N-terminal Truncation of Prion Protein Affects Both Formation and Conformation of Abnormal Protease-resistant Prion Protein Generated in Vitro*

Transmissible spongiform encephalopathy (TSE) diseases are a family of fatal neurodegenerative disorders, which affect both humans and animals. These diseases are characterized by conversion of the normal protease-sensitive host prion protein, PrP-sen, to an abnormal protease-resistant form, PrP-res. In the current study, deletions were introduced into the flexible tail of PrP-sen (23–124) to determine if this region was required for formation of PrP-res in a cell-free assay. PrP-res formation was significantly reduced by deletion of residues 34–94 relative to full-length hamster PrP. Deletion of another nineteen amino acids to residue 113 further reduced the amount of PrP-res formed. Furthermore, the presence of additional proteinase K cleavage sites indicated that deletion to residue 113 generated a protease-resistant product with an altered conformation. Conversion of PrP-res deletion mutants was also affected by post-translational modifications to PrP-sen. Conversion of unglycosylated PrP-sen appeared to alter both the amount and the conformation of protease-resistant PrP-res produced from N-terminally truncated PrP-sen. The N-terminal region also affected the ability of hamster PrP to block mouse PrP-res formation in scrapie-infected mouse neuroblastoma cells. Thus, regions within the flexible N-terminal tail of PrP influent interactions required for both generating and disrupting PrP-res formation.

Experimental Procedures

Clones—N-terminal deletion mutants of hamster PrP (HaPrP) were generated from the 5'-end of the gene encoding mouse PrP and the 3'-end of the gene encoding HaPrP cloned into Bluescript KS (+) (Stratagene). Briefly, plasmid 2-17 containing 1.0 kb of HaPrP including the

*The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

†To whom correspondence should be addressed: Laboratory of Persistent Viral Diseases, Rocky Mountain Laboratories, NIAID, National Institutes of Health, Hamilton, Montana 59840

‡The abbreviations used are: TSE, transmissible spongiform encephalopathy; PrP, prion protein; PK, proteinase K; HaPrP, hamster PrP; PrP-sen, protease-sensitive prion protein; PrP-res, protease-resistant prion protein; GPI, glycosphatidylinositol; PAGE, polyacrylamide gel electrophoresis.

Victoria A. Lawson, Suzette A. Priola, Kathy Wehrly, and Bruce Chesebro*†‡

From the Laboratory of Persistent Viral Diseases, Rocky Mountain Laboratories, NIAID, National Institutes of Health, Hamilton, Montana 59840

Received for publication, April 27, 2001, and in revised form, July 18, 2001

Published, JBC Papers in Press, July 20, 2001, DOI 10.1074/jbc.M103799200

Published, JBC Papers in Press, July 20, 2001, DOI 10.1074/jbc.M103799200

This paper is available on line at http://www.jbc.org
OFR (26) was digested with KpnI and NsiI, and a synthetic oligonucleotide polynucleotide encoding restriction enzyme sites KpnI, XhoI, EcoRI, NheI, AgeI, MluI, NsiI was inserted. The resulting plasmid was digested with AgeI and EcoRI, and a 0.2-kb EcoRI-AgeI fragment from the 5′ end of mouse PrP (27) was inserted to create plasmid p22-7. The amino acids of this portion of the PrP was cloned into the 3′ end of the PrP. Deletions were then produced by annealing four to six overlapping oligonucleotide sequences of the upper and lower strand of HaPrP between the AgeI (amino acid residue 34) and NsiI (amino acid residue 138) sites to reconstitute clones deleted between residues 34–94 (Ha3494), 34–115 (Ha1133), 34–120 (Ha120), and 34–124 (Ha124) (sequence requested). The resulting clones were then inserted into p22-7. PrP deletion mutants were sequenced, cloned into the pSSF retroviral packaging cell lines (p22 and PA317) as described previously (28, 29). To create GPI-anchored deletion mutants, clones in Bluescript KS (+) were cloned into pSSFF at BamHI and XhoI sites. To create GPI-negative deletion mutants, the StuI to Xhol region of each clone in Blue script was replaced with the StuI/XhoI fragment of a mouse PrP clone that lacked the GPI signal sequence in the 3′-untranslated region and that shared sequence homology with hamster PrP. The resulting clones were subcloned into pSSFF as described above. GPI-positive and GPI-negative wild type PrP (HaWT) clones have been described previously (19, 29). The pSSFF vector was used as a negative control for the immunoprecipitated protein used in the cell-free assays and for transduction of scrapie-infected mouse neuroblastoma (Sc′+N2a) cells.

Labeling and Purification of PrP-sen—The 35S labeling and purification of PrP-sen was performed as described previously (23). Briefly, a 25-cm² flask of 70–90% confluent cells was washed and preincubated in 1.5 ml of methionine/cysteine-free RPMI (ICN), supplemented with 1% dialyzed FBS and glucose at 36°C for 30 min. The cells were labeled with 1 mCi of [35S]methionine/cysteine (PerkinElmer Life Sciences) in the presence or absence of tunicamycin (Roche Molecular Biochemicals; 10 µg/ml) for 2 hours at 37°C. The monolayer was washed, lysed (0.5% Triton X-100, 0.5% sodium deoxycholate, 150 mM NaCl, 0.05 mg Tris-HCl, pH 7.4, 7.0, 0.008 m EDTA), and methanol-precipitated at −20°C. The resulting protein precipitate was sonicated into DLPC (4.2 mg/ml α-phosphatidyethanolamine, 50 mM Tris-HCl, pH 7.5, 2% sarkosyl in normal saline) and incubated overnight at 4°C with anti-PrP antibodies. HaWT and Ha3494 were precipitated with the hamster PrP-specific monoclonal antibody 3F4 (30, and Ha1133, Ha120, and Ha124 were precipitated with the C-terminal rabbit anti-peptide polyclonal antibody R20 (31). After incubation with protein A-Sepharose for 2 hours at 4°C in 4°C, the immunoprecipitated protein was eluted with 0.1 M acetic acid at 4°C. GPI-negative PrP was precipitated from the supernatant of 35S-labeled p22PA317 cells after the addition of 0.5% Nonidet P-40, 10 mg EDTA, 20 mg Tris-HCl, pH 7.4. PrP-sen expression in Sc′+N2a cells was detected by radiolabelling of a 25-cm² flask with 0.1 mCi of 35S. The final preparation of protein A-Sepharose-bound protein was resuspended in 20 µl of sample buffer (62.5 mM Tris-HCl, pH 6.8, 5% glycerol, 3 mM β-mercaptoethanol, 0.02% bromophenol blue, 4% SDS-PAGE gel) and run on a 16% SDS-PAGE gel (NOVEX).

Cell-free Conversion Assay—Cell-free conversion of PrP-sen to PrP-res was performed previously (19). Briefly, 200 ng of PrP-sen, purified from the brains of hamsters infected with the 263K strain as described previously (10), was pretreated for 1 hour at 37°C in 2.5 mM guanidine hydrochloride and then incubated with 25,000 cpm of 35S-labeled PrP-sen in conversion buffer (0.75 mM guanidine hydrochloride, 50 mM sodium citrate buffer, pH 6.0, 5 mM cysteiydylamin chloride, 1.2% sarkosyl) at 37°C for 48 hours. After incubation, one-tenth of the reaction was precipitated in methanol, and the remaining nine-tenths was treated with 80 µl of PK (12 µg/ml) for 1 hour at 37°C. These samples represented PK- and PK+ samples, respectively. PK digestion was stopped by the addition of thyroglobin and Pefabloc (Roche Molecular Biochemicals) and precipitated with 4 volumes of cold methanol. The resulting precipitate was resuspended in sample buffer and run on a 16% SDS-PAGE gel. The PK– and PK+ samples were quantified by phosphor autoradiographic image analysis of the dried gel (Molecular Dynamics Storm PhosphorImager system). PrP-res was considered to be all PK-resistant products not found in a control lane lacking PrP-res. The amount of PK-resistant PrP-res in the cell-free assay was calculated as a percentage of the input PrP-sen.

Binding Analysis—The amount of 35S-labeled PrP-sen bound to PrP-res was determined under conditions of the cell-free assay. After incubation for 48 hours, the conversion reaction was centrifuged at 14,000 g for 10 minutes at room temperature. The supernatant was removed, methanol-precipitated in the presence of a carrier protein (thyroglobin), and saved as the unbound fraction. The pellet was washed in wash buffer (50 mM sodium citrate buffer, pH 6.0, 5 mM cysteiydylamin chloride, 1.2% sarkosyl) and centrifuged again. The wash was saved and methylanol precipitated. The final pellet was resuspended in wash buffer and methylanol-precipitated. The bound (pellet) and unbound fractions were analyzed on a 16% SDS-PAGE gel, and the amount of 35S-labeled PrP-sen recovered in each fraction was quantified using the Storm PhosphorImager system. To calculate the percentage of total PrP-sen bound, the amount of bound 35S-labeled PrP-sen (all glycoforms) was divided by the total (bound and unbound) 35S-labeled PrP-sen recovered. The amount of unglycosylated 35S-labeled PrP-sen that was bound to the PK-resistant PrP-sen was also calculated. All values were normalized for background aggregation by subtracting the percentage of total or unglycosylated 35S-labeled PrP-sen found in the pellet in the absence of PrP-res.

Transduction and Analysis of Scrapie-infected Mouse Neuroblastoma Cells Expressing Deletion Mutants of Hamster PrP—Expression of hamster PrP molecules in scrapie-infected mouse neuroblastoma (Sc′+N2a) cells by transduction with the retroviral expression vector pSSFF and detection of PrP-res expression in these cells has been described previously (29, 32–34). Transduction efficiency was measured by immunofluorescence using the 18.8 monoclonal antibody (35), which reacts with the retroviral gag gene expressed by the pSSFF vector used in this study. Mouse PrP-res and foreign PrP-res derived from HaWT and Ha3494 were detected using rabbit anti-antibody R20 (31). Expression of foreign HaWT and Ha3494, but not mouse PrP, was detected with the hamster-specific monoclonal antibody 3F4. Foreign PrP-res derived from Ha1133, Ha120, and Ha124 was detected using the rabbit anti-antibody R20 and was distinguished from endogenous mouse PrP-res by the size shift associated with the deletion mutants. The Western blot was developed with the enhanced chemiluminescence reagent system as described by the manufacturer (Amersham Pharmacia Biotech).

Statistical Analysis—Statistical comparisons between groups were performed using one-way analysis of variance and the Newman-Keuls Multiple Comparison test using the PRISM™ software package.

RESULTS

Deletion of Amino Acid Residues within the Flexible N-terminal Tail of PrP Reduces Cell-free Conversion of Hamster PrP-sen—The role of the flexible N-terminal tail of PrP-sen in conversion of protease-sensitive PrP to the protease-resistant PrP was determined using a biochemical cell-free conversion assay. The conversion of hamster PrP with progressive deletions within the N-terminal tail was compared with conversion of HaWT (Fig. 1). All PrP-sen deletion mutants were converted to a protease-resistant form under the conditions used (Fig. 1b). However, conversion of PrP lacking residues 34–94, Ha3494, was reduced by −35% relative to HaWT (Fig. 1c), and deletion of an additional nineteen residues (residues 94–113), Ha1133, reduced conversion by a further 25%. Conversion was not significantly affected by further deletions within the N-terminal tail to reside 120 (Ha120) or 124 (Ha124). Thus, it appears that residues 34–94 and 34–113 influence conversion. This is in agreement with the proposed importance of residues in the vicinity of residue 94 in the conversion of PrP-sen to PrP-res (18).

Following PK digestion of the conversion products, multiple protease-resistant PrP molecules were detected between 12 and 33 kDa. The distribution of these products was affected by deletion of amino acids within the N-terminal tail (Fig. 1b). The conversion products of HaWT and Ha3494 were similar in molecular mass, giving two groups of bands at 19–22 and 25–33 kDa. This PK pattern was consistent with digestion of full-length PrP and little to no digestion of Ha3494, which has previously been shown for N-terminally truncated PrP (17). In contrast, smaller products of 12 and 14 kDa as well as a 19–22-kDa form were associated with conversions of Ha1133, Ha120, and Ha124. The 12- and 14-kDa conversion products appeared to result from PK cleavage at one or two new sites within the PrP polypeptide. Since the 12- and 14-kDa products were only a minor component of conversions of HaWT and
Ha394, the new proteolytic sites appear to be more accessible in PrP-res derived from PrP-sen with at least residues 34–113 deleted from the N-terminal tail.

Conversion of HaWT and Ha394 in the cell-free assay resulted in a prominent protease-resistant product between 25 and 33 kDa. The prominence of this conversion product is associated with conversion of a 60-kDa isoform of PrP that has been described previously to represent a dimerized form of PrP (19, 36). We were concerned that the reduced conversion of Ha113, Ha120, and Ha124 relative to HaWT and Ha394 may have been due to the reduced ability of these truncated molecules to efficiently convert the dimer isoform. However, conversion of HaWT, Ha394, and Ha113 without the dimer isoform resulted in a quantitative pattern of conversion consistent with that reported for conversion in the presence of the dimer (data not shown).

Glycosylation Affects the Generation of Protease-resistant PrP from PrP-sen with Deletions within the N-terminal Tail—Glycosylation may affect the solubility and structure of PrP-sen and could therefore influence its conversion to PrP-res. The effect of glycosylation on cell-free conversion of N-terminal deletion mutants was investigated with unglycosylated PrP derived from tunicamycin-treated cells (Fig. 2). In contrast with the effect of deletions within the N-terminal tail of glycosylated PrP-sen (Fig. 1c), the amount of protease-resistant PrP derived from unglycosylated PrP-sen was unchanged by deletions within the N-terminal tail (Fig. 2c). However, the distribution of the conversion product was very different (Fig. 2b). The main protease-resistant product of HaWT conversion had a molecular weight of ~21 kDa, which was again consistent with digestion of 89 amino acids from the N terminus of the 30-kDa unglycosylated protein. Protease-resistant Ha1394 had a similar molecular weight to protease-resistant HaWT. The lower molecular weight conversion products previously described for PrP with deletions Ha113, Ha120, and Ha124 were also present in the protease-resistant products of HaWT and Ha394. This suggested that the putative internal PK digestion sites may be more accessible in the unglycosylated than in the glycosylated PrP conversion product. Ha113 and Ha120 were converted to a protease-resistant form that appeared to have the same molecular mass as the input PrP-sen, ~16 kDa, whereas the majority of this fully protease-resistant PrP was absent from conversions using Ha124. The lower molecular mass bands at 12 and 14 kDa were also detected in conversions of Ha113, Ha120, and Ha124.

The low molecular mass bands at 12 and 14 kDa may represent an alternative conformation of PrP-res that is more susceptible to PK digestion. To assess the influence of the N-terminal tail on the formation of the fully protease-resistant form, we quantified the amount of PrP-res with a molecular mass greater than or equal to 15 kDa (Fig. 2d). By this method of analysis, PrP-res formation was decreased following deletion of residues 34–94 and 34–113. This result was consistent with the effect of deletion of these residues from glycosylated PrP-sen (Fig. 1c).
Cell-free Conversion of N-terminal Deletion Mutants of PrP-sen Lacking the GPI Anchor—PrP-sen is located on the external surface of the cell membrane, where it is anchored by a GPI moiety added to the C terminus of the protein at the time of translation. The GPI anchor is made hydrophobic by the presence of fatty acid chains, which may influence PrP solubility and conversion in an aqueous solution. To investigate the effect of deletions within the flexible N-terminal region on conversion of PrP in the absence of this modification, constructs were made that lacked the signal for the GPI anchor. GPI-negative PrP-sen was not heavily glycosylated and was found predominantly in a nonglycosylated form (Fig. 3c). However, unlike conversion of unglycosylated GPI-anchored PrP (Fig. 2c), in the absence of the GPI anchor, deletion of residues 34–94 (HaΔ94) resulted in a 36% decrease in conversion relative to HaWT (Fig. 3c). Progressive deletion to residues 113, 120, and 124 had a slight, but not significant, cumulative effect on conversion with the mean percentage conversion reduced relative to HaWT by 46, 49, and 63%, respectively. The pattern of protease-resistant PrP bands ranged in size from 7 to 21 kDa and was similar to that observed for unglycosylated PrP (Figs. 2b and 3b). Therefore, in addition to the effect of deglycosylation, removal of the GPI anchor also influenced conversion of N-terminal deletion mutants of PrP.

The N-terminal Region of Glycosylated PrP-sen Modifies the Binding of PrP to PrP-res—Conversion of PrP-sen to PrP-res is a two-stage process, which begins with binding of PrP-sen to PrP-res, followed by conversion of the bound PrP-sen to a protease-resistant form (21, 37, 38). To investigate whether deletions within the N-terminal tail affected the solubility of PrP-sen and its ability to bind to PrP-res, [35S]PrP-sen was incubated in the absence or presence of PrP-res under the cell-free assay conditions. The reaction was centrifuged, and the amount of PrP-sen found in the pellet fraction was quantified (Fig. 4). In the absence of PrP-res (Fig. 4a, solid bars), more than 20% of [35S]HaWT was detected in the pellet fraction (Fig. 4b). In the absence of PrP-res (Fig. 4c), samples of GPI-negative hamster PrP-sen deletion mutants after conversion with hamster PrP-res (263K) were calculated from the pixel volume of protease-resistant PrP found between 7 and 21 kDa relative to input PrP-sen (c). Under these conditions, the mean percentage conversion ± S.E. of GPI-negative HaWT was 47.3 ± 3.6%.

Binding of PrP to PrP-res—The ability of PrP-sen with deletions within the N-terminal region to bind to PrP-res is influenced by glycosylation. The amount of [35S]-labeled PrP-sen that was found in the pellet fraction (bound) of a conversion reaction after centrifugation at 14,000 × g for 10 min was calculated as a percentage of bound and unbound [35S]-labeled PrP-sen. The contribution of unglycosylated (open bars) and total (solid bars) PrP to binding was determined in the absence (a) or presence (b) of PrP-res (263K). The amount of PrP-sen found in the pellet in the presence of PrP-res (b) represents binding above the background of binding in the absence of PrP-res.

Expression of N-terminal Deletion Mutants of PrP-sen in Scrape-infected Mouse Neuroblastoma Cells—We have demonstrated the importance of the N-terminal region of hamster...
PrP-sen in its conversion to PrP-res in a cell-free assay. There is currently no assay available to study conversion of hamster PrP-sen mutants in live cells. However, HaWT has been shown to block PrP-res formation in scrapie-infected mouse neuroblastoma cells (32). We therefore investigated the function of the N-terminal region by expressing the deletion mutants in Sc+/-N2a cells chronically infected with mouse scrapie (Chandler/RML strain). Under conditions in which complete interference was observed in cells transduced with HaWT, only partial interference was mediated by HaN94, and no interference with PrP-res formation was observed in cells transduced with Ha113, Ha120, or Ha124, relative to the pSFF vector alone (Fig. 5). This was unlikely to have been a result of altered cellular processing of the truncated PrP-sen molecules, because all of the mutated molecules were detected on the surface of transduced Sc+/-N2a cells (data not shown). Therefore, hamster PrP residues 34–94 and 34–113 appeared to be important for interference with the generation of mouse PrP-res in these scrapie-infected mouse cells.

The expression of a foreign PrP-sen species may block PrP-res formation by binding PrP-res and blocking further conversion. Therefore, to determine if the reduced interference by the N-terminal deletion mutants was due to a decreased ability to bind mouse-derived PrP-res, we repeated the binding experiments using PrP-res from the Chandler/RML strain of mouse scrapie. The Chandler/RML strain of scrapie-infected neuroblastoma cells (Sc+/H9023) were transduced with infectious retrovirus supernatant from F2/Pa317 fibroblast cells expressing HaWT or HaPrP N-terminal deletion mutants. Transduction efficiency was 60–80% by indirect immunofluorescence using an antibody to the Gag protein of pSFF, and expression of the HaWT or deletion mutants was confirmed by radioimmunoprecipitation (data not shown). The immunoblot of transduced cell lysates after PK treatment was probed with the R30 anti-peptide antibody. No PK-resistant PrP was detected with the hamster-specific antibody 3F4, and no truncated conversion products were detected with the C-terminal antibody R20 (data not shown). Duplicate lanes have been spliced from the phosphor image.

**DISCUSSION**

Deletion of residues 34–94 and 34–113 from within the N-terminal tail of PrP-sen influenced the quantity and conformation of PrP-res generated in a cell-free assay. It is unclear how these residues may be influencing PrP conversion. However, the primary amino acid sequence within the 34–94 deletion is composed of five octapeptide repeats that may influence intramolecular and intermolecular interactions between PrP molecules and/or other cellular factors that are required for efficient conversion (39, 40). Extra copies of the octapeptide repeat are associated with heritable TSE disease in both humans (41) and transgenic mice (42, 43) and have been shown to induce PrP aggregation and alter PrP processing in tissue culture cells (39, 44, 45). In the current study, a considerable amount of HaWT PrP was pelleted by centrifugation in the absence of PrP-res, whereas deletion of the octapeptide repeat region in deletion mutant HaN94 significantly reduced the amount of this PrP-sen self-aggregation (Fig. 4a). The reduced ability of PrP to form self-aggregates was associated with a parallel decline in conversion efficiency. The role of these residues might be to enhance PrP polymerization, which might be beneficial for conversion to a protease-resistant form (40). Deletion of additional residues 95–113 (Ha113) caused a further decrease in PrP polymerization and a concurrent decrease in cell-free conversion. Therefore, residues within the N-terminal tail required for PrP-PrP interactions and efficient conversion include both the octapeptide repeat region and amino acids between residue 95–113. Interestingly, insertions and point mutations within this region (residues 95–113) are known to lead to genetic TSE diseases in humans (41) and have been shown to confer biochemical properties reminiscent of PrP-res on the mutated PrP-sen molecule (46).

In our experiments, deletion of residues 95–113 influenced not only the efficiency of PrP conversion in the cell-free assay but also the nature of the PrP-res produced. There was no apparent shift in molecular weight of the largest bands derived from PK digestion of PrP-res generated from unglycosylated Ha113, Ha113, and Ha120 (Fig. 2b). Therefore, in the presence of PrP-res, these constructs could adopt a fully protease-resistant conformation, which probably reflects the absence of the primary digestion site at residue 89. However, PK digestion of Ha1113, Ha120, and Ha124 also resulted in the formation of lower molecular mass bands between 7 and 14 kDa. These bands are likely to be the result of protease digestion at multiple sites downstream of the primary digestion site, since antibody mapping indicated that they were a result of progres-

---

**Fig. 5.** Transduction of scrapie-infected neuroblastoma cells with N-terminal deletion mutants of PrP-sen. Scrapie-infected mouse neuroblastoma cells (Sc+/N2a cells) were transduced with infectious retrovirus supernatant from F2/Pa317 fibroblast cells expressing HaWT or HaPrP N-terminal deletion mutants. Transduction efficiency was 60–80% by indirect immunofluorescence using an antibody to the Gag protein of pSFF, and expression of the HaWT or deletion mutants was confirmed by radioimmunoprecipitation (data not shown). The immunoblot of transduced cell lysates after PK treatment was probed with the R30 anti-peptide antibody. No PK-resistant PrP was detected with the hamster-specific antibody 3F4, and no truncated conversion products were detected with the C-terminal antibody R20 (data not shown). Duplicate lanes have been spliced from the phosphor image.

**Fig. 6.** Truncations within the N-terminal tail of PrP-sen do not affect the ability of HaPrP to bind to mouse PrP-res. HaPrP or mouse PrP with residues 108 and 111 changed to methionine to generate the 3F4 epitope (Mo3F4) were mixed with the Chandler/RML strain of PrP-res in the conditions described for the cell-free assay. The amount of 35S-labeled PrP-sen that was found in the pellet fraction (bound) of a conversion reaction after centrifugation at 14,000 × g for 10 min was calculated as a percentage of bound and unbound 35S-labeled PrP-sen (mean ± S.E.).
N-terminal Truncation of PrP Affects PrP-res Formation

In PrP-res derived from HaWT, residues 95–113 might protect these secondary cleavage sites from protease digestion. Alternatively, deletion of these residues may alter the conformation of the final protease-resistant product such that these secondary sites are more readily exposed to protease digestion. The significance of these altered forms of protease-resistant PrP in neurodegeneration and clinical disease remains to be determined. However, their formation in the cell-free conversion assay was template-dependent, since they were not detected in the absence of a PrP-res seed. Furthermore, similarly sized low molecular weight protease-resistant PrP forms have been detected in inherited TSE diseases of humans and in brain homogenates of scrapie-infected rodents (13, 47–50), suggesting that these proteins and/or the PrP-res from which they are generated may play a role in TSE pathogenesis in vivo.

The conversion efficiency of unglycosylated PrP-sen was not decreased by deletion of residues within the N-terminal region (Fig. 2c). This was in contrast to the effect of these same deletions in the glycosylated protein (Fig. 1c). Interestingly, the binding of the N-terminally truncated PrP-sen to PrP-res was also improved in the absence of glycosylation. However, conversion of unglycosylated PrP appeared to result in a larger proportion of the lower molecular weight species in the protease-resistant PrP. Quantification of the percentage conversion excluding these lower molecular weight species gave a similar pattern for conversion of the PrP deletion mutants (Fig. 2d) as was seen previously for glycosylated PrP (Fig. 1c). Glycosylation may therefore modulate interactions between PrP-sen and PrP-res and promote conversion to a more protease-resistant conformation. Furthermore, the presence of a longer N-terminal region appears to overcome this glycosylation effect, since HaWT and HaA94 were predominantly converted to higher molecular weight protease-resistant forms regardless of glycosylation state. Interactions of the flexible N-terminal tail of PrP-sen with antibodies (51) or other parts of PrP-sen itself (7) have been reported to alter the conformation of the glycosylated C-terminal domain of PrP. Therefore, residues 34–113, which affected the conversion of glycosylated PrP-sen, might act by modulating the conformation of the C-terminal domain, thus enabling efficient interactions between PrP-sen and PrP-res.

A further post-translational modification of PrP-sen involved expression of the molecule without the signal sequence for the GPI anchor. This modification resulted in a molecule that was mostly unglycosylated, which may have contributed to the improved conversion efficiency of HaΔ113, HaΔ120, and HaΔ124. However, efficient conversion of GPI-negative PrP was still dependent on residues 34–94, which supported our earlier hypothesis that intermolecular interactions between residues in the PrP octapeptide repeat region may be necessary for efficient conversion of PrP-sen. However, for unglycosylated PrP containing a GPI anchor, residues 34–94 were not required for efficient conversion (Fig. 2c), suggesting that in the absence of glycosylation, the hydrophobic fatty acids of the GPI anchor of PrP-sen might promote the PrP-PrP interactions required for conversion. Thus, in the absence of a GPI anchor, the N-terminal region may be required to mediate interactions between PrP-sen molecules to promote efficient conversion to a protease-resistant form.

Residues 34–93 are not required for scrapie susceptibility, since transgenic mice expressing PrP-sen lacking these residues remain susceptible to scrapie infection, albeit with delayed kinetics, reduced levels of detectable PrP-res, and an altered pattern of neurodegeneration (17). The decrease in PrP-res produced from cell-free conversion of HaΔ94 could correspond with the decreased PrP-res formation and delayed onset of disease observed in these transgenic mice. However, the cause of the altered pattern of neurodegeneration is unknown. Transgenic mice expressing further truncated PrP-sen lacking residues 34–106 are reportedly not susceptible to scrapie infection (18). This is in contrast with the current study, in which PrP-sen truncated between residues 34 and 113 could be converted to a protease-resistant form. This discrepancy may reflect a simple difference between sequences of PrP-res that are essential for conversion of mouse PrP versus the hamster PrP conversion presented here. Certainly, different amino acid residues have been shown to influence species-specific conversion of mouse and hamster PrP (52, 53). However, a more attractive explanation is that residues between positions 94 and 106 are required for initial infection with a TSE agent but are not essential for the subsequent formation of PrP-res. We have shown that the protease-resistant products associated with conversion of N-terminally deleted PrP-sen lack a large portion of the N-terminal region. Thus, it may also be that the antibodies used to detect PrP-res in the transgenic mouse model were specific to regions of PrP not present in the lower molecular weight protease-resistant products described here (17).

Expression of a foreign PrP species in mouse scrapie-infected neuroblastoma cells can block the generation of mouse PrP-res (32). In the present studies, interference with mouse PrP-res formation in Sc+ N2a cells was reduced by deletion of residues 34–94 from hamster PrP-sen and completely eliminated by deletion of residues 34–113. There was a strong correlation between the effect of deletion of these regions of the PrP molecule on interference in Sc+ N2a cells and conversion in the cell-free assay (Fig. 1). It is therefore possible that residues 34–113 play a similar role in both processes. Previous cell-free conversion studies suggested that interference with PrP-res generation by the expression of a foreign PrP species was primarily caused by inhibition of the acquisition of protease resistance rather than by preventing binding of the homologous PrP (54). Consistent with this conclusion, the ability of the hamster-derived PrP-sen to bind to mouse PrP-res was unaffected by the deletions within the N-terminal tail. This suggests that residues in the flexible N-terminal tail of PrP-sen may prevent the homologous PrP species from acquiring protease resistance by interfering with a step of conversion process subsequent to binding. Interestingly, in the system described here, molecules that have been shown to interfere with PrP-res generation in Sc+ N2a cells have contained one or both residues of the 3F4 epitope (32, 52), and deletion of this epitope in the HaΔ113 mutant ablated the ability of HaPrP to induce interference. The noninterfering PrP-sen molecules may also be less able to interact with other components of the conversion process, such as glycosaminoglycans. Zulianello et al. (55) proposed that PrP residues 23–34 may bind to an auxiliary molecule required for conversion. However, as residues 23–34 were present in both interfering and noninterfering deletion mutants described in the present paper, these residues do not appear to account for the interference observed here.

REFERENCES
1. Meyer, R. K., McKinley, M. P., Bowman, K. A., Braunfeld, M. B., Barry, R. A., and Prusiner, S. B. (1986) Proc. Natl. Acad. Sci. U. S. A. 83, 2310–2314
2. Riek, R., Hornemann, S., Wider, G., Gockshuber, R., and Wuthrich, K. (1997) FEBS Lett. 413, 282–288
3. Donne, D. G., Viles, J. H., Groth, D., Mehlhorn, I., James, T. L., Cohen, F. E., Prusiner, S. B., Wright, P. E., and Dyson, H. J. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 13452–13457
4. Caughey, B., Race, R. E., Ernst, D., Buchmeier, M. J., and Chesebro, B. (1989) J. Virol. 63, 175–181
5. Bolton, D. C., Meyer, R. K., and Prusiner, S. B. (1985) J. Virol. 53, 596–606

V. Lawson, unpublished observations.
