# Transmission of Prions

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**The "protein only" hypothesis holds that the infectious agent causing transmissible spongiform encephalopathies is a conformational isomer of PrP, a host protein that is predominantly expressed in the brain. This hypothesis is strongly supported by many lines of evidence. To date, prion diseases are unique among conformational diseases in that they are transmissible—experimentally and 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 sterilization 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.**

Transmissible spongiform encephalopathies (TSEs), or prion diseases, are degenerative disorders of the central nervous system (CNS) that lead to motor dysfunction, dementia, and death. Prion diseases include scrapie of sheep, bovine spongiform encephalopathy (BSE) in cattle, and such human diseases as Creutzfeldt-Jakob disease (CJD), Gerstmann-Sträussler-Scheinker syndrome, and fatal familial insomnia. More recently, variant CJD (vCJD), ascribed to consumption of BSE-contaminated products [1], has claimed over 100 victims. Neither humoral nor cellular immunologic responses have been detected in prion diseases.

Transmissibility of scrapie was first demonstrated in 1939 [2]. The remarkable resistance of the causative agent, later designated prion, was revealed when 10% of a flock of Scottish sheep developed scrapie after injection with a vaccine against looping ill prepared from formaldehyde-treated sheep brain extract [3]. The agent's unusual resistance to UV irradiation suggested that it might be devoid of nucleic acid [4]. The "protein only" hypothesis [5] in its updated version [6] proposes that the prion is a conformational isoform of the normal host protein $PrP^{C}$ [7, 8], which is found predominantly on the outer surface of neurons, attached by a glycosylphosphatidyl inositol anchor. The abnormal conformer, when introduced into the organism, would 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. $PrP^{Sc}$ is believed to be the principal or only constituent of the prion [6]. No differences in the primary structure of $PrP^{C}$ and $PrP^{Sc}$ have been detected, suggesting that they differ in their conformation [9]. While the tertiary structure of $PrP^{C}$ has been elucidated [10], that of $PrP^{Sc}$ has not; however the $\beta$-sheet content of $PrP^{Sc}$ is high while that of $PrP^{C}$ is low [11, 12]. 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 [13, 14]. The finding that PrP knockout *($Prnp^{o/o}$)* mice were completely protected against scrapie disease and failed to propagate prions [15, 16] and that introduction of murine *Prnp* transgenes into these mice restored susceptibility to prions [17] provides primary support for the protein only hypothesis.

Within the framework of the protein only hypothesis, the "refolding model" (figure 1*A*) proposes that $PrP^{C}$ unfolds to some extent and refolds under the influence of a $PrP^{Sc}$ molecule and that an activation energy barrier separates the two states [18]. The "nucleation model" (figure 1*B*) proposes that $PrP^{C}$ is in equilibrium with $PrP^{Sc}$ (or a precursor thereof) and that $PrP^{Sc}$ is only stable when it forms a multimer. Once such a multimer or seed is present, monomer addition ensues rapidly [19]. "Breakage" of aggregates must be postulated to explain the exponential increase of $PrP^{Sc}$ during infection [20]. Conversion in vitro of $PrP^{C}$ to a $PrP^{Sc}$-like product has been achieved by incubating $^{35}S$-labeled $PrP^{C}$ with $PrP^{Sc}$. Results showed the appearance of a partly protease-resistant radioactive product that, after protease treatment, had the mobility of protease-treated authentic $PrP^{Sc}$ [21]. This in vitro conversion exhibited the "species specificity" [22] and strain specificity [23] observed in vivo. However, because the yield was less than stoichiometric with regard to the $PrP^{Sc}$ used as seed, it has not been possible to determine whether there was an increase in infectivity. Perhaps the recently reported "cyclic amplification" procedure will lead to this goal [24].

Although it has been possible to convert recombinant $PrP^{C}$ into a $\beta$-sheet–rich partially protease-resistant structure by

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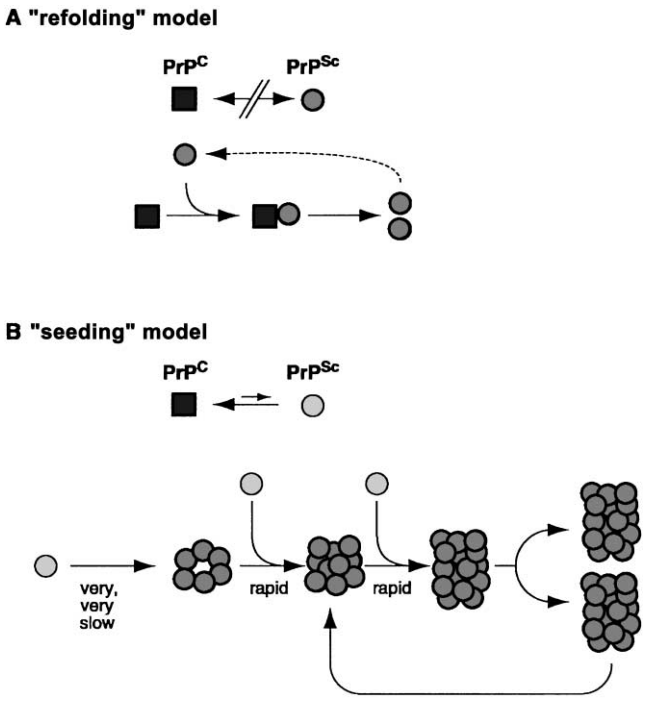


**Figure 1.** Models for the conformational conversion of PrP ($PrP^C$) to the protease-resistant aggregated form of PrP ($PrP^{Sc}$). *A,* Refolding model. Conformational change is kinetically controlled; a high activation energy barrier prevents spontaneous conversion at detectable rates. Interaction 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. With certain mutations in $PrP^C$, spontaneous conversion to $PrP^{Sc}$ may be rare, explaining why familial Creutzfeldt-Jakob disease (CJD) or Gerstmann-Sträussler-Scheinker syndrome arises spontaneously, albeit late in life. Sporadic CJD is extremely rare (occurring in ~1 of 1 million persons/year) and leads to spontaneous conversion of $PrP^C$ to $PrP^{Sc}$. *B,* Seeding model. $PrP^C$ and $PrP^{Sc}$ (or a $PrP^{Sc}$-like molecule, light symbols) 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 symbol). Although seed formation is rare, once a seed is present, monomer addition ensues rapidly. To explain exponential conversion rates, aggregates must be continuously fragmented, generating increasing surfaces for accretion.

physicochemical procedures [25, 26], there have been no reports that such material gives rise to transmissible prion disease [27–29]. Also, to date, it has not been possible to renature completely denatured prion preparation to an infectious state [30, 31], although the infectivity of partially inactivated material can be increased by certain procedures [32, 33]. One group [29] reported that intracerebral injection of a synthetic 55-residue peptide corresponding to region 89–143 of mouse PrP with a P101L substitution can induce neurologic prion-like disease, but only in transgenic mice expressing PrP with the same mutation. 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 [34]. They differ by incubation times in various inbred mouse lines, by lesion patterns in affected brains, and by the physicochemical 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 2 different prion strains, this proposal has not been experimentally supported [35].

The conformational hypothesis proposes that each strain is associated with a different conformation of $PrP^{Sc}$ and that each can convert the $PrP^C$ of its host into a likeness of itself. Indeed, $PrP^{Sc}$ species associated with 2 hamster-adapted scrapie strains, HY and DY, are cleaved to products of different lengths by proteinase K [36]; 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 [37, 38]. Moreover, $PrP^{Sc}$ of certain strains differs in the ratio of the diglycosylated to the monoglycosylated form [39]. It has been claimed that $PrP^{Sc}$ molecules of as many as 8 different strains can be differentiated by virtue of their relative affinity for a monoclonal antibody directed against an epitope that is fully available in $PrP^C$ but partially occluded in $PrP^{Sc}$ [40]. Some strains differ in their susceptibility to denaturation by guanidinium chloride, further supporting the conformational hypothesis of strain specificity [40, 41].

## Experimental Prion Transmission

Experimental transmission of TSEs is done most efficiently by intracerebral injection. Intraocular, intraspinal, intraperitoneal, and subcutaneous injections [42–44] or scarification [45] are less efficient. Peroral infection has been demonstrated in many animal species [46–52]. 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 [53, 54] to which they are normally resistant. Similarly, $Prnp^{o/o}$ mice transgenic for bovine, ovine, and human PrP genes became susceptible to prions from the cognate donors [55–57].

However, 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 that found with sCJD prions [38] (Asante et al., personal communication).

Even within a species, prion transmission 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 [39, 58]. Similarly, susceptibility of sheep to scrapie is determined by the polymorphic *PrP* genotype [59].

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 [60, 61] nor is PrP expression the only feature required for susceptibility of N2a neuroblastoma cells to prions in vitro [62]. This shows that other essential cellular components are required for prion uptake and/or replication; the conjectured "protein X" is a candidate for this role [63]. In addition, loci other than *PrP* contribute to the incubation time in mice [64–67].

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 accumulate PrP$^{Sc}$ and infectivity, albeit only very late after inoculation [68, 69]. Whether 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-1980s and led to about 180,000 clinically diagnosed cases (and likely to many more undiagnosed cases) was fueled by the feeding of BSE prion-contaminated bone-and-meat meal to cattle [70]. The kuru epidemic that developed in the first half of the twentieth century in Papua New Guinea was caused by ritualistic cannibalism [71] and is believed to have originated from a case of sporadic CJD. vCJD is thought to come about by ingestion of BSE prion-contaminated foodstuff.

Mice [46], sheep [49], calves [50], and nonhuman primates [51, 52] can be experimentally infected with the BSE agent by the oral route. It seems likely that sheep scrapie spreads by ingestion of the infectious agent, and although the source has not been established, infected placenta has been suggested [72]; however, scrapie prion-contaminated feces are a possibility that merits investigation. Perhaps the appearance of vCJD in predominantly young persons 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 hamsters [73].

How do prions make their way from the digestive tract to the CNS? The relative resistance of prion infectivity to protease digestion [74] probably allows a significant proportion of the infectious agent to survive passage through the digestive tract [46]. It is not clear how prions pass through the intestinal mucosa. M cells, which are portals for antigens and pathogens [75–77], can mediate transport of prions, at least in an experimental setting [78]. However, because only a few percent of animals in a herd of cattle exposed to the same feed develop BSE, the possibility that additional factors (e.g., lesions in the mucosa of the digestive tract) contribute to or are essential for prion uptake cannot be excluded.

After oral uptake, the infectious agent is found early on in Peyer's patches [46] and the enteric nervous system [79]. Depending on the host, other lymphoreticular tissue, in particular the spleen but also lymph nodes [80], are sites in which prions replicate and accumulate. This occurs in sheep scrapie, experimental BSE in sheep, vCJD in humans, and experimental mouse scrapie, but not in BSE in cattle [81]. Recent reports suggest that myeloid dendritic cells mediate transport within the lymphoreticular system [82, 83].

Mature B cells (with or without PrP$^{C}$ expression) are required for amplification of prions in spleen [84], not because they harbor or multiply prions [61], but because they are required for the maturation of follicular dendritic cells, the cells in which prion amplification and PrP$^{Sc}$ accumulation occurs [85, 86]. Nonetheless, neuroinvasion is possible even in the absence of follicular dendritic cells, suggesting that other cell types in the periphery also can amplify prions [80, 87]. 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 [88] or via the spinal cord, under involvement of the sympathetic nervous system [89]. If a sufficiently high dose of prions is administered intraperitoneally, neuroinvasion can occur without participation of the lymphoreticular system [90].

The biosynthesis of prions and 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 [91] or intraperitoneal inoculation [92]. Even 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 blood [92] and underlining the requirement of an interposed PrP-expressing compartment (shown to be the peripheral nervous system) [90]. In the case of experimental mouse scrapie, prion infectivity could not be detected in leukocytes [93] nor was infectivity detected in the blood of BSE-infected cattle [81]. However, a low but

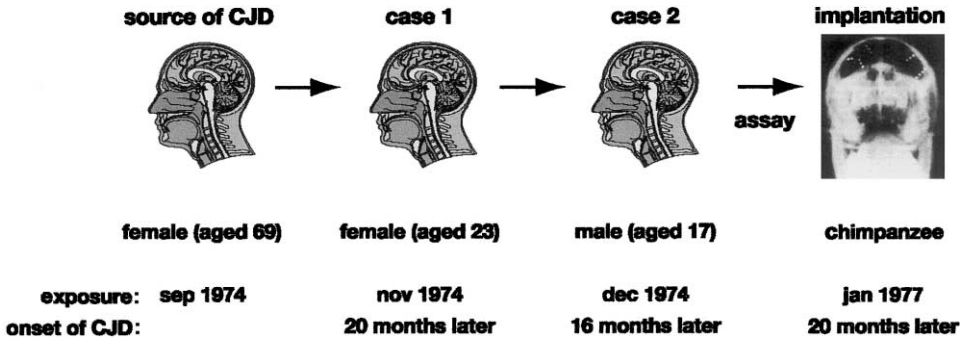


**Figure 2.** Accidental transmission of sporadic Creutzfeldt-Jakob disease (CJD) into 2 persons via intracerebral electrode. An electrode that had been inserted into the cortex of an unrecognized CJD patient and decontaminated after each use with benzene, 70% ethanol, and formaldehyde vapor was used in succession on 2 additional patients who subsequently developed CJD. After these events, the tip of the electrode was implanted into the brain of a chimpanzee, where it again caused lethal spongiform encephalopathy [98, 99].

reproducible titer of prions was detected in blood of scrapie-infected hamsters [94]. The preliminary report that 1 of 19 sheep transfused with blood from experimentally orally BSE-infected sheep came down with prion disease needs to be extended [95].

### Iatrogenic Transmission of Prions

Nearly 300 cases of involuntary transmission of CJD by medical interventions have been reported [96]. Most cases were 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 were attributed to surgical instruments previously used on CJD patients [97]; however, causality was proven only in 1 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 vapor (figure 2). It was then used in succession on 2 young patients and cleaned as above after each use. Within 2 years both patients developed CJD. After these events, the tip of the electrode was implanted into the brain of a chimpanzee where it caused lethal spongiform encephalopathy, proving that the electrode had retained infectious prions over several years and despite repeated attempts at sterilization [98, 99].

### 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 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 an initial experiment, wires were incubated overnight with brain homogenate from a terminally ill murine scrapie-infected mouse, washed exhaustively with PBS, and permanently implanted into brains of indicator mice. Scrapie disease resulted within about 70 days, an incubation time only slightly longer than that obtained by injecting 30 $\mu$L of 1% brain homogenate [100]. In order to more closely mimic real-life conditions, stain-

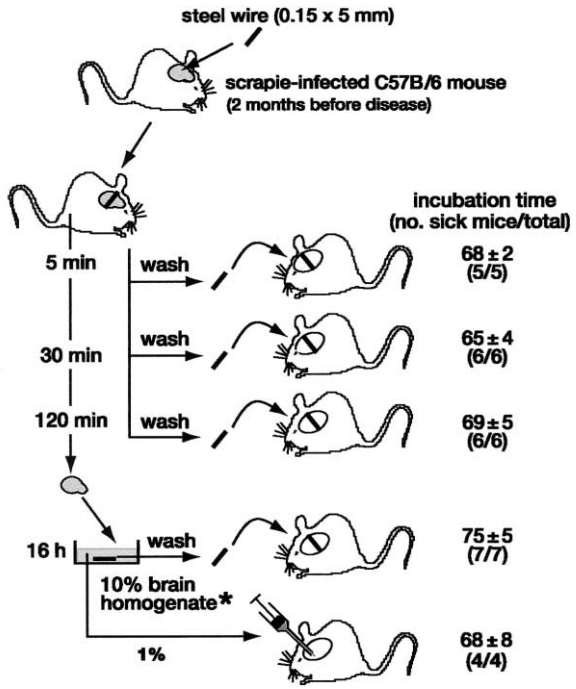


**Figure 3.** Transmission of mouse scrapie prions by stainless steel wire. Wires were inserted into the brains of scrapie-infected mice for 5, 30, or 120 min, washed exhaustively, and introduced permanently into brains of indicator mice. In all, 5 min of contact was sufficient for the wire to acquire a maximum load of infectivity, equivalent to the injection of 30 $\mu$L of 1% homogenate of the same brain. (Data from [101]).* Titer: 6.8 log $LD_{50}$ units/mL.

**Table 1.** Transient insertion of infectious wires into brains of indicator mice.

| Inoculation | No. sick/total | Incubation time (days) ± SD |
|---|---|---|
| Wires infected by exposure to scrapie brain | | |
| Transient insertion into indicator mice | | |
| 30 min | 4/4[a] | 94 ± 10 |
| 120 min | 2/2[b] | 87 ± 113 |
| 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 |
| Controls | | |
| Wires exposed to brain homogenate | 6/6 | 76 ± 3 |
| Brain homogenate (1%, 0.03 mL) | 3/3 | 69 ± 3 |

NOTE. Infectious wires were prepared by insertion for 5 min into scrapie-infected mouse brain. After a wash, wires were inserted into brains of 6 deeply anesthetized Tga20 indicator mice for the times indicated. Recovered wires were washed and implanted into Tga20 mice. As controls, wires incubated with 10% homogenate (6.8 log $LD_{50}$ U/mL) of the same brain and the homogenate itself were introduced into indicator mice. (Modified from [101]).

[a] Two of 6 mice died on the day of the intervention.

[b] Four of 6 mice died within 1 day of the intervention.

less steel wires were inserted directly into the brains of scrapie-infected, clinically still healthy, mice for various time periods, washed exhaustively, and assayed by permanent insertion into brains of indicator mice. Surprisingly, 5 min of contact was sufficient for the wire to acquire a maximum load of infectivity, equivalent to the injection of 1% homogenate of the same brain (figure 3).

A second important question is the length of time an infectious wire must remain in contact with brain tissue in order to initiate disease. Rather than leave the infectious wires permanently in the indicator mouse, they were inserted transiently, for 30 or 120 min to mimic possible conditions during a surgical operation. As shown in table 1, a contact time of 30 min was sufficient to elicit disease, albeit with lower efficiency than 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 another set of indicator mice (table 1) [101], 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 that 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 [102–104]. The unexpected high infectivity of steel wires could be due to selective binding of infectious particles or a higher potency of surface-bound infectivity. Despite the resistance of $PrP^{Sc}$ and scrapie infectivity to treatment in vitro with proteinase K, prion titers in brain after intracerebral inoculation decrease to below the level of delectability within ≤4 days [15]; however, infectious wires left in brain for 5 days still retained infectivity [101]. Perhaps metal-bound prions may be 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. Prion-coated gold wires exhibit intracerebral infectivity similar to steel wires [101] and plastic surfaces, such as polystyrene (figure 4), polypropylene, or polyethylene also tightly bind prions and transmit scrapie infectivity to adherent susceptible cultured cells (unpublished data).

We attempted to elute PrP from infectious steel wires with 2 *M* NaOH but failed to detect either protein (detection limit, 50 ng/wire) or PrP (detection limit, 15 pg/wire). Yet PrP immunoreactivity can be detected at the surface of prion-coated wires by chemiluminescence [101]. This raises the question as to whether infection of brain tissue elicited by infected wires results from 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 [62]. After 1–14 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 onto nitrocellulose membranes and assayed for the presence of protease-resistant PrP, the surrogate marker of prion infection [105]. Only the cells derived from the infected wire, but not from the residual monolayer, were $PrP^{Sc}$ positive (figure 5) and contained infectivity (unpublished data). This experiment shows that intimate contact between the prion-carrying surface and susceptible cells greatly

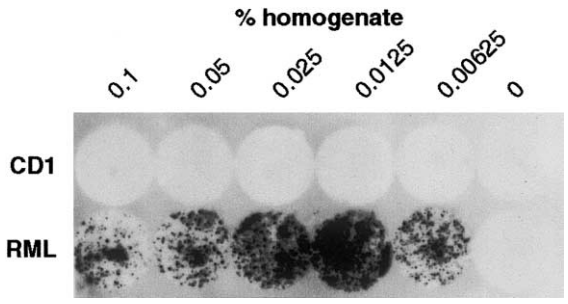


**Figure 4.** Infection of mouse neuroblastoma cells by plastic-bound prions. Polystyrene 96-well microtiter plate wells were exposed to various dilutions of a homogenate of scrapie prion-infected mouse brain, washed exhaustively, and dried. In total, 10,000 N2a/Bos2 mouse neuroblastoma cells [62] were cultured in wells for 3 days before transfer to 24-well plates where they were cultured for 4 weeks, splitting 1:10 twice a week. The cells were then transferred to coverslips and assayed for the presence of the protease-resistant aggregated form of PrP [105]. Optimal infectivity resulted when plates were coated with 0.0125% homogenate. High concentrations of brain proteins bound to the plastic appeared inhibitory for cell infection (unpublished data). RML, Rocky Mountain Laboratory strain of mouse-adapted scrapie prions.

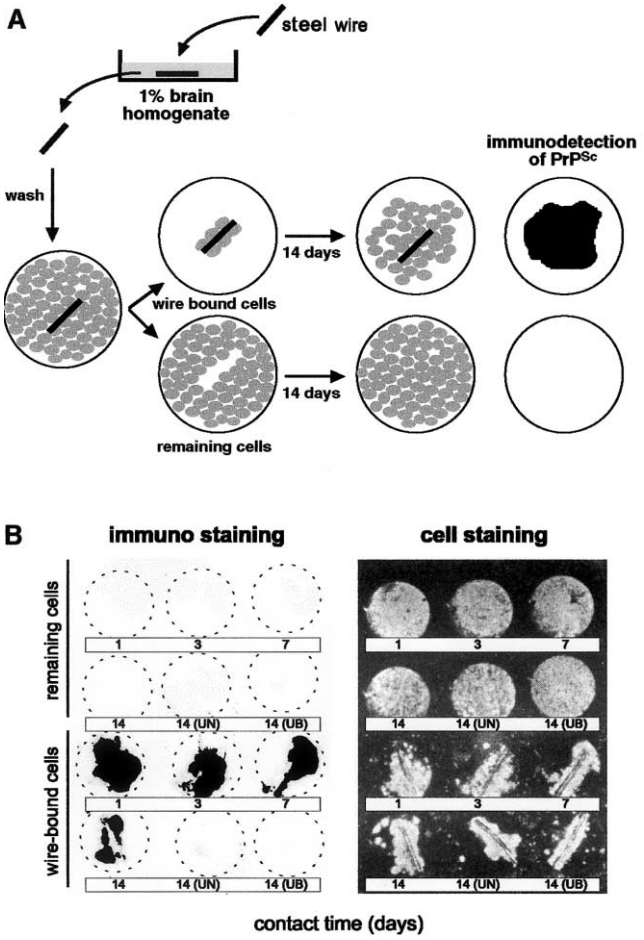


**Figure 5.** Neuroblastoma cells are infected by contact with prion-coated stainless steel wires. *A,* Wires were exposed to scrapie-infected brain homogenates, washed, and placed on a confluent layer of neuroblastoma cells. After 1–14 days, wires, to which a few cells had attached, were placed on a coverslip in a separate well and cultured another 14 days. Cells remaining in the original dish (remaining cells) and those derived from the cells clinging to the wire (wire-bound cells) were assayed for the protease-resistant aggregated form of PrP ($PrP^{Sc}$) by cell blot assay [105] and mouse bioassay. *B,* Left, panels show that cultures derived from wire-bound cells had been infected as evidenced by the accumulation of $PrP^{Sc}$; residual cells remained uninfected. Right, panels show the location of cells stained with ethidium bromide. UN, blank wire; UB, wire treated with uninfected brain homogenate (unpublished data).

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 [106].

The availability of prion-coated steel wires mimicking contaminated surgical instruments makes it possible to assess the efficacy of sterilization conditions on surface-bound prions. Preliminary results (table 2) confirm that treatment with formaldehyde is insufficient to sterilize infectious wires, while treatment with sodium hydroxide, guanidinium thiocyanate [101], or by autoclaving at 121°C for 20 min is efficacious (unpublished data). It is inappropriate to derive from these experiments recommendations for the sterilization of surgical instruments. It will first be necessary to validate the procedures by scaling up the contact surface between metal and brain tissue and, of importance, by using vCJD prions in a susceptible host, preferably a nonhuman primate.

## Conclusions

At least 20 human diseases are associated with the deposition of $\beta$-sheet–rich protein aggregates or amyloid [107, 108]. They are frequently designated "conformational diseases," although it is not clear in all cases whether or to what extent the misfolded proteins are the cause of the disease rather than the consequence. Prion diseases so far are 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 [109], 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, utilizing intermediate tissues for amplification. In the case of microbes and viruses such sophisticated behavior 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.

Prion protein is encoded by the genome of its host, so the question remains, 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 physiologic function but on the other has a rarely realized malignant potential that was not selected against because evolutionary pressure does not operate efficiently at the post-reproductive age. Possibly, in yeast a "prion-like" phenomenon involving Sup35 may confer selective advantage on yeast growing under fluctuating environmental conditions [110]. Another

**Table 2.** Effect of various treatments on the infectivity of wire-bound prions.

| Wire type, treatment | No. sick/total | Incubation time (days) ± SD |
|---|---|---|
| Uninfected, untreated | 0/3 | >260 |
| Infected | | |
| Untreated | 6/6 | 76 ± 5 |
| NaOH (1 *M,* 1 h, 25°C) | 0/6 | >260 |
| Formaldehyde (10%, 1 h, 25°C) | 6/6 | 92 ± 8 |
| Guanidinium thiocyanate (4 *M,* 16 h, 25°C) | 0/6 | >260 |
| Autoclaving (121°C, 20 min) | 0/6 | >170 |

NOTE. Data are from [101] and (unpublished).

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 minimized. More trivially, mammalian prion disease could result from the natural propensity of proteins to assume a $\beta$-sheet–rich conformation [111], 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.

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