The effects of prion protein proteolysis and disaggregation on the strain properties of hamster scrapie

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Native mammalian prions exist in self-propagating strains that exhibit distinctive clinical, pathological and biochemical characteristics. Prion strain diversity is associated with variations in PrPSc conformation, but it remains unknown precisely which physical properties of the PrPSc molecules are required to encipher mammalian prion strain phenotypes. In this study, we subjected prion-infected brain homogenates derived from three different hamster scrapie strains to either (i) proteinase K digestion or (ii) sonication, and inoculated the modified samples into normal hamsters. The results show that the strain-specific clinical features and neuropathological profiles of inoculated animals were not affected by either treatment. Similarly, the strain-dependent biochemical characteristics of the PrPSc molecules (including electrophoretic mobility, glycoform composition, conformational stability and susceptibility to protease digestion) in infected animals were unaffected by either proteolysis or sonication of the original inocula. These results indicate that the infectious strain properties of native prions do not appear to be altered by PrPSc disaggregation, and that maintenance of such properties does not require the N-domain (approximately residues 23–90) of the protease-resistant PrPSc molecules or protease-sensitive PrPSc molecules.

INTRODUCTION

Prion diseases are fatal neurodegenerative illnesses that occur in genetic, sporadic and infectious forms (Glatzel et al., 2005). From a public health perspective, prion diseases are challenging to control because infectious prions are highly resistant to environmental degradation (Brown & Gajdusek, 1991) and can potentially be transmitted by several different routes (Holada et al., 2000; Ligios et al., 2005; Mathiason et al., 2006; Seeger et al., 2005). The critical molecular event in the pathogenesis of prion diseases is the misfolding of the host-encoded prion protein (PrPC) into an infectious isoform (PrPSc), but the mechanism of this conformational change remains unknown (Prusiner, 1982). Mature PrP molecules contain 208 aa (residues 23–231), two N-linked glycosylation sites, an intramolecular disulfide bond and a C-terminal glycosylphosphatidylinositol anchor (Endo et al., 1989; Locht et al., 1986; Stahl et al., 1987; Turk et al., 1988). Recently, purified native PrPC molecules containing only prion protein and co-purified lipids have been converted into infectious PrPSc molecules de novo, through an in vitro reaction requiring accessory polyanions (Deleault et al., 2007).

Interestingly, mammalian prions occur in a variety of different ‘strains’. Strains are defined as natural isolates of infectious prions characterized by distinctive clinical and neuropathological features, which are faithfully recapitulated upon serial passage within the same animal species (Bruce, 1993; Carlson, 1996). Strain diversity is associated with variations in PrPSc conformation (Bessen & Marsh, 1992; Collinge et al., 1996; Peretz et al., 2001; Safar et al., 1998; Telling et al., 1996), but it remains unknown precisely which PrPSc conformers or domains are required to encode mammalian prion strain phenotypes.

Studies using a conformation-dependent immunoassay (CDI) technique have shown that prion-infected brains contain both protease-sensitive and protease-resistant PrPSc molecules (denoted sPrPSc and rPrPSc, respectively), and the ratio of sPrPSc:rPrPSc molecules generated in
infected animals is characteristic for each prion strain (Safar et al., 1998). These observations have raised the possibility that sPrPSc molecules may be essential for encoding the strain properties of mammalian prions. In addition, other studies have demonstrated that a variety of physical and chemical treatments alter the strain-dependent relationship between infectious titre and incubation time (Dickinson & Fraser, 1969; Somerville & Carp, 1983; Taylor & Fernie, 1996), raising the possibility that such treatments might also modify other strain properties, possibly by disrupting PrPSc aggregates.

Currently, the molecular structure of PrPSc molecules is unknown. Protease digestion experiments indicate that approximately 70 N-terminal amino acids of the rPrPSc molecules are accessible to proteolytic enzymes, and that the precise length of the protease-sensitive domain varies between different prion strains (Bessen & Marsh, 1992). It is not known whether this externally accessible domain might play a critical role in encoding strain properties.

Here, we performed a series of experiments with native mammalian prions to investigate the physical basis of prion strain variation in greater depth. Specifically, we studied whether this externally accessible domain is not known whether this externally accessible domain is accessible to proteolytic enzymes, and that the precise length of the protease-sensitive domain varies between different prion strains (Bessen & Marsh, 1992). It is not known whether this externally accessible domain might play a critical role in encoding strain properties.

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METHODS

Preparation of brain homogenates. All procedures were done at 4 °C. To avoid cross-contamination of various prion strains, all work was done in laminar flow biosafety cabinets, using disposable surface liners and aerosol barrier tips. Between each brain homogenate and inoculum preparation, safety cabinets were disinfected using 10 % acetic acid (Peretz et al., 2006). Inocula were prepared by adding 50 μl each sample (adjusted so that each group had similar concentrations of PK-resistant PrP as determined by Western blotting) to 450 μl diluent containing sterile PBS plus 1 mg BSA ml⁻¹.

Scrapie inoculation and diagnosis. Three-week-old female Syrian golden hamsters were injected with 50 μl of each inoculum using 28G disposable hypodermic needles inserted into the parietal lobe. After inoculation, hamsters were examined daily for neurological dysfunction, and standard diagnostic criteria were used to identify animals exhibiting signs of scrapie (Fraser & Dickinson, 1967). Animals at the end stage of disease were killed, and their brains were removed for histological and biochemical analyses.

PrPSc detection. Unless otherwise specified, protease-digested (+PK) samples were incubated with 50 μg PK ml⁻¹ for 1 h at 37 °C. An equal volume of 2 × SDS sample buffer was then added, and samples were boiled for 10 min at 95 °C. SDS-PAGE was performed on 1.5 mm 12 % polyacrylamide gels with an acrylamide/bisacrylamide ratio of 29:1 (Bio-Rad). After electrophoresis, the proteins were transferred to a methanol-charged, buffer-equilibrated PVDF membrane (Millipore) by using a Transblot SD SemiDry Transfer Cell (Bio-Rad) set at 2 mA cm⁻² for 30 min.

To visualize PrP signals, Western blot membranes were first treated with 3 M GdnSCN (Roche) at room temperature for 30 min. Membranes were then rinsed with TBST (10 mM Tris pH 7.2, 150 mM NaCl, 0.1 % Tween 20) and blocked for 1 h in skimmed milk buffered with TBST. The blocked membrane was incubated overnight at 4 °C with 3F4 monoclonal antibody (Signet Laboratories) diluted 1:5000 in TBST. After this incubation, the membrane was washed three times for 10 min in TBST and incubated for 1 h at 4 °C with horseradish peroxidase-labelled anti-mouse IgG secondary antibody conjugate (GE Healthcare) diluted 1:5000 in TBST. The membrane was washed four times for 10 min with TBST. The blot was developed by using West Femto chemiluminescence substrate (Pierce), sealed in plastic covers, and captured digitally by using a Fuji (Fujifilm) LAS-3000 chemiluminescence documentation system. Digital images were captured using Image Reader version 2.0 (Fujifilm). Relative molecular masses were based on migration of pre-stained standards from either Fermentas or Bio-Rad.

Enzymic deglycosylation. To deglycosylate PrPSc molecules, 50 μl of each sample was incubated with 40 μl PK in PBS, 1 % (final concentration of 10 μg ml⁻¹) for 30 min at 37 °C. A stock solution of 0.3 M PMSF dissolved in ethanol was added to each sample to achieve a final concentration of 1 mM. Samples were then diluted to a final

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volume of 750 µl containing PBS and 10% Sarkosyl (Sigma), and centrifuged at 100 000 g for 1 h at 4 °C. Supernatants were removed and pellets were resuspended in 100 µl 1x glycoprotein denaturing buffer (New England Biolabs) and boiled at 95 °C for 10 min. After boiling, 10 x G7 reaction buffer and 10% NP-40 (both from New England Biolabs) were added to a final concentration of 1 x and 1%, respectively. Next, 3 µl PNGase F enzyme or storage buffer (20 mM Tris/HCl pH 7.5, 50 mM NaCl, 5 mM EDTA) was added to tubes and samples were incubated at 37 °C for 2 h. Reactions were stopped by boiling samples in SDS sample buffer.

**Protease resistance assay.** Three scrapie-infected brains from each group were homogenized as described above and pooled for use in protease resistance and guanidine denaturation assays. Semi-quantitative Western blots were run to estimate the amount of PrPSc present in each brain pool, and samples were adjusted to normalize PrPSc concentration by using 10% Prnp0/0 brain homogenate as a diluent.

To determine protease resistance, normalized homogenates were digested for 1 h at 37 °C with increasing amounts of PK. The following concentrations were used: 0.316, 1, 3.16, 10, 31.6, 100, 316 and 1000 µg ml⁻¹. Digestion was terminated by boiling samples in SDS sample buffer. Following SDS-PAGE and Western blotting, PrP signals for samples in each group were normalized against the signal obtained at 0.316 µg ml⁻¹.

**Guanidine denaturation assay.** Guanidine denaturation assays were performed as described previously (Deleault et al., 2007). All centrifugation was done at 4 °C. Briefly, samples containing 40 µl normalized brain homogenate were brought up to 100 µl with 10% Triton X-100 and varying concentrations of GdnHCl to achieve a final concentration of 1% Triton X-100 and [GdnHCl] from 1–4 M (note that GdnHCl treatments started at 1 M due to inconsistencies of PrPSc recovery at lower concentrations). Samples were incubated for 90 min at 37 °C at 800 r.p.m. in an Eppendorf Thermomixer (Fisher), and 800 µl dilution buffer [10 mM Tris pH 8.0, 150 mM NaCl, 0.5% NP-40, 0.5% DOC (Sigma)] was added. The final GdnHCl concentration of each sample was adjusted to 0.4 M by adding 50 µl water or solutions containing varying concentrations of GdnHCl. Samples were then digested with 20 µg PK ml⁻¹ as described above, and PMSF (dissolved in 100% ethanol) was added to a final concentration of 1 mM to inactivate PK. Samples were then centrifuged at 100 000 g for 30 min. Supernatants were removed and pellets were resuspended in 1 ml dilution buffer. Samples were then vortexed, and centrifuged at 100 000 g for 20 min. Supernatants were removed, and the pellets were resuspended in 100 µl dilution buffer. An equal volume of 2x SDS sample buffer was added, and samples were boiled for 10 min. Following SDS-PAGE and Western blotting, PrP signals for samples in each group were normalized against the signal obtained at 1 M GdnHCl.

**PrPSc aggregation assay.** Samples were prepared by incubating 20 µl 10% (w/v) scrapie-infected brain homogenates with 0.65 ml PBS, 1% N-lauryl sarcosine with shaking at 800 r.p.m. at 37 °C for 20 min. Duplicate aliquots containing 0.3 ml were mixed with 0.7 ml PBS, 1% Triton X-100 and centrifuged at 100 000 g at 4 °C for 1 h. Supernatant fractions were discarded, the pellet was washed with 1 ml PBS, 1% Triton X-100, resuspended in 0.7 ml Triton X-100, and then either shaken at 800 r.p.m. at 37 °C for 1 h in an Eppendorf Thermomixer (control samples) or subjected to three times 30 s bursts of sonication separated by two times 30 min incubations in a Misonix 3000-MPD programmable sonicator (sonicated samples). Following each respective treatment, samples were vortexed vigorously for 20 s and layered over a 0.3 ml cushion of 30% sucrose, and centrifuged at 10 000 g for 30 min. Supernatant fractions were collected and precipitated with sodium phosphotungstic acid as described previously (Safar et al., 1998). Pellet fractions were resuspended by boiling in 60 µl SDS sample buffer for 10 min. For each sample, we calculated the per cent of PrPSc in the supernatant vs Western blot signal of PrPSc in supernatant/(PrPSc in supernatant + PrPSc in pellet).

**Quantification and statistical analyses of biochemical assays.** Digitally captured images of Western blots were subjected to densitometric analysis using the program Image Gauge version 4.22 (Fuji). For each condition, replicates were performed (n=3) and values were averaged. Mean values generated for the PK and GdnHCl curves were best fitted using the two parameter exponential decay equation and three parameter sigmoidal equation, respectively, by using the program SigmaPlot version 10.0 (Systat Software). Error bars indicate standard error of the mean (SEM).

**Neuropathology and statistical analyses.** Brains were removed at the time of sacrifice by using new, sterile-packaged dissection instruments and disposable surfaces to avoid cross-contamination. They were fixed by immersion in 10% buffered formalin for 1–2 days, cut into approximately 3 mm thick coronal sections, and placed in a tissue-processing cassette. Cassettes were treated with 88% formic acid for 1–1.5 h to disintegrate, and then switched back into 10% formalin for 1–3 days. The tissue was processed for paraffin embedding, and representative slides were stained with haematoxylin and eosin. Slides from adjacent sections were also prepared for antigen retrieval (Citraplus (Biogenex), pH 6.23 and pressure steaming to 126°C/16 p.s.i. (110.4 KPa) followed by immunostaining with 3F4 anti-PrP monoclonal antibody (DakoCytomation or Signet, final working concentrations 1 µg ml⁻¹) using a Biogenex 6000 automated stainer. Immunodetection was visualized using HRP/DAB MultiLink detection kit (Biogenex) following the manufacturer’s recommended protocol.

A single neuropathologist (B. Harris), who was blind to the identities of both experimental groups and the overall design of the study, examined all of the slides, which were labelled with arbitrary six digit numbers. Each brain was scored for a degree of vacuolation in five brain regions: frontal cortex, parietal cortex, hippocampus CA1/2, cerebellum and medulla. Scoring was done using ordinal variables as described previously (Deleault et al., 2007): 0, no lesions; 0.5, minimum vacuolation (2–3 vacuoles in half a x 40 objective field); 1.0, light vacuolation (3–5 vacuoles in half a field); 2.0, moderate vacuolation (several vacuoles evenly scattered); 3.0, extensive vacuolation (many vacuoles distributed in half a field); 4.0, severe vacuolation (numerous vacuoles often coalescing). 3F4 immunostaining was scored as follows in the same brain regions: 0, no deposition; 1.0, minimal deposition; 2.0, moderate deposition; 3.0, extensive deposition.

Data were analysed using Stata 9.0 (Stata Statistical Software: Release 9.0, College Station, TX). For each of the experimental groups, we compared vacuolation and immunohistochemistry characteristics in five brain regions using the non-parametric Mann–Whitney test. For each strain, we compared control versus sonicated and control versus PrPSc-30. We also compared incubation times for inoculation in each strain, we compared control versus sonicated and control versus PrPSc-30. We defined P < 0.05 as statistically significant.

**RESULTS**

We chose to use the hamster scrapie isolates Sc237, 139H and DY as starting materials for this study because PrPSc molecules have been most thoroughly characterized in hamster strains (Safar et al., 1998), and because these
strains have been previously characterized pathologically and biochemically (DeArmond et al., 1993; Peretz et al., 2001). To prepare experimental inocula, we separately subjected brain homogenates from all three strains to either digestion with PK or indirect sonication. To generate PrP27–30 samples, we chose to treat homogenates with 10 μg PK ml\(^{-1}\) at 37 °C for 1 h. For the purposes of this study, we define rPrP\(^{\text{Sc}}\) molecules as PrP\(^{\text{Sc}}\) molecules that resist proteolysis with 10 μg PK ml\(^{-1}\) at 37 °C for 1 h, and sPrP\(^{\text{Sc}}\) molecules as those that are degraded by this treatment. This arbitrary definition is operational; at a molecular level, there may exist a spectrum of PrP\(^{\text{Sc}}\) isoforms that exhibit finer differences in their levels of protease resistance. N-terminal truncation of the PrP27–30 molecules was confirmed by Western blot (Fig. 1), and based on densitometry measurements of PK-resistant bands, samples were subsequently adjusted so that the PrP concentrations in all inocula were similar. The disaggregation of sonicated PrP\(^{\text{Sc}}\) molecules was confirmed by a sucrose-cushion centrifugation assay (Supplementary Fig. S1 available in JGV Online).

Intracerebral inoculation of control and experimental samples into wild-type Syrian hamsters caused scrapie in all groups tested. For all three prion strains, the incubation periods produced by PrP27–30 and sonicated inocula did not differ statistically from those produced by control inocula (Table 1). As expected, hamsters inoculated with DY samples had incubation periods approximately twice as long as animals inoculated with 139H or Sc237 samples (Table 1).

The clinical signs displayed by hamsters inoculated with PrP27–30 or sonicated inocula were indistinguishable from the signs displayed by animals inoculated with the control inoculum for each strain. Specifically, all pre-terminal DY-inoculated animals displayed marked lethargy and diminished responsiveness to various stimuli, while all pre-terminal hamsters inoculated with Sc237 or 139H samples displayed ataxic gait, circling movements, trembling and hyper-responsiveness.

The strain-dependent regional distribution of neuropathological changes in scrapie-infected hamsters was measured by double blind scoring for vacuolation (Fig. 2a) and PrP immunohistochemistry (Fig. 2b). The results of these analyses show that the PrP27–30 and sonicated inocula produced patterns of vacuolation and PrP deposition that were very similar to the patterns produced by control inocula for all three strains (Fig. 2). We next performed a series of comparisons using the non-parametric Mann–Whitney test to determine whether any statistically significant differences could be identified between the different groups in individual brain regions. Among the 60 separate comparisons made, we found one statistically significant difference, between the cerebellar vacuolation of DY control compared with DY sonicated (\(P=0.04\)). The other nine comparisons between DY control and DY sonicated showed no statistically significant differences. Based on a significance level of \(P=0.05\), we would expect to see three statistically significant differences by chance alone, and we conclude that the difference in cerebellar vacuolation in DY control versus DY sonicated was most probably due to chance variation.

Next, we characterized the biochemical properties of PrP\(^{\text{Sc}}\) molecules present in the brains of scrapie-infected animals from each group. Western blotting revealed no differences in the glycoform distribution or electrophoretic mobility of PrP\(^{\text{Sc}}\) molecules produced by control and experimental inocula for all three strains (Fig. 3a). The relative electrophoretic mobility of PrP\(^{\text{Sc}}\) molecules was also examined with increased resolution by enzymic deglycosy-

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**Fig. 1.** Western blot analysis of brain homogenate samples inoculated into Syrian hamsters. Three different scrapie strains were used to generate samples: DY, 139H and Sc237. Control, PrP27–30 and sonicated samples were generated as described in Methods.

**Table 1.** Scrapie transmission data

<table>
<thead>
<tr>
<th>Strain</th>
<th>Inoculum</th>
<th>n/(n_o)</th>
<th>IP*</th>
<th>P value†</th>
</tr>
</thead>
<tbody>
<tr>
<td>DY</td>
<td>Control</td>
<td>6/7</td>
<td>259 ± 59</td>
<td>Ref</td>
</tr>
<tr>
<td></td>
<td>PrP27–30</td>
<td>8/8</td>
<td>246 ± 34</td>
<td>0.897</td>
</tr>
<tr>
<td></td>
<td>Sonicated</td>
<td>5/8</td>
<td>219 ± 16</td>
<td>0.518</td>
</tr>
<tr>
<td>139H</td>
<td>Control</td>
<td>7/7</td>
<td>106 ± 12</td>
<td>Ref</td>
</tr>
<tr>
<td></td>
<td>PrP27–30</td>
<td>7/8</td>
<td>150 ± 73†</td>
<td>0.158</td>
</tr>
<tr>
<td></td>
<td>Sonicated</td>
<td>7/7</td>
<td>110 ± 14</td>
<td>0.700</td>
</tr>
<tr>
<td>Sc237</td>
<td>Control</td>
<td>8/8</td>
<td>106 ± 26</td>
<td>Ref</td>
</tr>
<tr>
<td></td>
<td>PrP27–30</td>
<td>7/8</td>
<td>110 ± 21</td>
<td>0.559</td>
</tr>
<tr>
<td></td>
<td>Sonicated</td>
<td>8/8</td>
<td>116 ± 44</td>
<td>0.787</td>
</tr>
</tbody>
</table>

*Mean incubation period (IP) of scrapie sick animals ± SD.
†Mann–Whitney test comparing incubation period for each inoculum against controls within each strain.
‡The large SD in this group was attributable to two animals with incubation times of 214 and 288 days; excluding these animals, the incubation period of the remaining hamsters in this group was 110 ± 5 days.
lation of samples with PNGase F prior to Western blot. The results confirmed that all DY-derived samples (control, PrP27–30 and sonicated) contained PrPSc molecules with a slightly increased mobility compared with PrPSc molecules from all Sc237- and 139H-derived samples (Fig. 3b). To measure the structural stability of PrPSc molecules in scrapie-infected animals from each group, we performed PrPSc protease resistance (Fig. 4a) and guanidine denaturation assays (Fig. 4b). For all three strains, no statistically significant deviations in the protease resistance or guanidine stability of PrPSc molecules were found between the control, PrP27–30 and the sonicated groups. All DY-derived samples displayed greater susceptibility to protease digestion (Fig. 4a) and guanidine denaturation (Fig. 4b) than all Sc237- and 139H-derived samples. The (Hopkins)1/2 values for the control samples were approximately 1.9, 2.1 and 2.2 M for DY, 139H and Sc237, respectively. This rank order of stability to GdnHCl denaturation (DY<139H<Sc237) mirrored the GdnHCl stability rank order of the same strains previously determined by ELISA (Peretz et al., 2001).

**DISCUSSION**

Many advances in our understanding of prion disease pathogenesis have been gained over the past few decades, especially with regard to the central role of PrP conformational change. Furthermore, studies of fungal prions in model systems have confirmed the basic principle that self-propagating protein structure can encode heritable and infectious phenotypes (reviewed by Wickner et al., 2007). However, the intriguing question as to how specific PrPSc conformers structurally encode strain-specific mammalian prion disease phenotypes currently remains unanswered. The experiments described in this manuscript allow us to...
make several specific conclusions about the physical basis of scrapie strain variation.

**sPrP^{Sc} molecules are not required to maintain strain properties**

Using a CDI, Safar *et al.* (1998) found that native prions are composed of a mixture of sPrP^{Sc} and rPrP^{Sc} molecules. Furthermore, CDI analysis revealed that different strains of native hamster scrapie prions contain different ratios of sPrP^{Sc} : rPrP^{Sc} molecules (Safar *et al.*, 1998). However, the relative contributions of sPrP^{Sc} and rPrP^{Sc} molecules to prion infectivity and strain specificity have remained unknown.

Our results show that proteolytic removal of sPrP^{Sc} molecules does not alter the clinical, pathological or biochemical characteristics of three different strains of hamster prions. In addition, physical disruption of PrP^{Sc} aggregates by sonication also did not affect prion strain properties. Taken together, these results indicate that rPrP^{Sc} molecules are sufficient to encode all the known strain-specific characteristics of mammalian prions. Thus, variations in sPrP^{Sc} : rPrP^{Sc} ratio between different prion strains appear to be a by-product rather than a determinant of strain properties.

It is worth noting that our experiments necessarily defined rPrP^{Sc} and sPrP^{Sc} molecules operationally as infectious prions that are either resistant or sensitive to treatment with 10 μg PK ml{−1} for 1 h, respectively. It is certainly possible that PrP^{Sc} molecules with intermediate levels of protease resistance exist, and additional studies would be needed to study whether such forms might be necessary to maintain prion strain properties. We also advise that our studies do not provide any confirmation or information about the existence, infectious properties or strain-encoding capabilities of sPrP^{Sc} molecules. Recent developments in isolating autocatalytic sPrP^{Sc} molecules should facilitate characterization of this conformer (Pastrana *et al.*, 2006). Studies in prion-infected Tg(MoPrP P101L) mice have shown that high titres of prion infectivity can be generated in the absence of either sPrP^{Sc} or rPrP^{Sc} conformers (Barron *et al.*, 2001, 2007). Interestingly, it was observed that strain-dependent incubation times and neuronal targeting were significantly altered in Tg(MoPrP P101L) mice lacking PrP^{Sc} molecules compared with wild-type mice containing PrP^{Sc} molecules (Barron *et al.*, 2001), suggesting that PrP^{Sc} conformers may be required to maintain strain-specific information. Alternatively, the loss of strain information upon passage in Tg(MoPrP P101L) mice may be attributable to mutation in the primary sequence of PrP, and additional studies will be required to distinguish between these two possibilities.

**The N-terminal domain of PrP^{Sc} molecules is not required to maintain strain properties**

Digestion of rPrP^{Sc} molecules with PK forms truncated PrP27–30 molecules in which approximately 70 N-terminal
residues are removed (Prusiner et al., 1984). In full-length PrP\(^C\), this unstructured, polybasic region contains multiple octapeptide domains, and binds both copper ions and polyanionic molecules (Burns et al., 2003; Jackson et al., 2001; Jones et al., 2005; Viles et al., 1999; Warner et al., 2002). In addition, the extreme N-terminal residues 23–27 (KKRPK) are required for dominant-negative inhibition of PrP\(^S\) formation by mutant PrP\(^C\) molecules in neuroblastoma cells (Zulianello et al., 2000), and for interaction with LDLR1 during endocytosis of PrP\(^S\) (Taylor & Hooper, 2007). Samples containing non-denatured PrP27–30 molecules are known to be infectious (Prusiner et al., 1984), but it was previously unknown whether these truncated molecules can maintain prion strain properties. Our results show, for the first time, that the protease-resistant core of rPrP\(^S\) (i.e. PrP27–30) molecules is sufficient to encipher strain properties, including native patterns of neurotropism. Thus, we can conclude that the accessible approximately 70 N-terminal residues of PrP do not participate in coding hamster scrapie strain properties.

**Disaggregation of PrP\(^S\) molecules by sonication does not alter prion strain properties**

Finally, our results also showed that PrP\(^S\) disaggregation by sonication did not alter the infectious strain properties of the three hamster prion strains tested, despite disrupting PrP\(^S\) aggregates. Thus, we can conclude that strain variation is likely not to be encoded by differences in the quaternary state of PrP\(^S\) aggregates. These results are consistent with previous work showing that the strain properties of hamster 263K prions are not altered by
protein misfolding cyclic amplification, a prion amplification technique that employs intermittent sonication (Castilla et al., 2005).

**Conclusion**

Using animal bioassays, we have formally shown that neither protease digestion nor sonication of three different hamster scrapie isolates alters the infectious strain properties of the treated prions. These results indicate that PrPSc aggregation, sPrPSc molecules and the N terminus of native PrPSc molecules are all unnecessary for the maintenance of prion strain properties in vivo. We conclude that prion strain variation is most likely to be encoded by PrP27–30 molecules, either alone or in conjunction with accessory non-proteinaceous molecules.

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