Arch Virol (2007) 152: 603–609
DOI 10.1007/s00705-006-0858-0
Printed in The Netherlands

Archives of Virology

Both host prion protein 131–188 subregion and prion strain characteristics regulate glycoform of PrP^{Sc}

T. Yokoyama[1], **K. Shimada**[1], **K. Masujin**[1], **Y. Iwamaru**[1], **M. Imamura**[1], **Y. K. Ushiki**[1,2], **K. M. Kimura**[1], **S. Itohara**[3], and **M. Shinagawa**[1]

[1] Prion Disease Research Center, National Institute of Animal Health, Tsukuba, Ibaraki, Japan
[2] Nippi Research Institute of Biomatrix, Toride, Ibaraki, Japan
[3] Brain Science Institute, RIKEN, Wako, Saitama, Japan

Received March 5, 2006; accepted August 16, 2006; published online November 16, 2006

Summary

Prion proteins (PrPs) contain 2 N-linked glycosylation sites and are present in cells in 3 different forms. An abnormal isoform of prion protein (PrP^{Sc}) has different glycoform patterns for different prion strains. However, the molecular basis of the strain-specific glycoform variability in prions has remained elusive. To understand the molecular basis of these glycoform differences, we analyzed PrP^{Sc} in 2 lines of transgenic mice (MHM2 and MH2M with PrP null background) that expressed a chimeric PrP. Our result indicated that PrP 131–188 (substitutions at I139M, Y155N, and S170N) contributed to both PrP^{C} and PrP^{Sc} glycoform ratios. Furthermore, the PrP^{Sc} glycoform pattern within these transgenic mice showed a subtle difference depending on the inoculated prion. This study indicated that the PrP^{Sc} glycoform ratio was influenced by both host PrP^{C} and the prion strain.

Author's address: Takashi Yokoyama, Prion Disease Research Center, National Institute of Animal Health, Tsukuba, Ibaraki 305-0856, Japan. e-mail: tyoko@affrc. go.jp

Introduction

Transmissible spongiform encephalopathies (TSEs), also called prion diseases, are fatal neurodegenerative diseases that include scrapie in sheep and goats, bovine spongiform encephalopathy (BSE), and Creutzfeldt-Jakob disease (CJD) in humans [13]. The nature of the infectious agent prion has not been fully elucidated. The central event in prion pathogenesis is the conversion of the cellular isoform of a prion protein (PrP^{C}) into an abnormal isoform of the prion protein (PrP^{Sc}). PrP^{Sc} is the only known disease-specific marker and is closely associated with infectivity. Conformational differences are observed between PrP^{C} and PrP^{Sc}. PrP^{Sc} has a large number of β sheets and a diminished α-helical content compared with PrP^{C} [5, 10, 15]; hence, PrP^{Sc} is relatively resistant to protease digestion. The protease resistance of PrP^{Sc} has been widely accepted as the physico-chemical basis for distinguishing between PrP^{C} and PrP^{Sc}.

PrP^{C} is a glycoprotein and contains 2 N-linked glycosylation sites. It is present in cells in 3 different glycosylated forms (diglycosylated, monoglycosylated, and unglycosylated forms). It has been

reported that PrP^{Sc} has different glycoform patterns with different ratios of 3 bands for different prion strains [6]. Analysis of the PrP^{Sc} glycoform ratio has been used to discriminate prion strains and has become increasingly important in the differential diagnosis of human prion diseases. However, the molecular basis of strain-specific glycoform variability in prions has remained elusive. Clarifying the prion protein (PrP) glycosylation mechanism might lead to the understanding of prion strain variations. It has been proposed that the PrP^{C} glycoform ratio differs according to the brain regions, and the differential targeting of neurons by prion strains results in the differences in the PrP^{Sc} glycoform ratio [7, 17]. Furthermore, different scrapie prions can induce the formation of different PrP^{Sc} glycosylation patterns in the same cell line [3, 19]. These observations have raised the possibility that the direct influence of a prion strain on the posttranslational glycosylation modification of PrP^{C} or on PrP^{Sc} itself dictates the strain-specific glycosylation [3, 17, 19].

In this study, we examined the PrP^{Sc} glycoform transition that changes the PrP^{Sc} glycoform ratio, which depends on the adaptation of prions in interspecies transmission. The PrP^{Sc} glycoform ratio was altered depending on the shortening of incubation periods. To understand the molecular basis of this phenomenon, we analyzed mice and hamster chimeric PrP expressed by transgenic mice. The result obtained by using these mice indicated that PrP 131–188 influences the PrP^{C} and PrP^{Sc} glycoform patterns. Furthermore, the PrP^{Sc} glycoform ratio was also modified by the prion strain.

Materials and methods

Scrapie prions and animals

The mouse-adapted scrapie prion Obihiro, and hamster-adapted scrapie prion Sc237 were passaged in CD-1 mice (SLC, Japan) and Syrian hamster (SLC, Japan), respectively [21, 22]. Transgenic mice that expressed mouse and hamster chimeric PrP (MH2M and MHM2) were kindly provided by Dr. S. B. Prusiner [16]. Amino acid substitutions at positions L108M and V111M are present in the MHM2 mice. In addition to these amino acid substitutions, another 3 substitutions (I139M, Y155N, and S170N) are present in the MH2M mice. These mouse lines have been maintained by crossing with PrP0/0 mice [22] as PrP null background. Genotypes of the mice were determined by polymerase chain reaction (PCR) analysis of DNAs prepared from tail samples of the mice. The primers used were 5′-TCGGACGACAA GAGACAATC-3′ and 5′-TAGGGGCCACACAGAAAACA-3′ for chimeric PrP genes, and the primer combination was as previously described for the mouse PrP genotype analysis [22]. These transgenic mice (MH2M and MHM2 with PrP0/0 background) were also used for the transmission experiment.

Transmission experiment

Brains were stored at −80 °C, and brain homogenates (10% w/v) were prepared in phosphate-buffered saline (PBS). The homogenate was centrifuged at 3000 rpm for 5 min, and 20 μL of the supernatant was inoculated intracerebrally into the animals.

Western blot

The brains (brain stem) were homogenized (10%, w/v) in 0.5% Nonidet P-40, 0.5% sodium deoxycholate, 100 mM NaCl, 10 mM EDTA, and 10 mM Tris–HCl (pH 7.5) and then centrifuged at 3000 × *g* for 5 min. The supernatant was incubated with 50 μg/ml of proteinase K (PK) for 30 min at 37 °C. The sample was incubated with 4-(2-aminoethyl)-benzenesulfonyl fluoride hydrochloride (Pefablock, Roche; final concentration: 1 mM) for 5 min at 37 °C and then mixed with an equal volume of sodium dodecyl sulfate (SDS) sample buffer. Western blot (WB) analysis was carried out as described previously, and the mouse polyclonal antibody Ab. Tg was used as the primary antibody [22]. PrP signal intensity was calculated using ChemiImager (Alpha Innotech Co.). For PrP^{C} detection, the supernatant of the brain homogenate was subjected to WB without PK digestion, and the detection was carried out with biotinylated Ab. Tg as described previously [22].

Results

PrP glycoform in interspecies transmission

Mouse and hamster PrP^{C} glycoforms were analyzed. The brain homogenates of normal mouse and hamster were subjected to WB without PK digestion. As shown in Fig. 1A, in addition to the major band of diglycosylated PrP, 2 other bands (monoglycosylated and unglycosylated PrPs) were detected from the mouse brain homogenate. However, the diglycosylated PrP band was the main band in hamster PrP^{C} and the other 2 PrP bands were either not observed or were of low intensity.

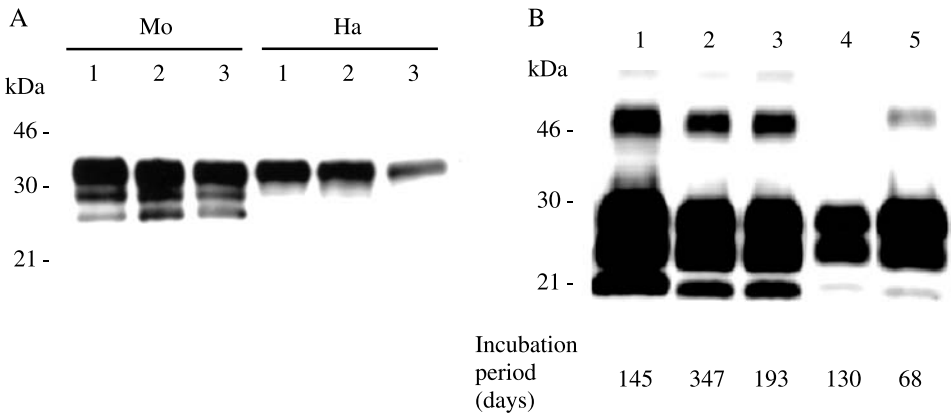

Fig. 1. PrP glycoform analysis in interspecies transmission. **A**, Western blot (WB) analysis of PrP^{C} in mouse (*Mo*) and hamster (*Ha*). Homogenates of cerebrum (*1*), cerebellum (*2*), and medulla (*3*) were subjected to WB without proteinase K (PK) digestion. **B**, WB analysis of PrP^{Sc} in hamsters inoculated with mouse-passaged scrapie prion. *1* Obihiro-strain-inoculated mouse brain; *2* Obihiro-strain-inoculated hamster brain (primary passage); *3* second-passaged hamster brain; *4* third-passaged hamster brain; *5* hamster passaged Sc237 strain inoculated hamster brain. The incubation periods of the examined animals are indicated below. Molecular weights are indicated on the left

This result shows that PrP^{C} glycoform differed depending on the animal species. A difference in the PrP^{C} glycoform ratio was not observed among the 3 brain regions that were examined (Fig. 1A).

Mouse and hamster brains that were inoculated with host-adapted prion showed a different PrP^{Sc} glycoform ratio (Fig. 1B, lanes 1 and 5); it was similar to that of the host PrP^{C}. In interspecies transmission, the glycoform ratio of accumulated PrP^{Sc} was analyzed after adaptation to another host. For this purpose, mouse-adapted Obihiro prion was transmitted intracerebrally into hamsters, and the diseased hamster brain homogenate was subsequently inoculated into other hamsters. At primary and secondary passages, the PrP^{Sc} glycoform ratio was similar to that of the Obihiro-inoculated mouse (Fig. 1B, lanes 1–3). In contrast, at the third passage, the PrP^{Sc} glycoform was altered and was similar to that of the Sc237-inoculated hamster (Fig. 1B, lanes 4 and 5).

Incubation periods and PrP^{Sc} glycoform correlation in interspecies transmission

Widely ranging incubation periods were observed in interspecies transmission, particularly in the primary and secondary passage [9]. The relationship between the PrP^{Sc} glycoform ratio and incubation periods was examined. The incubation periods of Obihiro-inoculated hamsters from primary to third passages were classified into 3 groups – below 200 days, between 200 and 300 days, and over 300 days. PrP^{Sc} glycoform was compared among these 3 groups. As shown in Fig. 2, when the incubation period was below 200 days, the PrP^{Sc} glycoform ratio of the Obihiro-inoculated hamsters was similar to that of the Sc237-inoculated hamsters. In

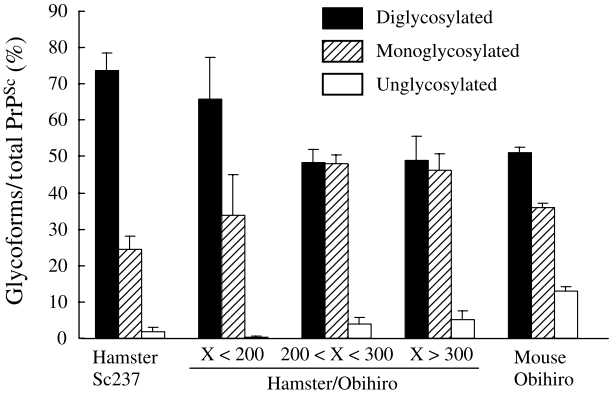

Fig. 2. Correlation between PrP^{Sc} glycoform and incubation periods. Obihiro-strain-inoculated hamsters (from primary to third passage) were categorized by their incubation periods (X; 3 hamsters, below 200 days; 3 hamsters, between 200 and 300 days; and 14 hamsters, over 300 days). Glycoform ratio of PrP^{Sc} was calculated. Means and SDs are shown

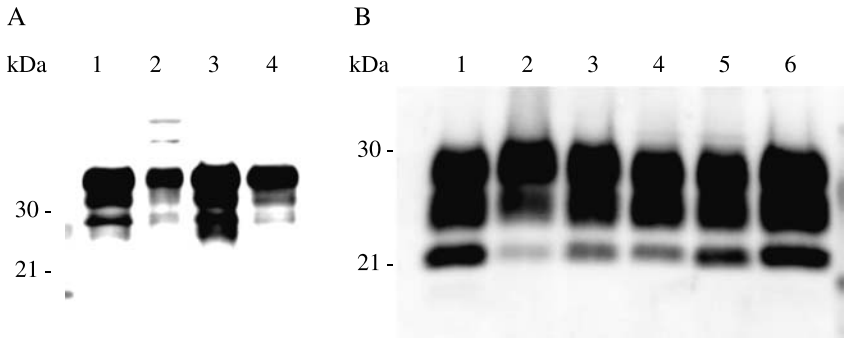

Fig. 3. PrP glycoform analysis in mouse and hamster chimeric PrP expression mice. **A**, Western blot (WB) analysis of PrP^C in mouse (*1*), hamster (*2*), MHM2 mouse (*3*), and MH2M mouse (*4*). Brain homogenate of each animal was subjected to WB without PK digestion. **B**, WB analysis of PrP^{Sc} in Obihiro- and Sc237-affected transgenic mice. *1* Mouse (Obihiro); *2* hamster (Sc237); *3* MH2M (Sc237); *4* Obihiro (MH2M); *5* MHM2 (Sc237); *6* MHM2 (Obihiro). Molecular weight markers are indicated on the left

contrast, in the case of the other 2 groups of longer incubation periods, the PrP^{Sc} glycoform ratios of the Obihiro-inoculated hamsters were similar to that of the Obihiro-inoculated mice. The PrP^{Sc} glycoform ratio was altered depending on the prion adaptation.

PrP glycoform ratio in transgenic mice

To understand the molecular mechanisms of glycoform transition with prion adaptation, we examined the PrP glycoform ratio in transgenic mice that expressed mouse and hamster chimeric PrP (MH2M and MHM2). Three PrP bands were detected in PrP^C of MHM2 mice, and the glycoform ratio of the MHM2 mouse PrP^C was similar to that of the mouse PrP^C (Fig. 3A, lanes 1 and 3). In contrast, the glycoform ratio of MH2M mouse PrP^C was similar to that of the hamster PrP^C (Fig. 3A, lanes 2 and 4). This result showed that these transgenic mice expressed different glycomodified PrP^C.

Mouse- and hamster-adapted scrapie prions were inoculated intracerebrally into both transgenic mice. The incubation periods of these mice are shown in Table 1. The MHM2 mice, which expressed the mouse-type PrP^C, were susceptible to the Obihiro prion; on the other hand, the MH2M mice, which expressed the hamster-type PrP^C, were susceptible to the Sc237 prion. The PrP^{Sc} glycoform ratio of these transgenic mice was analyzed. As shown in Figs. 3 and 4, a difference in the unglycosylated PrP band ratio was observed between the MH2M and MHM2 mice. In the MHM2 mice, there was a resemblance in the PrP^{Sc} glycoform ratio between

Table 1. Incubation periods in prion-inoculated mice and hamsters

Animal	PrP^C glycoform	Prion strain[a]	
		Obihiro	Sc237
Mouse	Mo	146.0 ± 1.0[b]	no transmission
MHM2	Mo	164.7 ± 5.9	420.5 ± 17.2
MH2M	Ha	187.4 ± 7.5	102.3 ± 3.6
Hamster	Ha	385.2 ± 15.7	69.0 ± 1.0

[a] Mice-adapted "Obihiro" strain or hamster-adapted "Sc237" strain was intracerebrally inoculated.
[b] Incubation periods (days); Mean ± SD.

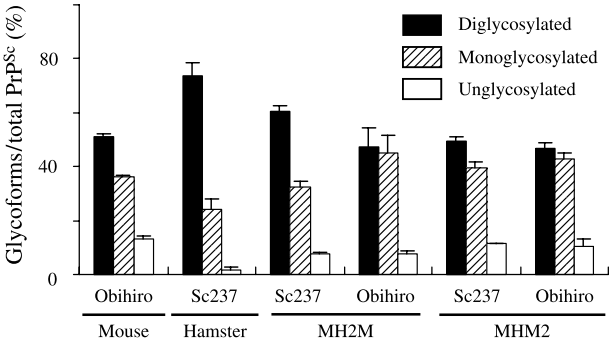

Fig. 4. Glycoform ratio of PrP^{Sc} in transgenic mice. Western blot (WB) signal intensity of diglycosylated, monoglycosylated, and unglycosylated PrP bands are indicated. Means and SDs are shown

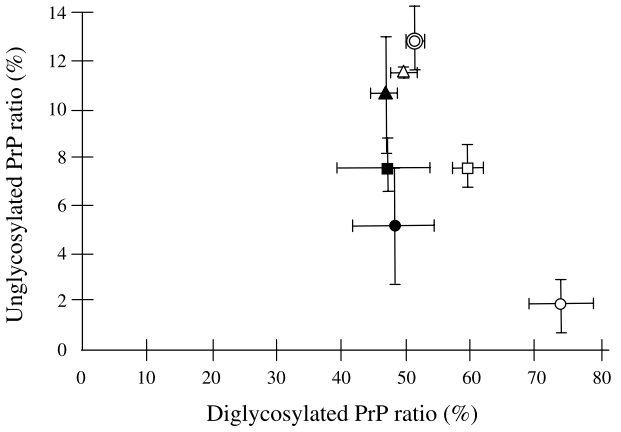

Fig. 5. Glycoform ratio of PrP^{Sc} in animals were plotted with their means and SDs. ○, Hamster (Sc237); □, MH2M (Sc237); △, MHM2 (Sc237); ●, hamster (Obihiro) (first passage); ■, MH2M (Obihiro); ▲, MHM2 (Obihiro); and ◎, mouse (Obihiro). X-axis, diglycosylated PrP band ratio; Y-axis, unglycosylated PrP^{Sc} band ratio

the Obihiro strain and the Sc237 strain. In the MH2M mice, the PrP^{Sc} glycoform ratio changed depending on the prion strain (Fig. 4). Subsequently, the unglycosylated and diglycosylated PrP^{Sc} ratio of each mouse was plotted. The ratio of unglycosylated PrP^{Sc} increased in the following order: hamster, MH2M, MHM2, and mouse. In the MH2M and MHM2 mice, the unglycosylated PrP^{Sc} ratio was constant irrespective of the prion strains. However, the diglycosylated PrP^{Sc} ratio changed depending on the prion strain, and this characteristic was obvious in the MH2M mice (Fig. 5).

Discussion

Prion infection in interspecies transmission has caused a phenomenon of ''species barrier.'' Amino acid substitution in the host PrP and the resulting conformational differences between the invading PrP^{Sc} and host PrP^{C} is thought to be an explanation for this phenomenon. Different PrP glycoform ratios among host species and strain variations may be due to different PrP conformations. To clarify the relationship between scrapie susceptibility and PrP glycoform ratio, hamsters were intracerebrally inoculated with mouse-passaged scrapie prions. The PrP^{Sc} glycoform transition was observed depending on the degree of prion adaptation (Figs. 1, 2). In early passage, the PrP^{Sc} glycoform ratio was similar to that of PrP^{Sc} in the inoculum, and the glycoform ratio gradually shifted to that of the prion-acquired host PrP with prion adaptation. This result indicated that the PrP^{Sc} glycoform ratio was influenced by both the host PrP amino acid sequence and by prion strain characteristics. In early passage, prion characteristics (inoculum) predominantly influenced the PrP^{Sc} glycoform ratio. Following subsequent passages, the glycoform ratio was altered to match the host PrP profile with shortening of the incubation periods. PrP^{Sc} in primary passaged animals might retain most characteristics of the original prion strain.

PrP^{Sc} glycosylation profiles may be indicative of the PrP^{C} glycoform pattern of the brain area in which PrP^{Sc} is formed. PrP^{C} and PrP^{Sc} showed different glycoform patterns depending on the tissue differences [14]. However, our result also showed a similar PrP^{C} glycoform ratio in different brain regions (Fig. 1). To prevent any unexpected influence in different brain areas, we used the medulla region in this experiment.

Investigating the PrP^{Sc} glycoform ratio in primary passaged animals might provide important information regarding prion strain diversities [6]. It has also been reported that MH2M mice inoculated with Sc237 generated a different PrP^{Sc} conformer, resulting in the emergence of a new prion strain [12]. Our result also showed that subsequent passages altered the PrP^{Sc} characteristics. In the case of BSE, pandemic occurrence was observed, and the BSE prion may already have several passage histories among cattle populations. Therefore, it might be difficult to determine the origin of BSE using the PrP^{Sc} glycoform ratio. It has been reported that there were no significant differences among PrP^{Sc} glycoforms of natural scrapie, but that was apparently different from that of BSE in cattle and experimentally BSE-affected sheep [18]. In contrast, 2 different prions, scrapie strain CH1641 and BSE, showed similarities in the PrP^{Sc} glycoform ratio, indicating that it has a limited usefulness in strain differentiation in natural hosts [8]. Many types of amino acid polymorphisms exist among sheep PrP, and some of them have been linked to scrapie susceptibility [1]. PrP^{C} conforma-

tional differences, which depend on the amino acid substitutions, also have to be considered while analyzing the PrP^{Sc} glycoform ratio in sheep scrapie. Recently, an atypical BSE, which showed the accumulation of a different glycoform of PrP^{Sc} with a different molecular weight of PK-resistant PrP, has been reported [2, 4, 20]. To investigate the different phenotype of BSE, it is necessary to clarify the PrP^{Sc} glycomodification mechanisms. In addition, successful transmission is expected to clarify whether these different PrP^{Sc} glycopatterns are linked to the different BSE prion strains.

To clarify the molecular basis of glycoform transition, transgenic mice (MHM2 and MH2M with PrP0/0 background) were used. As reported previously [16], scrapie prion susceptibility was influenced by the third subregion of PrP (131–188). A high-molecular-weight band was predominantly observed in both mouse and hamster PrP^{C}s. However, mouse PrP^{C} showed 3 different bands; in contrast, almost no unglycosylated band existed in hamster PrP^{C}. MHM2 mice, which have amino acid substitutions in PrP at L108M and V111M, expressed PrP^{C} with a mouse-type glycoform ratio, and these mice were susceptible to the mouse-adapted prion. In contrast, MH2M mice, which have 3 other amino acid substitutions (I139M, Y155N, and S170N) in addition to those of MHM2 mice, expressed PrP^{C} with a hamster-type glycoform ratio and were susceptible to the hamster-adapted prion (Table 1). Analysis of transgenic mice revealed that 3 amino acid substitutions located at the subregion of PrP 131–188 may contribute to both prion susceptibility and PrP glycoform ratio.

Glycosylation is initiated in the endoplasmic reticulum (ER). In the ER, glycans play a common role in promoting protein folding, quality control and cellular trafficking, and individual glycosylation patterns; these processes are important for specific functions of the mature glycoproteins that are subsequently processed in the Golgi complex [11]. According to the protein-only hypothesis, the conversion of PrP^{C} to PrP^{Sc} occurs at lipid rafts on the plasma membranes, where PrP^{C} has already been glycomodified in the host tissue. The host-adapted prion generates PrP^{Sc} in a pattern that is similar to host PrP^{C} glycosylation and requires shorter incubation periods. It may be the result of efficient conversion of PrP^{C} to PrP^{Sc}, and glycoform similarity might indicate the degree of prion adaptation. Furthermore, there is a possibility that PrP^{Sc} may directly influence the PrP^{C} glycomodification. Clarifying the mechanisms involved in altering the PrP^{Sc} glycoform ratio might provide insights into the conversion process *in vivo*.

Although PrP^{Sc} of the Obihiro strain showed a similar ratio of diglycosylated PrP^{Sc} within the examined rodent species, the ratio of unglycosylated PrP^{Sc} changed in different animal species. In contrast, in the case of the hamster-passaged prion Sc237, the molecular ratios of both diglycosylated and unglycosylated PrP^{Sc}s changed depending on the host species (Fig. 5). This difference in the PrP^{Sc} glycoform pattern in these transgenic mice also revealed the prion strain diversity.

Acknowledgments

We thank Dr. Stanley B. Prusiner for providing the MHM2 and MH2M mice and Dr. Shigeru Katamine and Dr. Shinichiro Atarashi for their efforts in acquiring these mice and Dr. Jim Hope for reading the manuscript. We thank Ms. Hiroko K. Hayashi, Yuka Ookubo, and Naoko Tabeta for their technical help. We also thank Ms. Junko Yamada for her general assistance and the animal laboratory staff at the National Institute of Animal Health for maintaining the mouse colony. This study was supported in part by grants from the Ministry of Agriculture, Forestry, and Fisheries; the Ministry of Health, Labor, and Welfare; and the Ministry of Science, Technology, and Education, Japan.

References

1. Baylis M, Goldmann W, Houston F, Cairns D, Chong A, Ross A, Smith A, Hunter N, McLean AR (2002) Scrapie epidemic in a fully PrP-genotyped sheep flock. J Gen Virol 83: 2907–2914
2. Biacabe A-G, Laplanche J-L, Ryder S, Baron T (2004) Distinct molecular phenotypes in bovine prion diseases. EMBO Rep 5: 110–114
3. Birkett CR, Hennion RM, Bembridge DA, Clarke MC, Chree A, Bruce ME, Bostock CJ (2001) Scrapie strains maintain biological phenotypes on propagation in a cell line in culture. EMBO J 20: 3351–3358
4. Casalone C, Zanusso G, Acutis P, Ferrari S, Capucci L, Tagliavini F, Monaco S, Caramelli M (2004) Iden-

tification of a second bovine amyloidotic spongiform encephalopathy: molecular similarities with sporadic Creutzfeldt-Jakob disease. Proc Natl Acad Sci USA 101: 3065–3070
5. Caughey BW, Dong A, Bhat KS, Ernst D, Hayes SF, Caughey WS (1991) Secondary structure analysis of the scrapie-associated protein PrP 27–30 in water by infrared spectroscopy. Biochemistry 30: 7672–7680
6. Collinge J, Sidle KC, Meads J, Ironside J, Hill AF (1996) Molecular analysis of prion strain variation and the aetiology of 'new variant' CJD. Nature 383: 685–690
7. DeArmond SJ, Qiu Y, Sànchez H, Spilman PR, Ninchak-Casey A, Alonso D, Daggett V (1999) PrP^{C} glycoform heterogeneity as a function of brain region: implications for selective targeting of neurons by prion strains. J Neuropathol Exp Neurol 58: 1000–1009
8. Hope J, Wood SCER, Birkett CR, Chong A, Bruce ME, Cairns D, Goldmann W, Hunter N, Bostock CJ (1999) Molecular analysis of ovine prion protein identifies similarities between BSE and an experimental isolate of natural scrapie, CH1641. J Gen Virol 80: 1–4
9. Kimura K, Kubo M, Yokoyama T (2000) Characteristics of prion protein (PrP^{Sc}) in the brains of hamsters inoculated serially with a mouse-passaged scrapie strain. J Comp Pathol 122: 123–130
10. Pan K-M, Baldwin M, Nguyen J, Gasset M, Serban A, Groth D, Mehlhorn I, Huang Z, Fletterick RJ, Cohen FE, Prusiner SB (1993) Conversion of α-helices into β-sheets features in the formation of the scrapie prion proteins. Proc Natl Acad Sci USA 90: 10962–10966
11. Parodi AJ (2000) Protein glucosylation and its role in protein folding. Annu Rev Biochem 69: 69–93
12. Peretz D, Williamson RA, Legname G, Matsunaga Y, Vergara J, Burton DR, DeArmond SJ, Prusiner SB, Scott MR (2002) A change in the conformation of prions accompanies the emergence of a new prion strain. Neuron 34: 921–932
13. Prusiner SB (1991) Molecular biology of prion diseases. Science 252: 1515–1522
14. Russelakis-Carneiro M, Saborio GP, Anderes L, Soto C (2002) Changes in the glycosylation pattern of prion protein in murine scrapie. J Biol Chem 277: 36872–36877
15. Safar J, Roller PP, Gajdusek DC, Gibbs CJ Jr (1993) Conformational transitions, dissociation, and unfolding of scrapie amyloid (prion) protein. J Biol Chem 268: 20276–20284
16. Scott M, Groth D, Foster D, Torchia M, Yang S-L, DeArmond SJ, Prusiner SB (1993) Propagation of prions with artificial properties in transgenic mice expressing chimeric PrP genes. Cell 73: 979–988
17. Somerville RA (1999) Host and transmissible spongiform encephalopathy agent strain control glycosylation of PrP. J Gen Virol 80: 1865–1872
18. Thuring CMA, Erkens JHF, Jacobs JG, Bossers A, Van Keulen LJM, Garssen GJ, Van Zijderveld FG, Ryder SJ, Groschup MH, Sweeney T, Langeveld JPM (2004) Discrimination between scrapie and bovine spongiform encephalopathy in sheep by molecular size, immunoreactivity, and glycoprofile of prion protein. J Clin Microbiol 42: 972–980
19. Vorberg I, Priola SA (2002) Molecular basis of scrapie strain glycoform variation. J Biol Chem 277: 36775–36781
20. Yamakawa Y, Hagiwara K, Nohtomi K, Nakamura Y, Nishijima M, Higuchi Y, Sato Y, Sata T, The Expert Committee for BSE Diagnosis, Ministry of Health, Labour and Welfare of Japan (2003) Atypical proteinase K-resistant prion protein (PrP^{res}) observed in an apparently healthy 23-month-old Holstein steer. Jpn J Infect Dis 56: 221–222
21. Yokoyama T, Itohara S, Yuasa N (1996) Detection of species specific epitopes of mouse and hamster prion proteins (PrPs) by anti-peptide antibodies. Arch Virol 141: 763–769
22. Yokoyama T, Kimura KM, Ushiki Y, Yamada S, Morooka A, Nakashiba T, Sassa T, Itohara S (2001) *In vivo* conversion of cellular prion protein to pathogenic isoforms, as monitored by conformation-specific antibodies. J Biol Chem 276: 11265–11271