Cell, Vol. 121, 49–62, April 8, 2005, Copyright ©2005 by Elsevier Inc. DOI 10.1016/j.cell.2005.03.008

Mechanism of Cross-Species Prion Transmission: An Infectious Conformation Compatible with Two Highly Divergent Yeast Prion Proteins

Motomasa Tanaka,[1] Peter Chien,[1,2,4] Koji Yonekura,[3] and Jonathan S. Weissman[1,2,*]

[1]Howard Hughes Medical Institute
Department of Cellular and Molecular Pharmacology
[2]Graduate Group in Biophysics
University of California, San Francisco
San Francisco, California 94143
[3]The W.M. Keck Advanced Microscopy Laboratory
Department of Biochemistry and Biophysics
University of California, San Francisco
San Francisco, California 94158

Summary

Efficiency of interspecies prion transmission decreases as the primary structures of the infectious proteins diverge. Yet, a single prion protein can misfold into multiple infectious conformations, and such differences in "strain conformation" also alter infection specificity. Here, we explored the relationship between prion strains and species barriers by creating distinct synthetic prion forms of the yeast prion protein Sup35. We identified a strain conformation of Sup35 that allows transmission from the *S. cerevisiae* (Sc) Sup35 to the highly divergent *C. albicans* (Ca) Sup35 both in vivo and in vitro. Remarkably, cross-species transmission leads to a novel Ca strain that in turn can infect the Sc protein. Structural studies reveal strain-specific conformational differences in regions of the prion domain that are involved in intermolecular contacts. Our findings support a model whereby strain conformation is the critical determinant of cross-species prion transmission while primary structure affects transmission specificity by altering the spectrum of preferred amyloid conformations.

Introduction

The prion hypothesis was originally proposed to account for the unusual nature of the infectious agent that causes mammalian transmissible spongiform encephalopathies (Prusiner, 1982) but has now been expanded to explain a number of non-Mendelian traits such as [*PSI*$^+$] and [*URE3*] in *Saccharomyces cerevisiae* and HET-s in filamentous fungi (Wickner, 1994; Uptain and Lindquist, 2002; Tuite and Cox, 2003). Although the primary structures of the infectious proteins in these various phenomena are unrelated, they all form similar β sheet-rich fibrillar aggregates commonly referred to as amyloid (Prusiner et al., 1998; Dobson, 2001; Chien et al., 2004). The self-propagating nature of such aggregates has long been postulated to be responsible for prion inheritance (Aguzzi and Polymenidou, 2004; Weissmann, 2004). It has recently been shown in filamentous fungi, yeast, and mammals that pure recombinant proteins can act as synthetic prions and that infection depends on the prion protein misfolding into an amyloid state (Sparrer et al., 2000; Maddelein et al., 2002; King and Diaz-Avalos, 2004; Tanaka et al., 2004; Legname et al., 2004). These results established the validity of the "protein-only" hypothesis and indicate that self-propagating amyloids are the infectious form of prion proteins.

Having established amyloid as the infectious protein form, we can now begin to explain several universal and otherwise puzzling features of prion inheritance in terms of the biochemical/biophysical properties of amyloid-based prion replication. For instance, self specificity in the amyloid propagation results in barriers that limit prion transmission between different species (Kocisko et al., 1995; Santoso et al., 2000; Vanik et al., 2004). Similarly, the ability of a single protein to misfold into more than one amyloid conformation underlies the prion strain phenomena in which prion particles composed of the same protein lead to distinct infectious states (King and Diaz-Avalos, 2004; Tanaka et al., 2004; Legname et al., 2004). While the primary structure of a prion protein plays critical role in determining its propensity for cross-species transmission, the specific infectious conformation or strain that a prion protein adopts also appears to be a major determinant of its ability to be transmitted between species (Collinge, 2001; Chien et al., 2004). However, the extent to which and mechanism by which distinct strain conformations modulate cross-species transmission remain a critical open question.

The yeast prion [*PSI*$^+$] provides a powerful system for investigating the molecular basis of prion inheritance. The [*PSI*$^+$] state results from self-propagating aggregates of an essential translation termination factor Sup35 and leads to a nonsense suppression phenotype (Uptain and Lindquist, 2002; Tuite and Cox, 2003; Chien et al., 2004). In yeast containing a nonsense mutation in the *ade1* gene, [*PSI*$^+$] colonies are white or pink and grow on media lacking adenine, while [*psi*$^-$] colonies are red and require adenine (Chernoff et al., 1995). [*PSI*$^+$] propagation is mediated by a modular, N-terminal Gln/Asn-rich sequence and, to a lesser extent, by a highly charged middle domain (Ter-Avanesyan et al., 1994; Glover et al., 1997; DePace et al., 1998; Liu et al., 2002; Bradley and Liebman, 2004). Transient overexpression of a fusion between the Sup35 N-terminal and middle domains (Sup-NM) leads to protein aggregation and de novo appearance of [*PSI*$^+$]. Sup-NM also forms self-seeding amyloid fibers in vitro (Glover et al., 1997; King et al., 1997), and introduction of these pure fibers into yeast causes conversion to [*PSI*$^+$], thus demonstrating the prion nature of the Sup-NM amyloid state (King and Diaz-Avalos, 2004; Tanaka et al., 2004).

Like mammalian prions and other yeast prions, [*PSI*$^+$] exhibits barriers that restrict interspecies transmission (Chernoff et al., 2000; Kushnirov et al., 2000a; Santoso et al., 2000). For example, Sup35 from *S. cerevisiae* (Sc) or *C. albicans* (Ca) each form self-propagating amyloids in vitro and adopt a heritable prion state in vivo, but prion forms of the Sc and Ca Sup35 proteins do not

*Correspondence: weissman@cmp.ucsf.edu
[4]Present address: Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139.

appear to cross-seed each other, presumably due to their low homology (~40% similarity in the N-terminal prion domains). However, species barriers are not absolute, as cross-seeding between heterologous proteins can be observed in some instances (Derkatch et al., 2001; Derkatch et al., 2004; Nakayashiki et al., 2001; Osherovich and Weissman, 2001; Vanik et al., 2004). The primary structure of prion proteins is a critical determinant of the specificity of prion transmission, with interspecies infectivity being strongly influenced by the degree of similarity between the sequences of two prion proteins.

Prion strains also play a major role in determining the specificity of [*PSI*$^+$] transmission (Chien and Weissman, 2001; King, 2001; Chien et al., 2003). Like mammalian prions and other yeast prions, [*PSI*$^+$] exhibits a range of heritable phenotypic strain variants (Derkatch et al., 1996). These strains differ in mitotic stability (Derkatch et al., 1996), dependence on the cellular chaperone machinery (Kushnirov et al., 2000b), and solubility and activity of Sup35 (Derkatch et al., 1996; Zhou et al., 1999; Kochneva-Pervukhova et al., 2001; Uptain et al., 2001) and lead to differences in the *ade1* color phenotype as well as in their specificity of transmission. The link between prion strains and transmission barriers is likely to be general, as the ability of prions to jump species barriers appears to differ substantially among mammalian prion strains (Collinge, 2001; Chien et al., 2004). The importance of understanding the role of strains in modulating specific cross-species transmission has been highlighted by the conclusion that a new variant of Creutzfeldt-Jacob disease resulted from the cross-species transmission to humans of the prion strain responsible for bovine spongiform encephalopathy (mad cow disease) (Bruce et al., 1997; Hill et al., 1997). Because prion strain variability can result from differences in the conformation of the infectious protein (King and Diaz-Avalos, 2004; Tanaka et al., 2004), the observed role of strains in modulating specificity of prion transmission suggests that amyloid conformation may be an important determinant of a prion's ability to be transmitted across species barriers. However, until recently, the difficulty in creating distinct infectious amyloid forms in vitro and in introducing such synthetic prions into an organism has limited the ability to directly explore this relationship.

In the present study, we take advantage of our ability to create in vitro and infect yeast with distinct prion forms (Tanaka et al., 2004) in order to investigate the role of Sup35 amyloid conformations in permitting cross-species transmission. Remarkably, we identified a subset of strain conformations that allow prion transmission back and forth between the *S. cerevisiae* and *C. albicans* Sup35 proteins, thus establishing that the propensity of a prion for cross-species transmission is determined by its strain conformation rather than its primary structure.

Results

Specificity of Prion Transmission Is Determined by Strain Conformations of a Sc/Ca Chimeric Prion

We first explored the role of prion strain conformation in determining specificity of prion transmission using a chimeric Sup-NM (Sup-NM$_{chim}$), which comprises the first 40 residues of Sup35 from *S. cerevisiae*, followed by residues 47–141 from *C. albicans* and the highly charged middle domain (124–253 residues) from *S. cerevisiae* (Figure 1A) (Santoso et al., 2000). Sup-NM$_{Chim}$ was previously shown to bridge the species barrier between *S. cerevisiae* and *C. albicans* (Chien and Weissman, 2001). In vivo, *S. cerevisiae* yeast in which the endogenous Sup-NM domain was replaced by one coding for Sup-NM$_{Chim}$ (Chim yeast) could be induced to form a prion state by transient overexpression of either *S. cerevisiae* Sup-NM (Sup-NM$_{Sc}$) or *C. albicans* Sup-NM (Sup-NM$_{Ca}$). However, the strain phenotypes of the resulting prion states differed markedly depending on the species used to induce prion formation. Similarly, in vitro, when seeded with fibers formed from the different species of Sup35, Sup-NM$_{Chim}$ formed two distinct self-propagating amyloid forms, termed Sup-NM$_{Chim[Sc]}$ (seeded by Sup-NM$_{Sc}$ fibers) and Sup-NM$_{Chim[Ca]}$ (seeded by Sup-NM$_{Ca}$ fibers). These conformations dictate subsequent seeding specificity. Sup-NM$_{Chim[Sc]}$ efficiently seeds conversion of Sup-NM$_{Sc}$ but not Sup-NM$_{Ca}$, while the converse holds for Sup-NM$_{Chim[Ca]}$. These observations are consistent with a model in which Sup-NM$_{Chim}$ bridges the species barriers in vivo by adopting two amyloid conformations, one compatible with *S. cerevisiae* and the other compatible with *C. albicans*.

To experimentally test the above model, we used our recently developed protocol to infect yeast with the two Sup-NM$_{Chim}$ conformations (Tanaka et al., 2004) by introducing them into prion-free [*psi*$^-$] yeast expressing either wild-type Sc Sup35 (Sc yeast) or a Sup35 variant, in which the Sc NM domain had been replaced by the Ca sequence (Ca yeast). Specificity in the infection experiment directly mirrored that seen in the earlier in vitro seeding experiments (Chien and Weissman, 2001) (Figure 1B and see Figures S1 and S2 in the Supplemental Data available with this article online). Sup-NM$_{Chim[Sc]}$ amyloid showed a markedly greater ability to induce prion formation in Sc yeast than did Sup-NM$_{Chim[Ca]}$ amyloid. Consistent with the previous in vitro cross-seeding experiment (Chien and Weissman, 2001), Sup-NM$_{Chim[Ca]}$ can infect Sc yeast with low efficiency. Conversely, Sup-NM$_{Chim[Ca]}$ but not Sup-NM$_{Chim[Sc]}$ amyloids could infect Ca yeast (Tanaka et al., 2004). Importantly, the two Sup-NM$_{Chim}$ amyloid conformations infected Chim yeast with similar efficiency but induced different prion strains (Figure 1B, inset), in agreement with our previous results in which prion formation in Chim yeast induced by transient overexpression of Sup-NM$_{Sc}$ or Sup-NM$_{Ca}$ led to distinct strains (Chien and Weissman, 2001). Taken together, these studies establish that the conformation of infectious Sup-NM$_{Chim}$ amyloid determines both the resultant prion strain phenotype and its propensity to infect yeast expressing Sup35 from distinct species.

Specific Amyloid Conformations Allow Cross-Seeding between the Highly Divergent Sc and Ca Sup35 In Vitro

The above studies used an artificial chimeric Sup35 protein that included sequences derived from both Sc and Ca. Potentially, the modular architecture of this protein rather than the inherent ability of a single se-

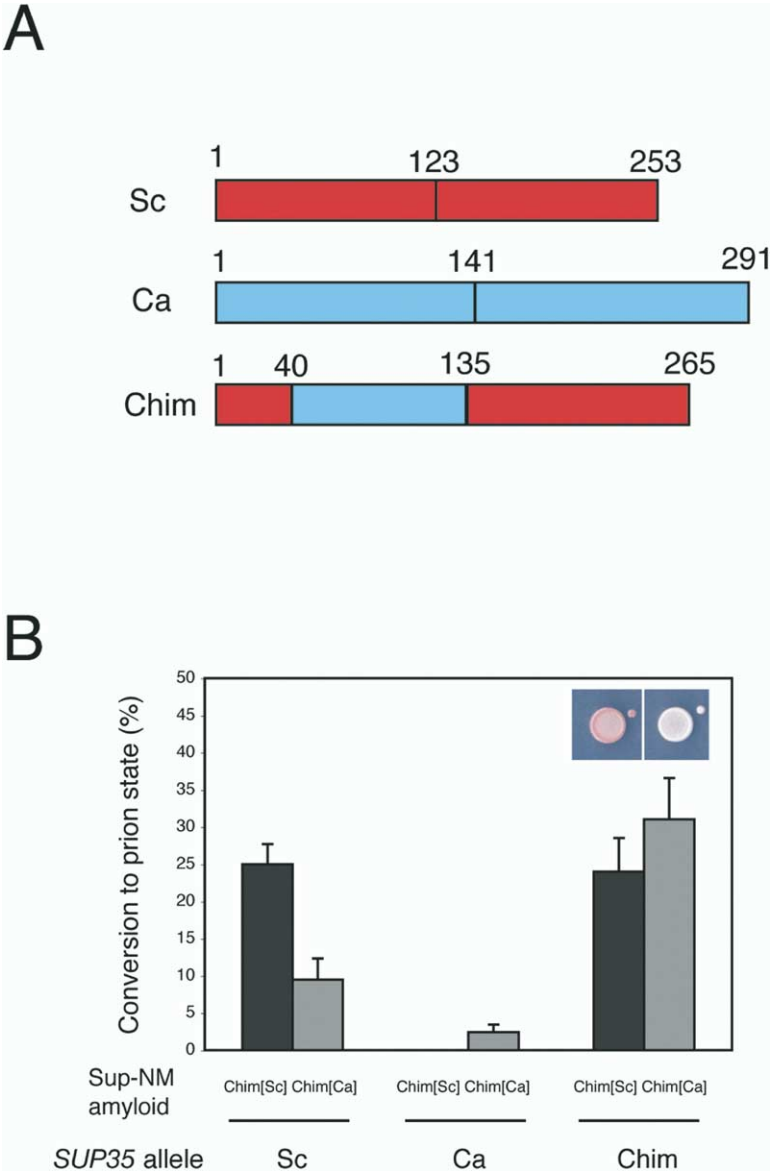

Figure 1. Conformation-Dependent, Species-Specific Induction of the Prion State by In Vitro-Converted Sup-NM_{Chim} Amyloid

(A) Color-coded schematic of Sup-NM_{Sc} (top), Sup-NM_{Ca} (middle), and Sup-NM_{Chim} (bottom) sequences.

(B) Infection of yeast expressing the indicated allele of *SUP35* prion domain with Sup-NM_{Chim} amyloid (5 μM) formed in the presence of 5% (w/w) Sup-NM_{Sc} (Sup-$NM_{Chim[Sc]}$) or Sup-NM_{Ca} (Sup-$NM_{Chim[Ca]}$) fiber seed. The inset shows examples of typical Chim prion strain phenotypes induced by infection with Sup-$NM_{Chim[Sc]}$ (left) or Sup-$NM_{Chim[Ca]}$ amyloid (right) on YEPD plates. Throughout, values with error bars are expressed as mean ± standard deviation. Note, as observed previously, experiments involving Ca yeast show a lower overall infection efficiency than those involving Sc yeast (Tanaka et al., 2004).

quence to adopt distinct conformations was responsible for the cross-seeding and unusual transmission properties observed. It was therefore important to test the effect of a prion's strain conformation on its propensity for cross-species transmission using naturally occurring prion domains. To this end, we produced two different amyloid conformations of Sup-NM_{Sc} by spontaneous polymerization at 4°C (Sc4) and 37°C (Sc37), as previously described (Tanaka et al., 2004). These two amyloids differ in their thermal stability, protease susceptibility, and local structure and produce distinct prion strains when used to infect yeast.

We first explored in vitro whether these two amyloid conformations differed in their ability to seed polymerization of pure Sup-NM_{Ca} protein. Sc37 amyloid failed to accelerate polymerization of Sup-NM_{Ca}, consistent with the previously reported strong species barrier between Sc and Ca Sup35 (Figure 2A) (Santoso et al., 2000). In contrast, addition of Sc4 amyloid shortened the lag time of Sup-NM_{Ca} polymerization, suggesting that Sc4 amyloid is capable of cross-seeding Sup-NM_{Ca}. In vivo, spontaneous polymerization is strongly inhibited, and, thus, much greater differences in the rate of polymerization in the presence and absence of an effective seed are expected in cells. Furthermore, following infection of seeds, cells will have several hours to recover so that the rate of polymerization seen in the cross-seeding experiments in vitro is expected to be sufficient to allow crossinfection in vivo. Finally, if the observed acceleration in Sup-NM_{Ca} polymerization were due to cross-seeding of Sup-NM_{Ca} by Sc4 fibers, we would expect the resulting Sup-NM_{Ca} amyloid to have a conformation distinct from that of spontaneously formed Sup-NM_{Ca} fibers. Consistent with this expectation, we found that the Sup-NM_{Ca} fiber formed by seeding with Sc4 amyloid (Ca[Sc4] amyloid) has lower melting (T_m) and broader transition (W) temperatures (T_m = 73 ± 3°C, W = 24 ± 3°C) than spontaneously

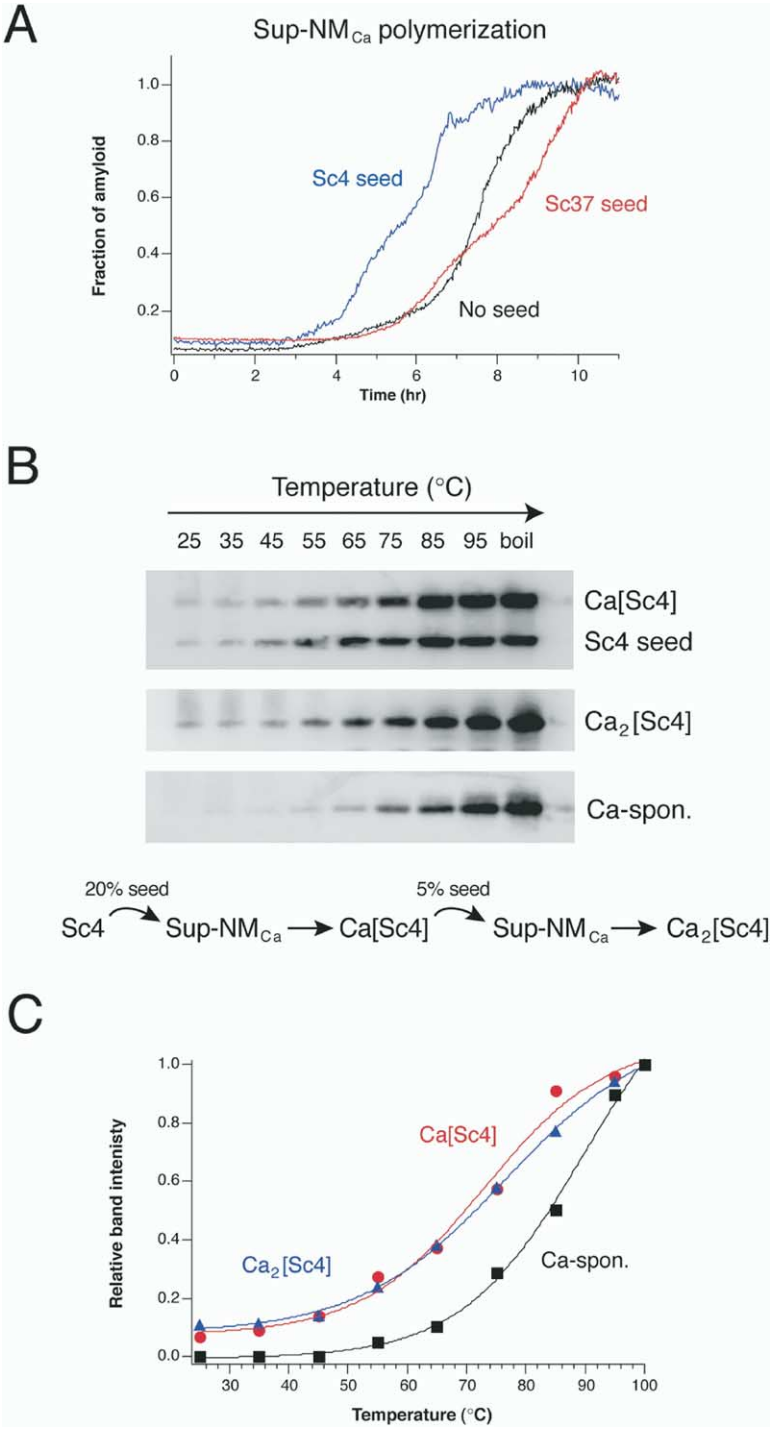

Figure 2. A Specific Conformation of Sup-NM$_{Sc}$ Amyloid Can Cross-Seed Sup-NM$_{Ca}$

(A) Amyloid formation of Sup-NM$_{CA}$ monomer in the absence or presence of 20% (w/w) Sc4 or Sc37 fiber seeds as monitored by thioflavine T fluorescence.

(B) Thermal stability of Ca[Sc4], Ca$_2$[Sc4], and spontaneously formed Sup-NM$_{Ca}$ (Ca-spon.) amyloid fibers as determined by pretreatment of the fibers at the indicated temperatures in the presence of SDS, followed by SDS-PAGE and Western blot analysis. Note that Sup-NM$_{Sc}$ and Sup-NM$_{Ca}$ monomers can be separated by SDS-PAGE, and their relative positions are indicated (top). Below is a schematic of how Ca[Sc4] and Ca$_2$[Sc4] amyloids are produced.

(C) The band intensities, which reflect susceptibility of Ca[Sc4], Ca$_2$[Sc4], and Ca-spon. amyloid fibers to thermal solubilization, are plotted against temperature and fit to a sigmoidal function.

formed Sup-NM$_{Ca}$ amyloid (T_m = 85 ± 5°C, W = 15 ± 2°C) (Figures 2B and 2C). Once templated, the Ca[Sc4] amyloid conformation is stable and can be propagated to a second generation of fibers, termed Ca$_2$[Sc4], that are formed by seeding polymerization of Sup-NM$_{Ca}$ with Ca[Sc4] amyloid: Ca$_2$[Sc4] fibers showed melting and transition temperatures (T_m = 74 ± 5°C, W = 23 ± 5°C) indistinguishable from those of Ca[Sc4] amyloid (Figures 2B and 2C). These results establish that Sc4 amyloid is able to cross-seed Sup-NM$_{Ca}$, leading to a novel self-propagating amyloid conformation.

Next, we examined the seeding specificity of the novel Sup-NM$_{Ca}$ conformation. For these studies, we used fibers, termed Ca$_3$[Sc4], made by three successive rounds of seeding and back dilution in order to eliminate any effect of residual Sc4 amyloid in the Ca[Sc4] fiber preparations (see Supplemental Data for details). As previously observed, spontaneously gener-

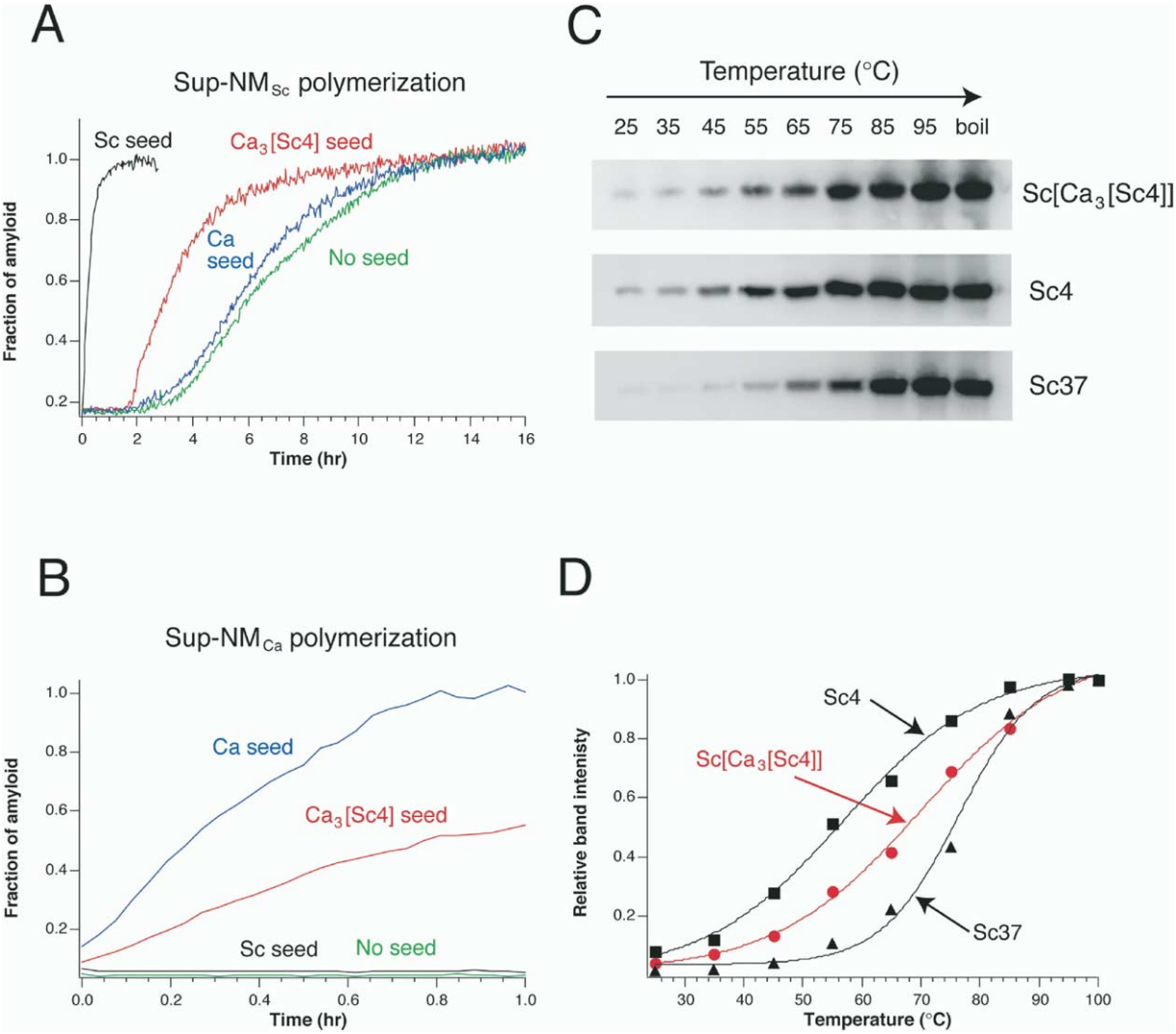

Figure 3. Sup-NM$_{Ca}$ Amyloid Fibers Can Cross-Seed Sup-NM$_{Sc}$ in a Conformation-Dependent Manner

(A) Amyloid formation of Sup-NM$_{Sc}$ in the absence or presence of 5% (w/w) spontaneously formed Sup-NM$_{Sc}$ (Sc), spontaneously formed Sup-NM$_{Ca}$ (Ca), or Ca$_3$[Sc4] fiber seeds as monitored by thioflavine T fluorescence.

(B) Amyloid formation of Sup-NM$_{Ca}$ in the absence or presence of 5% (w/w) Sc, Ca, or Ca[Sc4] fiber seeds as monitored by thioflavine T fluorescence.

(C) Thermal stability of Sc[Ca$_3$[Sc4]], Sc4, and Sc37 amyloid fibers.

(D) As in Figure 2C, the band intensities of monomeric Sup-NM$_{Sc}$ produced following treatment of Sc[Ca$_3$[Sc4]], Sc4, and Sc37 amyloids at the indicated temperature in the presence of SDS are plotted against temperature and fit to a sigmoidal function.

ated Sc and Ca amyloid forms are species specific in their seeding capabilities, and, as expected, Ca$_3$[Sc4] amyloid is able to seed polymerization of Sup-NM$_{Ca}$ monomer (Figures 3A and 3B). Remarkably, addition of Ca$_3$[Sc4] seed to Sup-NM$_{Sc}$ monomer accelerated the polymerization of Sup-NM$_{Sc}$ (Figure 3A), suggesting that Ca$_3$[Sc4] amyloid is capable of cross-seeding Sup-NM$_{Sc}$. Consistent with this proposal, the melting and transition temperatures (T_m = 71 ± 2°C, W = 22 ± 4°C) of the resulting Sup-NM$_{Sc}$ amyloid, termed Sc[Ca$_3$[Sc4]], are distinct from those of either the Sc4 or Sc37 fibers (T_m = 56 ± 2°C, W = 27 ± 2°C for Sc4 amyloid, T_m = 77 ± 2°C, W = 14 ± 1°C for Sc37 amyloid) (Figures 3C and 3D), indicating that Sc[Ca$_3$[Sc4]] amyloid represents yet another distinct Sup-NM$_{Sc}$ amyloid conformation. The conformational differences between Sc[Ca$_3$[Sc4]], Sc4, and Sc37 amyloid fibers were further confirmed by differences in the spectrum of induced prion strain phenotypes observed when these amyloids were introduced into Sc yeast (M.T. and J.S.W., unpublished data). Taken together, these studies indicate that, as a result of their conformations, the Sc4 and Ca$_3$[Sc4] fibers are able to overcome the species barrier that normally prevents cross-seeding between Sup-NM$_{Sc}$ and Sup-NM$_{Ca}$ proteins. In addition, our studies establish that such cross-seeding leads to the generation of novel amyloid conformations that are compatible with both Sup-NM$_{Sc}$ and Sup-NM$_{Ca}$ proteins.

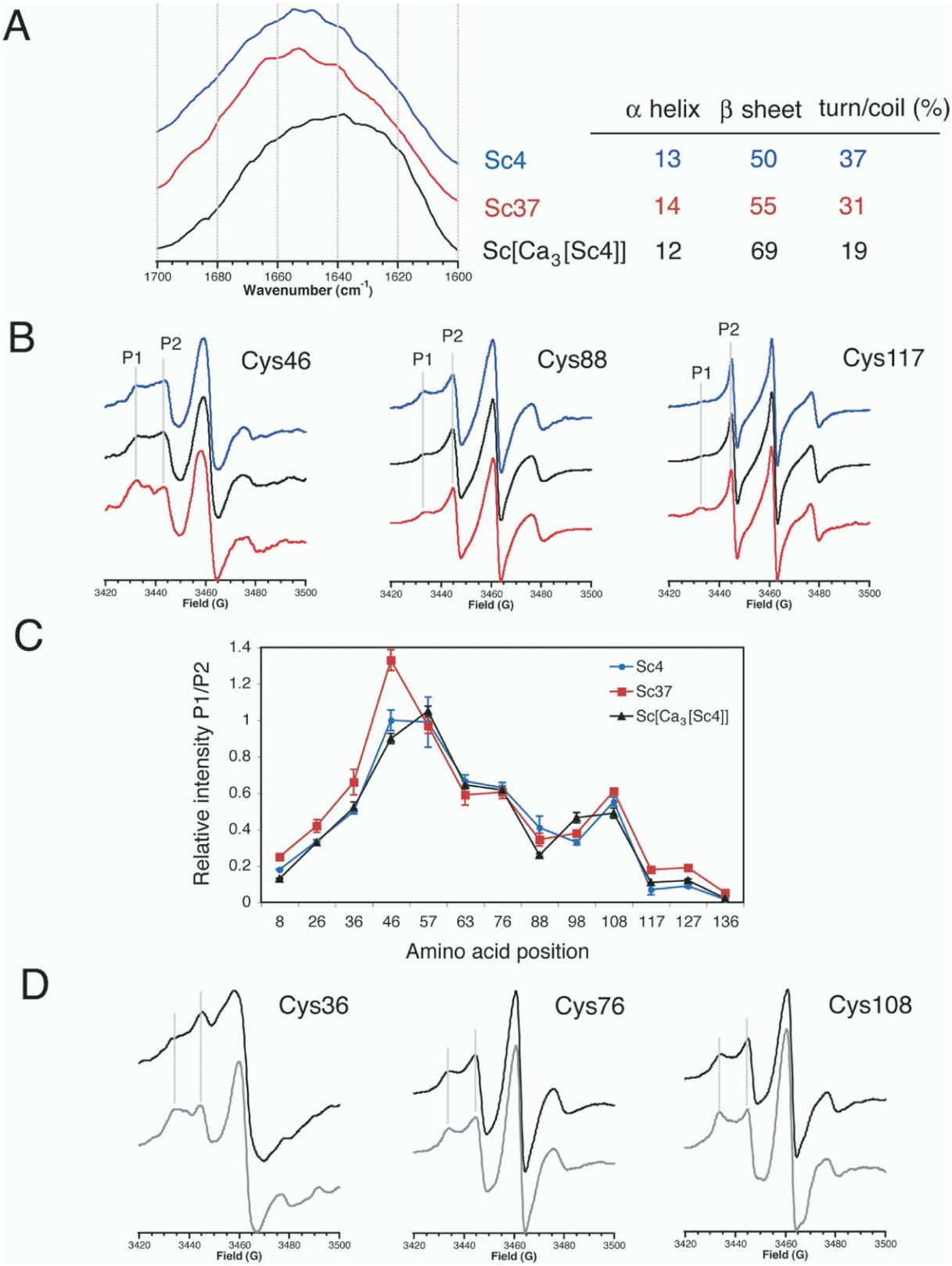

Figure 4. Structural Analysis of the Various Sup-NM Amyloid Conformations

(A) IR spectra (amide I region) of Sc4 (blue), Sc37 (red), and Sc[Ca_3[Sc4]] (black) amyloid fibers. Estimation of secondary structure contents calculated from each self-deconvoluted spectrum is shown on the right. See Supplemental Data for details.

(B) EPR spectra of Sc4 (blue), Sc37 (red), and Sc[Ca_3[Sc4]] (black) amyloid fibers spin labeled at positions 46 (left), 88 (middle), and 117

Structural Analysis of the Three Different Sup-NM Amyloid Conformations

We probed the conformations of Sc4, Sc37, and Sc[Ca_3[Sc4]] amyloid fibers by a range of different biophysical methods that report on both global and local structural features. These studies both demonstrated that the Sc[Ca_3[Sc4]] amyloid represents a novel fiber conformation and provided structural insights into the strain-dependent differences in the specificity of prion propagation. First, we used electron microscopy of negatively stained amyloid fibers to explore the overall dimensions of the different strain conformations. Although Sc[Ca_3[Sc4]] amyloid tended to have a smoother appearance, all amyloid fibers exhibited similar morphology (Figure S3), suggesting that there are not large differences in the quaternary structures of the various Sup-NM fiber forms. Second, we used Fourier Transform Infrared (FT-IR) spectroscopy to estimate secondary structure contents in the amyloid fibers. The IR spectral shape of Sc4 fibers was similar to that of Sc37 fibers, and deconvolution of the spectra indicated that they have a similar secondary structure content (Figure 4A). In contrast, there were marked differences in the IR spectrum of the Sc[Ca_3[Sc4]] amyloid indicative of a higher β sheet content in Sc[Ca_3[Sc4]], compared to Sc4 and Sc37 fibers.

Next, we employed site-directed spin labeling (SDSL) together with electron paramagnetic resonance (EPR) spectroscopy to examine localized conformational differences at specific amino acids in the Sc4, Sc37, and Sc[Ca_3[Sc4]] amyloid fibers. Earlier studies using SDSL and EPR revealed structural differences between the Sc4 and Sc37 fibers (Tanaka et al., 2004). Here, we were able to substantially improve the quality of the spectra by using a smaller spin probe ([1-Oxyl-2,2,5,5,-tetramethyl-Δ3-pyrroline-3-methyl] methanethiosulfonate, MTSL) (Hubbell et al., 2000) and a more sensitive instrument (see Experimental Procedures). We prepared Sup-NM mutants in which a single cysteine residue was substituted into wild-type Sup-NM (which lacks cysteines) approximately every tenth residue and labeled them with the cysteine-specific MTSL spin probe. We then polymerized each of the labeled cysteine mutants into the three different Sup-NM fiber conformations. EPR spectra revealed a broadening of peaks as well as the appearance of a new low field peak (P1) indicative of immobilized side chains and/or spin-spin interactions in a manner that was strongly dependent on the position of the labeled cysteine residue (Figures 4B–4D). The degree of immobilization could be quantitated by taking the ratio of P1 to a second peak (P2), which is present in the spectra of mobile spin probes. We observed general structural features common to both Sc4 and Sc37 fibers as well as local differences characteristic of specific fiber conformations. In particular, the P1/P2 ratio of residues in the N terminus (residues 8–46) and end (residues 117–127) of the prion domain was higher in Sc37 amyloid fibers than in Sc4 amyloid fibers, suggesting that the amyloid core contains stronger interactions and extends further into both the N terminus and the C terminus in the Sc37 conformation than in the Sc4 conformation. The SDSL-EPR analysis also revealed that Sc[Ca_3[Sc4]] fibers were in a conformation distinct from both Sc4 and Sc37 amyloids (Figures 4B and 4C). The most highly immobilized region was shifted from residue 46 to 57 in the Sc[Ca_3[Sc4]] conformation. Furthermore, differences between Sc[Ca_3[Sc4]] and both SC4 and Sc37 fibers were observed for residues 88–108.

The spectral broadening seen in the various amyloid forms was also suggestive of spin-spin interactions that occur when spin labels from different monomers are within 5–25 Å of each other (Serag et al., 2002). To explore this possibility, we performed spin dilution experiments in which fibers were prepared with a 1:1 ratio of labeled and unlabeled Sup-NM focusing on three residues (36, 76, and 108) from the N-terminal, middle, and C-terminal regions of the structured core. These residues showed similar P1/P2 ratios (Figure 4C) so that the dilution experiments allowed us to distinguish the relative contribution of spin-spin interactions and immobilization to P1/P2 values. Spectral differences between diluted and undiluted samples revealed that residues 36 and 108 are in close proximity with the corresponding residue from other Sup-NM molecules in the fibers, whereas residue 76 showed no evidence of spin-spin interactions (Figure 4D). Thus, both the N terminus and the C terminus of the amyloid core are in proximity to corresponding regions from other Sup-NM molecules in the fibers. Taken together, these studies establish that the Sc4, Sc37, and Sc[Ca_3[Sc4]] amyloid forms represent three distinct amyloid conformations and that at least some of these conformational differences are in regions likely to be involved in intermolecular interactions between Sup-NM molecules and thus are well suited to modulate prion specificity.

Specific Amyloid Conformations of Sup35 Jump Transmission Barriers In Vivo

Having demonstrated conformation-dependent cross-seeding and conformational differences of Sc Sup-NM amyloid fibers in vitro, we proceeded to test the ability of specific Sup-NM amyloid conformations to crossinfect in live yeast. Consistent with earlier studies that found a strong transmission barrier between Sc and Ca Sup35 (Santoso et al., 2000), transient overexpression of Sup-NM_{Sc}-GFP and Sup-NM_{Ca}-GFP fusion proteins efficiently induced prion formation only in yeast expressing Sup35 of the same species (Figure 5A). A drawback of such overexpression experiments, however, is that they do not allow for control of the amyloid conformation produced in the cell and are conse-

(right). P1 results from highly immobilized spin probes and P2 results from mobile spin probes.
(C) The relative ratio of the intensities of P1 and P2 (P1/P2) in EPR spectra is plotted against the amino acid position for Sc4 (blue), Sc37 (red), and Sc[Ca_3[Sc4]] (black) amyloid fibers.
(D) EPR spectra fully labeled (black) and 50% labeled (gray) Sc37 amyloid fibers. Spectra from fibers labeled at position 36 (left), 76 (middle), and 108 (right) are shown. EPR spectra were normalized by double integration to the same number of spins.

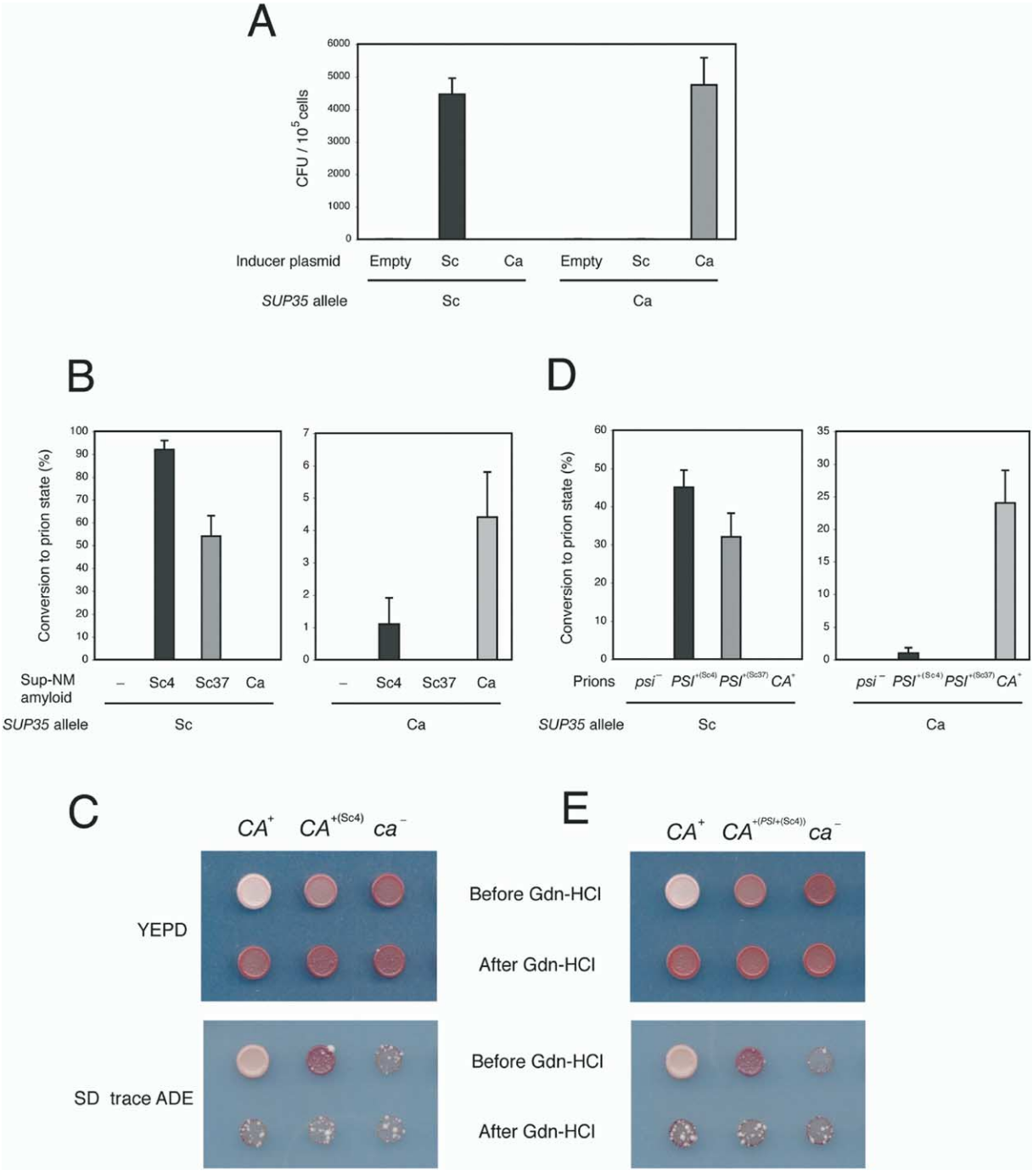

Figure 5. Jumping of Prion Transmission Barriers by the Sc4 Sup35 Amyloid Conformation In Vivo

(A) Species-specific induction of prion state expressing the indicated allele of *SUP35* following transient overexpression of Sup-NM_{Sc}-GFP or Sup-NM_{Ca}-GFP inducer protein. Shown is the number of [*PSI*$^+$] colony forming units (CFU) per 10^5 cells plated.

(B) Efficiency of infection of yeast expressing the indicated allele of *SUP35* by Sc4, Sc37, and spontaneously formed Sup-NM_{Ca} (Ca) amyloid (10 μM).

(C) Illustration of the prion strain phenotypes of Ca yeasts that are either prion-free (*ca*$^-$) or contain prions formed spontaneously (*CA*$^+$) or by infection with Sc4 amyloid (*CA*$^{+(Sc4)}$). The top and bottom show growth on YEPD and SD trace ADE plates, respectively. In each plate, top and bottom spots show the strain phenotypes before or after passage on YEPD plates containing 3 mM Gdn-HCl.

(D) Infection of yeast expressing the indicated allele of *SUP35* with partially purified prions (0.4 mg/ml) from the [*PSI*$^{+(Sc4)}$] or [*PSI*$^{+(Sc37)}$] strains produced by the infection of Sc yeast with Sc4 or Sc37 amyloid, respectively.

(E) Illustration of the prion strain phenotypes of Ca yeasts that are either prion-free (*ca*$^-$) or contain prions formed spontaneously (*CA*$^+$) or by infection with *PSI*$^{+(Sc4)}$ prions (*CA*$^{+(PSI+(Sc4))}$) as in (D).

quently ill suited to test the role of prion conformations on interspecies infectivity in vivo. To overcome this limitation, we directly tested by protein infection the ability of distinct amyloid conformations to cross the Sc-Ca prion transmission barrier. As observed previously, both Sc4 and Sc37 but not Sup-NM_{Ca} amyloid fibers are able to infect Sc yeast (Figure 5B, left). In excellent agreement with the in vitro results described above, we found that the Sc4 amyloid was indeed able to infect Ca yeast (Figure 5B, right), while introduction of the Sc37 amyloid did not induce prion conversion in Ca yeast even at the maximal attainable fiber concentration (40 μM). Moreover, infection of Ca yeast strain with Sc4 fibers led to a novel weak prion strain, termed [$CA^{+(Sc4)}$], characterized by a pink color phenotype on YEPD plates and intermediate growth on adenine deficient media, and, as expected for a prion state, was curable by transient growth in the presence of guanidine hydrochloride (Gdn-HCl) (Figure 5C).

In order to confirm the observed strain-specific jumping of the prion transmission barrier between the highly divergent *S. cerevisiae* and *C. albicans* yeast proteins, we infected yeast with different conformations of prion particles purified from an in vivo source. We first infected Sc yeast with in vitro-produced Sc4 or Sc37 amyloid yielding two different [PSI^{+}] strains, termed [$PSI^{+(Sc4)}$] and [$PSI^{+(Sc37)}$], respectively. Previously, we established that Sup35 prion particles in the [$PSI^{+(Sc4)}$] and [$PSI^{+(Sc37)}$] strains maintain the physical properties of the in vitro-generated parent fibers (Tanaka et al., 2004). We then partially purified Sup35 prion particles from cell extracts of [$PSI^{+(Sc4)}$] and [$PSI^{+(Sc37)}$] strains and examined their abilities to infect Sc and Ca yeast. As we observed previously (Tanaka et al., 2004), prions purified from both [$PSI^{+(Sc4)}$] and [$PSI^{+(Sc37)}$] strains can infect Sc yeast (Figure 5D). As predicted by the above in vitro seeding experiments, [$PSI^{+(Sc4)}$] prions but not [$PSI^{+(Sc37)}$] prions could infect Ca yeast, although the infection was less efficient than that seen with [CA^{+}] prions. Moreover, infection of Ca yeast with the [$PSI^{+(Sc4)}$] prions leads to a novel weak prion strain (Figure 5E), consistent with the results observed with pure Sc4 fibers.

The in vitro seeding experiments suggest that templating of Ca Sup35 prions by Sc4 prions would lead to a Ca conformation able to crosstransmit the infected state back to Sc yeast. To directly test this prediction, we created a prion state in Ca yeast, termed [$CA^{+(Sc4)}$], that had been templated in the Sc4 conformation by infecting Ca yeast with pure Sc4 fibers as described above. For comparison, we also prepared prions from a Ca strain, [$CA^{+(Ca)}$], which had been produced by infection of Ca yeast with spontaneously generated Sup-NM_{Ca} amyloid. As expected, prions from the [$CA^{+(Ca)}$] strain could readily infect Ca but not Sc yeast. In striking contrast, prions from [$CA^{+(Sc4)}$] strain were able to infect both Sc and Ca yeast with comparable efficiency (Figure 6A). Infection of Sc yeast with [$CA^{+(Sc4)}$]-derived prions resulted in a novel strain markedly different from that observed in Sc yeast directly infected with Sc4 fibers (Figure 6B). The ability of [$CA^{+(Sc4)}$] prions to infect Sc yeast is particularly remarkable, as [$CA^{+(Sc4)}$] is a relatively weak strain (compared to [$CA^{+(Ca)}$] strains, [$CA^{+(Sc4)}$] strains have a weak translation read-through phenotype and decreased ability to infect Ca yeast [Figures 5C and 6A]). Thus, the propensity of a given prion strain to be transmitted to distantly related prion proteins is independent of the strength of its strain phenotype or its prion titer. Neither the Sc4 nor the Ca[Sc4] prions were able to induce conversion in F, a prion protein derived from an unrelated asparagines-rich prion domain found in the yeast New1 protein (Osherovich et al., 2004) (Figure 6C), thus arguing that crossinfection does not result from a nonspecific increase in the propensity of the yeast to form prions. Taken together, these results establish that the Sc4 and Ca[Sc4] prion strains—by allowing cross-seeding between highly divergent proteins—result in conformation-specific breaching of the previously characterized strong Sc-Ca transmission barrier.

Discussion

Barriers inhibiting transmission of the infectious state between different prion proteins are a characteristic feature of prion inheritance. Both because of their central role in prion biology and because of the practical importance of preventing interspecies disease transmission, how such barriers arise and are breached has been the subject of intense interest. Many of the early efforts to understand transmission barriers focused on primary structure as a determinant of specificity. This focus now appears naive in light of the existence of the strain phenomenon. Infectious particles composed of identical prion proteins can have markedly different properties, including their propensity for cross-species transmission (Collinge 2001; Chien et al., 2004). The existence of distinct prion strains initially posed a challenge to the protein-only hypothesis (Soto and Castilla, 2004). We now know that such strain diversity can arise solely as a result of differences in the infectious conformation of the prion protein (King and Diaz-Avalos, 2004; Tanaka et al., 2004). Several studies point to an intimate relationship among prion strains, conformation of the infectious protein, and propensity for cross-species transmission (Barron et al., 2001; Chien and Weissman, 2001; Peretz et al., 2002; Chien et al., 2003). Despite the existence of these studies, a clear mechanistic understanding of how and why these phenomena are related has remained obscure, largely due to the difficulty of creating synthetic prions and testing their infectivity.

In the present study, we have taken advantage of our recently developed protein-infection protocol together with the ability to produce distinct, self-propagating infectious forms of Sup35, the protein determinant of the yeast prion [PSI^{+}], to directly explore the role of the Sup35 amyloid conformations in interspecies prion transmission. A major finding here is that particular amyloid conformations allow prion transmission back and forth between distantly related *S. cerevisiae* and *C. albicans* Sup35 proteins. In strong support for a central role of amyloid conformation in prescribing transmission specificity, we found that the propensity of a given prion strain to be transmitted to highly divergent species is independent of either the strength of its strain phenotype or its prion titer. This cross-species infection

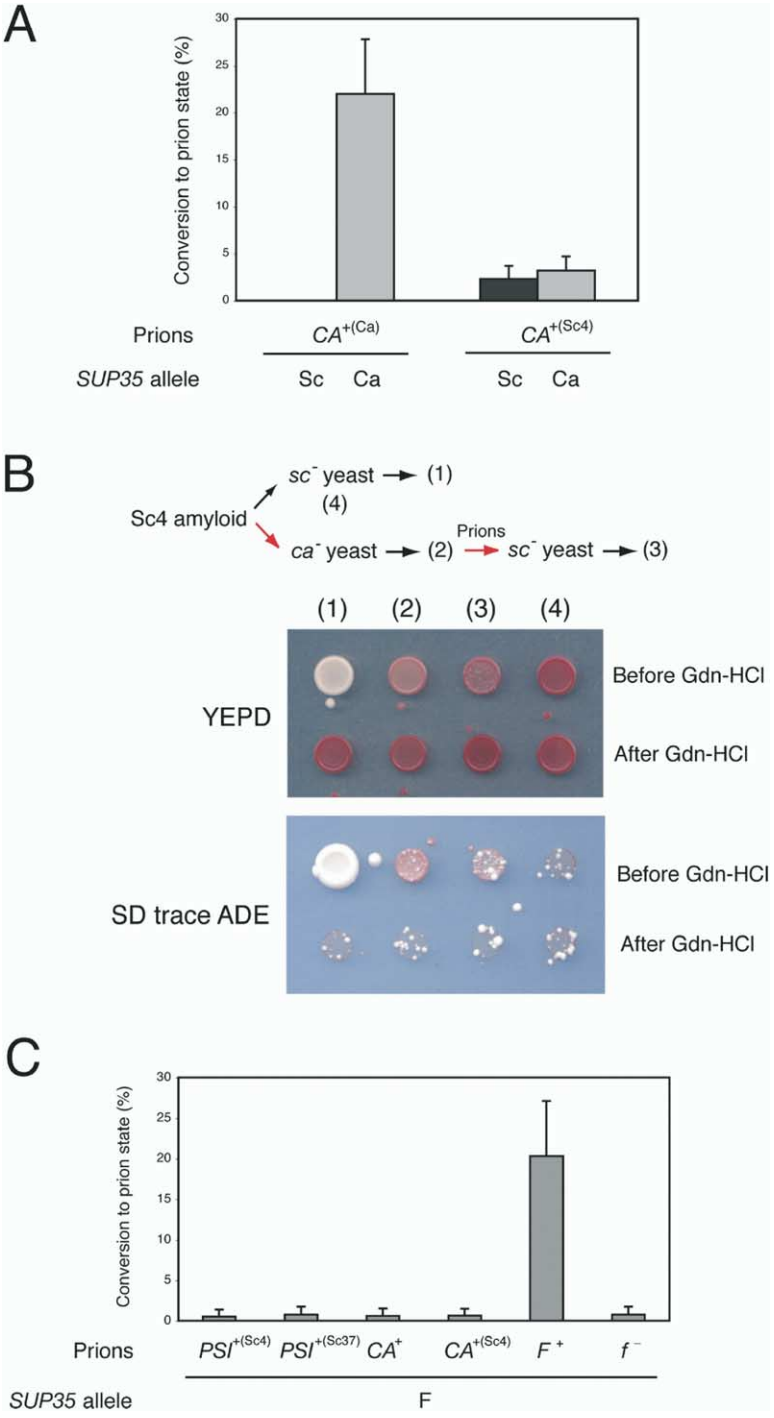

Figure 6. The Ca[Sc4] Strain Conformation Can Crossinfect Sc Yeast In Vivo

(A) Infection of yeast expressing the indicated allele of *SUP35* with yeast extracts (0.8 mg/ml) derived from $CA^{+(Ca)}$ and $CA^{+(Sc4)}$ strains.

(B) In vivo phenotypes of resulting prion strains. In the schematic, red arrows indicate steps involving transmission between Sc and Ca species, and black arrows indicate intraspecies transmission. Typical phenotypes of prion strains resulting from the transmission event indicated by the numbers were examined as described in Figures 5C and 5E. Infection of Ca yeast with Sc4 fibers exclusively induced a weak strain phenotype, shown in (2), while infection of Sc yeast with Ca[Sc4] prions predominantly (~80%) led to a very weak strain, shown in (3), as well as intermediate strength similar to that shown in (2) (~20%).

(C) Infection of yeast expressing a New1/Sc-Sup35 chimeric prion protein, termed F (Osherovich et al., 2004), with yeast extracts (0.8 mg/ml) derived from $PSI^{+(Sc4)}$, $PSI^{+(Sc37)}$, CA^{+}, $CA^{+(Sc4)}$, and F yeasts that are prion-free (f^{-}) or contain prions formed spontaneously (F^{+}).

was unexpected in light of both the strong divergence (~40% similarity in the prion domains) between the two Sup35 proteins and the findings of earlier studies showing that spontaneously generated prion forms of these two proteins show no apparent ability to crossinfect each other (Santoso et al., 2000; Chien and Weissman, 2001). Specifically, we find that a distinct amyloid conformation of Sc Sup35 prion protein formed in vitro is able to infect yeast expressing Ca Sup35. Remarkably, this cross-species transmission generates a novel prion strain, which can in turn be transmitted back to Sc yeast. In vitro studies with pure protein established that this cross-species infectivity is a direct result of the ability of a specific conformation of Sc Sup35 amyloid to seed polymerization of the Ca protein and vice versa. Thus, even strong species barriers can be bridged by specific prion strain conformations.

Our structural studies provide insights into the biological differences between the different prion strain conformations. EPR analysis, in particular, revealed lo-

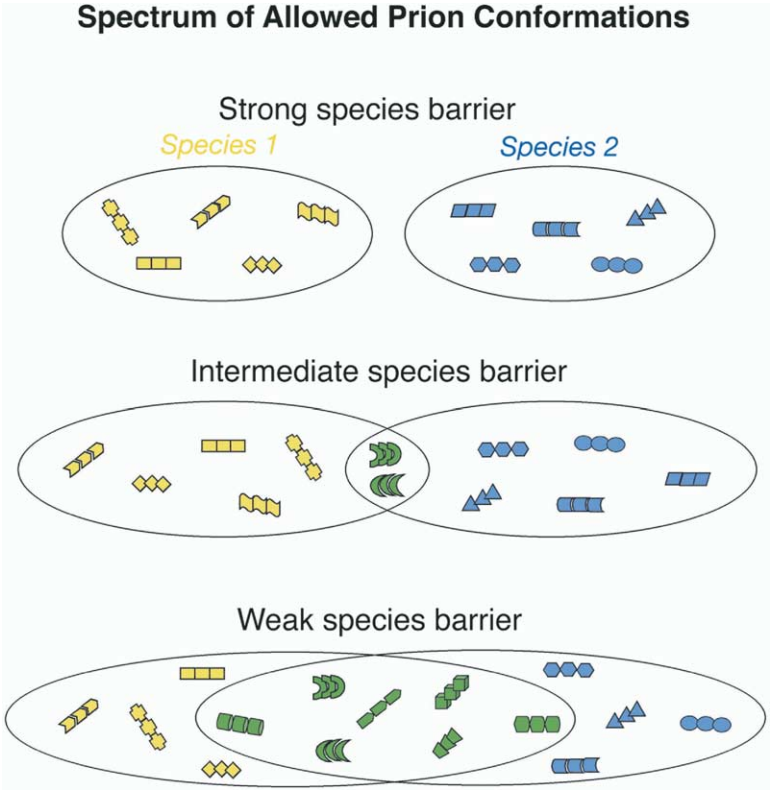

Figure 7. Venn Diagram Illustrating Relationship between Primary Structure of Prion Proteins, Spectrum of Preferred Prion Conformations and Capacity for Cross-Species Transmission

Each prion protein will have a spectrum of allowed prion conformations. For distantly related proteins, there may be no overlapping conformations resulting in absolute transmission barriers (top). For more related sequences, the degree of overlap increases as the similarity between the two proteins increases, thereby leading to an increase in the number of strain conformations that allow cross-species transmission (middle and bottom).

calized differences between Sc4 and Sc37 strain conformations in the beginning and end of the prion domain and suggested that, in the Sc37 conformation, the amyloid core is more highly ordered and that the structured region extends further into both the N terminus and C terminus. This increase in structure is consistent with the increased rigidity and thermal stability of the Sc37 fibers relative to Sc4 amyloid, which in turn is responsible for the differences in the strain phenotypes seen in vivo. The extended structure in the Sc37 conformation also helps explain the earlier observation (Bradley and Liebman, 2004) that propagation of strain differences requires residues beyond amino acid 124 even though this region is dispensable for prion inheritance. Finally, the structural difference in the N- and C-terminal regions may play a critical role in altering the ability of a given prion strain to cross-species barriers, as the EPR studies indicate that these regions are involved in intermolecular contacts in the Sup35 prion.

Given the ability of different prion strain conformations to alter the specificity of prion transmission, what, then, is the role of primary structure in determining the propensity of transmission between donor and host prion proteins? The critical importance of a given strain conformation in modulating a prion's seeding specificity together with the ubiquitous nature of prion strains suggests a model in which the primary structure acts predominantly to modulate the range of infectious prion conformations, and the specific conformation in turn determines the ability to be transmitted across a species barrier (Figure 7). A common feature of amyloid-forming proteins is that a single protein can adopt multiple distinct, self-propagating amyloid conformations with the spectrum of misfolded forms being determined by the protein's primary structure (Dobson 2001; Chien et al., 2004; Petkova et al., 2005). Thus, an absolute transmission barrier between two divergent species of prion proteins would exist only in the extreme case when two proteins have completely nonoverlapping spectra of allowed prion conformations. More generally, it would be expected that related but divergent proteins share a subset of conformations. This model predicts that crosstransmission will be possible for some subset of the accessible prion strain conformations, whereas, for the other conformations, a robust species barrier will prevent interspecies infection. As two protein sequences become more divergent, the number of overlapping conformations will decrease, and thus the probability of crossing a species barrier will decrease.

This view that primary structure determines prion specificity in large part by altering the spectrum of allowed prion conformations provides insight into a number of otherwise puzzling features of cross-species prion transmission. For example, this model helps to explain why in both yeast and mammalian prions point mutations or allelic variants can have dramatic effects on the specificity of prion transmission in a strain-specific manner (Bossers et al., 1997; Manson et al., 1999; Barron et al., 2001; King, 2001; Chien et al., 2003; Baylis and McIntyre, 2004), as the range of conformations favored by a prion protein can be strongly influenced by even small changes in the primary structure. In support of this, it has recently been shown that the M129/V129

codon in human prion protein is critical in determining strain phenotypes and susceptibility to BSE-derived prions (Wadsworth et al., 2004). This model also suggests an explanation for the observed ability of Sup35 derived from some non-Sc species to cross-seed Sc protein (Chernoff et al., 2000; Nakayashiki et al., 2001). Of particular interest was the finding that overexpression of *Pichia methanolic* Sup35 at lower but not elevated temperatures can induce prion formation in Sc yeast (Chernoff et al., 2000). *P. Methanolica* Sup35 is known to support a range of different prion strains (Kushnirov, et al., 2000a). In light of the above considerations, it is possible that temperature is acting to constrain the amyloid conformations formed spontaneously by *P. Methanolica* Sup35. The resulting range of strains would in turn determine whether cross-species transmission to the Sc protein is favored. More generally, even with noninfectious protein aggregates, such as those associated with a range of neurodegenerative diseases, the specific amyloid conformation adopted by a protein is likely to play an important role in determining its abilities to recruit heterologous proteins, thereby modulating the toxic effects of accumulating misfolded forms.

In addition to elucidating the link between prion strains and transmission barriers, the present studies illustrate the existence of an intimate link between interspecies transmission of prions and the development of novel prion strains. Passage of a specific Sc amyloid to Ca yeast resulted in the emergence of a novel strain in vivo and the formation of a distinct amyloid conformation in vitro. This newly generated Ca conformation can infect both Sc and Ca species of Sup35 proteins and induces yet another Sc prion strain distinct from the strain of the originally infected Sc yeast. Structural analyses including FT-IR and EPR spectroscopies directly demonstrated that passage of the prion from Sc to Ca and back to Sc resulted in a novel infectious conformation. These results are analogous to the observation that the infrequent cross-species transmission of mammalian prions between species with distinct prion protein genes leads to the emergence of new prion strains with distinct conformations (Peretz et al., 2002). More speculatively, cross-species prion infections may play an important role in promoting especially virulent forms of prions, such as those responsible for mad cow disease or new variant of Creutzfeldt-Jacob disease, by generating more promiscuous prion conformations that are compatible with a wide range of related species.

Experimental Procedures

Construction of Plasmids and Yeast Strains

Construction of bacterial and copper-inducible expression vectors for the various forms of Sup-NM was reported previously (Santoso et al., 2000). Isogenic [*psi*$^-$][*pin*$^+$] and [*PSI*$^+$] derivatives of 74D-694 yeast (*his3*, *leu2*, *trp1*, *ura3*; suppressible marker *ade1-14*[UGA]) (Chernoff et al., 1995; Santoso et al., 2000) were used for Sup-NM-GFP overexpression and infection experiments. Construction of Ca, Chim, and F yeasts that express Sup35 chimeric proteins fused to *S. cerevisiae* Sup35 middle and C-terminal domains from the wild-type *SUP35* chromosomal locus was described previously (Santoso et al., 2000; Osherovich et al., 2004).

In Vitro Analysis of Sup-NM Amyloid Fibers

Sup-NM proteins with polyhistidine tags at the C terminus were produced in bacteria and purified as reported previously (Santoso et al., 2000), except in the case of the cysteine mutants, in which 1 mM β-mercaptoethanol was included in all purification buffers. Preparation of Sup-NM amyloid fibers is described in Supplemental Data. Amyloid fiber formation was monitored by thioflavine T fluorescence using a 96-well plate reader (Chien et al., 2003). The thermal stability of amyloid fibers (2.5 μM) was examined by SDS-PAGE (7.5% or 4%–12% gradient gel [Invitrogen] [Chien et al., 2003]). Thermally solubilized monomeric Sup-NM was detected by Western blotting with a polyclonal anti-Sup-NM_{Sc} antibody (Santoso et al., 2000), which reacts with both Sup-NM_{Sc} and Sup-NM_{Ca}, followed by detection with chemiluminescence using a FluorChem 8800 (Alpha Innotech). The band intensities in Western blots were fitted to a sigmoidal curve with IgorPro3.0 (WaveMetrics, Inc.), using the following equation: y = A + B/(1 + 10^((C – x)/D)), where x, y, C, and D indicate temperature, band intensity, melting (T_m), and transition (W) temperatures, respectively. For FT-IR spectroscopy, Sc4 and Sc37 amyloids were spontaneously formed at 4°C and 37°C, respectively, and Sc[Ca_3[Sc4]] amyloid was prepared by polymerizing Sc Sup-NM with 5% (wt/wt) seed of Ca_3[Sc4] fibers. FT-IR spectra of the amyloid fibers (~100 μg) were recorded with a Perkin Elmer (Norwalk, CT) System FT-IR 2000 spectrophotometer with a microscope attachment at 23°C. Each spectrum was collected with a 2 cm^{-1} resolution and a 1 cm^{-1} interval from 1750 to 1550 cm^{-1}. Secondary structure contents were estimated as reported previously (Wille and Prusiner, 1999). See Supplemental Data for details. For EPR spectroscopy, Sup-NM single cysteine mutants were labeled by MTSL (5 equivalents) (Toronto Research Chemicals Inc.) (Hubbell et al., 2000) with >80% efficiency and purified as previously reported (Tanaka et al., 2004). Absence of labeling at noncysteine residues was confirmed using wild-type Sup-NM that lacks cysteine residues. Sc4, Sc37, and Sc[Ca_3[Sc4]] fibers were formed at 4°C, 37°C, and 23°C by polymerizing Sup-NM cysteine mutants with 5% (wt/wt) seed of spontaneously formed Sc4, Sc37 amyloids, and Sc[Ca_3[Sc4]] amyloid, respectively. Pelleted fibrillar Sup-NM (~50 μg) was loaded onto a flatcell, and EPR spectra were measured with a Bruker EMX EPR spectrometer at 23°C with 25 mW microwave power and a modulation of 1.0 G at 100 KHz over a scan range of 100 or 200 G. For dilution experiments, spin-labeled Sup-NM cysteine mutants were mixed with unlabeled wild-type protein in a 1:1 molar ratio. The use of MTSL, which is smaller than 4-Maleimido-2,2,6,6-tetramethyl-1-piperidinyloxy (Tanaka et al., 2004), together with a modulation of 1.0 G in the EPR spectrometer substantially improved the resolution of EPR spectra, compared with that in our earlier work (Tanaka et al., 2004).

In Vivo Analysis of Cross-Species Transmission of Sup-NM Prions

Induction experiments were carried out as previously described (Santoso et al., 2000). Briefly, Sup-NM_{Sc}-GFP and Sup-NM_{Ca}-GFP were overexpressed from a 2μ plasmid driven from a copper-inducible *CUP1* promoter (Santoso et al., 2000). Transformants were grown on SD-URA media containing 50 μM $CuSO_4$ for 24 hr. These cultures were plated on SD-ADE and SD complete plates, and visible colonies were counted after about 7 days. Protein infection experiments were carried out by the procedure of Tanaka et al. (2004). See Supplemental Data for details.

Supplemental Data

Supplemental Data include three figures and Supplemental Experimental Procedures and can be found with this article online at http://www.cell.com/cgi/content/full/121/1/49/DC1/.

Acknowledgments

We thank N. Naber and R. Cooke for help of the EPR experiments; H. Wille for assistance with the FT-IR experiments; and S. Collins, J. Newman, L. Osherovich, M. Schuldiner, K. Tipton, and members of the Weissman laboratory for helpful discussion and critical read-

ing of the manuscript. M.T. was supported by JSPS postdoctoral fellowships for research abroad. P.C. was supported by National Science Foundation Graduate Fellowships and the ARCS foundation. Funding was provided by Howard Hughes Medical Institute, The David and Lucile Packard Foundation, and the National Institutes of Health.

Received: December 30, 2004
Revised: February 22, 2005
Accepted: March 10, 2005
Published: April 7, 2005

References

Aguzzi, A., and Polymenidou, M. (2004). Mammalian prion biology: one century of evolving concepts. Cell *116*, 313–327.

Barron, R.M., Thomson, V., Jamieson, E., Melton, D.W., Ironside, J., Will, R., and Manson, J.C. (2001). Changing a single amino acid in the N-terminus of murine PrP alters TSE incubation time across three species barriers. EMBO J. *20*, 5070–5078.

Baylis, M., and McIntyre, K.M. (2004). Scrapie control under new strain. Nature *432*, 810–811.

Bossers, A., Belt, P.B.G.M., Raymond, G.J., Caughey, B., de Vries, R., and Smits, M.A. (1997). Scrapie susceptibility-linked polymorphisms modulate the in vitro conversion of sheep prion protein to protease-resistant forms. Proc. Natl. Acad. Sci. USA *94*, 4931–4936.

Bradley, M.E., and Liebman, S.W. (2004). The Sup35 domains required for maintenance of weak, strong or undifferentiated yeast [*PSI*$^+$] prions. Mol. Microbiol. *51*, 1649–1659.

Bruce, M.E., Will, R.G., Ironside, J.W., McConnell, I., Drummond, D., Suttie, A., McCardie, L., Chree, A., Hope, J., Birkett, C., et al. (1997). Transmissions to mice indicate that "new variant" CJD is caused by the BSE agent. Nature *389*, 498–501.

Chien, P., and Weissman, J.S. (2001). Conformational diversity in a yeast prion dictates its seeding specificity. Nature *410*, 223–227.

Chien, P., DePace, A.H., Collins, S., and Weissman, J.S. (2003). Generation of prion transmission barriers by mutational control of amyloid conformations. Nature *424*, 948–951.

Chien, P., Weissman, J.S., and DePace, A.H. (2004). Emerging principles of conformation-based prion inheritance. Annu. Rev. Biochem. *73*, 617–656.

Chernoff, Y.O., Lindquist, S.L., Ono, B., Inge-Vechtomov, S.G., and Liebman, S.W. (1995). Role of the chaperone protein Hsp104 in propagation of the yeast prion-like factor. Science *268*, 880–884.

Chernoff, Y.O., Galkin, A.P., Lewitin, E., Chernova, T.A., Newnam, G.P., and Belenkiy, S.M. (2000). Evolutionary conservation of prion-forming abilities of the yeast Sup35 protein. Mol. Microbiol. *35*, 865–876.

Collinge, J. (2001). Prion diseases of humans and animals: their causes and molecular basis. Annu. Rev. Neurosci. *24*, 519–550.

DePace, A.H., Santoso, A., Hillner, P., and Weissman, J.S. (1998). A critical role for amino-terminal glutamine/asparagine repeats in the formation and propagation of a yeast prion. Cell *93*, 1241–1252.

Derkatch, I.L., Chernoff, Y.O., Kushnirov, V.V., Inge-Vechtomov, S.G., and Liebman, S.W. (1996). Genesis and variability of [*PSI*$^+$] prion factors in *Saccharomyces cerevisiae*. Genetics *144*, 1375–1386.

Derkatch, I.L., Bradley, M.E., Hong, J.Y., and Liebman, S.W. (2001). Prions affect the appearance of other prions: the story of [PIN(+)]. Cell *106*, 171–182.

Derkatch, I.L., Uptain, S.M., Outeiro, T.F., Krishnan, R., Lindquist, S.L., and Liebman, S.W. (2004). Effects of Q/N-rich, polyQ, and non-polyQ amyloids on the de novo formation of the [*PSI*$^+$] prion in yeast and aggregation of Sup35 in vitro. Proc. Natl. Acad. Sci. USA *101*, 12934–12939.

Dobson, C.M. (2001). The structural basis of protein folding and its links with human disease. Philos. Trans. R. Soc. Lond. B. Biol. Sci. *356*, 133–145.

Glover, J.R., Kowal, A.S., Schirmer, E.C., Patino, M.M., Liu, J.J., and Lindquist, S. (1997). Self-seeded fibers formed by Sup35, the protein determinant of [*PSI*$^+$], a heritable prion-like factor of *S. cerevisiae*. Cell *89*, 811–819.

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

Hubbell, W.L., Cafiso, D.S., and Altenbach, C. (2000). Identifying conformational changes with site-directed spin labeling. Nat. Struct. Biol. *7*, 735–739.

King, C.Y. (2001). Supporting the structural basis of prion strains: induction and identification of [*PSI*$^+$] variants. J. Mol. Biol. *307*, 1247–1260.

King, C.Y., and Diaz-Avalos, R. (2004). Protein-only transmission of three yeast prion strains. Nature *428*, 319–323.

King, C.Y., Tittmann, P., Gross, H., Gebert, R., Aebi, M., and Wuthrich, K. (1997). Prion-inducing domain 2–114 of yeast Sup35 protein transforms in vitro into amyloid-like filaments. Proc. Natl. Acad. Sci. USA *94*, 6618–6622.

Kochneva-Pervukhova, N.V., Chechenova, M.B., Valouev, I.A., Kushnirov, V.V., Smirnov, V.N., and Ter-Avanesyan, M.D. (2001). [*PSI*$^+$] prion generation in yeast: characterization of the 'strain' difference. Yeast *18*, 489–497.

Kocisko, D.A., Priola, S.A., Raymond, G.J., Chesebro, B., Lansbury, P.T., Jr., and Caughey, B. (1995). Species specificity in the cell-free conversion of prion protein to protease-resistant forms: a model for the scrapie species barrier. Proc. Natl. Acad. Sci. USA *92*, 3923–3927.

Kushnirov, V.V., Kochneva-Pervukhova, N.V., Chechenova, M.B., Frolova, N.S., and Ter-Avanesyan, M.D. (2000a). Prion properties of the Sup35 protein of yeast *Pichia methanolica*. EMBO J. *19*, 324–331.

Kushnirov, V.V., Kryndushkin, D.S., Boguta, M., Smirnov, V.N., and Ter-Avanesyan, M.D. (2000b). Chaperones that cure yeast artificial [*PSI*$^+$] and their prion-specific effects. Curr. Biol. *10*, 1443–1446.

Legname, G., Baskakov, I.V., Nguyen, H.B., Riesner, D., Cohen, F.E., DeArmond, S.J., and Prusiner, S.B. (2004). Synthetic mammalian prions. Science *305*, 673–676.

Liu, J.J., Sondheimer, N., and Lindquist, S.L. (2002). Changes in the middle region of Sup35 profoundly alter the nature of epigenetic inheritance for the yeast prion. Proc. Natl. Acad. Sci. USA *99*, 16446–16453.

Maddelein, M.L., Dos Reis, S., Duvezin-Caubet, S., Coulary-Salin, B., and Saupe, S.J. (2002). Amyloid aggregates of the HET-s prion protein are infectious. Proc. Natl. Acad. Sci. USA *99*, 7402–7407.

Manson, J.C., Jamieson, E., Baybutt, H., Tuzi, N.L., Barron, R., McConnell, I., Somerville, R., Ironside, J., Will, R., Sy, M.S., et al. (1999). A single amino acid alteration (101L) introduced into murine PrP dramatically alters incubation time of transmissible spongiform encephalopathy. EMBO J. *18*, 6855–6864.

Nakayashiki, T., Ebihara, K., Bannai, H., and Nakamura, Y. (2001). Yeast [*PSI*$^+$] 'prions' that are crosstransmissible and susceptible beyond a species barrier through a quasi-prion state. Mol. Cell *7*, 1121–1130.

Osherovich, L.Z., and Weissman, J.S. (2001). Multiple Gln/Asn-rich prion domains confer susceptibility to induction of the yeast [*PSI*$^+$] prion. Cell *106*, 183–194.

Osherovich, L.Z., Cox, B.S., Tuite, M.F., and Weissman, J.S. (2004). Dissection and design of yeast prions. PLoS Biol. e86 2(4): DOI:10.1371/journal.pbio.0020086.

Peretz, D., Williamson, R.A., Legname, G., Matsunaga, Y., Vergara, J., Burton, D.R., DeArmond, S.J., Prusiner, S.B., and Scott, M.R. (2002). A change in the conformation of prions accompanies the emergence of a new prion strain. Neuron *34*, 921–932.

Petkova, A.T., Leapman, R.D., Guo, Z., Yau, W.M., Mattson, M.P., and Tycko, R. (2005). Self-propagating, molecular-level polymorphism in Alzheimer's beta-amyloid fibrils. Science *307*, 262–265.

Prusiner, S.B. (1982). Novel proteinaceous infectious particles cause scrapie. Science *216*, 136–144.

Prusiner, S.B., Scott, M.R., DeArmond, S.J., and Cohen, F.E. (1998). Prion protein biology. Cell *93*, 337–348.

Santoso, A., Chien, P., Osherovich, L.Z., and Weissman, J.S. (2000). Molecular basis of a yeast prion species barrier. Cell *100*, 277–288.

Serag, A.A., Altenbach, C., Gingery, M., Hubbell, W.L., and Yeates, T.O. (2002). Arrangement of subunits and ordering of beta-strands in an amyloid sheet. Nat. Struct. Biol. *9*, 734–739.

Soto, C., and Castilla, J. (2004). The controversial protein-only hypothesis of prion propagation. Nat. Med. *10*, S63–S67.

Sparrer, H.E., Santoso, A., Szoka, F.C., Jr., and Weissman, J.S. (2000). Evidence for the prion hypothesis: induction of the yeast [*PSI*$^+$] factor by in vitro-converted Sup35 protein. Science *289*, 595–599.

Tanaka, M., Chien, P., Naber, N., Cooke, R., and Weissman, J.S. (2004). Conformational variations in an infectious protein determine prion strain differences. Nature *428*, 323–328.

Ter-Avanesyan, M.D., Dagkesamanskaya, A.R., Kushnirov, V.V., and Smirnov, V.N. (1994). The *SUP35* omnipotent suppressor gene is involved in the maintenance of the non-Mendelian determinant [*PSI*$^+$] in the yeast *Saccharomyces cerevisiae*. Genetics *137*, 671–676.

Tuite, M.F., and Cox, B.S. (2003). Propagation of yeast prions. Nat. Rev. Mol. Cell Biol. *4*, 878–890.

Uptain, S.M., and Lindquist, S. (2002). Prions as protein-based genetic elements. Annu. Rev. Microbiol. *56*, 703–741.

Uptain, S.M., Sawicki, G.J., Caughey, B., and Lindquist, S. (2001). Strains of [*PSI*$^+$] are distinguished by their efficiencies of prion-mediated conformational conversion. EMBO J. *20*, 6236–6245.

Vanik, D.L., Surewicz, K.A., and Surewicz, W.K. (2004). Molecular basis of barriers for interspecies transmissibility of mammalian prions. Mol. Cell *14*, 139–145.

Wadsworth, J.D., Asante, E.A., Desbruslais, M., Linehan, J.M., Joiner, S., Gowland, I., Welch, J., Stone, L., Lloyd, S.E., Hill, A.F., et al. (2004). Human prion protein with valine 129 prevents expression of variant CJD phenotype. Science *306*, 1793–1796.

Weissmann, C. (2004). The state of the prion. Nat. Rev. Microbiol. *2*, 861–871.

Wickner, R.B. (1994). [URE3] as an altered URE2 protein: evidence for a prion analog in *Saccharomyces cerevisiae*. Science *264*, 566–569.

Wille, H., and Prusiner, S.B. (1999). Ultrastructural studies on scrapie prion protein crystals obtained from reverse micellar solutions. Biophys. J. *76*, 1048–1062.

Zhou, P., Derkatch, I.L., Uptain, S.M., Patino, M.M., Lindquist, S., and Liebman, S.W. (1999). The yeast non-Mendelian factor [*ETA*$^+$] is a variant of [*PSI*$^+$], a prion-like form of release factor eRF3. EMBO J. *18*, 1182–1191.