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Molecular biology of prions

Charles Weissmann[1], Masato Enari[2], Peter-Christian Klöhn[1], Daniela Rossi[3] and **Eckhard Flechsig[4]**

[1]MRC Prion Unit, Department of Neurodegenerative Disease, Institute of Neurology, Queen Square, London WC1N 3BG, UK; [2]National Cancer Center Research Institute, Radiobiology Division Tsukiji 5-1-1, Chuo-ku, Tokyo 104-0045, Japan; [3]Department of Pharmacological Sciences, Center for Excellence on Neurodegenerative Diseases, University of Milan, Via Balzaretti 9, 20133 Milan, Italy; [4]Institut für Virologie und Immunbiologie, Versbacherstrasse 7, D-97078 Würzburg, Germany

Abstract. The "protein only" hypothesis holds that the infectious agent causing transmissible spongiform encephalopathies is a conformational isomer of PrP, a host protein predominantly expressed in brain and is strongly supported by many lines of evidence. Prion diseases are so far unique among conformational diseases in that they are transmissible, not only experimentally but also by natural routes, mainly by ingestion. The pathway of prions to the brain has been elucidated in outline. A striking feature of prions is their extraordinary resistance to conventional sterilisation procedures, and their capacity to bind to surfaces of metal and plastic without losing infectivity. This property, first observed in a clinical setting, is now being investigated in experimental settings, both in animals and in cell culture.

The correspondence should be addressed to C. Weissmann, Email: charles.weissmann@prion.ucl.ac.uk

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INTRODUCTION

Transmissible spongiform encephalopathies (TSE's), or prion diseases, are degenerative disorders of the central nervous system leading to motor dysfunction, dementia and death. Prion diseases include scrapie of sheep, bovine spongiform encephalopathy (BSE) in cattle, and human diseases such as Creutzfeldt-Jakob disease (CJD), Gerstmann-Sträussler-Scheinker syndrome (GSS) and fatal familial insomnia (FFI). More recently, variant CJD (vCJD), ascribed to consumption of BSE-contaminated products, has claimed over 120 victims. Neither humoral nor cellular immunological responses have been detected in prion diseases.

Transmissibility of scrapie was first demonstrated in 1939 (Cuille and Chelle 1939). The remarkable resistance of the causative agent, later designated prion, was revealed early on when 10% of a flock of Scottish sheep came down with scrapie after having been injected with a vaccine against looping ill prepared from formaldehyde-treated sheep brain extract (Gordon 1946). The agent's unusual resistance to UV irradiation suggested that it might be devoid of nucleic acid (Alper et al. 1967). The "protein only" hypothesis (Griffith 1967) in its updated version (Prusiner 1989) proposes that the prion is a conformational isoform of the normal host protein PrP^C (Basler et al. 1986, Oesch et al. 1985), which is found predominantly on the outer surface of neurons, attached by a glycosylphosphatidyl inositol (GPI) anchor. The abnormal conformer, when introduced into the organism, is thought to cause the conversion of PrP^C into a likeness of itself.

In prion disease a largely protease-resistant, aggregated form of PrP designated PrP^{Sc}, accumulates, mainly in brain. It is believed to be the principal or only constituent of the prion (Prusine 1989). No differences in the primary structure of PrP^C and PrP^{Sc} were detected, suggesting that they differ in their conformation (Stahl et al. 1993). The tertiary structure of PrP^C has been elucidated (Riek et al. 1997) while that of PrP^{Sc} has not,

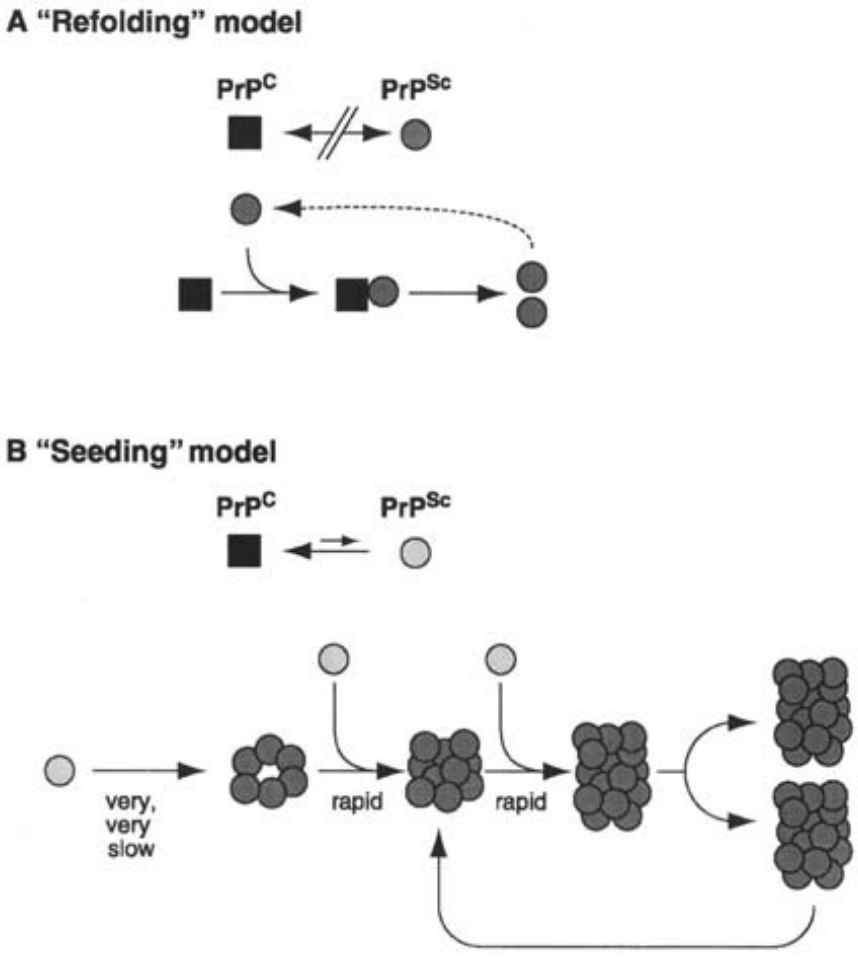


Fig. 1. Models for the conformational conversion of PrP^C to PrP^{Sc}. A, the "refolding" model: The conformational change is kinetically controlled, a high activation energy barrier preventing spontaneous conversion at detectable rates. Interaction with exogenously introduced PrP^{Sc} causes PrP^C to undergo an induced conformational change to yield PrP^{Sc}. This reaction could be facilitated by an enzyme or chaperone. In the case of certain mutations in PrP^C, spontaneous conversion to PrP^{Sc} may occur as a rare event, explaining why familial CJD or GSS arise spontaneously, albeit late in life. Sporadic CJD may come about when an extremely rare event (occurring in about one in a million individuals per year) leads to spontaneous conversion of PrP^C to PrP^{Sc}; B, the "seeding" model": PrP^C and PrP^{Sc} (or a PrP^{Sc}-like molecule, light) are in equilibrium, with PrP^C strongly favored. PrP^{Sc} is only stabilized when it adds onto a crystal-like seed or aggregate of PrP^{Sc} (dark). Seed formation is rare; however once a seed is present, monomer addition ensues rapidly. To explain exponential conversion rates, aggregates must be continuously fragmented, generating increasing surfaces for accretion.

however the ß-sheet content of PrP^{Sc} is high while that of PrP^{C} is low (Caughey et al. 1991, Pan et al. 1993). The conclusion that some form of PrP is the essential, perhaps only constituent of the infectious agent is based on compelling biochemical and genetic evidence (Prusine 1998, Weissmann 1999). The finding that PrP knockout (*Prnp$^{o/o}$*) mice are completely protected against scrapie disease and fail to propagate prions (Büeler et al. 1993, Sailer et al. 1994) and that introduction of murine *Prnp* transgenes into these mice restores susceptibility to prions is one of the main supports for the "protein only" hypothesis.

Within the framework of the "protein only" hypothesis, the "refolding model" (Fig. 1A) proposes that PrP^{C} unfolds to some extent and refolds under the influence of a PrP^{Sc} molecule and that the two states are separated by an activation energy barrier (Prusiner 1991). The "nucleation model" (Fig. 1B) postulates that PrP^{C} is in equilibrium with PrP^{Sc} (or a precursor thereof), that the equilibrium is largely in favour of PrP^{C} and that PrP^{Sc} is only stable when it forms a multimer. Once such a multimer or seed is present, monomer addition ensues rapidly (Jarrett and Lansbury 1993). "Breakage" of aggregates must be postulated to explain the exponential increase of PrP^{Sc} during infection (Orgel 1996). Conversion *in vitro* of PrP^{C} to a PrP^{Sc}-like product has been achieved by incubating ^{35}S-labeled PrP^{C} with PrP^{Sc} and demonstrating the appearance of a partly protease-resistant radioactive product which, after protease treatment, has the mobility of protease-treated authentic PrP^{Sc} (Kocisko et al. 1994). This *in vitro* conversion exhibits the species specificity (Raymond et al. 1997) and strain-specificity (Bessen et al. 1995) observed *in vivo*. However, because the yield is less than stoichiometric with regard to the PrP^{Sc} used as seed, it has not been possible to determine whether or not there was an increase in infectivity. Perhaps the "cyclic amplification" procedure reported recently will lead to this goal (Saborio et al. 2001). Whilst it has been possible to convert recombinant PrP^{C} into a β-sheet-rich, partially protease-resistant structure by physico-chemical procedures (Jackson et al. 1999, Lu and Chang 2001), there have so far been no reports that such material gives rise to transmissible prion disease (Hill et al. 1999, Kaneko et al. 2000, Shaked et al. 1999). Also, it has so far not been possible to renature completely denatured prion preparation to an infectious state (Post et al. 2000 Prusiner et al. 1993) although the infectivity of partially inactivated material can be increased by renaturation under certain conditions (McKenzie et al. 1998, Shaked et al. 2001). Prusiner and his colleagues have reported that intracerebral injection of a synthetic 55-residue peptide corresponding to region 89-143 of mouse PrP with a P101L substitution can induce neurological, prion-like disease, however this is achieved only in transgenic mice expressing PrP with the same mutation (Kaneko et al. 2000). The caveats here are that these transgenic mice show spontaneous disease even without inoculation, albeit only much later, and that transmissibility has yet to be demonstrated.

THE PUZZLE OF PRION STRAINS

Many distinct strains of scrapie prions have been derived from sheep scrapie isolates (Bruce et al. 1992). They differ by their incubation times in various inbred mouse lines, by the lesion patterns in affected brains and by the physico-chemical characteristics of the PrP^{Sc} generated. Because different strains can be propagated in a single inbred mouse line (homozygous with regard to its PrP gene) the same PrP molecule must be able to mediate different strain phenotypes. The targeting hypothesis assumes that strain specificity is associated with the glycosylation pattern of PrP^{Sc} and that this pattern is determined by the cell in which it is formed. However, inasmuch as a cloned cell line can propagate at least two different prion strains, this proposal has not been experimentally supported (Birkett et al. 2001). The conformational hypothesis proposes that each strain is associated with a different conformation of PrP^{Sc} and that each of these can convert the PrP^{C} of its host into a likeness of itself. Indeed, PrP^{Sc} species associated with two hamster-adapted scrapie strains, HY and DY, are cleaved to products of different length by proteinase K (Bessen and Marsh 1994); the different susceptibility to protease is attributed to different conformations of the cognate PrP^{Sc}. Similar findings were made with other prion strains propagated in the mouse (Hill et al. 1997, Telling et al. 1996). Moreover, PrP^{Sc} of certain strains differ in the ratio of the diglycosylated to the monoglycosylated form (Collinge et al. 1996). It has been claimed that PrP^{Sc} molecules of as many as eight different strains can be differentiated by virtue of their relative affinity for a monoclonal antibody directed against an epitope which is fully available in PrP^{C} but partially occluded in PrP^{Sc} (Safar et al. 1998). Some strains differ in their susceptibility to denaturation by guanidinium chloride, further supporting the

conformational hypothesis of strain specificity (Safar et al. 2000, Safar et al. 1998).

EXPERIMENTAL PRION TRANSMISSION

Experimental transmission of TSE's is done most efficiently by intracerebral injection. Intraocular, intraspinal, intraperitoneal and subcutaneous injections (Kimberlin et al. 1987, Kimberlin and Walker 1979, Kimberlin and Walker 1986) or scarification (Taylor et al. 1996) are less efficient. Peroral infection has been demonstrated in many animal species (Bons et al. 1999, Bradley 1996, Foster et al. 1993, Jeffrey et al. 2001, Maignien et al. 1999, Prusiner et al. 1985, Ridley and Baker 1996) . Transmission, as judged by onset of clinical disease and death, can be orders of magnitude more efficient within the same than between different species; this phenomenon defines the so-called species barrier. Seminal work by Prusiner and his group showed that introduction of the PrP transgene from the species in which the prions originated into the recipient host greatly increased susceptibility, both in regard to the proportion of animals succumbing to disease (attack rate) and time to appearance of clinical symptoms (incubation time). Thus, mice transgenic for Syrian hamster *PrP* genes, particularly in the absence of the mouse *PrP* gene, became very susceptible to hamster prions (Prusiner et al. 1990, Scott et al. 1993) to which they are normally resistant, as judged by clinical criteria. Similarly, $Prnp^{o/o}$ mice transgenic for bovine, ovine and human *PrP* genes became susceptible to prions from the cognate donors (Crozet et al. 2001, Mastrianni et al. 2001, Scott et al. 1999). However, interestingly, wild-type mice inoculated with vCJD prions have a shorter incubation time than transgenic $Prnp^{o/o}$ mice carrying human *PrP* genes, in contrast to what is found with sCJD prions (Hill et al. (1997), Asante et al. - personal communication).

Even within a species susceptibility to prion disease may be modulated by polymorphic variations of the *PrP* gene. For example, humans homozygous for the polymorphic *PrP* variant met129 are far more likely to contract sporadic CJD than the heterozygotes with the alleles met129/val129, and all cases of vCJD examined so far are homozygous for the met129 polymorphism (Collinge et al. 1996, Zeidler et al. 1997). Similarly, susceptibility of sheep to scrapie is determined by the polymorphic *PrP* genotype (Hunter et al. 2000).

Whereas the *PrP* gene is an essential determinant of susceptibility to prions, it is not the only one. For example, ectopic overexpression of *PrP* in T or B lymphocytes of $PrP^{o/o}$ mice does not render these cells susceptible to infection *in vivo* (Montrasio et al. 2001, Raeber et al. 1999b), neither is PrP expression the only feature required for susceptibility of N2a neuroblastoma cells to prions *in vitro* (Enari et al. 2001). This shows that other essential cellular components are required for

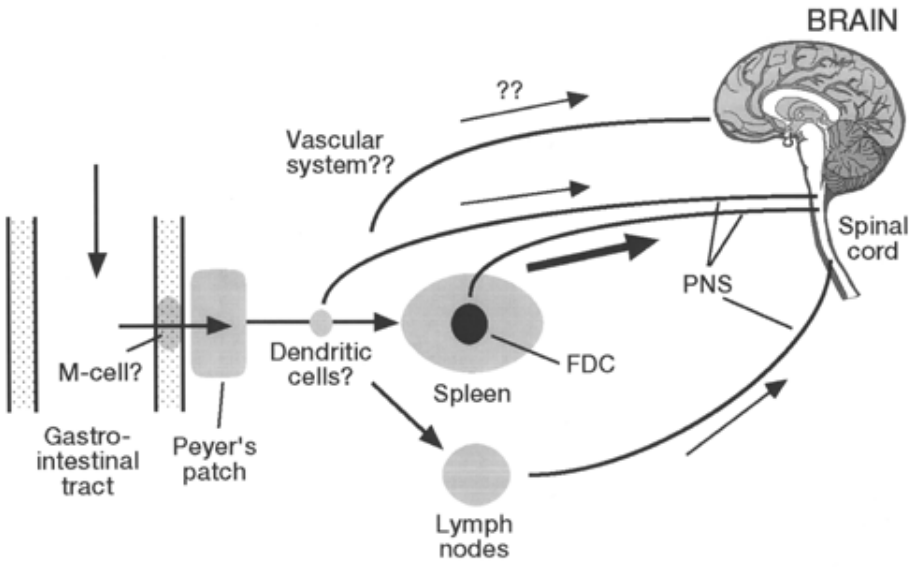


Fig. 2. Possible routes of propagation of ingested prions. After oral uptake, prions may penetrate the intestinal mucosa through M cells and reach Peyer's patches as well as the enteric nervous system. Depending on the host, prions may replicate and accumulate in spleen and lymph nodes. Myeloid dendritic cells are thought to mediate transport within the lymphoreticular system. From the lymphoreticular system and likely from other sites prions proceed along the peripheral nervous system to finally reach the brain, either directly via the vagus nerve or via the spinal cord, under involvement of the sympathetic nervous system.

prion uptake and/or replication; the conjectured "protein X" is a candidate for this role (Kaneko et al. 1997). In addition, loci other than PrP contribute to the incubation time in mice (Lloyd et al. 2001, Manolakou et al. 2001, Stephenson et al. 2000).

The interpretation of the so-called species barrier has been complicated by the finding that although mice inoculated with prions from another species fail to develop disease and thus appear to be resistant, they nonetheless accumulated PrP^{Sc} and infectivity, albeit only very late after inoculation (Hill et al. 2000, Race et al. 2001, Thackray et al. 2002). Whether or not such animals would succumb to clinical disease if they lived longer, i.e. beyond their normal life span, cannot be answered.

"NATURAL" TRANSMISSION OF PRIONS

While prion diseases are not contagious in the strict sense, i.e. by direct contact, they are transmissible perorally and parenterally. The BSE epidemic that emerged in the mid-eighties and led to about 180,000 clinically diagnosed cases (and likely to many times more non-diagnosed ones) was fueled by the feeding of BSE-prion-contaminated bone-and-meat meal to cattle (Kimberlin and Wilesmith 1994). The kuru epidemic that developed in the first half of the 20th century in Papua New Guinea was caused by ritualistic cannibalism (Alpers1979) and is believed to have originated from a case of sporadic CJD. Variant CJD is thought to come about by ingestion of BSE-prion-contaminated foodstuff and certainly mice (Maignien et al. 1999), sheep (Jeffrey et al. 2001), calves (Bradley 1996) and non-human primates (Bons et al. 1999, Ridley and Baker 1996) can be experimentally infected with the BSE agent by the oral route. It appears quite likely that sheep scrapie spreads by ingestion of the infectious agent, although the source has not been established; infected placenta has been suggested (Race et al. 1998), but scrapie prion-contaminated feces are a likely possibility that merits investigation. Perhaps the appearance of vCJD in predominantly young individuals is due to infection by contaminated foodstuff through wounds resulting from teething and tooth loss between early infancy and adolescence. Experimental transmission by the dental route has been shown in hamster (Ingrosso et al. 1999).

Somehow prions make their way from the digestive tract to the CNS (Fig. 2). The relative resistance of prion infectivity to protease digestion (Bolton et al. 1982) probably allows a significant proportion of the infectious agent to survive passage through the digestive tract (Maignien et al. 1999). It is not clear how prions pass through the intestinal mucosa. M cells, which are portals for antigens and pathogens (Hathaway and Kraehenbuhl 2000, Kaiserlian and Etchart 1999, Sansonetti and Phalipon 1999) are able to mediate transport of prions, at least in an experimental setting (Heppner et al. 2001). Thus, after oral uptake, the infectious agent may penetrate the mucosa through M cells and reach Peyer's patches, where they are found early on (Maignien et al. 1999) as well as the enteric nervous system (Beekes and McBride 2000). Depending on the host, other lymphoreticular tissue, in particular the spleen but also lymph nodes (Prinz et al. 2002), are sites in which prions replicate and accumulate; this is the case in sheep scrapie, experimental BSE in sheep, vCJD in man and experimental mouse scrapie, but not BSE in cattle (Bradley 1999). Recent reports suggest that myeloid dendritic cells mediate transport within the lymphoreticular system (Aucouturier et al. 2001, Huang et al. 2002). Interestingly, mature B cells (with or without PrP^{C} expression) are required for amplification of prions in spleen (Klein et al. 1998), however not because they themselves harbor or multiply prions (Montrasio et al. 2001), but because they are required for the maturation of follicular dendritic cells, the cells in which prion amplification and PrP^{Sc} accumulation occurs (Mabbott et al. 2000, Montrasio et al. 2000). Nonetheless, neuroinvasion is possible even in the absence of follicular dendritic cells, suggesting that other cell types in the periphery also can amplify prions (Oldstone et al. 2002, Prinz et al. 2002). From the LRS and likely from other sites, prions proceed along the peripheral nervous system to finally reach the brain, either directly *via* the vagus nerve (Beekes et al. 1998) or *via* the spinal cord, under involvement of the sympathetic nervous system (Bencsik et al. 2001). If a sufficiently high dose of prions is administered intraperitoneally, neuroinvasion can occur without participation of the LRS (Race et al. 2000).

Not only the biosynthesis of prions, but also their spread is dependent on PrP-containing cells. This was demonstrated by the finding that a PrP-expressing neuroectodermal graft in the brain of a $Prnp^{o/o}$ mouse could be infected by intracerebral injection of mouse prions but not by intraocular (Brandner et al. 1996) or intraperitoneal inoculation (Blättler et al. 1997). Even

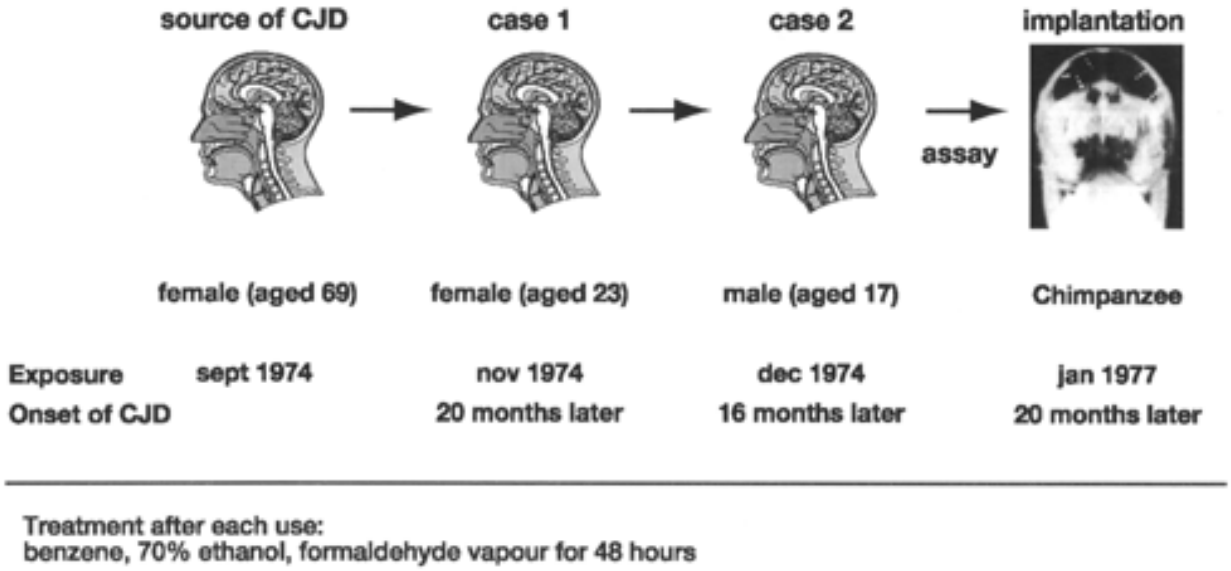
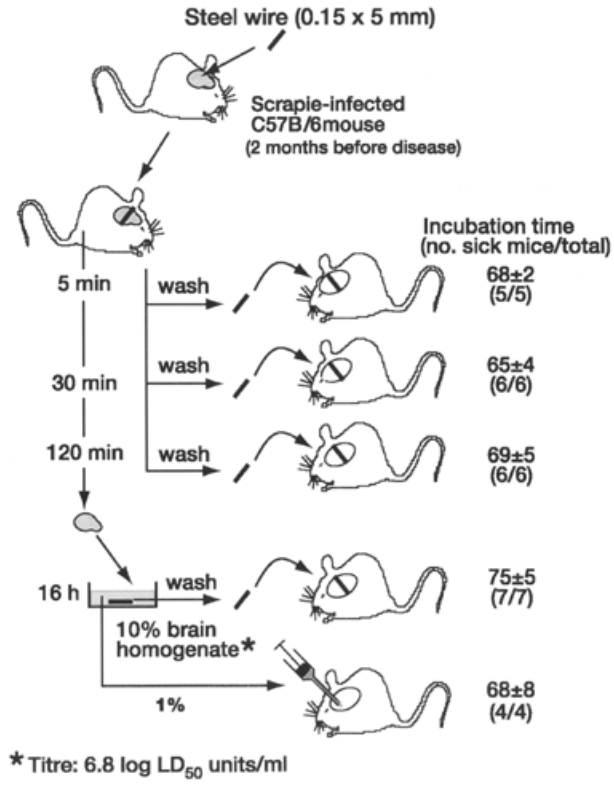


Fig. 3. Accidental transmission of sporadic CJD by an intracerebral electrode to two individuals. An electrode that had been inserted into the cortex of an unrecognized CJD patient was subjected to a decontamination procedure involving treatment with benzene, 70% ethanol and formaldehyde vapour after each use, and employed in succession on two additional patients who subsequently came down with CJD. Following these events, the tip of the electrode was implanted into the brain of a chimpanzee where it again caused lethal spongiform encephalopathy.



after irradiation and reconstitution with a PrP-expressing lymphohemopoietic system, prions failed to reach the graft after intraperitoneal or intravenous inoculation, showing that neuroinvasion, at least in the mouse, was not mediated by prion transport through the circulation (Blättler et al. 1997) and underlining the requirement of an interposed PrP-expressing compartment, shown to be the peripheral nervous system (Race et al. 2000) In the case of experimental mouse scrapie, prion infectivity could not be detected in leukocytes (Raeber et al. 1999a), nor was infectivity detected in the blood of BSE-infected cattle (Bradley 1999). However, a low but reproducible titre of prions was detected in blood of scrapie-infected hamsters (Holada et al. 2002). Also, one of 19 sheep transfused with blood from experimentally, orally BSE-infected sheep came down with prion disease (Houston et al. 2000).

Fig. 4. Transmission of mouse scrapie prions by stainless steel wire. Stainless steel wires were inserted into the brains of scrapie-infected mice for 5, 30 or 120 minutes, washed exhaustively and introduced permanently into brains of indicator mice. Five minutes of contact sufficed for the wire to acquire a maximum load of infectivity, equivalent to the injection of 30 µl 1% homogenate of the same brain. Data from Flechsig et al. (2001).

IATROGENIC TRANSMISSION OF PRIONS

Almost 300 cases of involuntary transmission of CJD by medical interventions have been reported (Brown et al. 2000). Most cases are due to injection of cadaveric human growth hormone or transplantation of dura mater, however a few incidents associated with cornea transplantation have been reported. Four instances of CJD following neurosurgical intervention have been attributed to surgical instruments that had previously been used on CJD patients (Brown 1996), however causality was proven only in one case. An electrode that had been inserted into the cortex of an unrecognized CJD patient was subjected to a decontamination procedure involving treatment with benzene, 70% ethanol and formaldehyde vapour (Fig. 3). It was then employed in succession on two young patients and cleaned as above after each use. Within 2 years both patients came down with CJD. Following these events, the tip of the electrode was implanted into the brain of a chimpanzee where it too caused lethal spongiform encephalopathy, proving that the electrode had retained infectious prions over several years and despite repeated attempts at sterilisation (Bernoulli et al. 1977, Gibbs et al. 1994).

EXPERIMENTAL TRANSMISSION OF SURFACE-BOUND PRIONS

The electrode described above had a complex structure: a steel shaft of about 6 mm diameter, with multiple silver contacts separated by rings of insulating plastic allowing for the existence of crevices into which infectious material might have penetrated. In order to clarify whether prions would bind to a homogeneous surface, we used fine stainless steel wires as model for a surgical instrument. In a first experiment wires were incubated overnight with brain homogenate from a terminally sick, murine-scrapie-infected mouse, washed exhaustively with PBS and permanently implanted into brains of indicator mice. This resulted in scrapie disease within about 70 days, an incubation time only slightly longer than that obtained by injecting 30 µl of 1% brain homogenate (Zobeley et al. 1999). In order to mimic more closely real-life conditions, stainless steel wires were inserted directly into the brains of scrapie-infected, clinically still healthy mice for various periods of time, washed exhaustively and assayed by permanent insertion into brains of indicator mice. Surprisingly, 5 minutes of contact sufficed for the wire to acquire a maximum load of infectivity, equivalent to the injection

Table I

Transient insertion of infectious wires into brains of indicator mice

Inoculation	Sick/total	Incubation time ± SD (days)
Wires infected by exposure to scrapie brain		
(a) Transient insertion into indicator mice		
30 min	4/4[$]	94 ±10
120 min	2/2[#]	87, 113
(b) Permanent insertion into indicator mice		
Wires not previously inserted	3/3	71 ± 2
Wires after transient insertion for:		
30 min	4/4	71 ± 3
120 min	5/5	68 ± 1
(c) Controls		
Wires exposed to brain homogenate	6/6	76 ± 3
Brain homogenate (1%, 0.03 ml)	3/3	69 ± 3

Infectious wires were prepared by insertion for 5 min into scrapie-infected mouse brain. After washing, they were inserted into brains of 6 deeply anaesthetised *Tga20* indicator mice for the times indicated. The recovered wires were washed and implanted into *Tga20* mice. As controls, wires incubated with 10% homogenate (6.8 log LD50 units/ml) of the same brain and the homogenate itself were introduced into indicator mice. Modified from Flechsig et al. (2001). [$], two of 6 mice died on the day of the intervention;[#], four of 6 mice died within a day of the intervention.

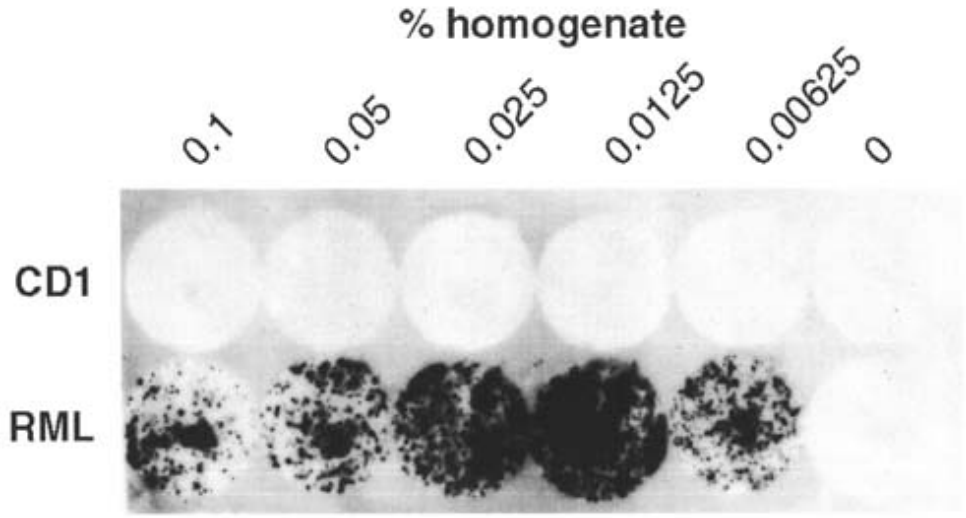


Fig. 5. Infection of mouse neuroblastoma cells by plastic-bound prions. Wells of a polystyrene 96-well microtiter plate were exposed to various dilutions of a homogenate of scrapie-prion-infected mouse brain, washed exhaustively and dried. Ten thousand N2a/Bos2 mouse neuroblastoma cells were cultured in the wells for 3 days, then transferred to 24-well plates and cultured 4 weeks, splitting 1:10 twice a week. The cells were then transferred to coverslips and assayed for the presence of PrP^{Sc}. Optimal infectivity resulted when plates were coated with 0.0125% homogenate. High concentrations of brain proteins bound to the plastic appear inhibitory for cell infection. Unpublished results (P.-C.K., CW)

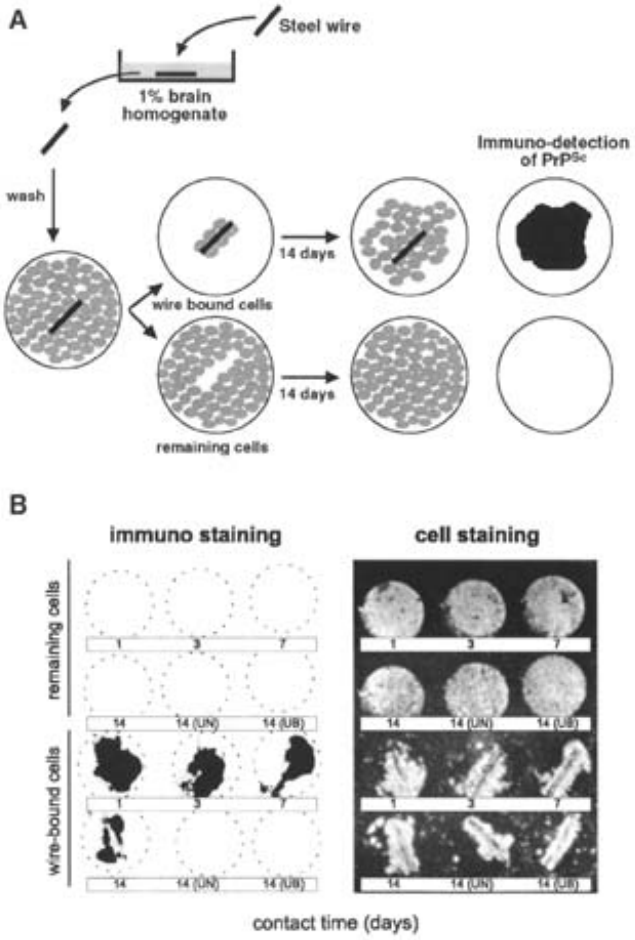


Fig. 6. Neuroblastoma cells are infected by contact with prion-coated stainless steel wires. A, stainless steel wires were exposed to scrapie-infected brain homogenates, washed and placed on a confluent layer of neuroblastoma cells. After periods ranging from 1 to 14 days the wire, to which a few cells had attached, were placed on a coverslip in a separate well and cultured for further 14 days. Both the cells remaining in the original dish ("remaining cells") and those derived from the cells clinging to the wire ("wire-bound cells") were assayed for PrP^{Sc} by the cell blot assay and the mouse bioassay. B, panels on the left show that the cultures derived from wire-bound cells had been infected, as evidenced by the accumulation of PrP^{Sc}, while residual cells remain uninfected. Panels on the right show the location of cells as revealed by ethidium bromide staining. UN, blank wire; UB, wire treated with uninfected brain homogenate. Unpublished results (M.E. and C.W.)

of 1% homogenate of the same brain (Fig. 4). A second important question regards the length of time an infectious wire must remain in contact with brain tissue in order to initiate disease. Rather than leave infectious wires permanently in the indicator mouse, they were inserted transiently, for 30 or 120 min, to mimic the conditions that might obtain during a surgical operation. As shown in Table I, a contact time of 30 min was sufficient to elicit disease, albeit with lower efficiency than was obtained after permanent insertion, as evidenced by the longer incubation time. The wires that had been inserted transiently into indicator mice remained fully infectious when introduced permanently into a further set of indicator mice (Table I) (Flechsig et al. 2001), reflecting the persistence of infectivity, as in the incident with the intracerebral electrode described above.

Why are wires exposed to infected brain or brain homogenates at least as infectious as injected homogenates, which contain far more protein than can be bound to a wire? The surfaces of steel and other metals tightly bind what appears to be a monolayer of protein (Eckert et al. 1997, Williams et al. 1985, Williams and Williams 1988). The unexpected high infectivity of steel wires could be due to selective binding of infectious particles or a higher potency of surface bound infectivity. It has been shown that despite the resistance of PrP^{Sc} and scrapie infectivity to treatment *in vitro* with proteinase K, prion titers in brain after intracerebral inoculation decrease below the level of detectability within 4 days or less (Büeler et al. 1993). On the other hand, infectious wires left for 5 days in brain still retained infectivity (Flechsig et al. 2001). Perhaps metal-bound prions are protected against rapid degradation in the brain and their apparently high specific infectivity may therefore be due to the long persistence of relatively low levels of infectivity. It can be mentioned in passing that prion-coated gold wires exhibit similar infectivity intracerebrally as steel wires (Flechsig et al. 2001), and that plastic surfaces, such as polystyrene (Fig. 5), polypropylene or polyethylene also tightly bind prions and transmit scrapie infectivity to adherent susceptible cultured cells (M.E., D.R., P.K. and C.W., unpublished data).

We attempted to elute PrP from infectious steel wires with 2M NaOH, but failed to detect either protein (detection limit, 50 ng/wire) or PrP (detection limit 15 pg/wire). On the other hand, PrP immunoreactivity could be detected at the surface of prion-coated wires by chemiluminescence (Flechsig et al. 2001). This raises the question as to whether infection of brain tissue elicited by infected wires comes about by direct contact with irreversibly surface-bound prions or whether it is due to a slow, so far undetected release of prions. This question is difficult to answer experimentally, however it would seem that intimate contact between the prion-loaded surface and target cells is a prerequisite for infection. Prion-coated wires were placed on monolayers of mouse neuroblastoma cells highly susceptible to mouse prions (Enari et al. 2001). After 1 to 14 days the wires, to which some cells had adhered, were transferred onto coverslips in the wells of a tissue culture plate and incubated for 14 days, allowing the cells to migrate off the wire and multiply. Cells derived from both the residual monolayer and the wire were blotted

Table II

Effect of various treatments on the infectivity of wire-bound prions

	Sick/Total	Incubation Time ± SD (days)
Uninfected Wires		
Untreated	0/3	
Infected Wires		
Untreated	6/6	76 ± 5
NaOH (1m, 1h, 25°C)	0/6	>260
Formaldehyde (10%, 1h, 25°C)	6/6	92 ± 8
Guanidinium thiocyanate (4M, 16h, 25°C)	0/6	>260
Autoclaving (121°C, 20 min)	0/6	>170

Modified from Flechsig et al. (2001) and unpublished results (E.F., C.W.).

onto nitrocellulose membranes and assayed for the presence of protease-resistant PrP, the surrogate marker of prion infection (Bosque and Prusiner 2000). Only the cells derived from the infected wire, but not from the residual monolayer were PrP^{Sc}-positive (Fig. 6) and contained infectivity (M.E., E.F. and C.W., unpublished data). This experiment shows that intimate contact between the prion-carrying surface and susceptible cells greatly promotes infection or is prerequisite. Similarly, cell-to-cell transmission of infectivity in cell culture is orders of magnitude more efficient than transmission by a prion preparation (Kanu et al. 2002).

The availability of prion-coated steel wires mimicking contaminated surgical instruments makes it possible to assess the efficacy of sterilisation conditions on surface-bound prions. Preliminary results (Table II) confirm that treatment with formaldehyde is insufficient to sterilize infectious wires, while treatment with sodium hydroxide, guanidinium thiocyanate (Flechsig et al. 2001) or autoclaving at 121°C for 20 min is efficacious (E.F., C.W. unpublished results). It is however not appropriate to derive from these experiments recommendations for the sterilisation of surgical instruments; it will first be necessary to validate the procedures scaling up the contact surface between metal and brain tissue and, importantly, using vCJD prions in a susceptible host, preferably a non-human primate.

CONCLUDING REMARKS

Twenty or more diseases of humans are associated with the deposition of beta-sheet-rich protein aggregates, or amyloid (Carrell and Lomas1997, Lansbury 1999). They are frequently designated "conformational diseases" although it is not in all cases clear whether or to what extent the misfolded proteins are the cause of the disease rather than the consequence. Prion diseases are so far unique conformational diseases, because they are transmissible by misfolded protein, not only under experimental conditions but also naturally, predominantly by ingestion. Although in certain cases the inception of an experimental amyloidosis can be accelerated by the injection of amyloid into a predisposed host (Johan et al. 1998), prions are exceptional in that they are able to enter their hosts by natural portals and make their way from the gut to the brain, utilising intermediate tissues for amplification. In the case of microbes and viruses such sophisticated behaviour is attributed to evolutionary processes, that is, genomic mutations and selection of mutants that most readily enter their host and find a suitable niche in which to replicate and/or perpetuate themselves. However, prion protein is encoded by the genome of its host, so what drives the prion to become more efficient in the destruction of its parent? We can only speculate. For example, the "misfolded" form of PrP may have originated as a "messenger" protein that on the one hand has or had a physiological function but on the other has a malignant potential that is rarely realised and was not selected against because evolutionary pressure does not operate efficiently at post-reproductive age. It has been proposed that in yeast a "prion-like" phenomenon involving Sup35 may confer selective advantage on yeast growing under fluctuating environmental conditions (True and Lindquist 2000). Another possibility is that PrP / PrP^{Sc} is derived from an ancient pathogen whose genetic material was integrated into the genome of its host and harnessed to fulfill a useful function while its pathogenic potential was minimised. More trivially, mammalian prion disease could be the result of the natural propensity of proteins to assume a -sheet rich conformation (Chiti et al. 1999), a failure of the organism to prevent their formation and accumulation in some cases, and the coincidental ability of the conformational isomer to penetrate organisms and cells through natural portals.

REFERENCES

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

Alpers M. (1979) Epidemiology and ecology of kuru. In: Slow transmissible diseases of the nervous system (Ed. S. B. Prusine). Academic Press, New York, p. 67.

Aucouturier P., Geissmann F., Damotte D., Saborio G.P., Meeker, H.C., Kascsak R., Carp R.I., Wisniewski T. (2001) Infected splenic dendritic cells are sufficient for prion transmission to the CNS in mouse scrapie. J. Clin. Invest. 108: 703-8.

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

Beekes M., McBride P.A. (2000) Early accumulation of pathological PrP in the enteric nervous system and gut-associated lymphoid tissue of hamsters orally infected with scrapie. Neurosci. Lett. 278: 181-184.

Beekes M., McBride P.A. Baldauf E. (1998) Cerebral targeting indicates vagal spread of infection in hamsters fed with scrapie. J. Gen. Virol. 79: 601-607.

Bencsik A., Lezmi S., Hunsmann G., Baron T. (2001) Close vicinity of PrP expressing cells (FDC) with noradrenergic fibers in healthy sheep spleen. Dev. Immunol. 8: 235-241.

Bernoulli C., Siegfried J., Baumgartner G., Regli F., Rabinowicz T., Gajdusek D.C., Gibbs C.J., Jr. (1977) Danger of accidental person-to-person transmission of Creutzfeldt-Jakob disease by surgery. Lancet 1: 478-479.

Bessen R.A., Kocisko D.A., Raymond G.J., Nandan S., Lansbury P.T., Caughey B. (1995) Non-genetic propagation of strain-specific properties of scrapie prion protein. Nature 375: 698-700.

Bessen R.A., Marsh R.F. (1994) Distinct PrP properties suggest the molecular basis of strain variation in transmissible mink encephalopathy. J. Virol. 68: 7859-7868.

Birkett C.R., Hennion R.M., Bembridge D.A., Clarke M.C., Chree A., Bruce M.E., Bostock C.J. (2001) Scrapie strains maintain biological phenotypes on propagation in a cell line in culture. EMBO J. 20: 3351-3358.

Blättler T., Brandner S., Raeber A.J., Klein M.A., Voigtlander T., Weissmann C., Aguzzi A. (1997) PrP-expressing tissue required for transfer of scrapie infectivity from spleen to brain. Nature 389: 69-73.

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

Bons N., Mestre-Frances N., Belli P., Cathala F., Gajdusek D.C., Brown P. (1999) Natural and experimental oral infection of nonhuman primates by bovine spongiform encephalopathy agents. Proc. Natl. Acad. Sci. USA 96: 4046-51.

Bosque P.J., Prusiner S.B. (2000) Cultured cell sublines highly susceptible to prion infection. J. Virol. 74: 4377-4386.

Bradley R. (1996) Bovine spongiform encephalopathy-distribution and update on some transmission and decontamination studies. In: Bovine spongiform encephalopathy- the BSE dilemma (Ed. C.J. Gibbs Jr). New York, NY, Springer, p. 11.

Bradley R. (1999) BSE transmission studies with particular reference to blood. Dev. Biol. Stand. 99: 35-40.

Brandner S., Raeber A., Sailer A., Blattler T., Fischer M., Weissmann C., Aguzzi A. (1996) Normal host prion protein (PrPC) is required for scrapie spread within the central nervous system. Proc. Natl. Acad. Sci. USA 93: 13148-13151.

Brown P. (1996) Environmental causes of human spongiform encephalopathy. In: Prion Diseases (Eds. H. Baker and N.J. Totowa). USA, Humana Press Inc., p. 139-154.

Brown P., Preece M., Brandel J.P., Sato T., McShane L., Zerr I., Fletcher A., Will R.G., Pocchiari M., Cashman N.R., d'Aignaux J.H., Cervenakova L., Fradkin J., Shonberger L.B., Collins S.J. (2000) Iatrogenic Creutzfeldt-Jakob disease at the millennium. Neurology 55: 1075-1081.

Bruce M.E., Fraser H., McBride P.A., Scott J.R., Dickinson A.G. (1992) The basis of strain variation in scrapie. In: Prion Diseases of Humans and Animals (Eds. S.B. Prusiner et al..). New York, London, Ellis Horwood, p. 497-508.

Büeler H., Aguzzi A., Sailer A., Greiner R.A., Autenried P., Aguet M., Weissmann C. (1993) Mice devoid of PrP are resistant to scrapie. Cell 73: 1339-1347.

Carrell R.W., Lomas D.A. (1997) Conformational disease. Lancet 350: 134-138.

Caughey B.W., Dong A., Bhat K.S., Ernst D., Hayes S.F., Caughey W.S. (1991) Secondary structure analysis of the scrapie-associated protein PrP 27-30 in water by infrared spectroscopy, Biochemistry 30: 7672-7680.

Chiti F., Webster P., Taddei N., Clark A., Stefani M., Ramponi G., Dobson C.M. (1999) Designing conditions for *in vitro* formation of amyloid protofilaments and fibrils. Proc. Natl. Acad. Sci. USA 96: 3590-94.

Collinge J., Sidle K.C., Meads J., Ironside J., Hill A.F. (1996) Molecular analysis of prion strain variation and the aetiology of "new variant" CJD. Nature 383: 685-690.

Crozet C., Bencsik A., Flamant F., Lezmi S., Samarut J., Baron T. (2001) Florid plaques in ovine PrP transgenic mice infected with an experimental ovine BSE. EMBO Rep. 2: 952-956.

Cuille J., Chelle P.L. (1939) Experimental transmission of trembling to the goat. C.R. Seances Acad. Sci. 208: 1058-1160.

Eckert R., Jeney S., Horber J.K. (1997) Understanding intercellular interactions and cell adhesion: lessons from studies on protein-metal interactions. Cell Biol. Int. 21: 707-713.

Enari M., Flechsig E., Weissmann C. (2001) Scrapie prion protein accumulation by scrapie-infected neuroblastoma cells abrogated by exposure to a prion protein antibody. Proc. Natl. Acad. Sci. USA 98: 9295-9299.

Fischer M., Rülicke T., Raeber A., Sailer A., Moser M., Oesch B., Brandner S., Aguzzi A., Weissmann C. (1996) Prion protein (PrP) with amino-proximal deletions restoring susceptibility of PrP knockout mice to scrapie. EMBO J. 15: 1255-1264.

Flechsig E., Hegyi I., Enari M., Schwarz P., Collinge J., Weissmann C. (2001) Transmission of scrapie by steel-surface-bound prions. Mol. Med. 7: 679-684.

Foster J.D., Hope J., Fraser H. (1993) Transmission of bovine spongiform encephalopathy to sheep and goats. Vet. Rec.133: 339-341.

Gibbs C.J., Jr., Asher D.M., Kobrine A., Amyx H.L., Sulima M.P., Gajdusek D.C. (1994) Transmission of Creutzfeldt-Jakob disease to a chimpanzee by electrodes contaminated during neurosurgery. J. Neurol. Neurosurg. Psychiatry 57: 757-758.

Gordon W.S. (1946) Advances in veterinary research- looping ill, tick-borne fever and scrapie. Vet. Rec. 58: 516-20.

Griffith J.S. (1967) Self-replication and scrapie. Nature 215: 1043-1044.

Hathaway L.J., Kraehenbuhl J.P. (2000) The role of M cells in mucosal immunity. Cell. Mol. Life Sci. 57: 323-332.

Heppner F.L., Christ A.D., Klein M.A., Prinz M., Fried M., Kraehenbuhl J.P., Aguzzi A. (2001) Transepithelial prion transport by M cells. Nat. Med. 7: 976-977.

Hill A.F., Antoniou M., Collinge J. (1999) Protease-resistant prion protein produced in vitro lacks detectable infectivity. J. Gen. Virol. 80: 11-14.

Hill A.F., Desbruslais M., Joiner S., Sidle K.C.L., Gowland I., Collinge J., Doey L.J., Lantos P. (1997) The same prion strain causes vCJD and BSE. Nature 389: 448-450.

Hill A.F., Joiner S., Linehan J., Desbruslais M., Lantos P.L., Collinge J. (2000) Species-barrier-independent prion replication in apparently resistant species. Proc. Natl. Acad. Sci. USA 97: 10248-10253.

Holada K., Vostal J.G., Theisen P.W., MacAuley C., Gregori L., Rohwer R.G. (2002) Scrapie infectivity in hamster blood is not associated with platelets. J. Virol. 76: 4649-4650.

Houston F., Foster J.D., Chong A., Hunter N., Bostock C.J. (2000) Transmission of BSE by blood transfusion in sheep. Lancet 356: 999-1000.

Huang F.P., Farquhar C.F., Mabbott N.A., Bruce M.E., MacPherson G.G. (2002) Migrating intestinal dendritic cells transport PrP(Sc) from the gut. J. Gen. Virol. 83: 267-271.

Hunter N., Goldmann W., Marshall E., O'Neill G. (2000) Sheep and goats: natural and experimental TSEs and factors influencing incidence of disease. Arch. Virol. Suppl. p. 181-188.

Ingrosso L., Pisani F., Pocchiari M. (1999) Transmission of the 263K scrapie strain by the dental route. J. Gen. Virol. 80: 3043-3047.

Jackson G.S., Hosszu L.L., Power A., Hill A.F., Kenney J., Saibil H., Craven C.J., Waltho J.P., Clarke A.R., Collinge J. (1999) Reversible conversion of monomeric human prion protein between native and fibrilogenic conformations. Science 283: 1935-1937.

Jarrett J.T., Lansbury P.J. (1993) Seeding "one-dimensional crystallization" of amyloid: a pathogenic mechanism in Alzheimer's disease and scrapie? Cell 73: 1055-1058.

Jeffrey M., Ryder S., Martin S., Hawkins S.A., Terry L., Berthelin-Baker C., Bellworthy S.J. (2001) Oral inoculation of sheep with the agent of bovine spongiform encephalopathy (BSE). 1. Onset and distribution of disease-specific PrP accumulation in brain and viscera. J. Comp. Pathol. 124: 280-289.

Johan K., Westermark G., Engstrom U., Gustavsson A., Hultman P., Westermark P. (1998) Acceleration of amyloid protein A amyloidosis by amyloid-like synthetic fibrils. Proc. Natl. Acad. Sci. USA 95: 2558-2563.

Kaiserlian D., Etchart N. (1999) Entry sites for oral vaccines and drugs: A role for M cells, enterocytes and dendritic cells? Semin. Immunol. 11: 217-224.

Kaneko K., Ball H.L., Wille H., Zhang H., Groth D., Torchia M., Tremblay P., Safar J., Prusiner S.B., DeArmond S.J., Baldwin M.A., Cohen F.E. (2000) A synthetic peptide initiates Gerstmann-Straussler-Scheinker (GSS) disease in transgenic mice. J. Mol. Biol. 295: 997-1007.

Kaneko K., Zulianello L., Scott M., Cooper C.M., Wallace A.C., James T.L., Cohen F.E. Prusiner S.B. (1997) Evidence for protein X binding to a discontinuous epitope on the cellular prion protein during scrapie prion propagation. Proc. Natl. Acad. Sci. USA 94: 10069-10074.

Kanu N., Imokawa Y., Drechsel D.N., Williamson R.A., Birkett C.R., Bostock C.J., Brockes J.P. (2002) Transfer of scrapie prion infectivity by cell contact in culture. Curr. Biol. 12: 523-530.

Kimberlin R.H., Cole S., Walker C.A. (1987) Pathogenesis of scrapie is faster when infection is intraspinal instead of intracerebral. Microb. Pathog. 2: 405-415.

Kimberlin R.H., Walker C.A. (1979) Pathogenesis of mouse scrapie: dynamics of agent replication in spleen, spinal cord and brain after infection by different routes. J. Comp. Pathol. 89: 551-562.

Kimberlin R.H., Walker C.A. (1986) Pathogenesis of scrapie (strain 263K) in hamsters infected intracerebrally, intraperitoneally or intraocularly. J. Gen. Virol. 67: 255-263.

Kimberlin R.H., Wilesmith J.W. (1994) Bovine spongiform encephalopathy. Epidemiology, low dose exposure and risks. Ann. N. Y. Acad. Sci. 6: 724.

Klein M.A., Frigg R., Raeber A.J., Flechsig E., Hegyi I., Zinkernagel R.M., Weissmann C., Aguzzi A. (1998) PrP expression in B lymphocytes is not required for prion neuroinvasion Nat. Med. 4: 1429-1433.

Kocisko D.A., Come J.H., Priola S.A., Chesebro B., Raymond G.J., Lansbury P.T., Caughey B. (1994) Cell-free formation of protease-resistant prion protein. Nature 370: 471-474.

Lansbury P.T.,Jr. (1999) Evolution of amyloid: what normal protein folding may tell us about fibrillogenesis and disease. Proc. Natl. Acad. Sci. USA 96: 3342-44.

Lloyd S.E., Onwuazor O.N., Beck J.A., Mallinson G., Farrall M., Targonski P., Collinge J., Fisher E.M. (2001) Identification of multiple quantitative trait loci linked to prion disease incubation period in mice. Proc. Natl. Acad. Sci. USA 98: 6279-6283.

Lu B.Y., Chang J.Y. (2001) Isolation of isoforms of mouse prion protein with PrP(SC)-like structural properties. Biochemistry 40: 13390-6.

Mabbott N.A., Mackay F., Minns F., Bruce M.E. (2000) Temporary inactivation of follicular dendritic cells delays neuroinvasion of scrapie. Nat. Med. 6: 719-720.

Maignien T., Lasmezas C.I., Beringue V., Dormont D., Deslys J.P. (1999) Pathogenesis of the oral route of infection of mice with scrapie and bovine spongiform encephalopathy agents. J. Gen. Virol. 80: 3035-3042.

Manolakou K., Beaton J., McConnell I., Farquar C., Manson J., Hastie N.D., Bruce M., Jackson I. J. (2001) Genetic and environmental factors modify bovine spongiform encephalopathy incubation period in mice. Proc. Natl. Acad. Sci. USA 98: 7402-7.

Mastrianni J.A., Capellari S., Telling G.C., Han D., Bosque P., Prusiner S.B., DeArmond S.J. (2001) Inherited prion disease caused by the V210I mutation: Transmission to transgenic mice. Neurology 57: 2198-2205.

McKenzie D., Bartz J., Mirwald J., Olander D., Marsh R., Aiken J. (1998) Reversibility of scrapie inactivation is enhanced by copper. J. Biol. Chem. 273: 25545-547.

Montrasio F., Cozzio A., Flechsig E., Rossi D., Klein M.A., Rulicke T., Raeber A.J., Vosshenrich C.A., Proft J., Aguzzi A., Weissmann C. (2001) B lymphocyte-restricted expression of prion protein does not enable prion replication in prion protein knockout mice. Proc. Natl. Acad. Sci. USA 98: 4034-4037.

Montrasio F., Frigg R., Glatzel M., Klein M.A., Mackay F., Aguzzi A., Weissmann C. (2000) Impaired prion replication in spleens of mice lacking functional follicular dendritic cells. Science 288: 1257-1259.

Oesch B., Westaway D., Wälchli M., McKinley M.P., Kent S.B., Aebersold R., Barry R.A., Tempst P., Teplow D.B., Hood L.E., Prusiner S.B., Weissmann C. (1985) A cellular gene encodes scrapie PrP 27-30 protein. Cell 40: 735-746.

Oldstone M.B., Race R., Thomas D., Lewicki H., Homann D., Smelt S., Holz A., Koni P., Lo D., Chesebro B., Flavell R. (2002) Lymphotoxin-alpha- and lymphotoxin-beta-deficient mice differ in susceptibility to scrapie: evidence against dendritic cell involvement in neuroinvasion. J. Virol. 76: 4357-4363.

Orgel L.E. (1996) Prion replication and secondary nucleation, Chem. Biol. 3: 413-414.

Pan K.M., Baldwin M., Nguyen J., Gasset M., Serban A., Groth D., Mehlhorn I., Huang Z., Fletterick R.J., Cohen F.E., Prusiner S.B. (1993) Conversion of alpha-helices into beta-sheets features in the formation of the scrapie prion proteins. Proc. Natl. Acad. Sci. USA 90: 10962-10966.

Post K., Brown D.R., Groschup M., Kretzschmar H.A., Riesner D. (2000) Neurotoxicity but not infectivity of prion proteins can be induced reversibly *in vitro.* Arch. Virol. Suppl.: 265-273.

Prinz M., Montrasio F., Klein M.A., Schwarz P., Priller J., Odermatt B., Pfeffer K., Aguzzi A. (2002) Lymph nodal prion replication and neuroinvasion in mice devoid of follicular dendritic cells. Proc. Natl. Acad. Sci. USA 99: 919-924.

Prusiner S.B. (1989) Scrapie prions. Annu. Rev. Microbiol. 43: 345-374.

Prusiner S.B. (1991) Molecular biology of prion diseases. Science 252: 1515-1522.

Prusiner S.B. (1998) Prions. Proc. Natl. Acad. Sci. USA 95: 13363-383.

Prusiner S.B., Cochran S.P., Alpers M.P. (1985) Transmission of scrapie in hamsters. J. Infect. Dis. 152: 971-978.

Prusiner S.B., Groth D., Serban A., Stahl N., Gabizon R. (1993) Attempts to restore scrapie prion infectivity after exposure to protein denaturants. Proc. Natl. Acad. Sci. USA 90: 2793-2797.

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., DeArmond S.J. (1990) Transgenetic studies implicate interactions between homologous PrP isoforms in scrapie prion replication. Cell 63: 673-686.

Race R., Jenny A., Sutton D. (1998) Scrapie infectivity and proteinase K-resistant prion protein in sheep placenta, brain, spleen, and lymph node: implications for transmission and antemortem diagnosis. J. Infect. Dis. 178: 949-953.

Race R., Oldstone M., Chesebro B. (2000) Entry versus blockade of brain infection following oral or intraperitoneal scrapie administration: role of prion protein expression in peripheral nerves and spleen. J.Virol. 74: 828-833.

Race R., Raines A., Raymond G.J., Caughey B., Chesebro B. (2001) Long-term subclinical carrier state precedes scrapie replication and adaptation in a resistant species: analogies to bovine spongiform encephalopathy and variant Creutzfeldt--Jakob disease in humans. J. Virol. 75: 10106-12.

Raeber A.J., Klein M.A., Frigg R., Flechsig E., Aguzzi A., Weissmann C. (1999a) PrP-dependent association of prions with splenic but not circulating lymphocytes of scrapie-infected mice. EMBO J. 18: 2702-2706.

Raeber A.J., Sailer A., Hegyi I., Klein M.A., Rulicke T., Fischer M., Brandner S., Aguzzi A., Weissmann C. (1999b) Ectopic expression of prion protein (PrP) in T lymphocytes or hepatocytes of PrP knockout mice is insufficient to sustain prion replication. Proc. Natl. Acad. Sci. USA 96: 3987-3992.

Raymond G.J., Hope J., Kocisko D.A., Priola S.A., Raymond L.D., Bossers A., Ironside J., Will R. G., Chen S.G., Petersen R.B., Gambetti P., Rubenstein R., Smits M.A., Lansbury P.T. Jr, Caughey B. (1997) Molecular assessment of the potential transmissibilities of BSE and scrapie to humans. Nature 388: 285-288.

Ridley R.M., Baker H.F. (1996) Oral transmission of BSE to primates [letter]. Lancet 348: 1174.

Riek R., Hornemann S., Wider G., Glockshuber R., Wüthrich K. (1997) NMR characterization of the full-length recombinant murine prion protein, *m*PrP(23-231). FEBS Letters 413: 282-288.

Saborio G.P., Permanne B., Soto C. (2001) Sensitive detection of pathological prion protein by cyclic amplification of protein misfolding. Nature 411: 810-813.

Safar J., Cohen F.E., Prusiner S.B. (2000) Quantitative traits of prion strains are enciphered in the conformation of the prion protein. Arch. Virol. Suppl. p. 227-235.

Safar J., Wille H., Itri V., Groth D., Serban H., Torchia M., Cohen F.E., Prusiner S.B. (1998) Eight prion strains have PrP(Sc) molecules with different conformations. Nat. Med. 4: 1157-1165.

Sailer A., Büeler H., Fischer M., Aguzzi A., Weissmann C. (1994) No propagation of prions in mice devoid of PrP. Cell 77: 967-968.

Sansonetti P.J., Phalipon A. (1999) M cells as ports of entry for enteroinvasive pathogens: mechanisms of interaction, consequences for the disease process. Semin. Immunol. 11: 193-203.

Scott M., Groth D., Foster D., Torchia M., Yang S.L., DeArmond S.J., Prusiner S.B. (1993) Propagation of prions with artificial properties in transgenic mice expressing chimeric PrP genes. Cell 73: 979-988.

Scott M.R., Will R., Ironside J., Nguyen H.O., Tremblay P., DeArmond S.J., Prusiner S.B. (1999) Compelling transgenetic evidence for transmission of bovine spongiform encephalopathy prions to humans. Proc. Natl. Acad. Sci. USA 96: 15137-15142.

Shaked G.M., Fridlander G., Meiner Z., Taraboulos A., Gabizon R. (1999) Protease-resistant and detergent-insoluble prion protein is not necessarily associated with prion infectivity. J. Biol. Chem. 274: 17981-17986.

Shaked G.M., Meiner Z., Avraham I., Taraboulos A., Gabizon R. (2001) Reconstitution of prion infectivity from solubilized protease-resistant PrP and nonprotein components of prion rods. J. Biol. Chem. 276: 14324-14328.

Stahl N., Baldwin M.A., Teplow D.B., Hood L., Gibson B.W., Burlingame A.L., Prusiner S.B. (1993) Structural studies of the scrapie prion protein using mass spectrometry and amino acid sequencing. Biochemistry 32: 1991-2002.

Stephenson D.A., Chiotti K., Ebeling C., Groth D., DeArmond S.J., Prusiner S.B., Carlson G.A. (2000) Quantitative trait loci affecting prion incubation time in mice. Genomics 69: 47-53.

Taylor D.M., McConnell I., Fraser H. (1996) Scrapie infection can be established readily through skin scarification in immunocompetent but not immunodeficient mice. J. Gen. Virol. 77: 1595-1599.

Telling G.C., Haga T., Torchia M., Tremblay P., DeArmond S.J., Prusiner S.B. (1996) Interactions between wild-type and mutant prion proteins modulate neurodegeneration in transgenic mice. Genes Dev. 10: 1736-1750.

Thackray A.M., Klein M.A., Aguzzi A., Bujdoso R. (2002) Chronic subclinical prion disease induced by low-dose inoculum. J. Virol. 76: 2510-2517.

True H.L., Lindquist S.L. (2000) A yeast prion provides a mechanism for genetic variation and phenotypic diversity. Nature 407: 477-483.

Weissmann C. (1999) Molecular genetics of transmissible spongiform encephalopathies, J. Biol. Chem. 274: 3-6.

Will R.G., Ironside J.W., Zeidler M., Cousens S.N., Estibeiro K., Alperovitch A., Poser S., Pocchiari M., Hofman A., Smith P.G. (1996) A new variant of Creutzfeldt-Jakob disease in the UK. Lancet 347: 921-925.

Williams D.F., Askill I.N., Smith R. (1985) Protein absorption and desorption phenomena on clean metal surfaces. J. Biomed. Mater. Res. 19: 313-320.

Williams R.L., Williams D.F. (1988) Albumin adsorption on metal surfaces. Biomaterials 9: 206-212.

Zeidler M., Stewart G., Cousens S.N., Estibeiro K., Will R.G. (1997) Codon 129 genotype and new variant CJD. Lancet 350: 668.

Zobeley E., Flechsig E., Cozzio A., Enari M., Weissmann C. (1999) Infectivity of scrapie prions bound to a stainless steel surface. Mol. Med. 5: 240-243

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