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The Emerging Principles of Mammalian Prion Propagation and Transmissibility Barriers: Insight from Studies in Vitro

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ABSTRACT
Self-perpetuating conformational conversion of the cellular prion protein PrP^C into the β-sheet-rich "scrapie" conformer (PrP^{Sc}) is believed to be the central molecular event in pathogenesis of a group of diseases known as transmissible spongiform encephalopathies. Recent advances provide growing support for the notion that a misfolded protein alone might act as an infectious agent. Furthermore, findings regarding the mechanism of prion protein structural rearrangement, the role of folding intermediates in conformational conversion, and "conformational adaptability" in the propagation of prion amyloids in vitro yield molecular-level insight into such phenomena as inherited prion diseases, prion transmission barriers, and prion strains.

I. Introduction: Prion Diseases and the "Protein-Only" Hypothesis

Few diseases have generated as much fascination, fear, and controversy in such a short time as the transmissible spongiform encephalopathies (TSEs or prion diseases) of mammals. The TSEs are a class of related neurodegenerative disorders including kuru, Creutzfeldt–Jakob disease (CJD), Gerstmann–Straussler–Scheinker disease, and fatal familial insomnia of humans, scrapie of sheep, bovine spongiform encephalopathy (BSE) of cattle, and chronic wasting disease of cervids, all of which are fatal.[1–4] These disorders may arise spontaneously, may be inherited, or may be acquired by infection. While usually very rare, under certain conditions TSEs can assume epidemic proportions. In recent years, an epidemic of BSE broke out among cattle in the United Kingdom, leading to serious concerns that animal prion diseases can be transmitted to humans.[1–4]

The most unusual feature of TSEs is the nature of the pathogenic agent. Certain characteristics of the disease, such as transmissibility and the existence of different TSE "strains", originally fostered the notion that these disorders are caused by "slow viruses". However, despite intensive effort, no conclusive evidence has been obtained for nucleic acid within the infectious TSE agent. Most researchers currently accept an alternative explanation, the "protein-only" hypothesis, according to which the infectious pathogen is a misfolded form of the cellular prion protein, PrP^C.[1,5] This rogue conformer, designated PrP^{Sc}, is believed to self-replicate by binding to PrP^C and inducing conversion of this protein to the PrP^{Sc} state. This model, implying that proteins alone may be infectious and carry heritable information, represents a new paradigm of molecular biology. While supported by recent discoveries regarding protein-based inheritance in yeast and other fungi,[6–8] the notion that PrP^{Sc} alone represents an infectious agent in TSEs still awaits a "final proof", tantamount to creating highly potent infectious material in vitro from pure protein. Tremendous progress in the past few years seems to have put this ultimate proof within reach (see below).

II. PrP^C and PrP^{Sc}: Two Forms of the Same Protein

The mature PrP^C is an ~210 amino acid protein containing a C-terminal glycosylphosphatidylinositol anchor, two asparagine-linked glycosylations, and a single disulfide bridge.[1–4] The N-terminal region contains characteristic glycine-rich octapeptide repeats involved in binding Cu^{2+} ions.[9] The protein has been implicated in several biological processes[3], but its normal physiological function remains unknown.

Spongiform encephalopathies are closely associated with cerebral accumulation of PrP^{Sc}.[1–5] The covalent structure of PrP^{Sc} appears indistinguishable from that of PrP^C. However, the two PrP isoforms have dramatically different physical properties. While PrP^C is readily degradable by proteinase K, PrP^{Sc} shows a remarkable resistance to proteolytic digestion, containing a protease-resistant core (PrP27-30) encompassing the C-terminal ~140 residues. Additionally, PrP^C is fully soluble in nonionic detergents, whereas PrP^{Sc} is not, forming aggregates with characteristics often similar to amyloid fibrils. These differences in physical properties reflect different conformations: low-resolution optical spectroscopic experiments indicate that PrP^C isolated from normal brain is rich in α-helical structure, whereas PrP^{Sc} contains mostly β-sheet.[1–4]

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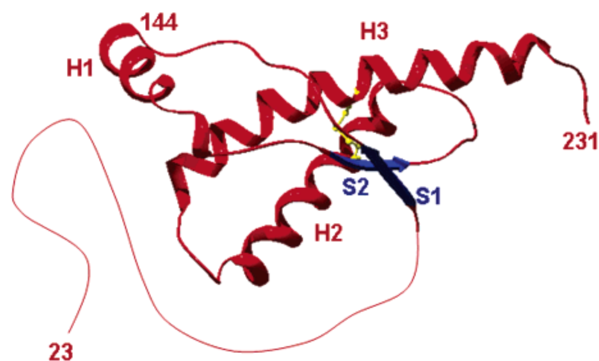


FIGURE 1. Solution structure of human prion protein as determined by NMR spectroscopy.[12] α-Helices are shown in red, β-strands are shown in blue, and the disulfide bond between Cys^{179} and Cys^{214} is indicated in yellow.

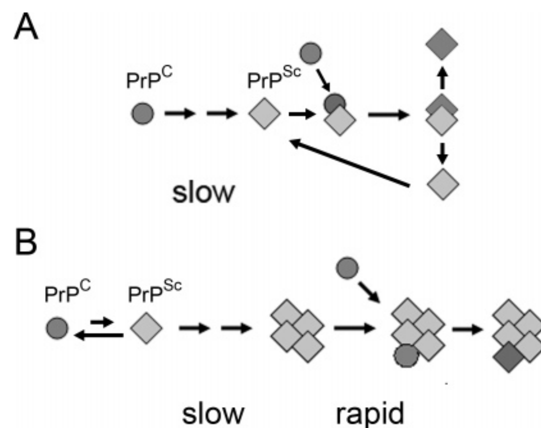


FIGURE 2. Schematic diagrams for different models of $PrP^C \rightarrow PrP^{Sc}$ conversion. Circles and diamonds represent molecules of PrP^C and PrP^{Sc}, respectively: (A) the monomer-directed heterodimer (HD) model; (B) the nucleation-dependent polymerization (NP) model. See text for details.

High-resolution NMR studies have shown that recombinant PrP, a nonglycosylated model of PrP^C, consists of a folded C-terminal domain and a largely unstructured N-terminus (Figure 1). The folded domain of PrP from different species is very similar, encompassing three α-helices and a short β-sheet.[10−12] X-ray crystallography has captured recombinant PrP as a domain-swapped dimer in which helix 3 from one monomer is linked to helix 2 from the other by an intermolecular disulfide bond.[13] However, the role of this dimer in the $PrP^C \rightarrow PrP^{Sc}$ conversion remains controversial. In contrast to the α-helical PrP^C, no high-resolution structural data is available for the β-sheet-rich PrP^{Sc} isoform.

III. Biophysical Mechanisms of Prion Protein Conversion

Within the framework of the protein-only hypothesis, the central molecular event in the pathogenesis of prion diseases is the conversion of PrP^C into PrP^{Sc}. This hypothesis is intriguing in light of Anfinsen's principle that the final fold of proteins is fully encoded in the amino acid sequence. However, the notion that PrP can exist in two (or more) stable conformations does not necessarily challenge this principle because other factors, such as the local environment and intermolecular interactions, may also influence protein conformation. Such conversion of proteins to an abnormal, β-sheet-rich structure is a common denominator of amyloid disorders.[14] However, the unique feature of prion diseases is transmissibility.

The two leading models proposed for prion propagation are the *heterodimer refolding* (HD) mechanism, also known as template-assistance or monomer-directed conversion,[5] and the *nucleated polymerization* (NP) mechanism.[15] These models differ with respect to the role of ordered prion protein oligomers in the conversion reaction. The HD model (Figure 2A) postulates that PrP^C is thermodynamically less stable than PrP^{Sc} but spontaneous conversion to PrP^{Sc} is kinetically limited. A critical step in the conversion would be formation of a heterodimer between PrP^C and PrP^{Sc} monomers. PrP^{Sc} in this complex would act as a template, inducing a conformational transition of PrP^C. Here, oligomerization is a *consequence*, not a cause, of conversion. A different view is presented by the NP model, according to which the infectious species is not the PrP^{Sc} monomer but a PrP^{Sc} aggregate (Figure 2B). This model postulates that monomeric PrP^C and PrP^{Sc} exist in an equilibrium far displaced toward PrP^C. Stabilization of PrP^{Sc} occurs only upon formation of an oligomer large enough to act as a stable nucleus. Monomeric PrP^C would subsequently add to the nucleus, adopting the structure of PrP^{Sc}. The rate-limiting step in the nucleated polymerization model is not conversion but nucleation. This step, responsible for the "lag phase" in the spontaneous conversion reaction, can be bypassed by addition of preformed PrP^{Sc} aggregates. While both these mechanisms are theoretically plausible, there is little evidence for the existence of a stable PrP^{Sc} monomer. Moreover, prion infectivity is associated with PrP^{Sc}-containing aggregates, not with PrP monomers.[16]

Cell-Free Conversion Studies. An important development in TSE research was the finding that PrP^C can be converted in vitro to a protease-resistant state (PrP-res) by incubating PrP-res from infected animals with normal PrP^C.[17] These cell-free experiments, pioneered by Caughey and co-workers, demonstrated that PrP conversion consists of two kinetically distinct steps: binding of PrP^C to PrP-res oligomer, followed by conversion of bound PrP^C to the PrP-res conformation.[18] In this in vitro conversion, formation of new PrP-res molecules invariably required the presence of oligomeric seeds, and the newly created PrP-res always remained tightly associated with the original PrP-res, indicating that aggregation is an inseparable aspect of PrP conversion. Remarkably, the cell-free conversion reaction was highly specific, reproducing certain elements of the species-barrier and strain specificity.[18] Originally these conversion experiments suffered low yield (below the level required for continuous propagation of the PrP-res state), but this yield may be improved by additional cofactors,[19] or using a procedure of protein misfolding cyclic amplification by sonication, PMCA.[20] In a recent landmark study,[21] the product of PMCA was

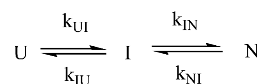
shown to be infectious in animals. However, these PMCA experiments used whole brain homogenate, which contains other components such as nucleic acids and lipids; the ultimate proof of the protein-only model will require synthesis of new infectious material in a well-defined system containing purified protein.

Conformational Conversion of the Recombinant Prion Protein and "Synthetic Prions". Considerable progress toward understanding the molecular mechanism of the $PrP^{C} \rightarrow PrP^{Sc}$ conversion has been made by studies using bacterially expressed recombinant prion protein produced by *Escherichia coli*. Our early experiments have shown that recombinant human PrP fragment 90−231 (huPrP90−231), when incubated at acidic pH in the presence of low concentrations of guanidine hydrochloride, undergoes a transition from α-helix to β-sheet structure.[22] Similar transition was reported for mouse PrP121−231 in the presence of urea and salt.[23] This acid-induced β-sheet-rich form of PrP was believed at that time to represent a "monomeric equilibrium intermediate". The notion that prion protein can exist as either an α-helical or β-sheet monomer seemed to be supported by a report that reduction of its disulfide bond could reversibly switch PrP between its native α-helical conformation and a monomeric β-structure, even in the absence of any denaturants.[24] This claim was considered support for the "monomer-directed" HD model of prion protein conversion. However, subsequent studies have conclusively demonstrated that the β-sheet-rich species populated at low pH in the presence of denaturant is not a monomeric equilibrium folding intermediate but, in fact, a PrP aggregate.[25,26] Furthermore, more recent data indicates that the disulfide-free monomeric PrP is not β-sheet rich but rather has molten globule-like properties.[27] While this partially unfolded conformer may form a β-sheet structure under some conditions, this transition is intimately associated with protein oligomerization.[27]

Prion protein oligomers formed during the conversion to β-sheet structure at acidic pH share certain physicochemical properties of PrP^{Sc}, such as increased resistance to proteinase K digestion and the presence of fibrillar particles (together with amorphous aggregates). Studies on the mechanism of this conversion reaction revealed that the formation of β-sheet oligomers is facilitated by low to medium concentrations of denaturants, whereas strongly unfolding conditions prohibit conversion, suggesting a role of partially folded intermediates in the conformational transition.[26] Importantly, the α-helix → β-sheet transition occurs concomitantly with oligomerization of the protein,[25,26] again arguing against the existence of a stable β-sheet-rich monomer of PrP.

While these early studies provided insight into the mechanism of prion protein α-helix → β-sheet transition, the conversion pathway at acidic pH appears to be non-autocatalytic; thus, it fails to mimic self-propagation of infectious prions. Autocatalytic (seeded) conversion of recombinant PrP was generated by disulfide oxidation−reduction,[28] leading to a model of prion propagation based on the domain-swapped crystal structure;[13] however, there

Scheme 1



$$U \underset{k_{IU}}{\overset{k_{UI}}{\rightleftharpoons}} I \underset{k_{NI}}{\overset{k_{IN}}{\rightleftharpoons}} N$$

is no evidence for disulfide reshuffling during PrP conversion in vivo. Baskakov and co-workers were first to describe a conversion of native (i.e., oxidized) recombinant PrP to classical amyloid fibrils at neutral pH.[29,30] This reaction had relatively poor seeding efficiency (resulting in only partial reduction of the lag phase upon addition of preformed amyloids) and required high denaturant concentration with vigorous shaking; nonetheless, identification of this pathway was an important step toward elucidating the mechanism of PrP conversion. In our research, we have recently found that some prion protein variants with TSE-associated point mutations can be converted to amyloid fibrils under milder, physiologically more relevant conditions.[31] Mechanistic studies of the conversion of one such mutant (D178N) indicate a nucleation-dependent polymerization mechanism involving three essential steps: nucleation, fibril elongation by recruitment of PrP monomer, and fibril fragmentation to create new ends (unpublished data).

Intracerebral injection of fibrils prepared by the procedure of Baskakov and colleagues into transgenic mice overexpressing PrP^{C} resulted in a neurological disease with very long incubation time, leading the authors to conclude that fibrils prepared from the recombinant PrP contain "synthetic prions".[32] Generation of infectious material from the recombinant PrP would constitute an irrefutable proof for the protein-only hypothesis. However, given that the experiments have been performed using mice that highly overproduce PrP, one cannot exclude alternative explanations of this fascinating data.[33]

The Role of Intermediates in Prion Protein Folding and Its Conformational Conversion. One of the controversies in prion research relates to the normal folding pathway of prion protein and the nature of the direct precursor of the oligomeric PrP^{Sc}. Partially structured folding intermediates are believed to play a key role in fibril formation by many classical amyloidogenic proteins.[14] However, such intermediates for prion protein have proved difficult to detect, leading some investigators to believe that prion protein conversion requires PrP to be fully unfolded for recruitment to PrP^{Sc}.[34,35]

The folding pathway of recombinant prion protein has been extensively studied by a kinetic stopped-flow method. Initial experiments revealed that PrP folds extremely fast, suggesting that the native structure (N) is acquired directly from the unfolded state (U) without sampling any intermediates.[34] However, more detailed stopped-flow experiments revealed that the folding of PrP is more complex and can be described by a three-state model involving a monomeric intermediate, I (Scheme 1).[36]

Formation of this early intermediate, which accumulates within the dead-time of the stopped-flow instrument (∼1 ms), was recently confirmed by continuous-flow measurements performed in collaboration with Dr. Hein-

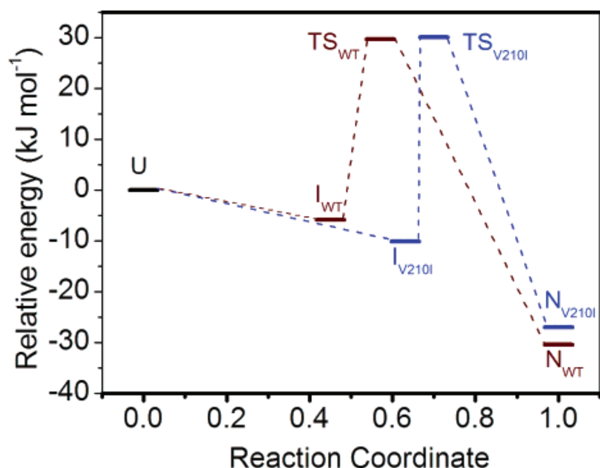


FIGURE 3. The reaction coordinate diagram for the refolding of the wild-type huPrP90−231 (red) and the V210I variant (blue). The diagram shows the free energy levels of the native (N) and intermediate (I) states relative to the unfolded state (U). TS represents the transition state between I and N. The difference in energy between states N and I for V210I is lower than that of the wild-type huPrP90−231, indicating a higher stability and, thus, population of the intermediate for the mutant protein.[39]

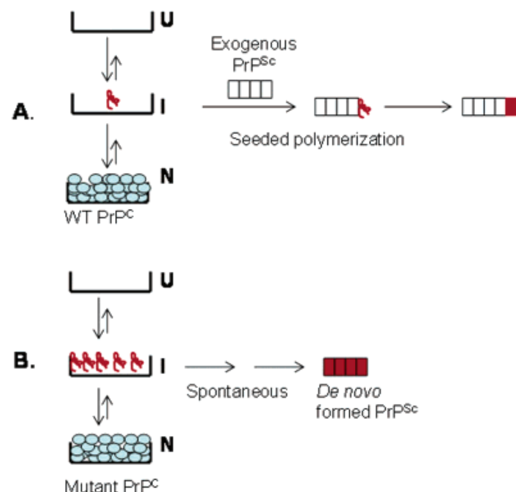


FIGURE 4. Schematic diagram illustrating the proposed role of partially structured monomeric intermediate in the $PrP^C \rightarrow PrP^{Sc}$ conversion for the wild type protein (A) and disease-associated mutants (B). For the wild-type protein, the population of the intermediate is very low (less than 0.01%). Thus, except for extremely rare sporadic cases, these species may be recruited into PrP^{Sc} oligomer only in the presence of exogenous PrP^{Sc} seeds. In contrast, for mutant proteins the concentration of partially folded intermediates may become sufficient to initiate the aggregation process in the absence of seed, leading to de novo formation of PrP^{Sc}. In each case, the population of fully unfolded state is negligible compared to both the native and intermediate states.[39]

rich Roder. The latter study enabled us to directly observe the two phases in PrP folding: the initial accumulation of the intermediate on a time-scale of ~100 μs, followed by a rate-limiting I → N transition on the millisecond time scale (unpublished data). Thermodynamic analysis of kinetic data revealed that while at neutral pH the intermediate is of relatively low stability, it becomes significantly more populated under acidic conditions.[36] The existence of a partially folded state for the prion protein is supported by hydrogen exchange NMR and high-pressure spectroscopy studies reported by other investigators.[37,38]

In addition to its involvement in normal prion protein folding, the partially structured intermediate could play a key role in the $PrP^C \rightarrow PrP^{Sc}$ conversion. Folding intermediates are usually characterized by a significant exposure of the polypeptide backbone to solvent, enabling increased intermolecular interactions. This, combined with much higher stability of the intermediate as compared with the fully unfolded protein,[36] renders the I state of PrP a particularly likely candidate for the direct monomeric precursor of PrP^{Sc}. To explore this possibility, we have recently extended stopped-flow studies to prion protein variants carrying mutations associated with inherited prion diseases.[39] For each mutant protein tested, the population of an intermediate was found to be at least 1 order of magnitude higher than that of the fully unfolded state. Furthermore, in the vast majority of cases, familial mutations resulted in major stabilization, and thus increased population, of the folding intermediate. This effect was observed even for mutations with only a minor effect on the global stability of the prion protein (e.g., V210I; see Figure 3). On the basis of these findings, we have proposed that most familial prion diseases may arise from a mutation-induced increase in the population of partially folded intermediates of PrP (see Figure 4).

IV. PrP23−144: An in Vitro Model for Studying the Molecular Basis of Prion Propagation, Species Barrier, and Strain Diversity

The difficulties in propagating an efficient autocatalytic conversion of the full length prion protein in vitro under physiologically relevant buffer conditions have prompted us to resort to a simpler model consisting of the recombinant polypeptide PrP23−144. This C-terminally truncated PrP variant, associated with a familial prion disease, encompasses the largely unstructured part of the prion protein, terminating just before the α-helical region (Figure 1).

PrP23−144 was found to spontaneously convert from a soluble monomer to amyloid fibrils under physiological (denaturant-free) buffer conditions[40] with a concentration-dependent lag phase. Addition of a small amount of preformed amyloid to soluble protein completely abolishes the lag phase, indicating an autocatalytic, nucleation-dependent character of the reaction. It is likely that the high efficiency of this in vitro conversion is due to the protein's largely unfolded structure (as opposed to the full-length PrP), which allows critical amyloid nucleation sites to be continually available for intermolecular contacts.[40] To locate these critical nucleation sites, a series of deletion variants was constructed, in which successive C-terminal fragments were removed from PrP23−144. It was concluded that the critical amyloidogenic region of PrP23−144 involves a short segment encompassing amino acid residues 138−141. This region may also be critical for the conversion of the full-length PrP, though in the latter case

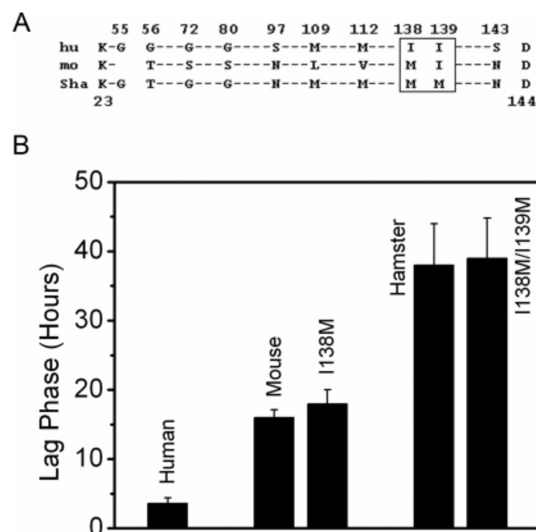


FIGURE 5. Comparison of species-specific PrP23−144 variants: (A) partial sequence alignment of PrP23−144 variants showing sequence differences with the critical amyloidogenic region boxed; (B) lag phases of PrP23−144 conversion (I138M, I138M huPrP23−144; I138M/I139M, I138M/I139M huPrP23−144). Adapted from ref 43.

it is likely "protected" by intramolecular interactions with the C-terminal domain.

Species Specificity in PrP23−144 Conversion and Seeding. Prion diseases are known to vary in pathology, infectivity, and time course depending on the host species.[1,2,4] However, the most interesting species-specific property of TSEs is the so-called species barrier, or the limited infectivity of prions from one species to another.[1,2,4] This barrier to transmission was traditionally attributed to specificity of a TSE virus. With the advancement of the protein-only model, this view was largely abandoned in favor of sequence similarity between host and donor PrP as the critical determinant of interspecies prion transmissibility.[18,41,42] Species with similar PrP sequences often do have lower barriers to cross-species TSE infection than do species with highly divergent prion proteins,[18,41,42] but the reason for this difference is unclear.

Encouraged by our findings regarding autocatalytic conformational conversion of PrP23−144, we have used this system as a model to explore the molecular basis of the species barrier in TSEs. To this end, PrP23−144 from three species—human (hu), mouse (mo), and Syrian hamster (Sha)—was purified, and the kinetics of conversion were monitored by the fluorescent ThT assay. As shown in Figure 5B, the three PrP23−144 variants show pronounced differences in the lag phase of amyloid formation. Comparison of PrP sequences from these three species reveals that they differ at residues 138 and 139, part of the critical 138−141 region identified in our earlier effort: human PrP contains isoleucine at both positions, in mouse protein I138 is replaced by methionine, and in ShaPrP methionine is present both at position 138 and at position 139 (Figure 5A). If the 138−141 region forms a critical amyloidogenic determinant of PrP23−144, the identity of residues in this region (rather than in other segments of PrP) would be expected to influence the conversion behavior of protein in a species-specific manner. To test this hypothesis, species-mimetic variants of human PrP23−144 were constructed, in which residues at the critical site were mutated to those of mouse (I138M) or Syrian hamster (I138M/I139M). These two mutant huPrP23−144 proteins converted with kinetics identical to mo- and ShaPrP23−144, respectively (Figure 5B). Conversely, ShaPrP23−144 with its critical residues mutated to those of human protein (i.e., M138I/M139I ShaPrP23−144) converted with kinetics essentially identical to human protein.[43] Thus, residues 138 and 139 appear to fully control species-specific kinetics of PrP23−144 conversion.

More importantly, we also found that PrP23−144 amyloids of different species have unique cross-seeding capabilities, a phenomenon reminiscent of species barriers. If preformed amyloid is added to soluble protein of the *same sequence*, immediate conversion with no lag phase takes place. In contrast, if the amyloid and soluble proteins are of different sequences, seeding does not always occur (Figure 6A). Amyloid of human PrP23−144 (designated [hu]), for instance, can seed conversion of soluble mouse protein but not ShaPrP23−144. Syrian hamster amyloid, on the other hand, can seed mouse protein but not huPrP23−144. Importantly, the same

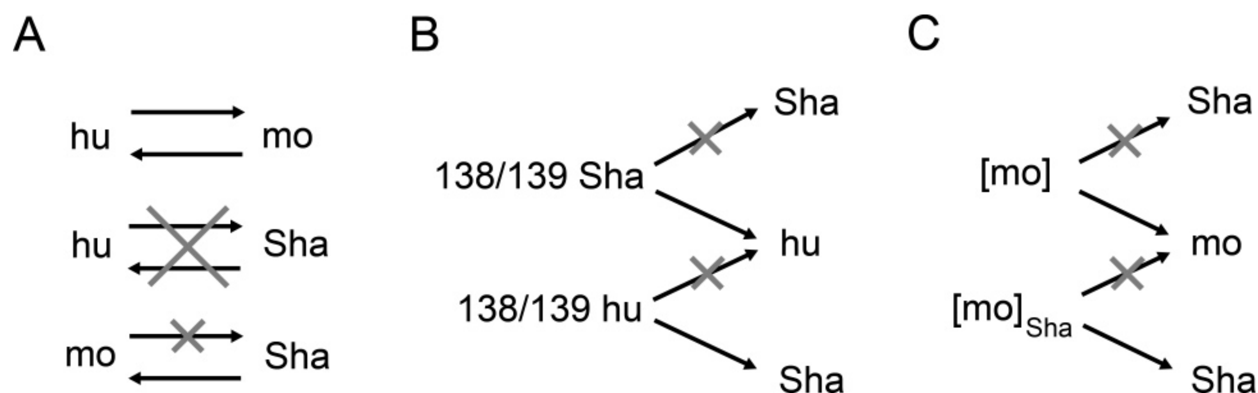


FIGURE 6. Schematic depiction of seeding capability of PrP23−144 amyloids: (A) cross-seeding pattern for species-specific PrP23−144 variants; (B) species-mimicking huPrP23−144 mutants (138/139 Sha, M138I/M139I ShaPrP23−144; 138/139 hu, I138M/I139M huPrP23−144) showing that mutation of critical residues in Syrian hamster protein transforms seeding specificity to match that of wild-type human protein (see panel A) and vice versa; (C) moPrP23−144 fibrils formed by seeding with Syrian hamster amyloid ($[mo]_{Sha}$) lose the seeding specificity of spontaneously formed mouse fibrils and gain that of Syrian hamster fibrils.

species-mimetic mutations at residues 138 and 139 that transform conversion kinetics are also sufficient to transform seeding properties, resulting in proteins with the seeding specificity corresponding to a different species (Figure 6B). Therefore, seeding barriers appear to be controlled not by global sequence similarity but by similarity only in the critical region.

How does the critical region determine seeding specificity? To address this question, the secondary structure and nanoscale morphology of PrP23−144 amyloid fibrils were examined using Fourier transform infrared (FTIR) spectroscopy and atomic force microscopy (AFM), respectively.[44] Human and mouse PrP23−144 fibrils, capable of seeding one another, display a segmented morphology in AFM images and characteristic secondary structure signature in the amide I region of FTIR spectra. Syrian hamster fibrils, on the other hand, which are mutually seeding incompatible with human protein, have a different conformation, with smooth fibrils displaying a distinct FTIR spectrum (Figure 7). The species-mimetic mutations that alter seeding specificity also alter fibril conformation to mimic that of the corresponding species;[44] for example, I138M/I139M huPrP23−144 forms smooth fibrils with an FTIR signature identical to Syrian hamster fibrils. Thus, seeding behavior is strongly correlated with amyloid fibril conformation.

The Link Between Species Barriers and Strains: Conformational Adaptation. The problem of TSE species barriers is confounded by the phenomenon of prion strains or variations of phenotype within a single species.[1,2,4] Unique strains of TSE can “emerge” in a host after repeated passage of TSE-infected brain extract originally taken from another species. The existence of strains has proven an exceptional challenge to the protein-only model; it is difficult to explain how a pathogen lacking nucleic acid could produce multiple phenotypes in one species. It was only recently that evidence began to emerge suggesting that distinct prion strains can be rationalized within this model, with individual strains representing different PrP^{Sc} conformers.[1,2,42,45]

The PrP23−144 conversion, which displays “species barriers” in seeding specificity, provided a unique opportunity to explore the molecular basis of prion strains. As shown in Figure 6A, there is an asymmetry in seeding capability between Syrian hamster and mouse proteins: Syrian hamster amyloid can seed conversion of soluble moPrP23−144, but mouse fibrils cannot seed Syrian hamster protein.[43] In animals, cross-species transmission of prions often results in emergence of a new TSE strain with different properties than the donor strain.[2] Might a corresponding adaptation take place in PrP23−144 conversion in vitro? To probe this question, we tested the seeding specificity of “second generation” moPrP23−144 fibrils formed by preseeding of mouse protein with 2% Syrian hamster amyloid. Remarkably, these second-generation mouse PrP fibrils (designated $[mo]_{Sha}$) lost the original seeding specificity of mouse fibrils and adopted that of Syrian hamster fibrils (Figure 6C). Thus, preseeding of mouse protein with Syrian hamster amyloids led to the emergence of a “Syrian hamster-like” strain of moPrP23−144 amyloid with the seeding specificity of the Syrian hamster template.[43]

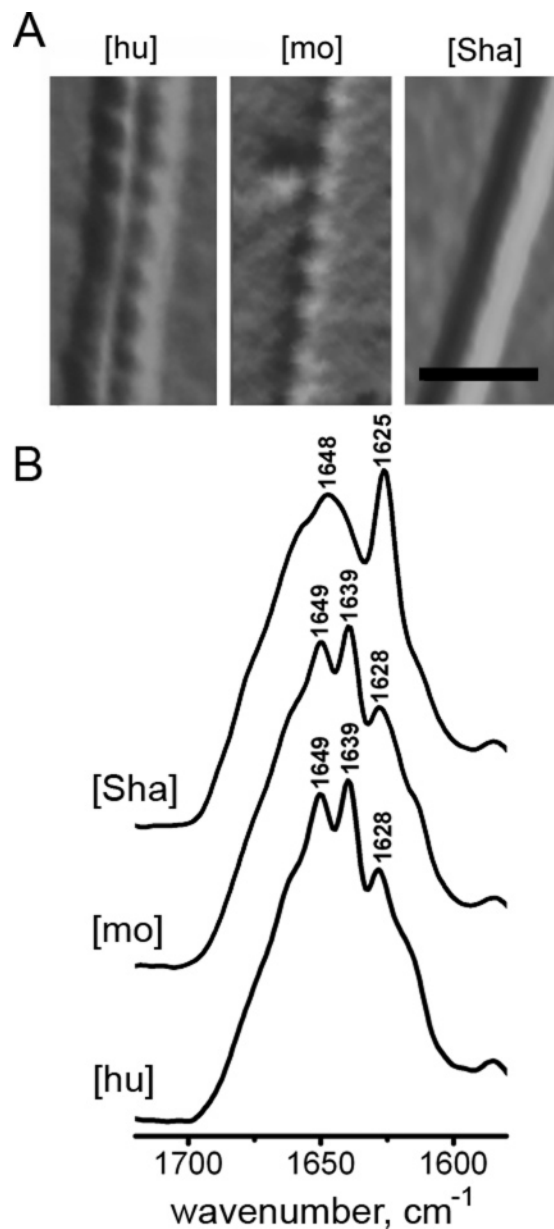


FIGURE 7. Species-specific conformations of PrP23−144 amyloid fibrils determined by AFM (A) and FTIR spectroscopy (B). Scale bar, 100 nm. Adapted from ref 44.

Examination by AFM revealed that these second generation $[mo]_{Sha}$ fibrils no longer had bead-like morphology of self-seeded mouse fibrils but were smooth and nonperiodic, exactly like Syrian hamster fibrils. Furthermore, the FTIR spectrum of $[mo]_{Sha}$ lost characteristics of self-seeded mouse amyloid and acquired those of Syrian hamster amyloid (Figure 8). Thus, the new strain of mouse PrP fibrils, created by cross-seeding with Syrian hamster fibrils, adopted the conformation of the parent seed. The concurrent acquisition of hamster-like pattern of seeding barriers strongly indicates that the seeding specificity does not merely correlate with but, rather, is fully encoded in amyloid fibril conformation.

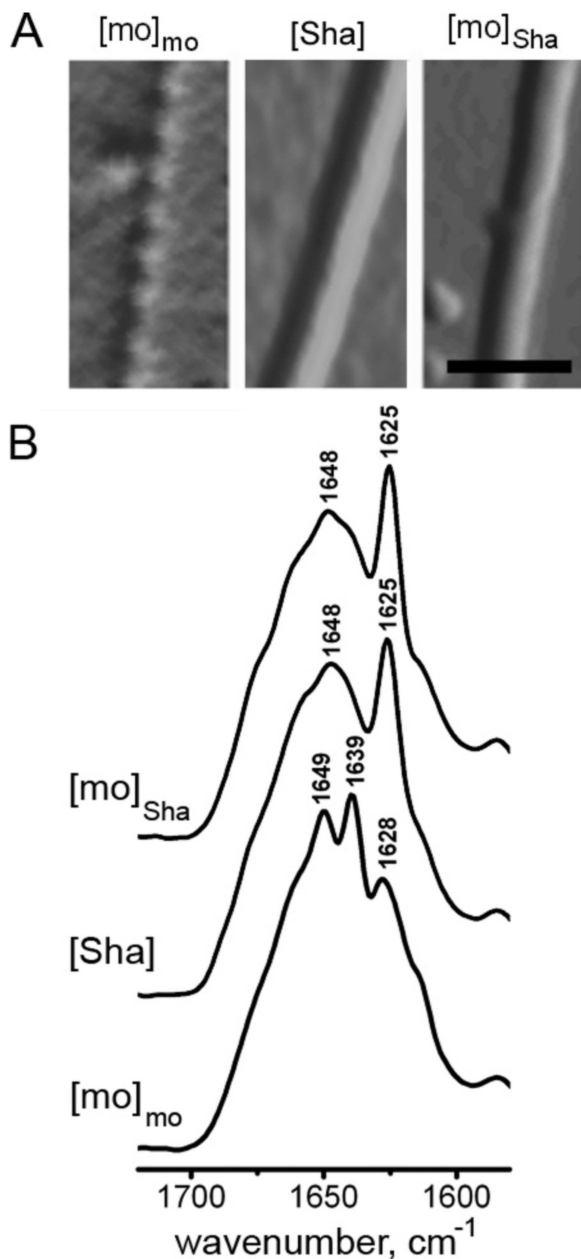


FIGURE 8. Cross-seeding of mouse PrP23−144 with Syrian hamster fibrils results in a strain of mouse fibrils ($[mo]_{Sha}$) that inherits amyloid conformation from the Syrian hamster seed, as indicated by AFM (A) and FTIR spectroscopy (B). Adapted from ref 44.

Despite considerable differences in the conformation between spontaneously formed Syrian hamster and mouse amyloids (Figure 6), Syrian hamster can seed the conversion of mouse protein, whereas mouse fibers cannot seed Syrian hamster protein (Figure 5). Our explanation for this asymmetry is that the conformation of Syrian hamster amyloid is within the range of conformers accessible to the moPrP23−144. On the other hand, the preferred conformation (i.e., acquired during spontaneous fibrillization) of mouse amyloid appears to be outside the spectrum of conformers accessible to ShaPrP23−144, accounting for the inability of mouse to seed the conversion of Syrian hamster protein.[44] Thus, cross-seeding requires monomeric protein to be *adaptable* to the

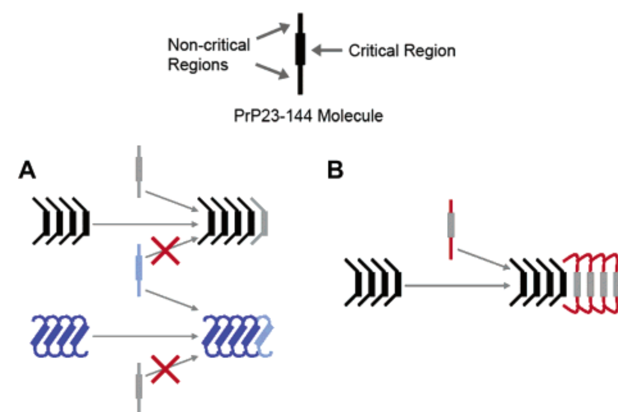


FIGURE 9. Schematic representation of conformational adaptability as the prerequisite for amyloid cross-seeding. Vertical lines represent soluble (monomeric) protein; the thick portion of a line represents the critical amyloidogenic region. (A) Recruitment of soluble protein (light colors) to the amyloid seed (dark colors) can occur only when the soluble protein is capable of adopting the conformation of the seed. Conformational adaptability is indicated by color similarity with respect to the seed. This adaptation need not involve the entire molecule but only the critical amyloidogenic region (B). Reproduced with permission from ref 46.

conformation of the amyloid seed. This *conformational adaptation* need not involve the entire PrP molecule but may take place only in a critical amyloidogenic region (Figure 9).[46]

The C-terminally truncated PrP23−144 obviously cannot mimic all aspects of the conversion of the full-length PrP, in which species barriers are further modulated by residues beyond the 23−144 region. However, a conceptually similar mechanism of conformational inheritance was inferred from experiments with yeast prion protein Sup35,[6,7,47] suggesting that the mechanistic principles established with PrP23−144 may be of general validity. Altogether, these studies indicate that barriers in prion transmissibility (seeding specificities) do not depend so much on species-dependent differences in PrP amino acid sequence as on the amyloid conformations associated with different prion strains. Amino acid sequence, on the other hand, dictates the spectrum of conformations that PrP^{C} of the host can adopt in the PrP^{Sc} state; transmission may occur only if this spectrum includes the conformation of the donor PrP^{Sc} strain. Thus, prion strains and species barriers represent different sides of a single coin.

V. Conclusions and Implications

While the idea, championed by Stanley Prusiner, that protein alone might act as an infectious agent has for many years been viewed with skepticism, a plethora of recent findings provides strong support for this protein-only model. Considerable advances have also been made in understanding biophysical properties of the prion protein and the mechanism of its folding, misfolding, and self-perpetuating conformational conversion. The findings regarding the roles of prion amyloid conformation and conformational adaptability in prion transmission can

explain at a molecular level the phenomenon of prion strains, providing a basis for understanding how variant CJD, a disease believed to be transmitted from "mad cows" to humans,[1,2,42] might have arisen as a new strain of human prions by conformational adaptation of human PrP to the structure of BSE prions. From the chemical perspective, the major current challenges include determination of PrP^{Sc} structure and defining at high resolution the conformational differences between prion strains. The latter task is of special importance since conformational polymorphism appears common to many amyloid fibrils, affecting their biological properties.[48]

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Note Added after Print Publication: The references from ref 9 to ref 32 were misnumbered in the version published on the Web May 19, 2006, and in the September 19, 2006 print issue. The correct electronic version of the paper with the references renumbered and two citations corrected in the text was published on September 27, 2006, and an Addition and Correction appears in the November 21, 2006 issue (Vol. 39, No. 11).

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