macromolecule (Bellinger-Kawahara *et al.* 1988).

Arguments in favor of a second prion component are 1) prion infectivity has not been recovered from denatured samples after attempts at renaturation (D. Groth and S. B. Prusiner, unpublished results), and 2) many "strains" of prions have been reported (Bruce and Dickinson 1987). The first argument raises the possibility of a second component, but it need not necessarily be a nucleic acid. The second argument focuses on prion diversity and offers a nucleic acid genome as the basis for this diversity. The convergence of experimental results from a wide variety of independent disciplines (i.e., protein chemistry, molecular genetics, immunochemistry, neuropathology, and experimental neurology) points to a pivotal role for PrP^{Sc} (or PrP^{CJD}) in animal and human prion diseases.

During purification, rod-shaped aggregates of prions are formed. The development of procedures to disperse infectious prion rods in DLPC and liposomes has led to many advances (Gabizon *et al.* 1987, 1988b, 1988c). Previously, purified prions were isolated as insoluble, rod-shaped aggregates that are indistinguishable from amyloids (Prusiner *et al.* 1982, 1983). Filamentous protein polymers that stain with Congo red dye show green-gold birefringence under polarized light; amyloids are often deposited in response to chronic destructive disease. Monoclonal antibodies raised against PrP 27-30 (Barry and Prusiner 1986) have been used to purify scrapie prion infectivity in DLPC (Gabizon *et al.* 1988b). Immunoaffinity-purified fractions contain PrP^{Sc} and high prion titers. Polyclonal antibodies to PrP 27-30 were found to neutralize scrapie infectivity. Determination of the N-terminal sequence of PrP 27-30 (Prusiner *et al.* 1984) made possible molecular cloning of the cognate PrP cDNA and the recognition that PrP 27-30 is derived from a larger protein (PrP^{Sc}) by limited proteolysis (Oesch *et al.* 1985). The observation that PrP mRNA does not change throughout the course of scrapie infection prompted the discovery of the cellular PrP isoform (PrP^{C}), which is protease-sensitive (Oesch *et al.* 1985).

By using a PrP cDNA clone, we found that PrP^{Sc} is encoded by a single-copy chromosomal gene (Basler *et al.* 1986) and not by a putative nucleic acid carried within the infectious scrapie prion particle (Oesch *et al.* 1985). This is a major feature distinguishing prions from both viruses and viroids. To date, no prion-specific nucleic acid that is required for transmission of disease has been identified (Bellinger-Kawahara *et al.* 1987a, 1987b, 1988; Gabizon *et al.* 1988c; Prusiner 1987, 1989; Meyer *et al.* 1991). PrP^{Sc} and PrP^{C} are thought to have the same amino acid sequence but differ due to some posttranslational process (Basler *et al.* 1986; Borchelt *et al.* 1990). Both PrP^{C} and PrP^{Sc} are glycoproteins that possess Asn-linked oligosaccharides and glycoinositol phospholipid (GPI) anchors (Stahl *et al.* 1987; Haraguchi *et al.* 1989). Whether the features that distinguish PrP^{Sc} from PrP^{C} arise from differences in their Asn-linked oligosaccharides or GPI anchors is unknown.

Molecular genetics of prion diseases. The results of genetic linkage and open reading frame sequencing studies in humans and mice suggest that amino acid substitutions in PrP may modulate the development of prion diseases. Recent studies have demonstrated that GSS is an autosomal dominant disorder and that a Pro→Leu substitution at codon 102 of the PrP gene is linked to the development of GSS (Hsiao *et al.* 1988, 1989a). Earlier investigations showed genetic linkage between an incubation time gene (*Prn-i*) and the PrP gene in inbred mice (Carlson *et al.* 1986). Mice with long incubation times have PrP genes (*Prn-p*^{b}) with Leu→Phe and Thr→Val substitutions at codons 108 and 189, respectively (Westaway *et al.* 1987). Several discordant mice in genetic crosses raised the possibility that the PrP gene and *Prn-i* are separate but tightly linked (Carlson *et al.* 1988), but recombinant capture studies (D. Westaway, G. Carlson, M. Torchia, and S. B. Prusiner, unpublished results) and Tg mouse studies described below argue for the congruency of the *Prn-p* and *Prn-i* genes. Recent studies suggest that these differences in murine PrP sequences produce distinct isolates of prions which show significantly different scrapie incubation times in a single host (Carlson *et al.* 1989).

Investigations with Tg mice expressing foreign PrP genes have brought a wealth of new knowledge about prions (Scott *et al.* 1989; Prusiner *et al.* 1990). Inoculation of Tg (HaPrP) 81 mice expressing the Syrian hamster (Ha) PrP with Ha prions caused scrapie in ~75 days; hamsters inoculated with Ha prions have the same incubation period, while non-Tg control mice failed to develop scrapie after >500 days. Inoculation of a second line, Tg (HaPrP) 71 mice, with Ha prions caused scrapie in ~170 days, similar to the time required for developing scrapie after inoculation with mouse prions. There appears to be an inverse relationship between the level of transgene HaPrP mRNA present in the transgenic animals and the length of the incubation time after inoculation with Ha prions (Prusiner *et al.* 1990). Tg 71 and 81 mice inoculated with Ha prions exhibited spongiform degeneration and reactive astrocytic gliosis (i.e., a proliferation of astrocytes in response to brain degeneration or necrosis), and Tg 81 mice also showed HaPrP amyloid plaques that are characteristic of hamster scrapie.

Both Tg 71 and 81 mice produced $HaPrP^{Sc}$ in their brains after inoculation with Ha prions. The brains of Tg 81 mice with scrapie were found to contain ~10^{9} ID_{50} units per gram of brain tissue based on Ha bioassays. Tg mice

carrying a HaPrP minigene failed to express $HaPrP^C$ and had scrapie incubation times after inoculation with Ha prions similar to those observed for non-Tg controls (i.e, >500 days). Our studies are the first demonstration of the synthesis of infectious scrapie prions programmed by a recombinant DNA molecule and argue that the PrP gene modulates susceptibility and the course and neuropathological aspects of the disease. It now seems feasible to determine first the domains of PrP and later the precise amino acids that are required for production of infectious scrapie prions, modulate the susceptibility of animals to prion diseases, and influence scrapie incubation times.

While the function of PrP^C is unknown, several observations suggested that it might be involved in cell recognition. PrP^C is bound to the external surface of cells by a GPI anchor (Stahl *et al.* 1987). The levels of PrP^C are highly regulated in the development of hamster brain and can be stimulated by a nerve growth factor (McKinley *et al.* 1987; Mobley *et al.* 1988). The Asn-linked oligosaccharides of PrP^{Sc}, and presumably PrP^C, contain the x-antigenic determinant (Galβ1→4 [Fuc 1→3] GlcNAc) associated with cell surface molecules involved in recognition (Endo *et al.* 1989). Recent studies on a protein showing acetylcholine receptor-inducing activity (ARIA) have shown that the chicken ARIA protein possesses ~32% homology with mammalian PrP (Harris *et al.* 1989). Whether mammalian PrP^C functions like chicken ARIA remains to be established. Interestingly, the synthesis of PrP^C and choline acetyltransferase is coordinately controlled in the developing hamster brain (Mobley *et al.* 1988).

Molecular mechanism of prion replication. That Tg mice containing a gene encoding hamster PrP synthesize *de novo* the prion homologous to that in the inoculum (Prusiner *et al.* 1990) may give some insight into the molecular mechanisms of prion replication. Synthesis of hamster prions in Tg mice must involve one or possibly more translation products of the HaPrP cosmid insert, because this is the only additional DNA that Tg (HaPrP) mice harbor compared to their non-Tg littermate controls. It is likely that $HaPrP^{Sc}$ in the prion inoculum interacts with the homologous transgene product (i.e., $HaPrP^C$ or a precursor) during the *de novo* synthesis of more hamster prions, and the minimum size of such a putative PrP^{Sc}-PrP^C complex would be a heterodimer. Interestingly, ionizing radiation studies (Bellinger-Kawahara *et al.* 1988) suggest that the infectious scrapie particle has a molecular weight of 55,000. A particle of this weight might be a homodimer composed of two PrP^{Sc} molecules, a multimer of PrP^{Sc} and PrP^C, or even a complex involving other cellular components (Oesch *et al.* 1990).

Whether the conversion of PrP^C or a precursor into PrP^{Sc} involves the addition or deletion of a chemical group, a tightly bound ligand, or only a conformational change remains to be established. In the absence of any evidence for either chemical modification or any ligand unique to PrP^{Sc}, it is possible that the difference between PrP^C and PrP^{Sc} is only conformational. The PrP^{Sc} molecule of the putative heterodimer might act as template for the conversion of PrP^C into a second PrP^{Sc} molecule. Precedents for such a process include propagation of conformational changes in oligomeric enzyme complexes by constrained interactions among protomers (Monod *et al.* 1965; Fox *et al.* 1986) and protein-catalyzed folding of protein molecules involving disulfide isomerases (Creighton *et al.* 1980), *cis/trans* prolyl isomerases (Lang *et al.* 1987; Evans *et al.* 1987), heat shock proteins (Ostermann *et al.* 1989), and a variety of other molecules that act as "chaperones" (Hemmingsen *et al.* 1988).

The involvement of a replication intermediate such as a heterodimer of PrP^C and PrP^{Sc} in prion synthesis might also explain the existence of "strains" or isolates of scrapie prions that breed true (Bruce and Dickinson 1987). A heterodimer model is also compatible with the view that GSS is both a genetic and infectious disease (Hsiao *et al.* 1989a; see below). Mutant PrP^C molecules might spontaneously fold into the appropriate conformation for PrP^{Sc} at some relatively low but finite frequency.

Infectious, sporadic, and genetic manifestations of prion diseases. The three human prion diseases (kuru, CJD, and GSS) illustrate three different manifestations of CNS degeneration: slow infection, sporadic disease, and genetic disorder (Prusiner 1987; Ridley *et al.* 1986). That these diseases can be transmitted to laboratory animals by inoculation is well-documented (Gajdusek 1977; Gajdusek *et al.* 1966; Masters *et al.* 1981a). Kuru is thought to have been spread exclusively through a slow infectious mechanism by means of ritualistic cannibalism (Alpers 1979, 1987). Although a few cases of CJD have been traced to inoculation with prions (e.g., injections of human growth hormone [Brown 1988; Gibbs *et al.* 1985; Goujard *et al.* 1988], transplantation of corneas, and implantation of cerebral electrodes), most appear to be sporadic despite considerable effort to implicate scrapie-infected sheep as an exogenous source (Harries-Jones *et al.* 1988; Malmgren *et al.* 1979; Kovanen and Haltia 1988). It is possible, although unlikely, that sporadic CJD results from prions that are ubiquitous in humans but have a very low efficiency of infection. In hamsters, scrapie infection by the oral route has been found to be 10^9 times less efficient than intracerebral inoculation (Prusiner *et al.* 1985).

Pedigree studies suggest that GSS and familial CJD (FCJD) may be inherited as autosomal dominant disorders (Baker *et al.* 1985; Masters *et al.* 1981b; Hsiao and Prusiner, 1990). The significance of this observation was uncertain until recently, when GSS was shown to be a genetic disease and its development was linked to a missense variant of the PrP (Hsiao *et al.* 1989a). Molecular cloning studies demonstrated a cytosine to thymine substitution in the second position of codon 102, which probably results from deamination of a methylated cytosine situated 5′ to guanine (Barker *et al.* 1984; Bird 1986). This mutation creates a *Dde*I restriction site, which was used to show genetic linkage between the PrP codon 102 amino acid substitution (Leu→Pro) and the development of GSS. To date, this mutation has been seen in one American (Hsiao *et al.* 1989a), one British (Hsiao *et al.* 1989a), two Japanese (Dohura *et al.* 1989; Hsiao *et al.* 1989b), and one German family (Goldgaber *et al.* 1989), all with ataxic GSS. About 10–15% of cases of CJD are familial, while most cases of GSS are inherited (Baker *et al.* 1985; Masters *et al.* 1981a; Ridley *et al.* 1986). FCJD has been reported in families with amino acid substitutions or insertions in the PrP gene (Owen *et*

al. 1989; Goldgaber *et al.* 1989; Collinge *et al.* 1989, 1990). GSS and FCJD are the only known human diseases that are both genetic and infectious.

The genetic linkage of a PrP gene mutation with GSS constrains further the possible structural models for the prion. If prions contain a small, as yet undetected, nucleic acid, then this molecule must be widespread throughout the world to explain the incidence of sporadic CJD; yet it must segregate in rare GSS families with the PrP mutation. It is noteworthy that Huntington's disease, another autosomal dominant genetic disorder, does not induce clinical illness until the fifth decade in most cases. The delayed onset of both Huntington's disease and GSS is not understood. The genetic linkage results also suggests an interesting mechanism for sporadic CJD if prions contain only PrP^{Sc} (or PrP^{CJD}). A somatic mutation (Hansen and Cavenee 1987) in the PrP gene or even an RNA editing error (Chen *et al.* 1987; Feagin *et al.* 1988; Powell *et al.* 1987; Shaw *et al.* 1988) or translational error in a single cell might lead to the generation of PrP^{CJD} in that cell; prions would then spread to neighboring cells when the PrP^{CJD} molecules exited. In contrast, the PrP mutation of GSS is a germ line mutation, consistent with its genetic transmission.

Natural scrapie of sheep and goats. The history of investigations of scrapie dates back to 1732. The unique clinical features of the disease in sheep make it readily recognizable in written records in several languages under a variety of names. Several early British and French investigators attempted to transmit scrapie from affected sheep to healthy sheep by injection of various tissues and body fluids, and the French investigators Cuillé and Chelle succeeded in 1936 with an intraocular injection of the spinal cord (Cuillé and Chelle 1939). Focusing on the experimental transmissibility of the disease, two subsequent groups of investigators came to view natural scrapie as either a virallike illness or genetic disorder that just happens to be experimentally transmissible.

Maternal (and lateral) contagious transmission of natural scrapie was first suggested by crosses of scrapie-affected and scrapie-free Suffolk sheep conducted by Dickinson and co-workers. Progeny of affected ewes rather than rams were about seven times more likely to develop the disease (Dickinson *et al.* 1965). In a subsequent study of Suffolks crossed with Scottish Blackface sheep, a similar but reduced tendency was again apparent; ratios of approximately 1.9 and 1.3 to 1 were obtained in experiments involving 54 and 66 offspring, respectively. A "background" scrapie incidence of up to 50% in the progeny of unaffected parents, interpreted in terms of lateral contagious transmission, was apparent in this later study (Dickinson *et al.* 1974).

By conducting crosses of Suffolk sheep, Parry reached quite a different conclusion. He deduced that scrapie was an autosomal recessive genetic disorder in which contagious spread of the infectious agent played little or no part. In Parry's data (Parry 1962), a tendency favoring maternal transmission on twins born of scrapie-affected ewes was noted by Dickinson and co-workers (Dickinson *et al.* 1965), although the numbers quoted for the progeny of unaffected ewes crossed with affected rams do not appear to correspond to the original data. However, the bulk of Parry's data on Suffolk sheep showed no overt tendency for ewes rather than rams to transmit the disease (Parry 1962). Parry's experiments also showed no evidence for contagious transmission, that is, the scrapie-free animals (designated "proven white") produced no affected offspring, even when crossed to scrapie-affected animals (Parry 1983).

Dickinson's hypothesis of maternal transmission can be directly tested by bioassay for infectivity in scrapie-affected ewes. In this regard, Hadlow and co-workers failed to detect infectious titers in the uterus (gravid or nongravid), ovary, or mammary gland of clinically affected Suffolk ewes (Hadlow *et al.* 1979). Investigations by Pattison and co-workers (Pattison *et al.* 1972, 1974) have been widely cited as indicating infectivity in the placentas of Swaledale ewes with scrapie. Unfortunately, negative controls were not described for these experiments, and the incubation times in the inoculated recipients were scattered; an alternative explanation is that the observed instances of scrapie represent cross-contaminated inocula (however, see Pattison 1988).

Although the viewpoints espoused by Dickinson and Parry seemed irreconcilable at the time, demonstration that the major or sole component of the scrapie prion is host-encoded (see below) suggests a resolution to the apparent conundrum of how a disease can be both genetic and infectious.

Experimental scrapie of sheep and goats. It was clear from the studies of scrapie in different breeds of sheep naturally affected with the disease that the genetic background of the host played a major part in the course of the disease. Early work on the influence of the host in experimental disease was done in England by Gordon who injected subcutaneously scrapie-affected brain extracts into 24 different breeds of British sheep. The published results showed incidences of disease ranging from 78% in Herdwicks and 72% in Dalesbreds to 0% in Dorset Downs. Subsequent studies revealed that the Dorset Downs were not fully resistant, but had a prolonged incubation period when compared to other breeds (Gordon 1966). Despite these apparently clear results, the genetic analysis of scrapie susceptibility in sheep has been complicated by the possibility of maternal transmission of the disease (Dickinson *et al.* 1974).

In 1961, Dickinson and associates began selecting two populations of Cheviot sheep, all of which were derived from a single foundation group presumed to be free from natural scrapie. One group was selected for increased incidence of scrapie following subcutaneous injection, while the other was selected for decreased incidence of disease. The two lines differ by approximately 90% in incidence of scrapie disease. Based on the response of each of these groups to either the subcutaneous or intracerebral inoculation of the SSBP/1 strain of the scrapie agent, the animals fell into a short incubation time (197 $\pm$ 7 days) group and a long incubation time (917 $\pm$ 90 days) group (Dickinson 1976).

When sheep with short incubation periods were mated with sheep with longer incubation periods, the results suggested that a single autosomal gene, which is dominant with respect to short incubation periods, controls the length of the incubation time. This gene was thought to have

two alleles, and these were designated SIP^{sA} and SIP^{pA}. It has been hypothesized that the action of SIP alleles may be to restrict the replication of certain "strains" or isolates while allowing others to replicate. This hypothesis predicts a differential response to different scrapie isolates, which seems to be the case (Dickinson and Fraser 1979).

Analysis of PrP gene polymorphisms in the two lines of Cheviot sheep suggests that the PrP gene may be linked genetically to the SIP gene. While the data suggest that sheep may be similar to mice where the PrP gene is linked to a gene controlling the length of the scrapie incubation time, additional samples are needed to clarify why four of 11 sheep thought to be homozygous for SIP^{sA} were heterozygous for PrP polymorphisms. Similarly, analysis of Suffolk and Île-de-France sheep has revealed all four permutations of the *Eco*RI and *Hin*dIII polymorphisms described previously (Hunter *et al.* 1989) (unpublished data of D. Westaway and J. Chatelain). Since the chromosomal phase between these polymorphisms does not appear to be fixed with respect to each other, the probability of linkage disequilibrium between these particular restriction fragment length polymorphisms and SIP alleles seems remote, precluding accurate SIP genotyping outside of the Cheviot breed. More hopeful in this regard is a recent molecular cloning study of PrP gene clones sequenced from a Suffolk sheep genomic library reporting two alleles (Goldman *et al.* 1990). A nucleotide change of G→A results in the substitution of arginine to glutamine in PrP.

The possibility of maternal transmission in the context of experimental scrapie was first addressed by Gordon (Gordon 1959, 1966). In the first report, there was an incidence of ~8% ($n = 123$) and in the second, where both the ewes and the rams were inoculated, an incidence of ~5% ($n = 63$). Dickinson and co-workers reported one affected animal ($n = 1$) in an experiment involving implantation of a fertilized egg into an inoculated recipient, and two scrapie-affected offspring ($n = 4$) in an experiment similar to Gordon's cited above, but using different breeds (Dickinson *et al.* 1966). Incubation times for many of the lambs in these experiments (Gordon 1966; Dickinson *et al.* 1966) were short, implying that they did not represent cryptic cases of natural scrapie. In contrast to these reports, a comparable study by Warren Foote and co-workers (personal communication) is essentially negative. Of a total of 86 embryos transplanted from inoculated donors into free recipients or from free donors into inoculated recipients, none of the resulting offspring developed the disease within an observation period greater than or equal to 5 yr. Direct inoculation of control animals in these experiments produced a scrapie incidence >51%. Similarly, negative results were obtained for maternal transmission in experimental scrapie of goats (Pattison 1964). Early reports (Eklund *et al.* 1963; Gibbs *et al.* 1965) suggesting maternal transmission of scrapie in mice were strongly challenged by subsequent investigators (Dickinson 1967; Field and Joyce 1970; Clarke and Haig 1971). A maternal effect is not apparent in the transmission of either familial or experimental CJD (Manuelidis and Manuelidis 1979; Amyx *et al.* 1981; Masters *et al.* 1981b), kuru (Alpers 1987), or GSS (Masters *et al.* 1981a; Hsiao *et al.* 1989a).

The bovine spongiform encephalopathy (BSE) epidemic. "Mad cows" dying of BSE present a major agricultural problem and may pose a potential public health dilemma. Since 1986, more than 30,000 cattle in Great Britain have been diagnosed with BSE (Anonymous 1989). Epidemiological studies suggest that the introduction of sheep offal into the diet of British cattle in 1981 may be the point source cause of BSE (Wilesmith *et al.* 1988; Winter *et al.* 1989; Wijeratne and Curnow 1990). Transmission of BSE to mice and cattle (Fraser *et al.* 1988; Dawson *et al.* 1990; Barlow and Middleton 1990), symptoms confined to the CNS, spongiform change in the brain (Wells *et al.* 1987), and protease-resistant PrP detected on immunoblots of affected brain extracts (Hope *et al.* 1988) all implicate scrapie prions in bone meal supplements derived from sheep carcasses.

Restrictions on imports of British beef products by France and West Germany present a major problem for British agriculture (Anonymous 1990). The possibility of bovine to human transmission of prions has resulted in the ban of beef being served to school children in Great Britain. Concern has escalated with reports of three domestic cats dying of a spongiform encephalopathy as well as the possibility that antelope in zoos may have developed CNS disorders by consuming prion-infected foodstuffs (Aldhous 1990a, 1990b).

Numerous epidemiological studies have attempted to link the development of CJD with the consumption of scrapie-infected sheep products (Goldberg *et al.* 1979; Malmgren *et al.* 1979; Matthews *et al.* 1985; Harries-Jones *et al.* 1988). Although such studies have failed to demonstrate a link between scrapie and CJD, the development of a related human disease, kuru, appears to be caused by consumption of prion-affected brains during ritualistic cannibalism (Gajdusek 1977). Whether bovine prions will prove pathogenic for humans remains an unanswered question of extreme importance. Pertinent to the question of whether consumption of bovine prions in beef products or bovine-derived pharmaceuticals will cause CNS dysfunction are the low efficiency of oral vertical transmission of prion infection, conflicting data about vertical transmission, and the "species barrier." Studies with Syrian hamsters have shown that oral transmission of experimental scrapie can be accomplished with regularity but the oral route is 10^9 times less efficient than the intracerebral one; parental inoculation is 10^5 times less efficient than intracerebral inoculation (Prusiner *et al.* 1985). Transmission between species is characterized by a stochastic process in which a few inoculated animals develop disease after extremely prolonged incubation periods (Pattison 1965; Pattison and Jones 1968). On the next passage in the homologous host, development of disease is a nonstochastic process with greatly shorter incubation times. Recent studies with Tg mice argue that the species barrier resides in the amino acid sequence of PrP (Scott *et al.* 1989).

Whether BSE occurs naturally or is due exclusively to the oral consumption of sheep scrapie prions is unknown. Although the N-terminal sequence of PrP recovered from cattle brains is similar to that of hamster PrP (Hope *et al.* 1988), it will be important to learn about the PrP genes from a variety of cattle and other animal species. Do cattle represent a dead-end host for prions as appears to be the

case for mink developing transmissible mink encephalopathy after consumption of scrapie-affected sheep meat, or will the disease spread horizontally among cattle? We do not know how scrapie spreads among sheep in flocks, and the controversy surrounding this issue reflects our ignorance about the origins, spread, and pathogenesis of natural scrapie.

Novel and unprecedented. In summary, a wealth of recently acquired experimental data has established that prions are novel and unprecedented pathogens. GSS and FCJD are unprecedented human illnesses because they are both genetic and infectious, and the etiologic prion particles themselves seem equally novel. Determining the chemical mechanisms responsible for converting PrP^{C} or a precursor into PrP^{Sc} will be extremely important and may open new avenues of research into mechanisms of cell homeostasis, recognition, and possibly differentiation. Once the events in PrP^{Sc} formation are known, therapeutic and preventive strategies for prion disease can be formulated.

The mechanism of prion replication appears to differ fundamentally from the nucleic acid-based mechanisms employed by conventional viruses and viroids. Biochemistry and molecular biology provide several precedents for posttranslational modifications of proteins (e.g., protein- or ligand-catalyzed changes in protein conformation), but prions appear to be the first example of a pathogen where nucleic acid-encoded information is not used to introduce pathogen-specific information into the host cell. Whether proteins other than PrP can be converted from benign, cellular isoforms into malignant, pathogenic molecules is unknown, but there is no obvious reason why such pathogens need be confined to the animal kingdom.

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LITERATURE CITED

Aldhous, P. 1990a. Antelopes die of "mad cow" disease. Nature (London) 344:183.

Aldhous, P. 1990b. Spongiform encephalopathy found in cat. Nature (London) 345:194.

Alper, T., Cramp, W. A., Haig, D. A., and Clarke, M. C. 1967. Does the agent of scrapie replicate without nucleic acid? Nature (London) 214:764-766.

Alper, T., Haig, D. A., and Clarke, M. C. 1966. The exceptionally small size of the scrapie agent. Biochem. Biophys. Res. Commun. 22:278-284.

Alpers, M. 1987. Epidemiology and clinical aspects of kuru. Pages 451-465 in: Prions – Novel Infectious Pathogens Causing Scrapie and Creutzfeldt-Jakob Disease. S. B. Prusiner and M. P. McKinley, eds. Academic Press, Orlando, FL.

Alpers, M. P. 1979. Epidemiology and ecology of kuru. Pages 67-90 in: Slow Transmissible Diseases of the Nervous System, Vol. 1. S. B. Prusiner and W. J. Hadlow, eds. Academic Press, New York.

Amyx, H. L., Gibbs, C. J., Jr., Gajdusek, D. C., and Greer, W. E. 1981. Absence of vertical transmission of subacute spongiform viral encephalopathies in experimental primates (41092). Proc. Soc. Exp. Biol. Med. 166:469-471.

Anonymous 1990. Mad cows and the Minister. Nature (London) 345:277-278.

Anonymous 1989. Report of the Working Party on Bovine Spongiform Encephalopathy. Department of Health, Ministry of Agriculture, Fisheries & Food, U.K. 35 pp.

Baker, H. F., Ridley, R. M., and Crow, T. J. 1985. Experimental transmission of an autosomal dominant spongiform encephalopathy: Does the infectious agent originate in the human genome? Br. Med. J. 291:299-302.

Barker, D., Schafer, M., and White, R. 1984. Restriction sites containing CpG show a higher frequency of polymorphism in human DNA. Cell 36:131-138.

Barlow, R. M., and Middleton, D. J. 1990. Dietary transmission of bovine spongiform encephalopathy to mice. Vet. Rec. 126:111-112.

Barry, R. A., and Prusiner, S. B. 1986. Monoclonal antibodies to the cellular and scrapie prion proteins. J. Infect. Dis. 154:518-521.

Basler, K., Oesch, B., Scott, M., Westaway, D., Wälchli, M., Groth, D. F., McKinley, M. P., Prusiner, S. B., and Weissmann, C. 1986. Scrapie and cellular PrP isoforms are encoded by the same chromosomal gene. Cell 46:417-428.

Bellinger-Kawahara, C. G., Kempner, E., Groth, D. F., Gabizon, R., and Prusiner, S. B. 1988. Scrapie prion liposomes and rods exhibit target sizes of 55,000 Da. Virology 164:537-541.

Bellinger-Kawahara, C., Cleaver, J. E., Diener, T. O., and Prusiner, S. B. 1987a. Purified scrapie prions resist inactivation by UV irradiation. J. Virol. 61:159-166.

Bellinger-Kawahara, C., Diener, T. O., McKinley, M. P., Groth, D. F., Smith, D. R., and Prusiner, S. B. 1987b. Purified scrapie prions resist inactivation by procedures that hydrolyze, modify, or shear nucleic acids. Virology 160:271-274.

Bird, A. P. 1986. CpG-rich islands and the function of DNA methylation. Nature (London) 321:209-213.

Bockman, J. M., Prusiner, S. B., Tateishi, J., and Kingsbury, D. T. 1987. Immunoblotting of Creutzfeldt-Jakob disease prion proteins: Host species-specific epitopes. Ann. Neurol. 21:589-595.

Bolton, D. C., McKinley, M. P., and Prusiner, S. B. 1984. Molecular characteristics of the major scrapie prion protein. Biochemistry 23:5898-5906.

Bolton, D. C., McKinley, M. P., and Prusiner, S. B. 1982. Identification of a protein that purifies with the scrapie prion. Science 218:1309-1311.

Borchelt, D. R., Scott, M., Taraboulos, A., Stahl, N., and Prusiner, S. B. 1990. Scrapie and cellular prion proteins differ in their kinetics of synthesis and topology in cultured cells. J. Cell Biol. 110:743-752.

Brown, P. 1988. The decline and fall of Creutzfeldt-Jakob disease associated with human growth hormone therapy. Neurology 38:1135-1137.

Bruce, M. E., and Dickinson, A. G. 1987. Biological evidence that the scrapie agent has an independent genome. J. Gen. Virol. 68:79-89.

Butler, D. A., Scott, M. R. D., Bockman, J. M., Borchelt, D. R., Taraboulos, A., Hsiao, K. K., Kingsbury, D. T., and Prusiner, S. B. 1988. Scrapie-infected murine neuroblastoma cells produce protease-resistant prion proteins. J. Virol. 62:1558-1564.

Carlson, G. A., Westaway, D., DeArmond, S. J., Peterson-Torchia, M., and Prusiner, S. B. 1989. Primary structure of prion protein may modify scrapie isolate properties. Proc. Natl. Acad. Sci. USA 86:7475-7479.

Carlson, G. A., Goodman, P. A., Lovett, M., Taylor, B. A., Marshall, S. T., Peterson-Torchia, M., Westaway, D., and Prusiner, S. B. 1988. Genetics and polymorphism of the mouse prion gene complex: The control of scrapie incubation time. Mol. Cell. Biol. 8:5528-5540.

Carlson, G. A., Kingsbury, D. T., Goodman, P. A., Coleman, S., Marshall, S. T., DeArmond, S. J., Westaway, D., and Prusiner, S. B. 1986. Linkage of prion protein and scrapie incubation time genes. Cell 46:503-511.

Chandler, R. L. 1961. Encephalopathy in mice produced by inoculation with scrapie brain material. Lancet 1:1378-1379.

Chen, S.-H., Habib, G., Yang, C.-Y., Gu, Z.-W., Lee, B. R., Weng, S.-A., Silberman, S. R., Cai, S. J., Deslypere, J. P., Rosseneu, M., Gotto, A. M., Li, W.-H., and Chan, L. 1987. Apolipoprotein B-48 is the product of a messenger RNA with an organ-specific inframe stop codon. Science 238:363-366.

Clarke, M. C., and Haig, D. A. 1971. An attempt to determine whether material transmission of scrapie occurs in mice. Br. Vet. J. 127:32-34.

Collinge, J., Owen, F., Poulter, M., Leach, M., Crow, T. J., Rossor, M. N., Hardy, J., Mullan, M. J., Janota, I., and Lantos, P. L. 1990. Prion dementia without characteristic pathology. Lancet 2:7-9.

Collinge, J., Harding, A. E., Owen, F., Poulter, M., Lofthouse, R., Boughey, A. M., Shah, T., and Crow, T. J. 1989. Diagnosis of Gerstmann-Sträussler syndrome in familial dementia with prion protein gene analysis. Lancet 2:15-17.

Creighton, R. E., Hillson, D. A., and Freedman, R. B. 1980. Catalysis by protein-disulphide isomerases of the unfolding and refolding of proteins with disulphide bonds. J. Mol. Biol. 142:43-62.

Cuillé, J., and Chelle, P. L. 1939. Experimental transmission of trembling to the goat. C.R. Seances Acad. Sci. 208:1058-1060.

Dawson, M., Wells, G. A. H., and Parker, B. N. J. 1990. Preliminary evidence of the experimental transmissibility of bovine spongiform encephalopathy to cattle. Vet. Rec. 126:112-113.

DeArmond, S. J., McKinley, M. P., Barry, R. A., Braunfeld, M. B., McColloch, J. R., and Prusiner, S. B. 1985. Identification of prion amyloid filaments in scrapie-infected brain. Cell 41:221-235.

Dickinson, A. G. 1976. Scrapie in sheep and goats. Pages 209-241 in: Slow Virus Diseases of Animals and Man. R. H. Kimberlin, ed. North-Holland Publishing, Amsterdam.

Dickinson, A. G. 1967. Ataxias and transmissible agents. Lancet 1:1166.

Dickinson, A. G., and Fraser, H. 1979. An assessment of the genetics of scrapie in sheep and mice. Pages 367-386 in: Slow Transmissible Diseases of the Nervous System, Vol. 1. S. B. Prusiner and W. J. Hadlow, eds. Academic Press, New York.

Dickinson, A. G., Stamp, J. T., and Renwick, C. C. 1974. Maternal and lateral transmission of scrapie in sheep. J. Comp. Pathol. 84:19-25.

Dickinson, A. G., Young, G. B., and Renwick, C. C. 1966. Scrapie: Experiments involving maternal transmission in sheep. Pages 244-247 in: Report of Scrapie Seminar. U.S. Dep. Agric. Agric. Res. Serv. ARS 91-53.

Dickinson, A. G., Young, G. B., Stamp, J. T., and Renwick, C. C. 1965. An analysis of natural scrapie in Suffolk sheep. Heredity 20:485-503.

Diener, T. O. 1987. PrP and the nature of the scrapie agent. Cell 49:719-721.

Doh-ura, K., Tateishi, J., Sasaki, H., Kitamoto, T., and Sakaki, Y. 1989. Pro→Leu change at position 102 of prion protein is the most common but not the sole mutation related to Gerstmann-Sträussler syndrome. Biochem. Biophys. Res. Commun. 163:974-979.

Eklund, C. M., Hadlow, W. J., and Kennedy, R. C. 1963. Some properties of the scrapie agent and its behavior in mice. Proc. Soc. Exp. Biol. Med. 112:974-979.

Endo, T., Groth, D., Prusiner, S. B., and Kobata, A. 1989. Diversity of oligosaccharide structures linked to asparagines of the scrapie prion protein. Biochemistry 28:8380-8388.

Evans, P. A., Dobson, C. M., Kautz, R. A., Hatfull, G., and Fox, R. O. 1987. Proline isomerization in staphylococcal nuclease characterized by NMR and site-directed mutagenesis. Nature 329:266-268.

Feagin, J. E., Abraham, J. M., and Stuart, K. 1988. Extensive editing of the cytochrome C oxidase III transcript in trypanosoma brucei. Cell 53:413-422.

Field, E. J., and Joyce, G. 1970. Evidence against transmission of scrapie by animal mouse fomites. Nature (London) 226:971-973.

Fox, R. O., Evans, P. A., and Dobson, C. M. 1986. Multiple conformations of a protein demonstrated by magnetization transfer NMR spectroscopy. Nature (London) 320:192-194.

Fraser, H., McConnell, I., Wells, G. A. H., and Dawson, M. 1988. Transmission of bovine spongiform encephalopathy to mice. Vet. Rec. 123:472.

Gabizon, R., McKinley, M. P., Groth, D. F., Kenaga, L., and Prusiner, S. B. 1988a. Properties of scrapie prion liposomes. J. Biol. Chem. 263:4950-4955.

Gabizon, R., McKinley, M. P., Groth, D. F., and Prusiner, S. B. 1988b. Immunoaffinity purification and neutralization of scrapie prion infectivity. Proc. Natl. Acad. Sci. USA 85:6617-6621.

Gabizon, R., McKinley, M. P., and Prusiner, S. B. 1988c. Properties of scrapie prion proteins in liposomes and amyloid rods. Pages 182-196 in: Novel Infectious Agents and the Central Nervous System, Ciba Foundation Symposium 135. G. Bock and J. Marsh, eds. John Wiley and Sons, Chichester, U.K.

Gabizon, R., McKinley, M. P., and Prusiner, S. B. 1987. Purified prion proteins and scrapie infectivity copartition into liposomes. Proc. Natl. Acad. Sci. USA 84:4017-4021.

Gajdusek, D. C. 1977. Unconventional viruses and the origin and disappearance of kuru. Science 197:943-960.

Gajdusek, D. C., Gibbs, C. J., Jr., and Alpers, M. 1966. Experimental transmission of a kurulike syndrome to chimpanzees. Nature (London) 209:794-796.

Gibbs, C. J., Jr., Joy, A., Heffner, R., Franko, M., Miyazaki, M., Asher, D. M., Parisi, J. E., Brown, P. W., and Gajdusek, D. C. 1985. Clinical and pathological features and laboratory confirmation of Creutzfeldt-Jakob disease in a recipient of pituitary-derived human growth hormone. N. Engl. J. Med. 313:734-738.

Gibbs, C. J., Jr., Gajdusek, D. C., and Morris, J. A. 1965. Viral characteristics of the scrapie agent in mice. Pages 195-202 in: Slow, Latent and Temperate Virus Infections, NINDB Monogr. 2. D. C. Gajdusek, C. J. Gibbs, Jr., and M. P. Alpers, eds. U.S. Government Printing Office, Washington, DC.

Goldberg, H., Alter, M., and Kahana, E. 1979. The Libyan Jewish focus of Creutzfeldt-Jakob disease: A search for the mode of natural transmission. Pages 195-211 in: Slow Transmissible Diseases of the Nervous System, Vol. 1. S. B. Prusiner and W. J. Hadlow, eds. Academic Press, New York.

Goldgaber, D., Goldfarb, L. G., Brown, P., Asher, D. M., Brown, W. T., Lin, S., Teener, J. W., Feinstone, S. M., Rubenstein, R., Kascsak, R. J., Boellaard, J. W., and Gajdusek, D. C. 1989. Mutations in familial Creutzfeldt-Jakob disease and Gerstmann-Sträussler-Scheinker's syndrome. Exp. Neurol. 106:204-206.

Goldman, W., Hunter, N., Foster, J. D., Salbaum, J. M., Beyreuther, K., and Hope, J. 1990. Two alleles of a neural protein gene linked to scrapie in sheep. Proc. Natl. Acad. Sci. USA 87:2476-2480.

Gordon, W. S. 1966. Review of work on scrapie at Compton, England, 1952-1964. Pages 19-36 in: Report of Scrapie Seminar. U.S. Dep. Agric. Agric. Res. Serv. ARS 91-53.

Gordon, W. S. 1959. Scrapie panel. Pages 286-294 in: Proc. Annu. Meet. U.S. Livestock Sanitary Assoc., 63rd.

Goujard, J., Entat, M., Maillard, F., Mugnier, E., Rappaport, R., and Job, J. C. 1988. Human pituitary growth hormone (hGH) and Creutzfeldt-Jakob disease: Results of an epidemiological survey in France, 1986. Int. J. Epidemiol. 17:423-427.

Hadlow, W. J. 1959. Scrapie and kuru. Lancet 2:289-290.

Hadlow, W. J., Race, R. E., Kennedy, R. C., and Eklund, C. M. 1979. Natural infection of sheep with scrapie virus. Pages 3-12 in: Slow Transmissible Diseases of the Nervous System, Vol. 2. S. B. Prusiner and W. J. Hadlow, eds. Academic Press, New York.

Hansen, M. F., and Cavenee, W. K. 1987. Genetics of cancer predisposition. Cancer Res. 47:5518-5527.

Haraguchi, T., Fisher, S., Olofsson, S., Endo, T., Groth, D., Tarantino, A., Borchelt, D. R., Teplow, D., Hood, L., Burlingame, A., Lycke, E., Kobata, A., and Prusiner, S. B. 1989. Asparagine-linked glycosylation of the scrapie and cellular prion proteins. Arch. Biochem. Biophys. 274:1-13.

Harries-Jones, R., Knight, R., Will, R. G., Cousens, S., Smith, P. G., and Matthews, W. B. 1988. Creutzfeldt-Jakob disease in England and Wales, 1980-1984: A case-control study of potential risk factors. J. Neurol. Neurosurg. Psychiatry 51:1113-1119.

Harris, D. A., Falls, D. L., Walsh, W., and Fischbach, G. D. 1989. Molecular cloning of an acetylcholine receptor-inducing protein. Soc. Neurosci. Abstr. 15:70.7.

Hemmingsen, S. M., Woolford, C., van der Vies, S. M., Tilly, K., Dennis, D. T., Georgopoulos, C. P., Hendrix, R. W., and Ellis, R. J. 1988. Homologous plant and bacterial proteins chaperone oligomeric protein assembly. Nature (London) 333:330-334.

Hope, J., Reekie, L. J. D., Hunter, N., Multhaup, G., Beyreuther, K., White, H., Scott, A. C., Stack, M. J., Dawson, M., and Wells, G. A. H. 1988. Fibrils from brains of cows with new cattle disease contain scrapie-associated protein. Nature (London) 336:390-392.

Hsiao, K., and Prusiner, S. B. 1990. Inherited human prion diseases. Neurology. 40:1820-1827.

Hsiao, K., Baker, H. F., Crow, T. J., Poulter, M., Owen, F., Terwilliger, J. D., Westaway, D., Ott, J., and Prusiner, S. B. 1989a. Linkage of a prion protein missense variant to Gerstmann-Sträussler syndrome. Nature (London) 338:342-345.

Hsiao, K. K., Doh-ura, K., Kitamoto, T., Tateishi, J., and Prusiner, S. B. 1989b. A prion protein amino acid substitution in ataxic Gerstmann-Sträussler syndrome. Ann. Neurol. 26:137.

Hsiao, K. K., Westaway, D. A., and Prusiner, S. B. 1988. An amino acid substitution in the prion protein of ataxic Gerstmann-Sträussler syndrome. Am. J. Hum. Genet. 43:A87.

Hunter, N., Foster, J. D., Dickinson, A. G., and Hope, J. 1989. Linkage of the gene for the scrapie-associated fibril protein (PrP) to the Sip

gene in Cheviot sheep. Vet. Rec. 124:364-366.
Hunter, N., Hope, J., McConnell, I., and Dickinson, A. G. 1987. Linkage of the scrapie-associated fibril protein (PrP) gene and Sinc using congenic mice and restriction fragment length polymorphism analysis. J. Gen. Virol. 68:2711-2716.
Kitamoto, T., Tateishi, J., Tashima, I., Takeshita, I., Barry, R. A., DeArmond, S. J., and Prusiner, S. B. 1986. Amyloid plaques in Creutzfeldt-Jakob disease stain with prion protein antibodies. Ann. Neurol. 20:204-208.
Kovanen, J., and Haltia, M. 1988. Descriptive epidemiology of Creutzfeldt-Jakob disease in Finland. Acta Neurol. Scand. 77:474-480.
Lang, K., Schmid, F. X., and Fischer, G. 1987. Catalysis of protein folding by prolyl isomerases. Nature (London) 329:268-270.
Malmgren, R., Kurland, L., Mokri, B., and Kurtzke, J. 1979. The epidemiology of Creutzfeldt-Jakob disease. Pages 93-112 in: Slow Transmissible Diseases of the Nervous System, Vol. 1. S. B. Prusiner and W. J. Hadlow, eds. Academic Press, New York.
Manuelidis, E. E., and Manuelidis, L. 1979. Experiments on maternal transmission of Creutzfeldt-Jakob disease in guinea pigs. Proc. Soc. Exp. Biol. Med. 160:233-236.
Masters, C. L., Gajdusek, D. C., and Gibbs, C. J., Jr. 1981a. Creutzfeldt-Jakob disease virus isolations from the Gerstmann-Sträussler syndrome. Brain 104:559-588.
Masters, C. L., Gajdusek, D. C., and Gibbs, C. J., Jr. 1981b. The familial occurrence of Creutzfeldt-Jakob disease and Alzheimer's disease. Brain 104:535-558.
Matthews, W. B., Acheson, E. D., Batchelor, J. R., and Weller, R. O. 1985. McAlpine's Multiple Sclerosis. Churchill Livingstone, Edinburgh. 358 pp.
McKinley, M. P., Hay, B., Lingappa, V. R., Lieberburg, I., and Prusiner, S. B. 1987. Developmental expression of prion protein gene in brain. Dev. Biol. 121:105-110.
McKinley, M. P., Bolton, D. C., and Prusiner, S. B. 1983. A protease-resistant protein is a structural component of the scrapie prion. Cell 35:57-62.
Meyer, N., Rosenbaum, V., Schmidt, B., Gilles, K., Mirenda, C., Groth, D., Prusiner, S. B., and Riesner, D. 1991. Search for a putative scrapie genome in purified prion fractions reveals a paucity of nucleic acids. J. Gen. Virol. 72:37-49.
Mobley, W. C., Neve, R. L., Prusiner, S. B., and McKinley, M. P. 1988. Nerve growth factor increases mRNA levels for the prion protein and the beta-amyloid protein precursor in developing hamster brain. Proc. Natl. Acad. Sci. USA 85:9811-9815.
Monod, J., Wyman, J., and Changeux, J.-P. 1965. On the nature of allosteric transitions: A plausible model. J. Mol. Biol. 12:88-118.
Oesch, B., Teplow, D. B., Stahl, N., Serban, D., Hood, L. E., and Prusiner, S. B. 1990. Identification of cellular proteins binding to the scrapie prion protein. Biochemistry 29:5848-5855.
Oesch, B., Westaway, D., Wälchli, M., McKinley, M. P., Kent, S. B. H., Aebersold, R., Barry, R. A., Tempst, P., Teplow, D. B., Hood, L. E., Prusiner, S. B., and Weissmann, C. 1985. A cellular gene encodes scrapie PrP 27-30 protein. Cell 40:735-746.
Ostermann, J., Horvich, A. L., Neupert, W., and Hartl, F.-U. 1989. Protein folding in mitochondria requires complex formation with *hsp60* and ATP hydrolysis. Nature (London) 341:125-130.
Owen, F., Poulter, M., Lofthouse, R., Collinge, J., Crow, T. J., Risby, D., Baker, H. F., Ridley, R. M., Hsiao, K., and Prusiner, S. B. 1989. Insertion in prion protein gene in familial Creutzfeldt-Jakob disease. Lancet 1:51-52.
Parry, H. B. 1983. Scrapie Disease in Sheep. Academic Press, New York. 192 pp.
Parry, H. B. 1962. Scrapie: A transmissible and hereditary disease of sheep. Heredity 17:75-105.
Pattison, I. H. 1988. Fifty years with scrapie: A personal reminiscence. Vet. Rec. 123:661-666.
Pattison, I. H. 1965. Experiments with scrapie with special reference to the nature of the agent and the pathology of the disease. Pages 249-257 in: Slow, Latent and Temperate Virus Infections, NINDB Monogr. 2. D. C. Gajdusek, C. J. Gibbs, Jr., and M. P. Alpers, eds. U.S. Government Printing Office, Washington, DC.
Pattison, I. H. 1964. The spread of scrapie by contact between affected and healthy sheep, goats or mice. Vet. Rec. 76:333-336.
Pattison, I. H., and Jones, K. M. 1968. Modification of a strain of mouse-adapted scrapie by passage through rats. Res. Vet. Sci. 9:408-410.
Pattison, I. H., Hoare, M. N., Jebbett, J. N., and Watson, W. A. 1974. Further observations on the production of scrapie in sheep by oral dosing with foetal membranes from scrapie-infected sheep. Br. Vet. J. 130:1xv-1xvii.
Pattison, I. H., Hoare, M. N., Jebbett, J. N., and Watson, W. A. 1972. Spread of scrapie to sheep and goats by oral dosing with foetal membranes from scrapie-affected sheep. Vet. Rec. 90:465-468.
Powell, L. M., Wallis, S. C., Pease, R. J., Edwards, Y. H., Knott, T. J., and Scott, J. 1987. A novel form of tissue-specific RNA processing produces apolipoprotein B-48 in intestine. Cell 50:831-840.
Prusiner, S. B. 1989. Scrapie prions. Annu. Rev. Microbiol. 43:345-374.
Prusiner, S. B. 1987. Prions and neurodegenerative diseases. N. Engl. J. Med. 317:1571-1581.
Prusiner, S. B. 1982. Novel proteinaceous infectious particles cause scrapie. Science 216:136-144.
Prusiner, S. B., Scott, M., Foster, D., Pan, K.-M., Groth, D., Mirenda, C., Torchia, M., Yang, S. L., Serban, D., Carlson, G. A., Hoppe, P. C., Westaway, D., and DeArmond, S. J. 1990. Transgenetic studies implicate interactions between homologous PrP isoforms in scrapie prion replication. Cell 63:673-686.
Prusiner, S. B., Cochran, S. P., and Alpers, M. P. 1985. Transmission of scrapie in hamsters. J. Infect. Dis. 152:971-978.
Prusiner, S. B., Groth, D. F., Bolton, D. C., Kent, S. B., and Hood, L. E. 1984. Purification and structural studies of a major scrapie prion protein. Cell 38:127-134.
Prusiner, S. B., McKinley, M. P., Bowman, K. A., Bolton, D. C., Bendheim, P. E., Groth, D. F., and Glenner, G. G. 1983. Scrapie prions aggregate to form amyloid-like birefringent rods. Cell 35:349-358.
Prusiner, S. B., Bolton, D. C., Groth, D. F., Bowman, K. A., Cochran, S. P., and McKinley, M. P. 1982. Further purification and characterization of scrapie prions. Biochemistry 21:6942-6950.
Ridley, R. M., Baker, H. F., and Crow, T. J. 1986. Transmissible and non-transmissible neurodegenerative disease: Similarities in age of onset and genetics in relation to aetiology. Psychol. Med. 16:199-207.
Roberts, G. W., Lofthouse, R., Brown, R., Crow, T. J., Barry, R. A., and Prusiner, S. B. 1986. Prion-protein immunoreactivity in human transmissible dementias. N. Engl. J. Med. 315:1231-1233.
Scott, M., Foster, D., Mirenda, C., Serban, D., Coufal, F., Wälchli, M., Torchia, M., Groth, D., Carlson, G., DeArmond, S. J., Westaway, D., and Prusiner, S. B. 1989. Transgenic mice expressing hamster prion protein produce species-specific scrapie infectivity and amyloid plaques. Cell 59:847-857.
Serban, D., Taraboulos, A., DeArmond, S. J., and Prusiner, S. B. 1990. Rapid detection of Creutzfeldt-Jakob disease and scrapie prion proteins. Neurology 40:110-117.
Shaw, J. M., Feagin, J. E., Stuart, K., and Simpson, L. 1988. Editing of kinetoplastid mitochondrial mRNAs by uridine addition and deletion generates conserved amino acid sequences and AUG initiation codons. Cell 53:401-411.
Stahl, N., Borchelt, D. R., and Prusiner, S. B. 1990. Differential release of cellular and scrapie prion proteins from cellular membranes by phosphatidylinositol-specific phospholipase C. Biochemistry 29:5405-5412.
Stahl, N., Borchelt, D. R., Hsiao, K., and Prusiner, S. B. 1987. Scrapie prion protein contains a phosphatidylinositol glycolipid. Cell 51:229-240.
Taraboulos, A., Serban, D., and Prusiner, S. B. 1990. Scrapie prion proteins accumulate in the cytoplasm of persistently-infected cultured cells. J. Cell Biol. 110:2117-2132.
Wells, G. A. H., Scott, A. C., Johnson, C. T., Gunning, R. F., Hancock, R. D., Jeffrey, M., Dawson, M., and Bradley, R. 1987. A novel progressive spongiform encephalopathy in cattle. Vet. Rec. 121:419-420.
Westaway, D., Goodman, P. A., Mirenda, C. A., McKinley, M. P., Carlson, G. A., and Prusiner, S. B. 1987. Distinct prion proteins in short and long scrapie incubation period mice. Cell 51:651-662.
Wijeratne, W. V. S., and Curnow, R. N. 1990. A study of the inheritance of susceptibility to bovine spongiform encephalopathy. Vet. Rec. 126:5-8.
Wilesmith, J. W., Wells, G. A. H., Cranwell, M. P., and Ryan, J. B. M. 1988. Bovine spongiform encephalopathy: Epidemiological studies. Vet. Rec. 123:638-644.
Winter, M. H., Aldridge, B. M., Scott, P. R., and Clarke, M. 1989. Occurrence of 14 cases of bovine spongiform encephalopathy in a closed dairy herd. Br. Vet. J. 145:191-194.