Published in final edited form as:

Nat Rev Microbiol. ; 9(11): 771–777. doi:10.1038/nrmicro2650.

De novo generation of prion strains

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Preface

Prions are self-replicating proteins that cause neurodegenerative disorders such as “mad cow” disease. These misprocessed protein conformations accumulate in the central nervous system, causing spongiform changes in the brain and eventually death. Since the inception of the protein-only hypothesis — which posits that misfolded proteins are the infectious agents that cause these diseases — researchers seeking to generate infectious proteins from defined components in the laboratory have had varying degrees of success. Here, we discuss several recent studies that have produced an array of novel prion strains *in vitro* that exhibit increasingly high titers of infectivity. These advances promise unprecedented insight into the structure of prions and the mechanisms by which they originate and propagate.

Prions are infectious proteinaceous agents that cause heritable, sporadic, and infectious neurological diseases in humans and other animals, including Creutzfeldt-Jakob disease in humans, bovine spongiform encephalopathy in cattle, scrapie in sheep, and chronic wasting disease in ungulates such as deer (Figure 1, for a recent broad review see Ref.¹). In all of these diseases, a conformational change occurs in the prion protein (PrP), from its normal or cellular form (PrP^C) to a disease-causing conformation (PrP^{Sc}). Once PrP^{Sc} becomes present in an individual — whether this occurs by spontaneous conformational conversion or by infection — PrP^{Sc} is capable of recruiting PrP^C and converting it to its own form. Definitive proof of the protein-only hypothesis, which posits that prions are composed solely of PrP^{Sc}, has called for the generation of infectious prions in the laboratory — a challenge that has proved elusive for decades. Several years ago, synthetically generated prions were initially reported², but these preparations harbored low infectious titers that affected only a single line of transgenic (Tg) mice that overexpressed an N-terminally truncated PrP of wild-type sequence corresponding to the protease-resistant core of PrP^{Sc}. Although these mice do not spontaneously generate prions³, the particular nature of the transgene was thought to render them more susceptible to synthetic prion infection. In this Progress article, we discuss several recent advances in the generation of synthetic prions, including reports of high-titer preparations⁴; infection of additional lines of mice, including wild-type mice; the ability to control strain properties by adjusting the conditions used for prion formation⁵; and novel synthetic prion strains that mimic rare forms of the disease³ (Table 1).

Prion strains

The existence of prion strains, which are isolates that generate distinct phenotypes in identical hosts, was an enigmatic discovery⁶. Because prions are composed only of protein and replicate using the PrP^C substrate present in the host, differences in prion strains cannot

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be attributed to genetic variability (which accounts for the existence of viral strains). To explain this phenomenon, a theory was posited that prion strains result from conformational variability — that is, PrP can assume several different, self-propagating conformations, each of which accounts for a distinct prion strain ⁷. Analysis of prions isolated from infected animals with different phenotypes showed distinct biochemical properties, which provided indirect evidence for this theory ^{8–10}.

While direct evidence for the conformational basis of mammalian prion strains has been difficult to obtain, *Saccharomyces cerevisiae* proteins with self-replicating conformations, known as yeast prions ^{11, 12}, have contributed significantly to our understanding of the molecular basis of prion strains. Sup35, a translation release factor that suppresses nonsense mutation phenotypes in the prion state, is one such protein. A recombinant Sup35 protein fragment containing the prion-forming domain (Sup35-NM) was refolded under laboratory conditions into two different amyloid conformations, that is, multimeric protein structures with a fibrillar morphology. Upon transduction into yeast, these conformations were shown to initiate two distinct [*PSI*⁺] strain phenotypes, one strong and the other weak ¹³. Propagation rates in of yeast prions are related to the strength of the phenotype, since weaker prions are more frequently lost during yeast division ^{14, 15}. The propagation rates for these yeast prion strains were inversely correlated to their conformational stabilities based on thermal denaturation ¹³, a finding that was later extended to mammalian prion strains ¹⁰. While discoveries with yeast prions may guide those of mammalian prions, it is important to note that there are significant differences between the two systems. Yeast prions do not cause disease and therefore cannot teach us about prion pathology on a scale above the molecular level. Furthermore, yeast prions propagate by a simple amyloid growth mechanism, in which monomers are added to the growing end of the fiber ¹⁶. This fact, coupled with cell division, allow for relevant insights from purely *in vitro* models ¹⁷. In contrast, mammalian prions are not necessarily composed of amyloid, although most are competent to form amyloid ¹⁸ and to seed amyloid growth ¹⁹.

Cell-free systems for creating prions

The development of cell-free *in vitro* models for mammalian prion propagation lag behind analogous yeast systems. For example, distinct [*PSI*⁺] strains, extracted from yeast and propagated *in vitro* by incubation with a recombinant Sup35 N-terminal fragment, were able to reconstitute their parental strains upon transduction into uninfected yeast ²⁰. In contrast, simply incubating PrP^{Sc} in the presence of PrP^C substrate results in a very low yield of “converted” protein ²¹. Furthermore, amyloid preparations obtained by seeding with purified prions seem to be far less infectious than brain-derived samples (D.W.C. & S.B.P., unpublished observations). The highest yields for *in vitro* propagation have been achieved by seeding normal brain homogenate with prions and applying repeated rounds of sonication, a process known as protein misfolding cyclic amplification (PMCA; Box 1) ²². Although sonication introduces a series of problems, such as heterogeneous protein denaturation and high well-to-well variability in the generation of PrP^{Sc}, PMCA is currently the only cell-free assay that converts PrP^C into an infectious form in substantial yields.

Box 1

Protein misfolding cyclic amplification (PMCA)

The protein misfolding cyclic amplification (PMCA) technique is used to amplify prions *in vitro* ⁶⁹ (see the figure). Normal prion protein (PrP^C) is mixed with smaller volumes of disease-causing prion protein (PrP^{Sc}). The samples are subjected to sonication, or short bursts of ultrasound, and incubation. Sonication is presumed to break apart large PrP^{Sc} aggregates and partially denature both PrP^{Sc} and PrP^C, facilitating growth of PrP^{Sc}

aggregates during incubation. Newly formed PrP^{Sc} is then used to seed subsequent PrP^{Sc} amplification using fresh PrP^C in multiple rounds of PMCA.

Several important discoveries laid the foundation for recent advances in the creation of prions in the laboratory. Tg mice expressing a PrP form with a P101L mutation (which corresponds to that which causes Gerstmann-Sträussler-Scheinker syndrome in humans) developed neurological dysfunction and exhibited brain lesions typical of prion disease, with disease onset times that were dependent on the level of transgene expression²³. A 55mer peptide, which spanned residues 89–143 of PrP with the P101L mutation and was folded into a beta-rich conformation, hastened disease onset when inoculated into young mice expressing low levels of the P101L transgene^{24, 25}. Later, Tg9949 mice, which overexpress MoPrP(89–231) and are not genetically predisposed to develop prion disease, were infected with recombinant PrP (wild-type PrP residues 89–230) refolded into an amyloid conformation². The prion strain recovered from the brains of these mice was denoted MoSP1 and was transmissible to wild-type mice by serial passage². MoSP1 was easily distinguished from naturally occurring prions due to its high conformational stability²⁶. During two subsequent rounds of serial passage, the incubation period (measured from inoculation to onset of neurological dysfunction) of MoSP1 isolates decreased from over 500 days to 177 days in Tg9949 mice¹⁰. Strikingly, each shortening of the incubation period was accompanied by a decrease in the conformational stability of PrP^{Sc}¹⁰. No evidence suggesting that Tg9949 mice spontaneously generate prions was found, despite extensive experimentation, including repeated serial passage of three aged Tg9949 mouse brains as well as the examination of over 50 Tg9949 brains by biochemical analysis and over 100 Tg9949 brains by neuropathologic analysis⁵.

New prion strains from amyloid fibers

Biochemical analysis of prions obtained from infected animals have given some insight into the structural variations that make up different strains. These variations include differences in glycosylation patterns, extent of protease resistance, electrophoretic mobility of proteolytic fragments, and conformational stability^{8, 27}. However, the ability to modulate purposefully prion strain phenotypes by altering the conformation of PrP has only recently been demonstrated: recombinant PrP folded into distinct amyloid conformations gave rise to distinguishable prion strains, with incubation periods that were dependent on the conformational stability of the recombinant PrP amyloid⁵. By altering the conditions used to refold recombinant PrP, amyloids with different conformational stabilities were generated. These amyloids were then inoculated into mice that overexpressed full-length PrP at four-fold compared to wild-type levels. This resulted in prion strains with incubation periods and conformational stabilities that were correlated to the stability of the amyloid

fibers used to inoculate the mice (Figure 2). The inability to infect wild-type mice directly with these preparations indicates that unidentified properties, in addition to conformational stability, modulate infectivity and incubation period. Conflicting results using hamster prion strains with conformational stabilities that cover a much narrower range, compared to the synthetic prions studies, also support this notion²⁸. Nonetheless, the direct demonstration of the conformational basis of prion strain diversity provides further evidence that synthetic prions originate from the recombinant amyloid preparations, and not from the host or from contamination. If prions were arising spontaneously in the host, one would expect the strain properties to be independent of the amyloid properties. Exhaustive negative controls, including inoculation of the host mice with control solutions, biochemical and neuropathological analysis of age-matched controls, and serial passage of aged brains from the host mice, also excluded spontaneous prion generation and contamination⁵.

While these studies clearly established that distinct conformations of synthetic prions can account for different phenotypes, they did not elucidate mechanisms of infectivity. Some amyloid conformations harbored infectivity whereas others did not⁵. Thus, how infectivity is enciphered in prions remains to be established.

Amyloid inoculation of Tg mice that overexpressed an N-terminally truncated PrP resulted in novel protease-sensitive synthetic prions³. In contrast, many naturally occurring prions contain some fraction of PrP^{Sc} in a conformation that resists protease digestion (protease-resistant PrP^{Sc}, or rPrP^{Sc})²⁹. This observation has led to the idea that protease resistance equates with prion infectivity and pathogenesis. However, many naturally occurring prion strains also contain PrP^{Sc} in a conformation that is sensitive to protease digestion (sPrP^{Sc})²⁷. The protease-sensitive, synthetic prion strains that were generated demonstrate that sPrP^{Sc} is transmissible and pathogenic, and can occur as a distinct entity from rPrP^{Sc}. Furthermore, repeated serial passage of these strains never resulted in the formation of rPrP^{Sc}, arguing that sPrP^{Sc} neither gives rise to nor results from rPrP^{Sc}.

In contrast to the clear relationship between amyloid stability, incubation period, and prion stability, the factors that influence the protease-sensitivity of synthetic prion strains are less clear. While initial findings of inoculation of Tg9949 mice with recombinant PrP amyloid fibers resulted in rPrP^{Sc} (Ref.²), the protease-sensitive synthetic prion strains were obtained with amyloid fibers prepared under similar conditions³. This finding suggests that the protease resistance of prions strains is determined by one or more subtle conformational differences that may occur stochastically.

Exposing amyloid fibers formed from recombinant PrP of hamster sequence to heat in the presence of brain homogenate or albumin also led to the generation of a novel prion strain named SSLOW (for 'Synthetic Strain Leading to OverWeight')³⁰. Hamsters inoculated with amyloid fibers prepared in this way did not show symptoms of disease during 661 days of observation following inoculation, but were found to have low levels of PrP^{Sc} in the brains upon termination of the experiment. A serial transmission caused disease in >480 days. Biochemically, SSLOW had a conformational stability comparable to naturally occurring prion strains. However, the disease phenotype reported was unusual, in that it included weight gain and an unusually slow disease progression. Given the biochemical similarities between SSLOW and naturally occurring prions, the conformational basis of this unusual strain phenotype remains to be determined.

New prion strains from sonication

A central issue in the generation of prions from recombinant protein has been the apparently low titers of amyloid fibers formed³¹, which results in long incubation times and often the inability to infect wild-type animals directly. If long incubation times do in fact result from

low titers, then this implies that synthetic prion preparations are heterogeneous mixtures containing many different conformations (which limits their usefulness for determining the structure of PrP^{Sc}). Alternatively, long incubation times may indicate that amyloid fibers require maturation or further adaptation of their conformation in order to become fully infectious.

Several laboratories have succeeded in creating prion infectivity under conditions developed for PMCA^{4, 32, 33}. The initial report for the spontaneous generation of infectivity came in the form of unexpected amplification of prions in negative controls during studies that aimed to identify minimal components necessary for prion amplification *in vitro*³³. The components required for amplification included polyanions in addition to PrP^C, which was accompanied by co-purified lipids. These results were verified by repeating the experiment in a laboratory that had never been used for prion research, reducing the potential for contamination. It was later shown that prions can be generated in a similar fashion using brain homogenate as the substrate, rather than minimal components, and that the newly created prion strain was distinct from a commonly used lab strain³². Prions created in these studies using either PrP^C or normal brain homogenate had titers that were sufficient to infect hamsters with incubation periods of 113 to 168 days, while naturally occurring hamster prion strains can have incubation periods of 60–300 days^{27, 34}.

Synthesis of highly infectious prions from recombinant PrP has recently been reported using sonication in the presence of lipids and RNA⁴. The infectivity of these preparations was comparable to naturally occurring strains, implying that the authors have achieved a level of purity several orders of magnitude higher than previously reported. Such high titers may be rapidly detected by bioassay, so the robustness and reproducibility of these findings shall soon become apparent. Despite this remarkable report, the possibility of contamination in PMCA cannot be eliminated — especially given reports of a billion-fold amplification of prion titers using PMCA²² — and no convincing control has been devised to do so. Even if this finding was attributable to contamination of the starting materials, the ability to amplify prions efficiently using recombinant protein is a significant breakthrough, as it may enable the use of labeled recombinant protein for structural studies.

In related work, recombinant PrP (rather than PrP^C contained in brain homogenates) has been employed with purified PrP^{Sc} seed, obtained from brain homogenate, to initiate the PMCA reaction³⁵. This study showed that polyanions and lipids are not required for prion amplification, although trace quantities of such cofactors in the brain-derived seed were not ruled out. In bioassays, the attack rate (i.e., the fraction of inoculated animals that developed prion disease) was consistent with low titers in these preparations, rather than a prion strain with a long-incubation period. Upon serial passage, the incubation periods and biochemistry resulting from these amplified prions were consistent with the naturally strain used as seed, though the pattern of brain lesions indicated some differences. If a novel prion strain was in fact created, this argues that prolonged sonication can alter the conformation of PrP^{Sc}.

Despite the exciting results, some caveats should be noted about the PMCA approach. This technique results in uneven amplification of prions — from well to well and from experiment to experiment — which produces great variability³⁶ and prevents the resulting data from being quantified. It is well documented that sonication causes an uneven distribution of energy, resulting in cavitation and high temperatures associated with cavitation³⁷ as well as the generation of free radicals³⁸. Protein conformation is sensitive to temperature denaturation, and free radicals may covalently alter proteins. Both cavitation and free-radical modification of proteins are stochastic processes and inherently difficult to control, potentially explaining the variability observed in PMCA experiments. In contrast, the denaturing agents urea and guanidine, used in the production of synthetic prions

elsewhere^{2, 3, 5}, result in comparatively even and well defined denaturation of protein throughout the solution. In some cases, sonication initiates amyloid formation with proteins that are usually monomeric³⁹, indicating denaturation. Furthermore, such alterations in protein conformation may result in the degradation of the protein⁴⁰. It is noteworthy that the durations of sonication used for PMCA greatly exceed those used in recombinant protein production, often by a factor of over 100. Degradation and denaturation may thus limit the usefulness of products produced by PMCA for structural studies.

Conformational diversity and other protein misfolding diseases

Recent findings implicate a prion-like spread of misfolded proteins in Alzheimer's disease⁴¹ and Parkinson's disease^{42, 43}, following on from earlier suggestions that the amyloid in Alzheimer's disease might be the cause of this disorder^{44–47}. The ability to model disease states using synthetic peptides and recombinant proteins refolded into pathogenic conformations can shed light on the molecular mechanisms involved in these and other diseases. For example, systemic amyloidosis caused by serum amyloid A (SAA) may also be transmitted by a prion-like mechanism^{48, 49}, and amyloid fibers composed of synthetic peptides corresponding to fragments of the SAA protein can accelerate the disease process⁵⁰.

Brain extracts containing misfolded A β peptides accelerated disease in Tg mouse models of Alzheimer's disease, in which the mice overexpressed disease-related mutations in the amyloid precursor protein (APP). However, synthetic peptides of A β 1–40 and A β 1–42, mimicking the short peptides found in Alzheimer's disease and aged to allow time for formation of fibrils, failed to accelerate disease in the same mouse model⁴¹. Notably, synthetic A β peptides prepared in a similar manner have been shown to be neurotoxic⁵¹. These findings suggest that different conformations may be required for neurotoxicity and self-propagation. In fact, earlier work with synthetic A β peptides suggested a neurotoxic mechanism that involved the initiation of tau misfolding within the cell⁵².

The prion-like propagation of extracellular proteins like A β and SAA is not entirely unexpected, given the ability of these proteins to interact with homologous proteins without having to cross a cell membrane; however, recent findings suggest that this may be the case for intracellular proteins as well. Intracellular aggregates have previously been thought to arise in individual cells during stochastic nucleation events⁵³. Now, aggregates of several proteins, including tau⁵⁴, α -synuclein⁵⁵, and polyglutamine^{56, 57}, have been shown to pass from the extracellular space into the cell or from one cell to another. Aggregates formed of truncated recombinant tau were shown to enter cells and seed the polymerization of endogenous tau. Furthermore, these tau aggregates were also shown to pass from cell to cell in culture⁵⁴. It seems plausible that aggregates are released into the extracellular space after a cell dies, break apart, and then infect other cells.

With α -synuclein, a protein whose overexpression and misfolding is associated with Parkinson's disease, endocytosis seems to play an important role in its prion-like spread. In cell culture, formation of fibrils of recombinant α -synuclein is required for the protein to enter the cell. These fibrils are internalized via endocytosis, while monomers diffuse passively across the membrane⁵⁵. Endocytosis of α -synuclein aggregates results in cell death⁵⁸, again releasing toxic aggregates into the extracellular space to infect other cells. Like tau, α -synuclein has also been shown to pass from cell to cell in culture⁵⁹.

The prion-like spread of protein aggregates has been independently demonstrated for polyglutamine peptides, which represent a key sequence motif in the huntingtin protein. Additional polyglutamine expansions in the huntingtin protein cause Huntington's disease, with the number of repeats generally related to the age of onset. Cells have been shown to

take up aggregates of chemically synthesized polyglutamine peptides, resulting in death when localized to the nucleus ⁵⁶. Following uptake, aggregates initiate conformational conversion of endogenous polyglutamine-containing proteins, resulting in a persistent protein misfolding ⁵⁷. The role of protein misfolding in Huntington's disease has been controversial ⁶⁰; these recent results suggest that the mechanism of conformation-dependent toxicity may be more complex than had been appreciated previously, as it is now apparent that the mechanism may involve both spontaneous misfolding within cells as well as cell-to-cell transmission of misfolded proteins.

Looking forward

While accumulating data have established the misprocessing of specific proteins in the pathogenesis of many disorders, including Alzheimer's, Parkinson's and Huntington's as well as the tauopathies and prion diseases as described above, the elucidation of atomic-level structures for these misfolded proteins has been nearly impossible, except in the case of short- to intermediate-length peptides ⁶¹. Major obstacles include insolubility, conformational heterogeneity, and difficulty in assembling misfolded proteins into crystals. Some structural insights have been achieved for fungal prions through solid-state NMR (ssNMR) ⁶² and hydrogen-exchange coupled with NMR (HXNMR) ⁶³. However, these observations depend on a high yield of the fungal prion proteins in their active state. Studies employing X-ray fiber diffraction, a technique that has yielded only low-resolution structural information, suggest that amyloid fiber preparations containing synthetic prions have different structural assemblies compared to naturally occurring prions ⁶⁴, implying that the process for converting recombinant PrP into a pathogenic form by amyloid formation will need to be improved before ssNMR or HXNMR can be applied. Whether the high-titer prions generated from recombinant PrP using sonication ⁴ are of sufficient quality and quantity to allow for structural determination remains to be determined. Structural determinations of misprocessed proteins should provide mechanistic insights into propagation and pathogenicity.

Like fungal prions ⁶⁵, mammalian prions likely replicate with the assistance of as-yet-unknown auxiliary factors ⁶⁶. Experiments with PMCA suggest that polyanions and lipids may facilitate conversion in a non-specific fashion ^{4, 33}. Undoubtedly, the increasing reliability of *in-vitro* models should shed light on the effect of cofactors on the conversion process.

The generation of prion infectivity from defined components in the laboratory is an objective as ambitious and complex as synthesizing a virus from nucleic acid ⁶⁷ or replicating DNA outside of the cellular context ⁶⁸. Although the foundations of this field were established several years ago ^{2, 24}, recent advances have improved the efficiency with which prions can be made ⁴ and the ability to modulate synthetic prion conformation ⁵. These advances provide the strongest argument to date that the infectivity of prions resides only in their proteinaceous component, evidence that should satisfy any reasonable skeptic. The ability to generate high-quality preparations of prions from recombinant protein and to control their properties should yield unprecedented insight into the structural and mechanistic biology of prions. Such insights should bring us closer to a cure for Creutzfeldt-Jakob disease and other neurodegenerative disorders.

GLOSSARY

A β

a short peptide cleaved from the amyloid precursor protein that forms the amyloid plaques found in Alzheimer's disease

Age of onset	the age at which an animal manifests first clinical signs of disease
Alpha-synuclein	a protein expressed primarily in neurons whose aggregation results in Lewy bodies found in Parkinson's disease, Alzheimer's disease, and dementia with Lewy bodies
Bovine spongiform encephalopathy (BSE)	a prion disease of cattle, also known as "mad cow" disease
Chronic wasting disease (CWD)	a highly transmissible prion disease of free- ranging cervids (elk, deer, moose) in North America
Gerstmann-Sträussler-Scheinker (GSS) disease	a genetic prion disease in humans caused by mutations in the <i>PRNP</i> gene
Huntingtin	a protein whose expanded polyglutamine repeats causes; Huntington's disease; the number of repeats inversely correlates with age of onset.
Incubation period	for animal studies, the interval of time, usually measured in days, between inoculation and the first signs of neurologic dysfunction
Prion protein (PrP)	A glycosylphosphatidyl inositol–anchored membrane protein that is expressed primarily in the central nervous system and whose expression is required for the development of prion diseases. The normal function of PrP is unknown
PrP^{Sc}	an aberrantly folded isoform of the normal prion protein (PrP ^C) and hypothesized to be the sole component of the infectious prion
Systemic amyloidosis	a disease that features amyloid deposition in various organs
Synthetic prion	an infectious protein created from minimal components in the laboratory
Tau	a protein expressed primarily in neurons whose aggregation causes the tauopathies
Tauopathies	neurodegenerative diseases caused by the misprocessing and aggregation of tau protein, which results in neurofibrillary tangles, paired helical filaments, and/or Pick bodies in the brain

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Figure 1.

Prions cause neurodegenerative diseases in humans and animals, including (A, from left to right) scrapie in sheep, bovine spongiform encephalopathy in cattle, and chronic wasting disease in deer. (B) The fundamental event in prion disease pathogenesis is the conformational conversion of the prion protein from its normal or cellular form (PrP^C) into a disease-causing form (PrP^{Sc}). Once formed, PrP^{Sc} replicates by inducing the conversion of PrP^C into additional PrP^{Sc} molecules. (C) Neuropathological features of prion disease include the formation of vacuoles (left) and PrP deposits (right). Brain sections of mice with prion disease were stained with hematoxylin and eosin to visualize vacuoles and anti-PrP antibodies to identify PrP.

Figure 2.

Diverse PrP conformations account for the phenotypes displayed by synthetic prion strains. PrP amyloids with high, intermediate, and low stability were formed by altering the length of the recombinant PrP (recPrP) construct and the conditions used for refolding. In mice, the incubation periods and conformational stability of the resulting prion strains were dependent on the conformational stability of the recPrP amyloid from which they originated⁵.

Table 1

Summary of evidence for prions generated *in vitro* in the absence of an infectious seed.^a

Inoculum	Source of inoculum	n	Host	Incubation period (d)	Bioassay controls	Resulting Strains	Ref.
PrP peptide with P101L mutation folded into a beta conformation ^b	Synthetic peptide	1	Transgenic mice harboring P101L mutation	360	PrP(P101L) peptide folded into a non-beta conformation	Unnamed	24
Recombinant PrP refolded into amyloid	<i>E. coli</i>	2	Transgenic mice overexpressing wild-type PrP(89–231) (Tg9949 mice) ^c	>500 ^c	Phosphate-buffered saline	MoSP1; MoSP2 ^d	2
PrP ^C sonicated with copurified lipids, RNA	Syrian hamsters	2	Syrian hamsters	134–168	Unsonicated PrP ^C ; lipids; RNA	Unnamed	33
Sonicated Syrian hamster brain homogenate	Syrian hamsters	1	Syrian hamsters	113	None	PGP-h1	32
Recombinant PrP folded into amyloid	<i>E. coli</i>	10	Transgenic mice overexpressing full-length, wild-type PrP (Tg4053 mice)	477–701	α -helical recPrP; phosphate-buffered saline (PBS) with bovine serum albumin (BSA); Tg4053 brain homogenate	MoSP5; MoSP6; MoSP7; MoSP9; MoSP12; MoSP13	5
Recombinant PrP amyloid fibers exposed to heat in the presence of albumin or brain homogenate (annealed fibers)	<i>E. coli</i>	2	Syrian hamsters	>661	Heated brain homogenate; α -helical recPrP heated with brain homogenate; untreated recPrP amyloid	SSLOW	30
Recombinant PrP folded into amyloid	<i>E. coli</i>	20	Tg9949 ^c	>500 ^c	α -helical recPrP; β -oligomeric recPrP; PBS/BSA; Tg9949 brain	MoSP3; MoSP4; MoSP19	3
Recombinant PrP sonicated with lipids, RNA	<i>E. coli</i>	1	Wild-type mice (FVB strain)	150	homogenate Sonicated lipids; RNA; unsonicated recPrP; lipids; recPrP; RNA incubated at 37 °C	Unnamed	4

^a n, number of independent preparations tested in bioassay and resulted in infectivity.

^b The PrP(P101L) peptide was deemed to have accelerated spontaneous disease rather than initiate a new prion strain.

^c Determination of the incubation period was complicated by late-onset, neurological dysfunction in this line of Tg mice.

^d Detailed characterization of MoSP2 is described in Ref. 3.