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Insights into Chronic Wasting Disease and Bovine Spongiform Encephalopathy Species Barriers by Use of Real-Time Conversion

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ABSTRACT

The propensity for transspecies prion transmission is related to the structural characteristics of the enciphering and new host PrP, although the exact mechanism remains incompletely understood. The effects of variability in prion protein on cross-species prion transmission have been studied with animal bioassays, but the influence of prion protein structure versus that of host cofactors (e.g., cellular constituents, trafficking, and innate immune interactions) remains difficult to dissect. To isolate the effects of protein-protein interactions on transspecies conversion, we used recombinant PrP^C and real-time quaking-induced conversion (RT-QuIC) and compared chronic wasting disease (CWD) and classical bovine spongiform encephalopathy (cBSE) prions. To assess the impact of transmission to a new species, we studied feline CWD (fCWD) and feline BSE (i.e., feline spongiform encephalopathy [FSE]). We cross-seeded fCWD and FSE into each species' full-length, recombinant PrP^C and measured the time required for conversion to the amyloid (PrP^{Res}) form, which we describe here as the rate of amyloid conversion. These studies revealed the following: (i) CWD and BSE seeded their homologous species' PrP best; (ii) fCWD was a more efficient seed for feline rPrP than for white-tailed deer rPrP; (iii) conversely, FSE more efficiently converted bovine than feline rPrP; (iv) and CWD, fCWD, BSE, and FSE all converted human rPrP, although not as efficiently as homologous sCJD prions. These results suggest that (i) at the level of protein-protein interactions, CWD adapts to a new species more readily than does BSE and (ii) the barrier preventing transmission of CWD to humans may be less robust than estimated.

IMPORTANCE

We demonstrate that bovine spongiform encephalopathy prions maintain their transspecies conversion characteristics upon passage to cats but that chronic wasting disease prions adapt to the cat and are distinguishable from the original prion. Additionally, we showed that chronic wasting disease prions are effective at seeding the conversion of normal human prion protein to an amyloid conformation, perhaps the first step in crossing the species barrier.

Prion diseases are characterized by the seeded misfolding and aggregation of the cellular prion protein, PrP^C, to an amyloid fibrillar state, PrP^{Res}. All mammals express PrP^C, with only minor polymorphisms within and between species (1). Prion disease has been identified in humans (Creutzfeldt-Jakob disease [CJD]), cattle (bovine spongiform encephalopathy [BSE]), cervids (chronic wasting disease [CWD]), felines (feline spongiform encephalopathy [FSE]), and sheep and goats (scrapie), as well as other mammals (2). The transmissibility of prion diseases makes them unique among other protein misfolding diseases, including Alzheimer's and Parkinson's diseases. Though prion diseases vary in their proclivity to transmit to new species, the classical BSE (cBSE) epidemic and subsequent transmission to humans (variant CJD [vCJD]), felines, and several ungulate species illustrate the importance of understanding zoonotic transmission (2–8).

The transspecies transmissibility of cBSE and CWD has been studied in natural hosts, conventional and transgenic rodents, and *in vitro* models. CWD has been transmitted experimentally to sheep, felines, cattle, and squirrel monkeys (9–14). BSE has been transmitted experimentally to sheep, European red deer, and macaques (15–17). Transgenic mice have been developed to explore the potential transmissibility of cBSE and CWD to humans. In four studies with mice expressing the human prion protein (TgHu), cBSE inoculation produced mixed results, with attack rates ranging from 0 to ~50% (18–21). Inoculation of TgHu mice with CWD has yielded no infections (21–24). Additionally, three distinct *in vitro* amyloid amplification methods have also shown that BSE is better able to convert human PrP than is CWD (25–31).

That prion characteristics are not solely defined by primary structure can be inferred by the existence of prion strains (25). Indeed, several studies have noted the similarity that transspecies-passaged cBSE prions (as represented by FSE, vCJD, and sheep BSE) have to the original cBSE agent, despite the variable primary structure of the passaged prions (19, 26, 32, 33). Therefore, it is logical to conclude that variability in prion conformation and the interactions of PrP^{Res} and PrP^C are essential to prion species barriers. We employ real-time quaking-induced conversion (RT-QuIC) to assess the complementarity of seed-substrate pairing and, by extension, the possibility of transspecies conversion, of

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TABLE 1 Sources of the prion seeds used in the study experiments[a]

Prion type	Species (*n*)	Prion disease status	Tissue	Inoculum	Inoculation	Sample ID
CWD	WTD (1)	+	Caudal brain sections	Colorado Department of Wildlife positive deer LA01	Oral	104
Exper. CWD	WTD (6)	+	Whole brain	CSU CWD-positive deer 700, 800 series	Oral or aerosol	CBP6
Negative deer	WTD (1)	−	Caudal brain sections	CSU CWD-negative deer UGA 1/2	Oral	123
Feline CWD	Feline (2)	+	Multiple brain sections	CSU CWD-positive deer	Intracranial	4137/4152
Negative cat	Feline (1)	−	Obex	CSU CWD-negative deer UGA 1/2	Oral	4141
FSE[b]	Feline (1)	+	Medulla	Natural infection	Assumed oral	FSE
BSE	Bovine (1)	+	Obex	Natural infection	Assumed oral	BSE
Negative cow	Bovine (1)	−	Brainstem	Not inoculated		Negative bovine
Field CWD 1[c]	WTD	+	Obex	Field isolate		H92
Field CWD 2[c]	WTD	+	Obex	Field isolate		98933968

[a] Abbreviations: CSU, Colorado State University; Exper., experimental; WTD, white-tailed deer.
[b] See reference 3.
[c] See reference 36.

two prions pertinent to human health, i.e., the agents causing CWD and BSE.

In the studies to follow, we hypothesized that in RT-QuIC, cBSE and passaged forms of cBSE would have similar transspecies seeding characteristics and would readily convert human rPrP, whereas passaged forms of CWD would maintain fewer CWD seeding tendencies and would convert human rPrP poorly.

MATERIALS AND METHODS

rPrP production. The coding regions for full-length (amino acids [aa] 23 to 231) recombinant prion protein (rPrP) from each species (bank vole, bovine, feline, human M129, and white-tailed deer) were kindly provided by Glenn Telling. Full-length constructs (bovine, feline, human M129, and white-tailed deer) were cloned into the pET100D expression system (Life Technologies). The truncated Syrian hamster construct in *Escherichia coli* BL21 was kindly provided by Byron Caughey. The bank vole *PRNP* coding region was truncated using specific primers to isolate the sequence for amino acids 90 to 231 and was cloned into the vector that contained the Syrian hamster construct. *E. coli* BL21 Star cells (Life Technologies) were used to express rPrP. Briefly, BL21 cells from a glycerol stock were spiked into 5 ml LB medium and 5 μg/ml ampicillin and grown overnight with shaking at 37°C. LB medium (1 liter) was inoculated with the 5-ml culture plus 5 μg/ml ampicillin and autoinduction reagents [final concentrations, 0.5 M $(NH_4)_2SO_4$, 1 M KH_2PO_4, 1 M Na_2HPO_4, 0.5% glycerol, 0.05% glucose, 0.2% α-lactose, 0.001 M $MgSO_4$]. Bacteria were harvested when the optical density at 600 nm (OD_{600}) reached approximately 3.0; cells were lysed and inclusion bodies isolated according to the manufacturer's protocol with Bug Buster and Lysonase (EMD-Millipore). Inclusion bodies were solubilized and rotated in 8 M guanidine hydrochloride (GdnHCl) and 100 mM Na_2HPO_4 for at least 1 h at room temperature. The denatured rPrP was bound to Superflow nickel resin (Qiagen) and refolded at a rate of 0.75 ml/min on the column with a gradient from 6.0 M GdnHCl–100 mM Na_2HPO_4–10 mM Tris (pH 8.0) to the same buffer with no GdnHCl. A gradient to 0.5 M imidazole–100 mM NaH_2PO_4–10 mM Tris (pH 5.5) with a flow rate of 2.0 ml/min resulted in rPrP elution. The eluted rPrP was filtered and dialyzed at a concentration of ~0.4 mg/ml in two changes of 4.0 liters 20 mM NaH_2PO_4 (pH 5.5). After dialysis, the rPrP was filtered and stored at 4°C, its concentration having been determined by A_{280}. Purity of the rPrP samples was tested by performing gel electrophoresis (12% bis-Tris gels with 1× MOPS [morpholinepropanesulfonic acid; Bio-Rad], 190 V, 55 min) and staining with Coomassie brilliant blue (Bio-Rad).

RT-QuIC. rPrP (0.1 mg/ml) was combined with premixed reaction buffer (final concentrations, 400 mM NaCl, 1 mM EDTA, 20 mM NaH_2PO_4) and freshly made thioflavin T (ThT; final concentration, 10 μM) for a final volume of 95 μl per well in a 96-well plate. Brain material (Table 1) was homogenized in 1× phosphate-buffered saline (PBS) as a 10% solution with a bead beater (Next Advance). Brain homogenate was aliquoted in single-use tubes and frozen at −80°C. Homogenate aliquots were thawed and diluted into 1× PBS plus 0.05% SDS (first set of conditions, here termed "condition 1") or 0.1% SDS ("condition 2"). Two microliters of diluted brain seed was added to 95 μl protein substrate mix in the well of an optical bottom 96-well plate. A real-time quaking-induced conversion (RT-QuIC) experiment consisted of 400 cycles (100 h) of shaking and incubation at 45°C; specifically, plates were shaken for 1 min (700 rpm, double orbital) followed by 1 min of rest. Fluorescence (450-nm excitation and 480-nm emission, 20 flashes/well) was recorded every 15 min using a gain of 1,700 (96-well plate).

In order to ensure that the results observed in our RT-QuIC experiments were not a result of specific experimental conditions, all the experiments in this study were completely repeated under two sets of conditions. For condition 1, one or two batches of each rPrP species (bovine, feline, white-tailed deer, human) were used and the concentration of SDS used to dilute the brain material was 0.05%. For condition 2, new batches (one or two) of each species of rPrP were used, with an SDS concentration of 0.1% in the sample dilution step. For both condition 1 and condition 2, there were 6 to 8 replicates (on 3 or 4 plates) of every reaction. Because the reaction conditions resulted in different kinetics (kinetics using 0.1% SDS were faster than 0.05% SDS), rates of amyloid conversion could not be compared between experiments. The data shown here are from condition 2, but these patterns were maintained in condition 1.

Artificial seeds. In order to assess each species' inherent propensity to convert from $rPrP^{C}$ to $rPrP^{Res}$ (amyloidogenicity), we created synthetic $rPrP^{ThT+}$ seeds from truncated Syrian hamster $rPrP^{C}$ (SH; amino acids 90 to 231) or truncated bank vole $rPrP^{C}$ (BV; amino acids 90 to 231). For the SH artificial seeds, 0.5 mg/ml SH $rPrP^{C}$ with 2 M GdnHCl was shaken at 37°C overnight. BV artificial seeds were made by shaking 0.3 mg/ml BV $rPrP^{C}$ at 37°C without GdnHCl. In both cases, 2 μl artificial seed (or 10-fold dilutions thereof) was added to the RT-QuIC reaction mixture in place of a brain-derived seed.

Western blotting and quantification. A volume of 5 μl, 9 μl, or 13 μl of a 5% homogenate of BSE or CWD (sample 104) was added to 1 μl of proteinase K (PK; 100 μg/ml; Invitrogen) and 1 μl of 2% SDS and brought to a volume of 15 μl with water. These samples were incubated at 37°C for 30 min and then at 45°C for 10 min. Five microliters of a 4× sample loading buffer/reducing agent (Life Technologies) was added to the samples, and they were incubated at 93°C for 3 min. Eighteen microliters was added to each well of a 12-well, 12% bis-Tris gel. The samples were electrophoresed for 10 min at 115 V and then for 1 h at 150 V. Transfers were performed in cold transfer buffer (20% MeOH, 192 mM glycine, 25 mM

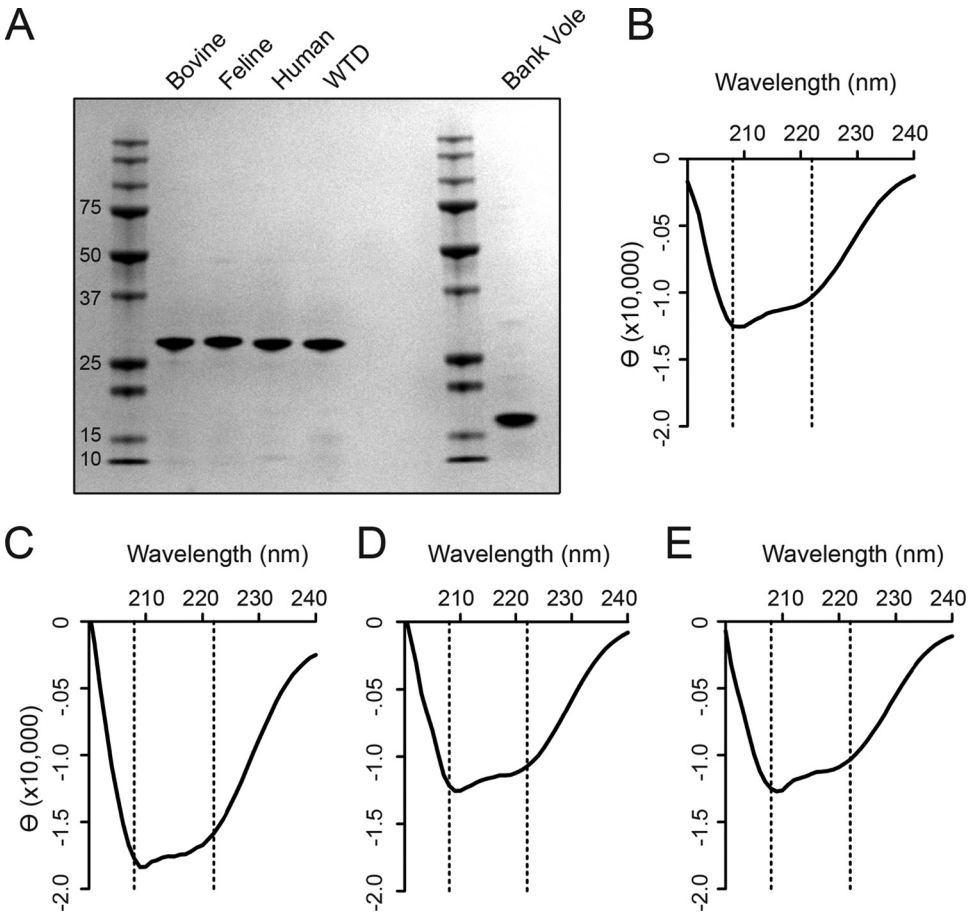

FIG 1 rPrP substrates are of comparable quality. (A) Coomassie blue visualization of 1.5 μg rPrP substrate indicates the purity of each rPrP. Bands represent, from left to right, ladder, bovine (amino acids 23 to 231), feline (aa 23 to 231), human M129 (aa 23 to 231), and white-tailed deer (aa 23 to 231) rPrP, ladder, and bank vole (aa 90 to 231) rPrP. (B) CD spectrum for bovine rPrP. (C) CD spectrum for feline rPrP. (D) CD spectrum for human M129 rPrP. (E) CD spectrum for white-tailed deer rPrP. The dotted lines indicate the anticipated minima for α-helical structure (B to F).

Tris base) for 1.5 h at 115 V, and the membranes were blocked overnight at 4°C in Tris-buffered saline plus 0.1% Tween (TBST) and dry milk. 6H4 was bound to the membrane in TBST plus milk for 1 h at a concentration of 1:5,000, and then the membrane was washed with TBST for 1 h. The secondary antibody, a Licor-tagged goat anti-mouse antibody, was bound to the membrane in TBST plus milk for 1 h at a concentration of 1:20,000. The membrane was again washed with TSBT for 1 h. Images were collected on the Odyssey CLx Infrared Imager (Li-Cor Biosciences, Inc.) and analyzed using Image Studio Lite Version 4.0 (Li-Cor Biosciences, Inc.). Briefly, the intensity was calculated for each lane of the Western blot and then divided by the number of microliters of 5% brain homogenate added to the well. Finally, the intensity per microliter for each PK^+ lane was divided by the intensity of the PK^- lane to determine the relative quantity of PK-resistant material.

Quantitative analysis. The lag phases of positive and negative samples were determined using MARS software (BMG Labtech) by calculating the time required to meet a threshold for positive samples (34). The threshold was defined as the average baseline fluorescence plus 5 standard deviations for each experiment. The rate is the inverse of the lag phase for each sample (1/lag phase), with units of $hours^{-1}$. We use the rate of amyloid conversion here instead of lag phase in order to account for the samples that never cross the threshold (lag phase for these samples is undefined, but the rate can be conservatively assigned a value of $0\ h^{-1}$). Despite their similar purity, conformation, and functionality, the rPrP substrates were not equally sensitive. In order to compare one seed's behavior across multiple substrates without confounding by the inherent amyloidogenicity differences between the substrates, relative rates were calculated and used for analysis. Specifically, all rates for a given substrate were divided by the highest rate in that substrate; the fastest sample has a rate of 1.0. Average relative rates of amyloid conversion from 6 to 12 replicates are displayed as the means, with error bars indicating the standard errors of the means. The difference between rates at each dilution was tested with the nonparametric Mann-Whitney U (MWU) test (when both samples had a nonzero median) in Prism 5.0 (Graphpad) or the nonparametric one-sample Wilcoxon signed-rank (WSR) test (when one of the samples had a median of zero) in R. A statistically significant difference was defined by a P value of <0.05 from the appropriate test. Significant differences by the MWU test are indicated in the figures by an asterisk (*), while significant differences by the WSR test are indicated by a pound sign (#).

RESULTS

Assessment of recombinant bovine, feline, human, and white-tailed deer PrP and quality. In order to compare the behavior of a given prion seed across multiple rPrP substrates, it was necessary to ensure that every batch of every substrate had similar purity, structure, and functionality. Each rPrP preparation was analyzed by gel electrophoresis and Coomassie blue staining to show that the substrates were of comparable purity (Fig. 1A). To ensure that the secondary structures from each preparation were comparable,

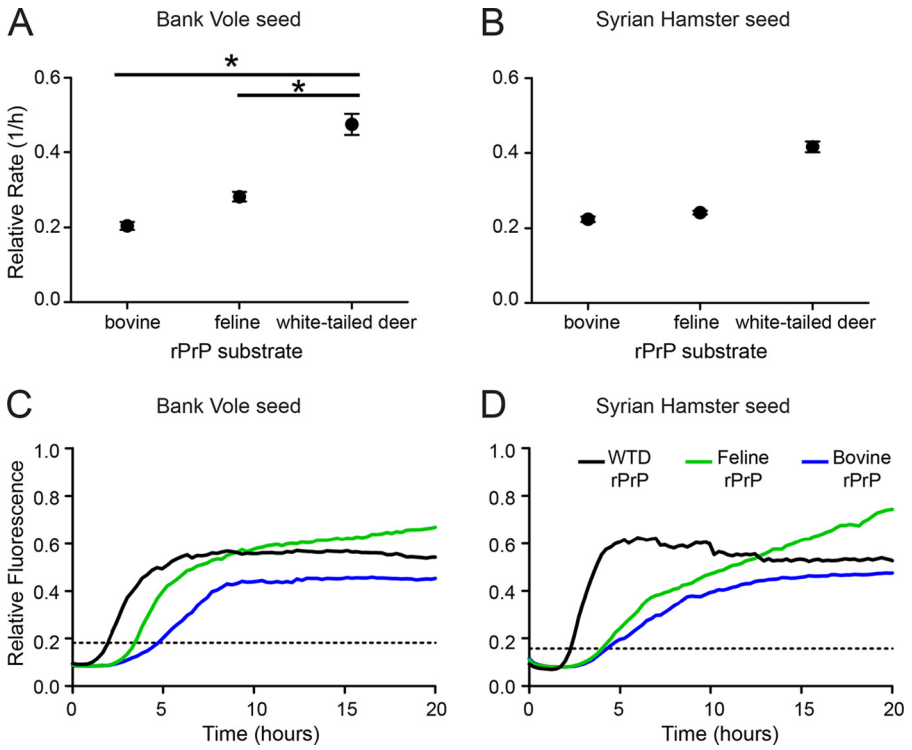

FIG 2 rPrP substrates have different inherent amyloidogenicity. (A) Points indicate the average rate of amyloid conversion for each substrate (bovine, feline, and white-tailed deer) upon addition of artificial truncated bank vole $rPrP^{ThT+}$. (B) Points indicate the rate of amyloid conversion for each substrate upon the addition of artificial truncated Syrian hamster $rPrP^{ThT+}$. Error bars represent the standard errors of the means (SEM) (A and B). (C and D) Each line represents the average ThT fluorescence for 4 replicates for each seed-substrate combination, and the dotted line indicates the threshold for determination of the lag phase.

we used circular dichroism (CD) spectroscopy (Fig. 1B to E). All substrates had minima at 208 nm and 222 nm, indicating a predominance of α-helical secondary structure, as expected. Each preparation of recombinant protein was analyzed with the homologous species' prion seed in RT-QuIC to verify its ability to form amyloid upon addition of seed. Homologous seeds (e.g., CWD brain samples in full-length white-tailed deer substrate) were considered positive controls, and negative brain samples (deer, bovine, and feline) were negative controls. A batch was considered functional if the wells containing positive-control seeds exhibited ThT fluorescence emission signals that crossed the threshold within 20 h (rate, $\geq 1/20$, 0.05 h^{-1}) and if the negative controls had an average rate less than 0.015 h^{-1}. By these metrics, we determined that all batches of rPrP were comparable and that seed behavior could consequently be compared among substrates.

rPrP species differ in inherent propensity for conversion, as reflected by the rate of amyloid conversion. Despite their similar purity, conformation, and functionality, the rPrP substrates did not have an equal propensity to convert to an amyloid conformation (amyloidogenicity), which may reflect inherent features from each species, as multiple purified batches of rPrP had the same behavior. With a variety of prion seeds (CWD, BSE, fCWD, FSE), the highest rate of amyloid conversion for full-length white-tailed deer rPrP (0.27 h^{-1}) was nearly twice the highest rate for full-length bovine (0.13 h^{-1}) or feline (0.17 h^{-1}) rPrP. We assessed the inverse of the lag phase, the rate of amyloid conversion, in order to include samples that never become positive (which were conservatively assigned a rate of 0).

To verify the observed differences among substrates, we used artificial seeds ($rPrP^{ThT+}$ created by shaking $rPrP^{C}$ with guanidine hydrochloride at 37°C) and compared the rates of amyloid conversion. Mimicking our observation with brain-derived prion seeds, white-tailed deer rPrP converted the fastest with the addition of truncated bank vole $rPrP^{ThT+}$, with a rate approximately twice the rate of bovine and feline rPrP conversion (Fig. 2A). We observed the same pattern when the seed was prepared from truncated Syrian hamster rPrP (Fig. 2B). The same trends were visualized with representative raw data (Fig. 2C and D). These experiments with artificial seeds confirmed observations in experiments with natural seeds, demonstrating the inherently different propensities for amyloid conversion in different species. In order to compare the behavior of a given seed among species with various levels of amyloidogenicity, we normalized the rates by considering the maximum rate in each substrate to be 1.0 h^{-1}.

RT-QuIC using full-length rPrP recapitulates BSE and CWD species barriers *in vitro*. We hypothesized that real-time conversion would reflect *in vivo* species barriers, as defined by preferential seeding of the native host species rPrP. By using quantitative RT-QuIC analysis, we found the native prion of a given species to be most compatible (the fastest conversion to amyloid) with its host substrate. To demonstrate an *in vitro* species barrier, we added cBSE or CWD PrP^{Res} to the nonhomologous rPrP substrate. As anticipated, CWD converted full-length white-tailed deer rPrP faster than it did full-length bovine rPrP (Fig. 3A). Likewise, cBSE seeded full-length bovine rPrP relatively faster than full-length white-tailed deer rPrP (Fig. 3B). Thus, RT-QuIC recapitulated the *in vivo* species-seeding proclivities of CWD and cBSE prions.

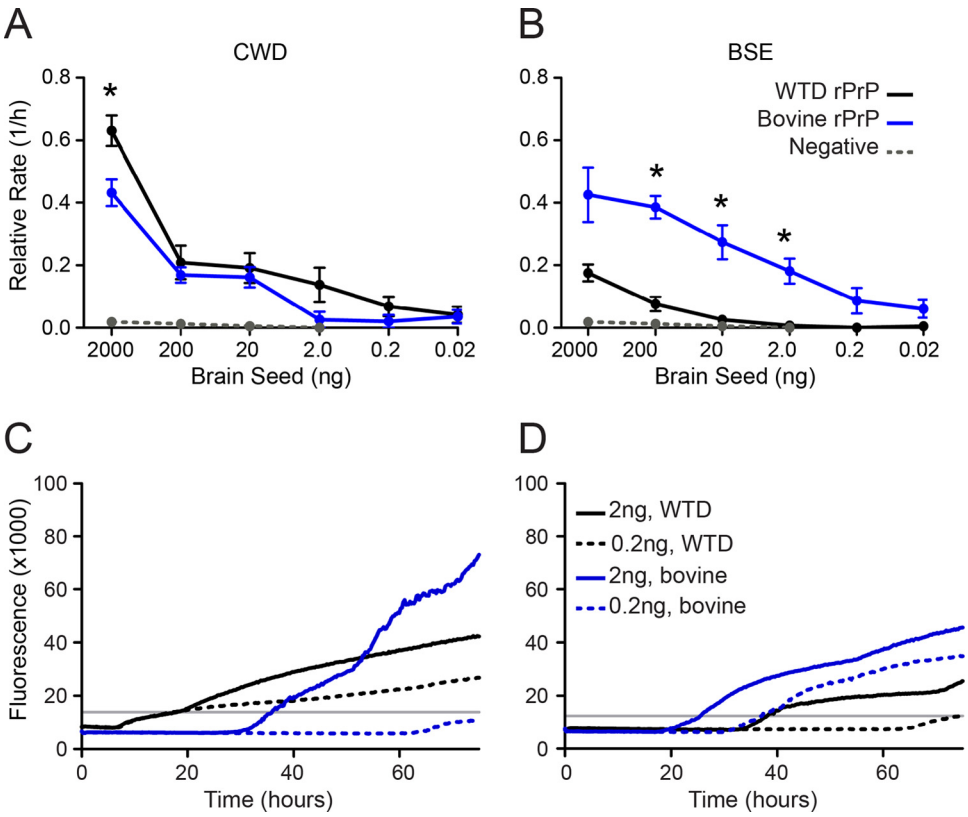

FIG 3 Preference for intraspecies conversion by BSE and CWD prions is recapitulated in RT-QuIC. (A) CWD was seeded into white-tailed deer rPrP (black line) or bovine rPrP (blue line). (B) BSE is seeded into white-tailed deer (WTD) rPrP (black line) or bovine rPrP (blue line). Each point represents the relative rate of amyloid conversion for each seed concentration (in nanograms), and the error bars represent SEM. The rate of spontaneous amyloid conversion is designated by the dotted gray line in panels A and B. Significant differences between substrates were tested by the MWU test (*, $P < 0.05$). (C and D) Each line represents the average ThT trace for 6 replicates of 3 dilutions of each seed-substrate combination, and the gray line indicates the threshold for determination of the lag phase.

Enciphering characteristics of cBSE and cBSE-derived prions are conserved after transspecies transmission. cBSE and CWD are prion diseases that have been naturally passaged in their respective species (cattle and deer), whereas feline spongiform encephalopathy (FSE) and feline chronic wasting disease (fCWD) are first-passage infections in a new host species (cat). To investigate the biochemical properties of cBSE and CWD after transspecies transmission to felines, we compared the amyloidogenicity of fCWD and FSE in the original host and in feline substrate. We found fCWD to be a more efficient seed for its new (feline) host, suggesting that adaptation to the new host had occurred (Fig. 4A). In contrast, FSE remained a more efficient seed for its enciphering (bovine) host, despite its derivation from feline brain PrP^C (Fig. 4B). Thereby, these cross-species seeding experiments in RT-QuIC indicated that the characteristics of cBSE were maintained upon passage to a new species whereas CWD had adapted to its new host. These findings in felids suggest that cBSE may retain its ability to cross species barriers even after transmission to a new host species and that CWD may change substantially upon transspecies transmission.

Human $rPrP^C$ can be converted by bovine, feline, and cervid prions. The threat of zoonotic transmission of prion disease is evident and well documented, yet such transmission is uncommonly observed and incompletely understood. We thereby explored the propensity of heterologous prions to convert human rPrP. In these human $rPrP^C$ experiments, we used sporadic CJD brain as a positive control and normal bovine, white-tailed deer, and feline brain as negative controls. sCJD, as expected, seeded human $rPrP^C$ most efficiently, so all other seeds were normalized to the rate of conversion of sCJD. We found human $rPrP^C$ to be a competent substrate in RT-QuIC for CWD, fCWD, cBSE, and FSE (Fig. 5A). Interestingly, CWD and fCWD converted human $rPrP^C$ more efficiently than did cBSE and FSE. These data suggest that at the level of PrP^C-PrP seed interaction, CWD has the ability to template the conversion of human $rPrP^C$ to ThT-positive amyloid. In order to assess whether CWD was faster than cBSE due to an increased concentration of prion seed, we performed Western blotting on the seed inocula. Western blots indicated that the cBSE sample had a higher concentration of PrP^{Res} than the CWD sample, indicating that CWD was not a better seed than cBSE due to PrP^{Res} content (Fig. 5B). Finally, we assessed the behavior of 8 CWD field isolates, brain samples from white-tailed deer infected naturally and verified to be positive using full-length white-tailed deer RT-QuIC (Fig. 5C). All 8 of these isolates converted human $rPrP^C$, confirming that our observations were not due to the use of experimentally CWD (Fig. 5D). In all, these experiments suggest that the CWD prions naturally circulating in the western United States have the capacity to convert human $rPrP^C$ in this assay of protein-protein interactions.

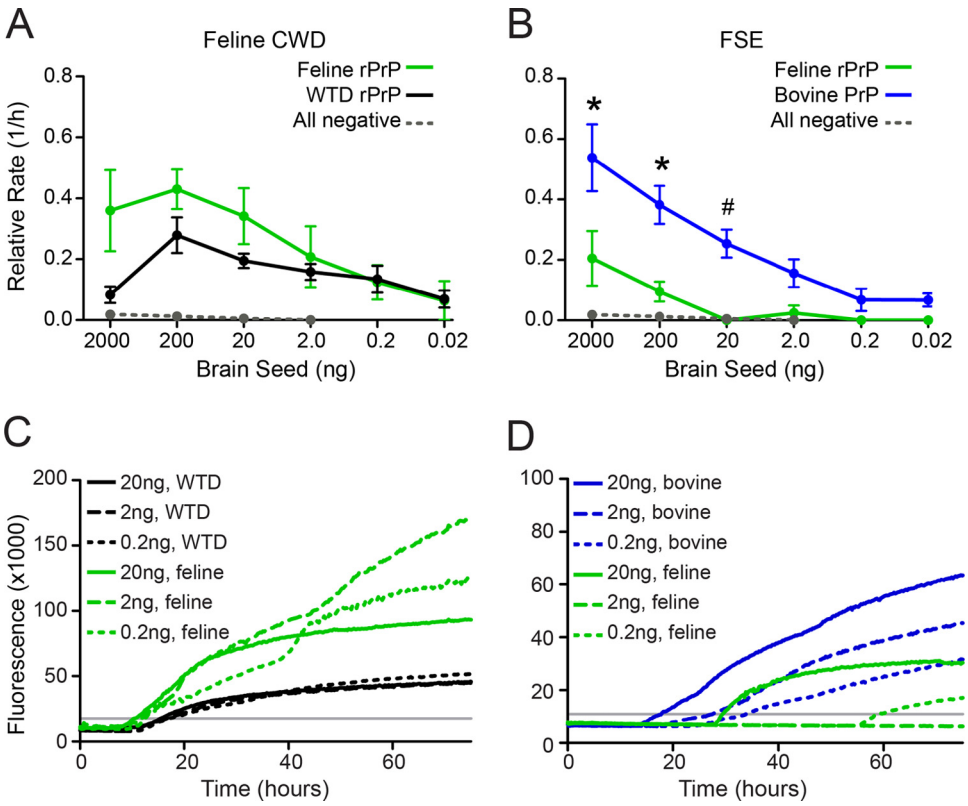

FIG 4 BSE and CWD prions passed into felines demonstrate either (i) maintenance of original species characteristics (BSE) or (ii) adaptation to the new species (CWD). (A) Points indicate the average rate of amyloid conversion for white-tailed deer rPrP (black line) or feline rPrP (green line) upon seeding with an fCWD seed. (B) Points indicate the rate of amyloid conversion for bovine rPrP (blue line) or feline rPrP (green line) upon addition of an FSE seed. The rate of spontaneous amyloid conversion is designated by the dotted gray line, and error bars represent SEM. Significant differences between substrates were tested by the MWU test (*, $P < 0.05$) or the WSR test (#, $P < 0.05$). (C, D) Each line represents the average ThT trace for 6 replicates of 3 dilutions of each seed-substrate combination, and the gray line indicates the threshold for determination of the lag phase.

DISCUSSION

Despite decades of investigation, a complete characterization of barriers to transspecies transmission of prion diseases remains elusive. Many animals, including multiple lines of PrP transgenic mice, have been inoculated with various prions to define prion disease species barriers and understand the effects of passage into a new host. Likewise, *in vitro* assays have been used to model propensities for transspecies PrP^C conversion. We have employed RT-QuIC since it permits observation of amyloid conversion in real time and, consequently, comparison of the conversion efficiency of seed (PrP^{Sc})-substrate ($rPrP^C$) combinations. Though RT-QuIC, as performed here, likely produced noninfectious amyloid products, we believe that RT-QuIC offers valuable insight regarding the efficiency of the initial amyloid conversion of a substrate by a seed.

We report that cBSE, after passage to felids in the form of FSE, remained a more efficient seed for the prion protein of the enciphering (bovine) host than for the new (feline) host (Fig. 4). This pattern has been observed in other contexts as well: cBSE-derived prions maintain many of their characteristics upon experimental or natural transmission to a new species (32, 33). We show here that these features are maintained as a result of the conformation of cBSE prions and not based solely on cellular cofactors. Conversely, fCWD was a more efficient seed for the new (feline) host than for the enciphering (white-tailed deer) host (Fig. 4). This suggests that when felids are infected with CWD, the resulting feline CWD has adapted to the new host. This appears to be an example of the difference between prions that adapt to new hosts upon passage and amplification that occurs without adaptation (J. Bian, V. Khaychuck, K. Bowling, K. Angers, N. Fernandez-Borges, E. Vidal, C. Meyerett-Reid, S. Kim, C. Calvi, J. Bartz, E. Hoover, U. Agrimi, J. Richt, J. Castilla, and G. C. Telling, submitted for publication) It would be interesting to test additional passaged BSE and CWD samples, particularly in light of the evidence for prion strains, but these samples are rare. It is important to note that the behavior of the fCWD and FSE in this paper may be dependent on the CWD and BSE that infected the cats.

We also assessed the seeded conversion of human $rPrP^C$ by BSE, CWD, FSE, and fCWD. Previous *in vitro* work using protein misfolding cyclic amplification (PMCA) and two seeded fibrillization assays found human (polymorphism M129) PrP^C to be a weakly competent substrate for conversion by CWD and cBSE (25, 28, 29). We demonstrate that both FSE and fCWD have the ability to seed human $rPrP^C$ as well. Our finding that cBSE was a poor, if not ineffective, seed for human $rPrP^C$ in RT-QuIC was also observed by Orrú et al. (35). In contrast, our finding that CWD is an efficient seed for human $rPrP^C$ (albeit not as efficient as human sCJD) differs from previous results using PMCA or other seeded fibrillization assays (25, 28, 29). Perhaps the disparities between these *in vitro* assays reflect that RT-QuIC measures the

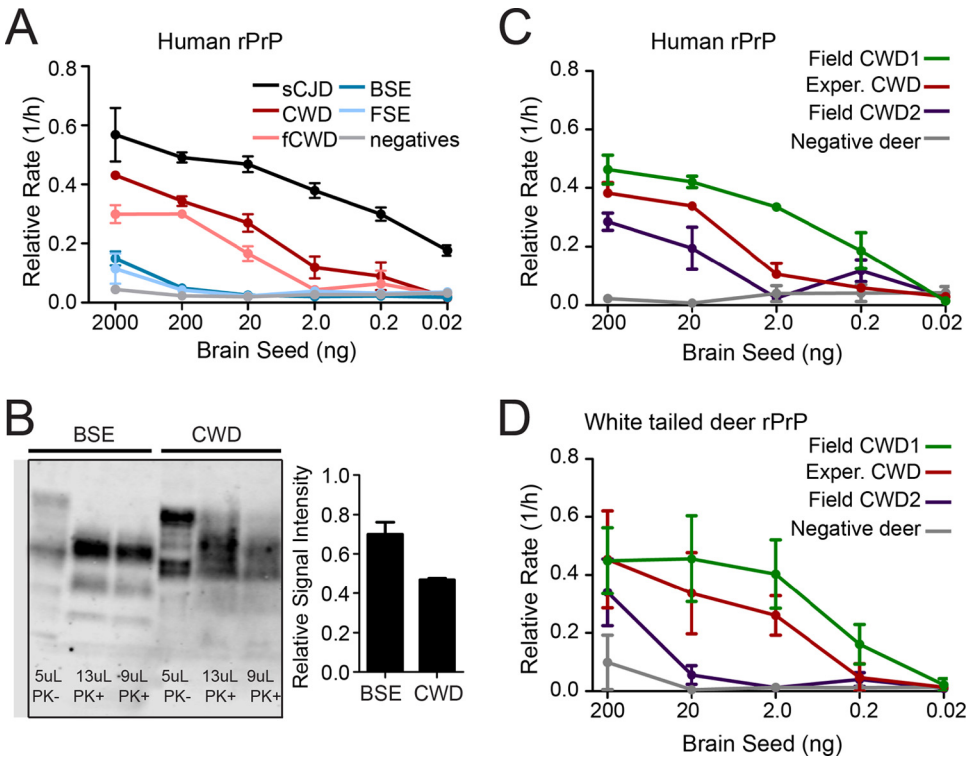

FIG 5 CWD is capable of efficiently seeding the conversion of human rPrP^{C}. (A) Points represent the average rate of conversion of full-length human M129 rPrP by sporadic CJD (black line), CWD (red line), fCWD (pink line), BSE (dark blue line), and FSE (light blue line). Error bars indicate SEM. The rate of spontaneous amyloid conversion is indicated by the gray line. (B) Western blot of BSE and CWD. PK-digested lanes indicate the presence of more PrP^{Res} in the BSE sample than in the CWD sample. Densitometry indicates that BSE has more PrP^{Res}/microliter relative to total undigested PrP/microliter than does CWD. (C) Points indicate the average rate of amyloid conversion for multiple field isolates of CWD in full-length human M129 rPrP^{C}. The gray line indicates the rate of spontaneous amyloid conversion. (D) Points indicate the average rate of amyloid conversion for multiple field isolates of CWD in full-length white-tailed deer rPrP^{C}. The gray line indicates the rate of spontaneous amyloid conversion.

rate of amyloid conversion (indicating the initial transspecies seeding) versus total PrP^{Res} after conversion (25, 26, 28–30). We understand the rate of amyloid conversion to depend on both the quantity of prions in the seed and the competence of the seed to convert the substrate (34). Because our cBSE brain seed had a higher concentration of PrP^{Res} relative to the total PrP than did our CWD brain sample, we interpreted the increased rate of amyloid conversion in human rPrP^{C} to reflect the relative compatibility of seed with substrate. Indeed, our analysis supports the notion that human rPrP^{C} is a competent substrate for other several nonhuman prions. Of course, we also understand that any *in vitro* estimation of prion species barriers carries the innate caveats of a reductionist model of complex *in vivo* processes.

In summary, real-time conversion demonstrates that CWD and BSE prions differ in their enciphering rigidity and plasticity across species barriers. One illustration is the conservation versus adaptation of enciphering prion characteristics upon passage to cats. These experiments also demonstrate that human rPrP can be converted to amyloid by both cBSE and CWD prions. These data point to the importance of deciphering the mechanisms by which prions infect and adapt to a new species and of prompt continued vigilance regarding indirect pathways that may facilitate transspecies prion transmission.

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