Autocatalytic Conversion of Recombinant Prion Proteins
Displays a Species Barrier*

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The most unorthodox feature of the prion disease is the existence of an abnormal infectious isoform of the prion protein, PrPSc. According to the “protein-only” hypothesis, PrPSc propagates its abnormal conformation in an autocatalytic manner using the normal isoform, PrPC, as a substrate. Because autocatalytic conversion is considered a key element of prion replication, in this study I tested whether in vitro conversion of recombinant PrP into abnormal isoform displays specific features of an autocatalytic process. I found that recombinant human PrP formed two distinct β-sheet rich isoforms, the β-oligomer and the amyloid fibrils. The kinetics of the fibrils formation measured at different pH values were consistent with a model in which the β-oligomer was not on the kinetic pathway to the fibrillar form. As judged by electron microscopy, an acidic pH favored to the long fibrils, whereas short fibrils morphologically similar to “prion rods” were formed at neutral pH. At neutral pH the conversion to the fibrils can be seeded with small aliquots of preformed fibrils. As small as 0.001% aliquot displayed seeding activity. The conversion of human PrP was seeded with high efficacy only with the preformed fibrils of human but not mouse PrP and vice versa. These studies illustrate that in vitro conversion of recombinant PrP displays specific features of an autocatalytic process and mimics the transmission barrier of prion propagation observed in vivo. I speculate that this model can be used as a rapid assay for assessing the intrinsic propensities of prion transmission between different species.

Spontaneous conversion of the normal cellular isoform of the prion protein, PrPC, into the abnormal pathological isoform, PrPSc, underlies sporadic forms of prion disease, including Creutzfeldt-Jakob disease in humans (1). The occurrence of the sporadic forms of prion diseases is very rare, about one person/year (2). Such a low occurrence is believed to be because of the extremely slow spontaneous conversion of PrPC into PrPSc (3). However, the conversion can be substantially accelerated by administration of an exogenous PrPSc. The “protein-only” hypothesis of prion propagation postulates that the abnormal isoform, PrPSc, acts as a transmissible agent of the disease and self-propagates its pathological conformation using PrPC as a substrate (4).

Substantial effort has been devoted to the development of a cell-free system for conversion of non-infectious prion protein (PrP) into the infectious PrPSc isoform (5–8). To monitor conversion in vitro, many studies exploited conformational differences between PrPC and PrPSc (5, 9–12) because PrPC is a proteinase K-resistant, aggregated β-sheet-rich multimer (13). Even though formation of β-sheet-rich proteinase K-resistant multimeric isoforms of PrP have been reported (6, 10, 11, 14, 15), the ultimate direct proof of the protein-only hypothesis, i.e. the in vitro reconstitution of PrPSc from non-infectious PrP, has not yet been achieved. Despite substantial differences between PrPC and PrPSc, it is still unclear which physical property can be used as a valid probe to monitor formation of infectious PrPSc in a cell-free system.

According to the protein-only hypothesis, an autocatalytic conversion from PrPC into PrPSc is a key feature that underlies the molecular basis of the transmissible form of prion diseases (16). Strong support for the idea that certain proteins are capable of self-propagating their abnormal conformations was provided by the prion biology of yeast and fungi (17–19). In vitro made fibrils of Sup35 and HET-s, the prion proteins of yeast and fungi, respectively, cause prion propagation in vivo and change the phenotype of cells (19, 20). The yeast model displays one of the key features that illustrate an autocatalytic mechanism of conversion, a seeding phenomenon in which abnormal isoforms produced during conversion are able to initiate and facilitate conversion in a fresh reaction. Furthermore, seeding of fibril formation of Sup35 in vitro mimics two other peculiar features of in vivo prion propagation, strain phenomenon and species barrier (21, 22), offering a powerful system to study the propagation of yeast prions. Development of similar in vitro models for conversion of mammalian PrPs would be beneficial to our understanding of the mechanisms of prion diseases.

Using C-terminal fragments of recombinant human and mouse PrPs that encompassed residues 90–231 and 89–230, respectively (designated Hu recPrP and Mo recPrP), I have developed an in vitro conversion system that displays attributes of an autocatalytic process. Thus, the conversion of monomeric recPrP into the amyloid fibrils can be seeded with small aliquots of preformed fibrils. I found that cross-seeding with preformed fibrils of heterologous recPrP was not as efficient as seeding with fibrils of homologous recPrP. The low efficiency of cross-seeding illustrated a high selectivity of the autocatalytic conversion in vitro in the absence of cellular environment. This phenomenon mimicked species barrier of prion transmission observed in vivo. I speculate that this model system can be used for assessing an intrinsic propensity of prion transmission between different species.

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The abbreviations used are: PrP, prion protein; Hu, human; Mo, mouse; ThT, thioflavin T; recPrP, recombinant prion protein; SEC, size-exclusion chromatography; GdnHCl, guanidine hydrochloride.
EXPERIMENTAL PROCEDURES

Protein Expression and Purification—The genes coding for Hu PrP-(90–231) and Mo PrP-(89–231) were amplified by PCR from pC1817 plasmids containing the full-length Hu PrP and the full-length Mo PrP genes, correspondingly. The amplified genes were cloned into the pET101/n-TOPO vector (Invitrogen) and transformed into Top10 cells (Invitrogen). The transformants were tested by PCR amplification, and the plasmids isolated from the proper clones were retransformed into BL21 Star (DE3) (Invitrogen). The insertion of the correct gene was confirmed by DNA sequencing (Biopolymer Laboratory, University of Maryland, Baltimore).

For the expression of Hu recPrP-(90–231) and Mo recPrP-(89–230), 2% of an overnight culture was inoculated in fresh LB medium that contained 50 μg/ml carbenicillin (Sigma) and was grown at 37 °C until the A600 nm reached 0.7. Isopropyl-β-D-thiogalactopyranoside (IPTG, Sigma) was then added to a final concentration of 1 mM, and the cultures were grown for an additional 16 h. The cells were harvested, resuspended in lysis buffer (50 mM potassium phosphate, pH 7.8, 400 mM NaCl, 100 mM KCl, 10% glycerol, 0.5% Triton X-100, 10 mMimidazole), and lysed by freezing in liquid nitrogen and then immediate thawing at 42 °C. The samples were then centrifuged at 10,000 × g for 20 min, and both proteins were purified as described by Mehlhorn et al. (23). The purified proteins were confirmed by SDS-PAGE and electrospray mass spectrometry to be single pure species with an intact disulfide bond.

Formation of Amyloid Fibrils in Vitro—The oxidized form of both Hu recPrP and Mo recPrP spontaneously converted into the fibrillar isoform upon continuous shaking at 800 rpm in conical plastic tubes (Eppendorf) in a reaction volume > 0.4 ml at 37 °C (24). When the reaction was carried out under the same experimental conditions but in a reaction volume < 0.3 ml, the conversion did not occur spontaneously. However, it was possible to induce the conversion by seeding. The conversion in reaction volume < 0.3 ml is considered to be at subthreshold conditions. The detailed mechanism of these volume-regulated subthreshold effects will be described elsewhere.

To monitor the kinetics of fibril formation, 10-μl aliquots were withdrawn and diluted into 80-fold into 50 mM sodium acetate buffer (pH 5.5) to a final concentration of recPrP 10 μg/ml. After the addition of thioflavin T (ThT) (Molecular Probes, Eugene, OR) to a final concentration of 5 μM, fluorescence spectra were recorded in 0.4-cm rectangular cuvettes with excitation at 445 nm on a FluoroMax-3 fluorimeter (Jobin Yvon, Edison, NJ); both excitation and emission slits were 4 nm. ThT fluorescence (Fig. 1a) showed a substantial lag-phase followed by a fast rate of elongation at these pH values.

Forming of the β-Oligomer versus the Amyloid Fibrils—Typical kinetics of spontaneous in vitro fibril formation of Hu recPrP at 37 °C showed a substantial lag-phase followed by a rapid increase of amyloid fibrils accumulation as measured by ThT fluorescence (Fig. 1a). The length of the lag-phase was a function of the pH value, ranging from 2 h at pH 6.1 and 7.2 to 26 h at pH 3.7 (Fig. 1c). In a previous study, using mouse and Syrian hamster recPrPs, we showed that acidic pH favored formation of the β-oligomer, which is off the kinetic pathway to the amyloid fibrils (24). Competition between two misfolding pathways, one of which leads to the β-oligomer and another to the fibrillar form, may explain the substantial differences in the length of the lag-phase.

To test whether the conversion of Hu recPrP to the β-oligomer interfered with the formation of the fibrils, I analyzed the composition of the reaction mixture using size-exclusion chromatography (SEC) (Fig. 1b). After 30 min of incubation at 37 °C, Hu recPrP was found predominantly in the β-oligomeric form at pH 3.7, whereas only 10% of the protein converted into the β-oligomer at pH 7.2. The length of the lag-phase of the fibril formation was a function of the relative amount of the monomeric recPrP: the larger the amount of the monomer presented at the initial stage of fibril formation, the shorter was the lag-phase (Fig. 1c).

The result of our experiments on the pH dependence of fibril formation was consistent with a model proposed earlier for the conversion of mouse and hamster recPrP (24). This model considers that the α-monomer exists in a slow equilibrium with the β-oligomer, and the β-oligomer is not on the kinetic pathway of the fibril formation (Fig. 1c). The equilibrium is shifted toward the β-oligomer at acidic pH, whereas the incubation of Hu recPrP at neutral pH leads to the formation of fibrils.

Because both misfolding pathways may co-exist, a simple experimental procedure for distinguishing between the two abnormal isoforms is needed. It is easy to confuse the β-oligomer with the amyloid fibrils using only CD spectroscopy because both abnormal isoforms have similar CD spectra and almost identical molar ellipticity at 222 nm (Fig. 2a). On the other hand, a ThT binding assay provided more definitive detection of the amyloid fibrils versus the β-oligomer (Fig. 2b). Even though both abnormal isoforms bind ThT, the binding capacity of the amyloid form is ~30-fold higher than the binding capacity of the β-oligomer.

The Elongation of Fibrils—Low abundance of the monomer at pH 3.7 and 4.2 determines the low rate of the initiation of fibril formation and explains the long lag-phase (see above). I also observed that the rate of conversion at pH 3.7 and 4.2 was much slower than the rate at pH 6.0 and 7.2 as judged from the increase in ThT fluorescence (Fig. 1a). For example, the change in fluorescence was 100 relative units/h at pH 6.0 and only 14 relative units/h at pH 3.7. The slow rate of conversion at acidic pH was consistent with the low population of the monomer observed throughout the time course of fibril formation (Fig. 1d). Interestingly, at acidic pH the concentration of the α-monomer remained relatively constant despite its ongoing recruitment into the fibrils. By decreasing the total population of soluble non-fibrillar forms (the α-monomer and the β-oligomer), the equilibrium between the α-monomer and the β-oligomer shifted back to the α-monomer, thereby maintaining its population (data not shown). On the other hand, the substantially higher level of the α-monomer at pH 6.0 and 7.2 explains the fast rate of elongation at these pH values.

As judged from electron microscopy the fibrils formed at pH 6.0 and 7.2 were substantially shorter than the fibrils formed at pH 3.7 (Fig. 3). Formation of long fibrils occurred under experimental conditions in which the initiation step was very inefficient, whereas the constant low level of the α-monomer provided the substrate for fibril elongation. In contrast to acidic pH, the high population of the α-monomer at neutral pH accelerated the step of nucleation. The formation of numerous centers of nucleation was followed by rapid elongation of fibrils and fast consumption of the α-monomer. The fast drop of the concentration of the α-monomer resulted in quick termination of elongation, which explains the short length of fibrils formed at neutral pH.

The fibrils formed at different pH values also had a distinct
morphology. The fibrils prepared at pH 6.0 and 7.2 resembled 
PrPSc “prion rod” and were lacking the distinct coil morphology, 
which was a characteristic of fibrils formed at pH 3.7.

Seeding of Fibril Formation—Because an autocatalytic con-
version is a key hallmark of prion propagation, I tested 
whether in vitro formation of fibrils shows features of the
autocatalytic process. When small aliquots of preformed fibrils 
(seeds) of Hu recPrP were transferred from a mature reaction 
into fresh reactions, I observed a significant reduction in the 
lag-phase (Fig. 4a). The extent to which the lag-phase was 
reduced depended on the amount of seed added to the fresh
reaction mixture. Under the experimental conditions em-
ployed, as little as an 0.001% aliquot of preformed fibrils dis-
played a seeding effect in fresh reactions. I also observed a 
seeding effect when the preformed fibrils of Mo recPrP were 
used to seed the conversion in a fresh reaction of Mo recPrP 
(Fig. 4d). These data illustrate that in contrast to the formation 
of the β-oligomer, the conversion of recPrP into fibrils exhibits 
specific features of an autocatalytic process.

As judged from the length of the lag-phase, the efficacy of 
cross-seeding of Mo recPrP with the fibrillar form of Hu recPrP 
was much lower than the efficacy of seeding of Mo recPrP with 
the fibrillar Mo recPrP (Fig. 4d). Similarly, preformed fibrils of 
Mo recPrP displayed low seeding activity toward Hu recPrP 
(Fig. 4b). Thus, 1% of preformed fibrils of Mo recPrP exhibited 
apparent seeding activity, which was an equivalent of the seed-
ing activity that would be displayed by 0.004% of preformed 
fibrils of Hu recPrP (Fig. 4c). This experiment illustrates that 
the differences in the amino acid sequence of recPrP from 
different species may substantially affect the ability of 
cross-seeding.

Autocatalytic Conversion under Sub-threshold Conditions—At 
sub-threshold conditions the process of amyloid formation did not 
occur spontaneously but was initiated only upon addition of seeds 
(Fig. 5a; the detailed mechanism of the conversion at sub-threshold 
conditions will be published elsewhere). Furthermore, the process 
of seeded conversion at sub-threshold conditions also displayed 
strong selectivity with respect to the primary structures of seeds 
and a substrate. Thus, the conversion of Hu recPrP was induced 
only upon the addition of Hu but not Mo seeds (Fig. 5a). Moreover, 
the presence of monomeric Mo polypeptides in a mixture with Hu 
polypeptides substantially reduced the rate of conversion seeded 
with fibrils of Hu recPrP (Fig. 5b). I observed this inhibition of the 
conversion rate despite high concentrations of total recPrP in the 
mixtures. Seeded conversion at sub-threshold conditions demon-
strated an autocatalytic mechanism for in vitro amyloid formation 
and displayed strong species selectivity of this process.
In a previous study (24) we established an experimental protocol for the refolding of Mo recPrP-(89-231) and Syrian hamster recPrP-(90-231) into two distinct β-sheet rich isoforms, the β-oligomer and amyloid fibrils. The amyloid fibrils of recPrP share several important physical properties with PrP-(27-30). Using a panel of specific Fabs, we showed that the amyloid of recPrP has an epitope presentation similar to that of PrP-(27-30), indicating their common overall architecture (24). To the extent that these biochemical tests capture important conformational properties of PrPSc, in vitro fibril formation can provide insight into the events underlying prion conversion. Current work demonstrated that the oxidized α-monomeric Hu recPrP can also be converted to the β-oligomer form and to amyloid fibrils. These data indicate that the folding behavior of Hu recPrP is similar to that of Mo recPrP and Syrian hamster recPrP (9, 24). Although the earlier studies of oxidized Hu recPrP reported that the protein can be refolded to a β-sheet-rich isoform, they failed to distinguish between the β-oligomeric and fibrillar forms (10).

The failure to distinguish two different misfolding pathways led to further confusion when the transition of the wild type was compared with transition of the disease-associated variant F198S of Hu recPrP. Thus, Vanik and Surewicz (25) reported that wild type Hu recPrP-(90-231) converts into amyloid fibrils at pH 5.0 without any lag-phase as monitored by CD. According to our data, the two misfolding pathways co-exist at pH 5.0, at which the formation of the amyloid fibrils requires shaking and displays the lag-phase, whereas the formation of the β-oligomer occurs without any lag-phase regardless of shaking. Because both the β-oligomeric and fibrillar forms have similar CD spectra (Fig. 2a), CD cannot be used as a valid probe to monitor the kinetics of amyloid formation. The ThT binding assay provides a better way for selective measurements of amyloid formation. The binding capacity of the amyloid fibrils is 30-fold higher than the capacity of the equivalent amount of β-oligomer (Fig. 3b). However, because ThT alsobinds to the β-oligomers, special precautions need to be taken for presentation of data. It is more informative to present the ThT binding as a change in absolute values rather than as a normalized percentage. Be-
cause a normalized percentage was used for the comparison of the wild type and the F198S variant (25), uncertainty remained as to whether the formation of the \( /H9252\)-oligomer or the amyloid fibrils was measured by ThT. According to our studies, incubation of wild type Hu recPrP-(90–231) under the experimental conditions described by Vanik and Surewicz (25) (1 M GdnHCl, pH 5.0, at 37°C without shaking) resulted in the formation of the \( /H9252\)-oligomer but not amyloid fibrils. Without GdnHCl, the authors observed polymerization only with the F198S variant but not with the wild type Hu recPrP (Ref. 25, Fig. 5 therein). These data are consistent with our earlier studies that demonstrated the conformational transition from the \( /H9251\)-monomer to the \( /H9252\)-oligomer was separated by a large energetic barrier associated with partial unfolding and oligomerization (9). Partially denaturing conditions destabilize the \( /H9251\)-monomer and facilitate the conversion to the \( /H9252\)-oligomer. Because the F198S variant is thermodynamically less stable than the wild type, the transition of the F198S variant to the \( /H9252\)-oligomeric form may occur even without denaturants, as described by Vanik and Surewicz (25).

In the current study I have shown that in vitro conversion of Hu recPrP to the fibrillar form involves an autocatalytic mech-

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**Fig. 4. Seeding of in vitro fibrillar formation.**

- **a**, the kinetics of fibril formation for Hu recPrP (0.4 mg/ml; reaction volume, 0.6 ml) in the absence (○) and presence of 0.001% (△), 0.01% (▽), 0.1% (■), and 1% (□) of preformed fibrils of Hu recPrP. Seeds are added at time zero.
- **b**, the kinetics of fibril formation for Hu recPrP (0.4 mg/ml; reaction volume, 0.6 ml) in the absence (○) and presence of 1% of preformed fibrils of Hu recPrP (□), and 1% of preformed fibrils of Mo recPrP (▽). The line represents the fit of the data to a linear function. The length of the lag-phase of fibril formation for Hu recPrP seeded with 1% of fibrillar Mo recPrP is shown (△). The kinetics of fibril formation for Mo recPrP (0.4 mg/ml; reaction volume, 0.6 ml) in the absence (○) and presence of 1% of preformed fibrils of Mo recPrP (■) and 1% of preformed fibrils of Hu recPrP (△). All experiments were carried out under standard conditions in phosphate buffer, pH 6.5, in the presence of 3.4 M urea and 1 M GdnHCl. The fibrils were assayed by ThT fluorescence.

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**Fig. 5. Seeded conversion under sub-threshold conditions.**

- **a**, the kinetics of fibril formation for Hu recPrP (0.3 mg/ml; reaction volume, 0.3 ml) seeded with 5% of fibrillar Hu recPrP at time zero (○), with 5% of fibrillar Mo recPrP at time zero and with 5% of fibrillar Hu recPrP added after 8 h of incubation as indicated by the arrow (△), and without seeding (▽). The kinetics of fibril formation for Hu recPrP (0.3 mg/ml) and for the mixtures of recPrP with the following ratios of Hu to Mo (0.3 to 0.075 mg/ml (△) and 0.3 to 0.15 mg/ml (▽)). The conversion was induced with 5% of fibrillar Hu recPrP added at time zero. The reaction volume was 0.3 ml. Without seeding, Hu recPrP (0.3 mg/ml) did not display any increases in ThT binding (■). All experiments were carried out in phosphate buffer, pH 6.5, in the presence of 3.4 M urea and 1 M GdnHCl.
anism, i.e. the ability of preformed fibrils of recPrP to initiate the process of conversion. The process of seeded conversion is consistent with the protein-only hypothesis, which postulates that PrPSc propagates its pathological conformation in an autocalytic manner and acts as a transmissible agent of the prion diseases. Seeded conversion of the reduced form of recombinant prion protein was recently described by Lee and Eisenberg (26). The conversion was achieved through reduction of the disulfide bond in the presence of dithiothreitol and GdnHCl at alkaline pH followed by removal of dithiothreitol and GdnHCl at acidic pH, which resulted in the formation of intermolecular disulfide bonds accompanied by polymerization into fibrils. The seeding led to linear elongation of preformed fibrils. Because the polypeptides assembled in the amyloid fibrils are linked to each other by intermolecular disulfide bonds, it is unlikely that multiplication of the catalytic centers can be achieved in the time course of polymerizing using this protocol unless repetitive cycles of reduction-oxidation are applied. Although the reduced form of recPrP has been shown to form a fibrillar structure (14, 26), it is the oxidized form that causes the prion disease (27, 28). The seeding phenomenon has never been observed before using the oxidized form of recPrP (90–231) folded into an abnormal conformation.

The seeding of in vitro conversion reaction with preformed fibrils described in current protocol was highly efficient. I observed the seeding effect even when the molar ratio of Hu recPrP polypeptides assembled to fibrils to the monomeric Hu recPrP was 1:100,000 (Fig. 4a). Our studies of the oligomerization states demonstrated that the multimeric isoforms of recPrP with a molecular mass >1 MDa possessed seeding activity. If one assumes that every seed consists of, on average, 100 polypeptides, the actual molar ratio of active centers of one species might be as high as 1:10,000,000. These data show that the autocalytic conversion is highly efficient when the reaction is seeded with fibrils of homologous recPrP. Furthermore, I found that the autocalytic conversion displayed species specificity. Preformed fibrils of Hu recPrP were capable of seeding the conversion of Hu recPrP, whereas the conversion of Mo recPrP with the same amount of fibrillar Hu recPrP was delayed and vice versa. This experiment illustrates the high selectivity of the autocalytic conversion with respect to the amino acid sequences of seeds and substrates. Using PrP-derived fragments, Kundu et al. (29) found that the residues within the 138–141 region of PrP are important for the formation of amyloid structure. Interestingly, one of the mismatches between Hu and Mo PrP is located at position 139 (methionine in Hu PrP and isoleucine in Mo PrP). Whether this or other positions within the region 90–231 influences the species selectivity of autocalytic conversion remains to be determined.

The high selectivity of autocalytic conversion was also confirmed using sub-threshold conditions (Fig. 5). Strong species selectivity of seeded conversion in vitro recapitulated the species barrier of prion propagation observed in vivo. Moreover, our data were in agreement with an earlier observation made on transgenic mice, which co-expressed both Hu and Mo PrP (30). Mice with high level of Hu PrPC expression were resistant to Hu PrPSc but became susceptible to human prions upon ablation of the Mo PrP gene (30). Similarly, the mixing of Mo and Hu polypeptides inhibited in vitro conversion induced by Hu seeds (Fig. 5b). Such inhibition can be caused by competition between Hu and Mo polypeptides for binding to the active centers of Hu seeds. It has yet to be determined whether the binding of Mo polypeptides to Hu seeds totally blocks the binding of Hu polypeptides to seeds or just slows down the rate of the binding and the conversion of Hu recPrP.

The species barrier limits transmission of prions across species (31) and is considered as one of the most peculiar feature of prion propagation. From studies in transgenic animals, two factors have been identified that contribute to the species barrier: (i) the difference in PrP sequences between the donor species and the recipient species and (ii) the conformational properties of individual strains of PrPSc (32–34). When the sequence of the PrPC of a recipient animal is identical to the sequence of PrPSc of a donor animal, the disease has the shortest incubation time. In contrast, if PrPSc is transmitted from one species to another, the incubation time is longer in the first passage, and the newly infected animals develop atypical clinical signs and unusual histopathology (31, 33).

Both features, the species barrier and the strain phenomenon, were studied extensively using an in vitro conversion system of the prion protein of yeast Sub35 (21, 35). Preformed amyloid fibrils of Sup35 were capable of seeding the formation of new fibrils in fresh solution and also provided templates that determined the conformation of the new fibrils. As a result, the strain and species-specific conformational properties of the seeds were conferred to the newly formed fibrils (21). For a variety of technical reasons a cell-free system has never been developed using mammalian recombinant PrP. Our in vitro system displayed high efficiency and species selectivity of the conversion in the absence of cellular environment.

In addition to the differences between the amino acid sequences of PrPSc and PrPSc, the conformational diversity of PrPSc strains seem to affect the ability to cross the species barrier in vitro (33, 36). Some strains of PrPSc overcome the species barrier more easily than others when transmitted to different species. Considering the conformational diversity of existing strains and the possibility of the emergence of new strains with novel conformational properties in the future (37), it would be difficult to predict the risk of transmission of different PrPSc strains from domestic and wild animals to human. Because no cellular factors and no strain-dependent templating are required for the in vitro conversion system presented in our study, the selectivity of seeding primarily reflects the differences in the amino acid sequences of a seed and a substrate. This system might be used perhaps as a rapid assay for assessing the intrinsic propensities of prion transmission between different species.

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