Replication of distinct scrapie prion isolates is region specific in brains of transgenic mice and hamsters

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Scrapie prions are composed largely, if not entirely, of PrP^{Sc} molecules. The prion isolates Sc237 and 139H exhibit markedly different incubation times in Syrian, Armenian, and Chinese hamsters, as well as in transgenic (Tg) 81 mice expressing Syrian hamster PrP (SHaPrP). Repassage of prions from transgenic mice or Chinese hamsters into Syrian hamsters revealed that the original properties of the prion isolates are retained. When Syrian hamsters were first inoculated with 139H prions and subsequently challenged with Sc237 prions, the incubation period was determined by the faster Sc237 isolate. Regional mapping studies demonstrated different kinetics and patterns of PrP^{Sc} accumulation for Sc237 and 139H prions in the brains of Syrian hamsters as well as Tg(SHaPrP)7 mice. That distinct prion isolates induce different region-specific accumulations of PrP^{Sc} in brain suggests a novel mechanism for propagation of isolates whereby they replicate in particular sets of neurons. The prion isolates could be targeted to specific CNS cells by differing conformations of PrP^{Sc}, post-translational modifications of PrP^{Sc} such as Asn-linked glycosylation, or an as yet undetected macromolecule complexed with PrP^{Sc} in the prion.

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Studies with transgenic mice expressing foreign and mutant prion protein (PrP) genes have provided a wealth of new knowledge about the structure of the prion particle, the pathogenesis of prion diseases, and the events that feature in the replication of prions (Scott et al. 1989; Hsiao et al. 1990; Prusiner et al. 1990; Westaway et al. 1991). Investigations of transgenic (Syrian hamster PrP) [Tg(SHaPrP)] mice expressing SHaPrP demonstrated that PrP^{Sc} in the inoculum dictates whether mouse prions (Mo prions) or Syrian hamster prions (SHa prions) will be produced. Furthermore, the scrapie incubation times and neuropathology are determined by the particular prion synthesized. Although no physical evidence is available, it seems likely that PrP^C and PrP^{Sc} transiently form a complex during the synthesis of nascent PrP^{Sc} molecules (Prusiner et al. 1990; Prusiner 1991).

Early studies of Dickinson and colleagues not only identified inbred strains of mice with short or long incubation times, but also distinct isolates of the scrapie agent distinguishable by their different incubation times and neuropathologic lesions (Dickinson et al. 1968; Fraser and Dickinson 1968, 1973; Dickinson and Meikle

1971; Dickinson 1976; Bruce and Dickinson 1987; Bruce et al. 1991). Distinct isolates of the scrapie agent have often been referred to as strains. Although earlier studies were complicated by the use of *Prn-p^a* and *Prn-p^b* mice (Carlson et al. 1986, 1988, 1989; Hunter et al. 1987; Westaway et al. 1987, 1991; Race et al. 1990), the existence of at least a few scrapie isolates in mice seems secure (Bruce and Dickinson 1987; Bruce et al. 1991). Derivation of new isolates in Syrian hamsters after passage from mice has also been reported (Kimberlin and Walker 1978; Kimberlin et al. 1987b, 1989). The existence of distinct prion isolates poses a conundrum because both the molecular structure and the mechanism responsible for the different biological properties of these isolates seem to be without precedent. Diversity in biology is generally encoded within nucleic acids; yet no chemical, biological, or physical evidence argues for a scrapie-specific polynucleotide (Prusiner 1991).

To investigate the molecular processes that feature in the replication of prions, we first asked whether Mo prions injected together with SHa prions or sequentially into Tg(SHaPrP) mice might influence the replication of SHa prions. The replication of SHa prions was found to be independent of Mo prions producing longer incubation times. Similarly, two distinct isolates of SHa prions designated Sc237 and 139H and causing disease in ~75

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Table 1. Scrapie incubation times in Tg(SHAPrP)81 mice inoculated with mixtures of mouse and Sc237 SHa prions

Inocula		Number	Incubation times	
Mo (log ID ₅₀ units)	SHa (units)		illness (days ± S.E.M.)	death
<i>A. Tg(SHAPrP)81 mice</i>				
2		6	277 ± 7	286 ± 5
6		8	192 ± 5	204 ± 5
	2	8	104 ± 2	107 ± 2
	4	6	81 ± 4	84 ± 4
	7	7	66 ± 2	71 ± 2
6	2	3	101 ± 2	108 ± 2
6	4	4	83 ± 2	87 ± 2
2	7	7	67 ± 1	75 ± 2
2	4	7	84 ± 2	90 ± 2
<i>B. Nontransgenic 81 mice</i>				
6		4	131 ± 1	145 ± 2
	7	8	>541	
6	2	9	131 ± 2	143 ± 3
6	4	5	145 ± 1	159 ± 3
2	4	3	147 ± 6	157 ± 8
2	7	6	166 ± 6	189 ± 4
6	1	4	135 ± 3	150 ± 3

and ~165 days, respectively, were inoculated sequentially into Syrian hamsters. The replication of the Sc237 isolate was independent of the 139H isolates, as judged by scrapie incubation times.

End-point titrations of Syrian hamster brain extracts showed that both the Sc237 and 139H isolates produced ~10⁹ ID₅₀ units of prions per gram of brain tissue. In the brains of inoculated hamsters, each isolate produced similar amounts of PrP^{Sc}, which was converted to PrP 27–30 after limited proteolysis in vitro. The two prion isolates produced different kinetics and patterns of PrP^{Sc} accumulation in Syrian hamsters and Tg(SHAPrP) mice, raising the possibility that these isolates are synthesized in different sets of cells in the central nervous system (CNS). Distinct isolates of prions might derive their biological properties from different conformations of PrP^{Sc}, post-translational chemical modifications, or non-covalently bound molecules (Prusiner 1991; Weissmann 1991).

Results

Synthesis of SHa prions in transgenic mice and Syrian hamsters

Tg(SHAPrP)81 and nontransgenic control mice were inoculated with mixtures of RML Mo and Sc237 SHa prions (Table 1), and the incubation times were measured. The incubation times in Tg81 mice were determined by SHa prions alone even if the dose of Mo prions in the inoculum was 10⁴-fold greater than that of SHa prions. Non-Tg81 mice inoculated with ~10⁶ ID₅₀ units of Mo prions had incubation times of ~130 days, whereas Tg81

mice inoculated with Mo prions had times of ~180 days (Table 1) (Prusiner et al. 1990).

A kinetic study was performed in which SHa prions were inoculated at increasing intervals after the inoculation of Mo prions (Table 2A). Again, the scrapie incubation times in Tg81 mice were determined by the SHa prions alone. No influence of SHa prions on the incubation times in non-Tg81 mice inoculated with Mo prions could be discerned (Table 2B).

To extend the experiments with SHa and Mo prions, Syrian hamsters were challenged with Sc237 prions at increasing intervals after the inoculation with 139H prions (Table 3). The scrapie incubation times were determined only by the Sc237 prions and, thus, were independent of the 139H prions. Even if the 139H prions were inoculated as much as 63 days before the inoculation with Sc237 prions, the incubation time was the same as that for animals inoculated with Sc237 prions alone. These results contrast with those reported by others where mouse isolates with long incubation times inoculated prior to short incubation time isolates produced long incubation times. Such observations were interpreted as competition among isolates for replication sites (Dickinson et al. 1972; Kimberlin and Walker 1985).

Biochemical and biological properties of two prion isolates

Because one infectious unit of Sc237 prions causes disease in hamsters ~140 days after inoculation (Marsh and Kimberlin 1975; Kimberlin and Walker 1977; Prusiner et

Table 2. Scrapie incubation times in Tg(SHAPrP)81 mice inoculated with Sc237 SHa prions at various intervals after inoculation with Mo prions

Time of inoculation with SHa prions ^a (day)	Number	Incubation time		Interval between inoculation with SHa prions and illness (day)
		illness (days ± s.e.m.)	death	
<i>A. Tg(SH_aPrP)81 mice</i>				
0	10	76 ± 2	79 ± 1	76
1	3	79 ± 4	81 ± 5	78
10	13	86 ± 1	90 ± 2	76
30	2	97 ± 0	102 ± 2	67
60	11	140 ± 2	146 ± 2	80
100	12	169 ± 2	175 ± 2	69
— ^b	20	194 ± 4	200 ± 3	
<i>B. Nontransgenic 81 mice</i>				
0	14	138 ± 3	154 ± 3	138
1	4	128 ± 6	141 ± 2	127
10	12	138 ± 3	154 ± 4	128
30	6	121 ± 4	139 ± 3	91
60	15	136 ± 3	156 ± 4	76

^aAll mice were inoculated with ~10⁶ ID₅₀ units of Mo prions at day 0 and with ~10⁷ ID₅₀ units of Sc237 SHa prions at the day designated.

^bNo SHa prions inoculated.

Table 3. Scrapie incubation times in Syrian hamsters inoculated with Sc237 prions at various intervals after inoculation with 139H prions

Time of inoculation with Sc237 prions ^a (day)	Number	Incubation time		Interval between inoculation with Sc237 and onset of illness (days)
		illness (days ± S.E.M.)	death (days ± S.E.M.)	
0	5	85 ± 3	94 ± 2	85
1	8	81 ± 2	91 ± 1	80
10	7	85 ± 4	94 ± 3	75
28	5	107 ± 2	120 ± 3	79
63	6	151 ± 0	178 ± 2	88
— ^b	6	159 ± 1	185 ± 3	

^aAll groups of Syrian hamsters were inoculated with 139H prions on day 0.

^bNo Sc237 prions inoculated.

al. 1982b), we considered the possibility that 139H prions were produced as a consequence of the defective synthesis of Sc237 prions. To assess this possibility, hamsters were inoculated with 139H prions and sacrificed 172 days after inoculation, and the concentration of infectious prion particles was measured by end-point titrations. There was no difference between prion titers in 10% (wt/vol) brain homogenates prepared from Syrian hamsters with clinical signs of scrapie after inoculation with either Sc237 (Prusiner et al. 1982b) or 139H prions. Prion titers ($\log ID_{50}$ U/ml $\pm$ S.E.M.) of 8.1 ± 0.33 , 8.6 ± 0.45 , and 7.8 ± 0.30 were found by end-point titrations for three brain extracts from hamsters inoculated with 139H prions. In Figure 1, results from end-point titrations of 139H and Sc237 in Syrian hamsters are plotted—the reciprocal of the incubation time as a function of the $\log_{10}$ of the dose. The slopes of the two lines are clearly different. A comparison of any two doses of 139H

gives a larger change in incubation time than those of Sc237.

Previous studies had shown an excellent correlation between PrP^{Sc} concentrations and Sc237 prion titers (McKinley et al. 1983; Jendroska et al. 1991). As expected, we found that the concentration of PrP^{Sc} in brains of terminally ill hamsters infected with 139H was similar to that with Sc237. Two different brain extracts prepared from Syrian hamsters with clinical scrapie after inoculation with Sc237 contained 70 and 80 μ g of PrP^{Sc} /gram of protein, whereas four different extracts from hamsters inoculated with 139H had 72, 86, 84, and 83 μ g of PrP^{Sc} /gram of protein.

To facilitate purification on a small scale, a discontinuous sucrose gradient ultracentrifugation (Prusiner et al. 1982a) was replaced by ultrafiltration with a membrane of 300-kD exclusion limit. The ultrafiltration was operated in a continuous dilution mode, that is, the volume lost in the course of the filtration was continuously replaced by the addition of fresh buffer. The prion rods were retained by the membrane, whereas most proteins, peptides, and other molecules with molecular mass <300 kD passed through the filter. After ~1000-fold dilution, the ultrafiltration was terminated, and the retentate was concentrated and analyzed by SDS-PAGE (Fig. 2A). Three major bands were found by silver staining: They correspond to PrP 27–30 with varying amounts of N-linked oligosaccharides (Oesch et al. 1985; Taraboulos et al. 1990a). The ultrafiltration procedure yields PrP 27–30 of a purity similar to that obtained with sucrose gradients (Prusiner et al. 1982b, 1983); in the silver-stained lanes in Figure 2A, no components other than PrP 27–30 are visible. PrP 27–30 is used to designate the protease-resistant portion of PrP^{Sc} ; the amino-terminal ~67 amino acids of PrP^{Sc} are hydrolyzed when PrP 27–30 is generated by limited proteolysis (McKinley et al. 1983; Prusiner et al. 1991).

Figure 1. Scrapie incubation times plotted as a function of the dose of inoculum for two distinct prion isolates. The $\log_{10}$ dose, which is calculated from the titer $\times$ dilution, is on the x-axis; the reciprocal of incubation time is on the y-axis. The curves were fitted by linear regression analysis. Sc237 regression coefficient = 0.88, slope = 1.11, and y intercept = 4.88; 139H regression coefficient = 0.91, slope = 0.51, and y intercept = 2.82.

Figure 2. Scrapie prion proteins in brains of Syrian hamsters inoculated with Sc237 or 139H prions. (A) Analysis of 139H prion rods from Syrian hamster brains after purification by ultrafiltration. A 12% SDS–polyacrylamide gel (Laemmli 1970) was stained with silver (Turk et al. 1988). (Lane 1) Molecular weight markers; (lane 2) denatured prion rods purified from 139H-infected hamster brain. The three bands represent PrP 27–30 with 2, 1, and no *N*-linked oligosaccharides. (B) Western blot of brain homogenates and purified PrP 27–30. PrP was visualized with the monoclonal antibody 13A5 (Barry and Prusiner 1986). (Lanes 1,2) 50 μ g of brain extract from Syrian hamsters infected with Sc237 and 139H, respectively. The three bands represent the double, single, and non-*N*-linked glycosylated forms of PrP 33–35. (Lanes 3,4) Purified prion rods purified from the brains of hamsters infected with Sc237 and 139H, respectively. The three bands represent the variations in extent of glycosylation for PrP 27–30, as described above.

We compared PrP^{Sc} and PrP 27–30 from the brains of hamsters infected with Sc237 and 139H. In Figure 2B a Western blot of brain homogenate from terminally ill Syrian hamsters infected with either Sc237 or 139H is shown. In lanes 1 and 2, the patterns of PrP immunoreactivity for Sc237 and 139H were indistinguishable. With both isolates, double, single, and nonglycosylated forms of PrP were found. No difference in molecular mass, glycosylation, and immunogenicity could be discerned between PrP molecules in the two isolates. In lanes 3 and 4, purified PrP 27–30 from Sc237 and 139H is shown; again, no differences could be discerned from the two isolates.

Phenotypic properties of prion isolates upon passage in different hamster species

To assess the variation and distribution of scrapie incubation for the two SHa prion isolates, we assembled the data from 48 Syrian hamsters infected with Sc237 prions. These animals had mean incubation periods from inoculation to onset of illness of 77 ± 1 (S.E.M.) days and to death of 89 ± 2 (S.E.M.) days, whereas, 94 Syrian hamsters inoculated with 139H prions developed neurologic dysfunction at 167 ± 1 (S.E.M.) and died at 206 ± 2 (S.E.M.) days. For Sc237 prions, the hamsters died ~ 12 days after the onset of illness while those hamsters with 139H prions showed a progressive neurologic disorder lasting ~ 39

days. Although other investigators state that 139H causes disease in Syrian hamsters in ~ 130 days (Kimberlin et al. 1987b, 1989), we have not been able to confirm their observations. They have not reported survival times so no objective comparison can be made.

Because the homology among the PrP gene products of three species of hamsters exceeds 96% (Lowenstein et al. 1990), we studied the behavior of two scrapie isolates in these animals. In Syrian hamsters, intracerebral inoculation of $\sim 10^7$ ID₅₀ units of 139H (Table 4) requires almost 100 days longer to produce clinical signs of scrapie than does the Sc237 isolate. 139H prions inoculated into Armenian hamsters required ~ 40 days longer to produce disease than did Sc237 (Table 4). Upon passage of the two isolates in Chinese hamsters, we found that the phenotypes of the two scrapie prion isolates were reversed: 139H prions required 100 days less than that needed for Sc237 prions to produce clinical signs of scrapie (Table 4). Furthermore, the duration of illness in Chinese hamsters for 139H is ~ 15 days in contrast to Syrian hamsters, where the animals deteriorate progressively over a period of ~ 40 days. Because of the species barriers between the three hamster species presumably owing to differences in PrP sequence (Lowenstein et al. 1990), it was necessary to acquire data on prions passaged repeatedly in each species and passaged directly from Syrian to Chinese hamsters (Table 4A). Although the incubation times are longer, as expected upon crossing the species barrier, the relative characteristics of the changes attendant with passage of each scrapie isolate in different hamster species are similar.

After passage of Sc237 and 139H prions into Chinese hamsters, these isolates were inoculated into Syrian hamsters to determine the incubation times for each isolate. 139H prions required ~ 100 days longer to produce clinical signs of scrapie than Sc237, showing that the phenotypic differences between the two strains are distinct for any given host (Table 4A). Although the difference in incubation times of 100 days for the two isolates is similar to that noted above for the Syrian to Syrian passage, the incubation times are longer owing to crossing of species barrier again. A second passage of either Sc237 or 139H in Syrian hamsters demonstrates that this is the case. Results similar to those with Chinese hamsters were obtained when Sc237 and 139H prions were passaged into Armenian hamsters (Table 4B). On first passage, the incubation time in Armenian hamsters was longer for Sc237 than for 139H prions; and on subsequent passage, the incubation times for both prions were similar. Passage of the two prion isolates from Armenian back into Syrian hamsters showed that each strain retained its original properties: 139H required ~ 100 days longer than Sc237 for the appearance of clinical signs of scrapie (Table 4B).

Characteristics of SHa prion isolates passaged in transgenic mice

We also studied the behavior of the two scrapie isolates Sc237 and 139H prions in Tg(SHaPrP)⁸¹ and Tg-

Table 4. Scrapie incubation times for Syrian, Armenian, and Chinese hamsters inoculated with either Sc237 or 139H prions

Inoculum ^a	Hamster ^b	Sc237 ^c			139H		
		number ^d	illness ^e	death ^e	number ^d	illness ^e	death
			(days ± S.E.M.)			(days ± S.E.M.)	
<i>A. Chinese hamsters</i>							
SHa → SHa	Syrian	48	77 ± 1	89 ± 2	94	167 ± 1	206 ± 2
SHa → SHa	Chinese	4	344 ± 7	358 ± 16	20	241 ± 3	284 ± 3
SHa → CHa	Chinese	9	265 ± 2	274 ± 3	8	226 ± 4	241 ± 7
CHa → CHa	Chinese	15	272 ± 3	307 ± 6	8	171 ± 5	181 ± 11
SHa → CHa	Syrian	16	121 ± 8	133 ± 6	9	229 ± 4	274 ± 7
CHa → SHa	Syrian	13	69 ± 2	79 ± 5	10	182 ± 3	194 ± 4
<i>B. Armenian hamsters</i>							
SHa → SHa	Armenian	4	174 ± 1	194 ± 2	7	146 ± 1	161 ± 1
SHa → AHa	Armenian	8	128 ± 3	153 ± 5	4	153 ± 0	154 ± 0
AHa → AHa	Armenian	16	125 ± 2	148 ± 3	8	165 ± 3	174 ± 3
SHa → AHa	Syrian	14	113 ± 2	129 ± 4	7	214 ± 7	232 ± 5

^aPassage history of the inoculum. (SHa) Syrian hamster; (AHa) Armenian hamster; (CHa) Chinese hamster.

^bSpecies of hamster inoculated intracerebrally.

^cSome of the data for Sc237 prions in A and B are taken from Lowenstein et al. (1990).

^dNumber of animals.

^eIncubation period in days $\pm$ S.E.

(SHaPrP)⁷ mice (Prusiner et al. 1990). The difference in incubation times for the two isolates was $\sim$ 35 days in Tg(SHaPrP)⁸¹ mice (Table 5A; Fig. 3A). In contrast, Tg(SHaPrP)⁷ mice had similar incubation times when inoculated with either Sc237 or 139H prions (Table 5B; Fig. 3B). Passage of Sc237 and 139H from transgenic mice into Syrian hamsters produced incubation times similar to those found upon repeated passage in Syrian hamsters (Tables 4 and 5). These observations argue that the phenotypic changes in scrapie incubation times for two prion isolates upon passage in Tg(SHaPrP) mice are modulated by the level of SHaPrP^C expression. It is noteworthy that the duration of illness was considerably shorter

in Syrian hamsters inoculated with 139H prions passed through Tg81 or Tg7 mice compared with the original SHa inoculum (Table 5).

Only a few nontransgenic mice inoculated with Sc237 prions develop scrapie after a prolonged incubation period (Table 5C) (Scott et al. 1989), but those inoculated with 139H prions uniformly developed CNS disease at $\sim$ 500 days. Passage of the Sc237 prions through Chinese hamsters to produce Chinese hamster PrP^{Sc} (CHaPrP^{Sc}) produced clinical scrapie in all of the CD-1 Swiss mice $\sim$ 325 days after inoculation. Of note, the amino acid sequence of CHaPrP is more similar to MoPrP-A than SHaPrP (Lowenstein et al. 1990).

Table 5. Scrapie incubation times for transgenic mice and Syrian hamsters inoculated with either Sc237 or 139H prions

Inoculum	Animal ^a	Sc237			139H		
		number	illness (days $\pm$ S.E.M.)	death	number	illness (days $\pm$ S.E.M.)	death
A. <i>Tg81 mice</i>							
SHa $\rightarrow$ SHa	Syrian	48	77 $\pm$ 1	83 $\pm$ 2	94	167 $\pm$ 1	206 $\pm$ 2
SHa $\rightarrow$ SHa	Tg81	22	75 $\pm$ 2	75 $\pm$ 2	19	110 $\pm$ 2	115 $\pm$ 2
SHa $\rightarrow$ Tg81	Tg81	8	81 $\pm$ 2	83 $\pm$ 3	7	106 $\pm$ 3	110 $\pm$ 3
SHa $\rightarrow$ Tg81	Syrian	5	77 $\pm$ 2	91 $\pm$ 2	3	205 $\pm$ 1	214 $\pm$ 3
B. <i>Tg7 mice</i>							
SHa $\rightarrow$ SHa	Tg7	26	48 $\pm$ 1	51 $\pm$ 1	11	40 $\pm$ 3	42 $\pm$ 3
SHa $\rightarrow$ Tg7	Tg7	6	54 $\pm$ 1	58 $\pm$ 2	6	59 $\pm$ 2	63 $\pm$ 3
SHa $\rightarrow$ Tg7	Syrian	11	81 $\pm$ 1	95 $\pm$ 1	7	181 $\pm$ 6	188 $\pm$ 4
C. <i>Nontransgenic mice</i>							
SHa $\rightarrow$ SHa	non-Tg81	9	>700		11	499 $\pm$ 15	523 $\pm$ 15
CHa $\rightarrow$ CHa	CD-1	7	325 $\pm$ 17	346 $\pm$ 1610	10	>420	

^aTransgenic mice, nontransgenic mice, and Syrian hamsters were inoculated intracerebrally.

Figure 3. Incubation times for two distinct prion isolates in transgenic mice expressing the SHaPrP. (A) Tg(SHaPrP)81; (B) Tg(SHaPrP)7 mice inoculated with Sc237 (open bars) or 139H (solid bars) prions.

Correlation of vacuolation and PrP^{Sc} for two prion isolates

In addition to incubation times, the distribution and amount of neuropil vacuolation have been used to define prion isolates (Fraser and Dickinson 1968, 1973). Consistent with this, vacuolation was coarser in gray matter and was found in all layers of the cerebral cortex of hamsters inoculated with 139H prions, whereas it was more delicate and subtle with Sc237 and was largely confined to layers 4–6 (Fig. 4). Figure 4, A and C, shows layer II of the cerebral cortex while Figure 4, B and D, shows layer IV. Shrinkage artifact around blood vessels and some cells (A) can be readily distinguished from disease-related vacuolation in the neuropil between neurons (B). In Figure 4, C and D, widespread vacuolation is evident along with increased cell number owing to proliferation of astrocytes and infiltration of microglia. Vacuolation was also present in the caudate nucleus and cerebellum of Syrian hamsters inoculated with 139H prions in contrast to those inoculated with Sc237, in which pathology was not significant in these structures (DeArmond et al.

1987). Reactive astrocytic gliosis and vacuolation colocalized with both isolates (Fig. 4). Additionally, amyloid plaques composed of SHaPrP were found in the same subpial, subependymal, and subcallosal locations with both 139H and Sc237 prions (DeArmond et al. 1985).

The regional distribution PrP^{Sc} in the brains of Syrian hamsters was compared for Sc237 and 139H prions by use of “histoblots” immunostained with α -PrP antibodies (Taraboulos et al. 1992) (Fig. 4). The histoblots were pretreated with proteinase K to eliminate PrP^C and then exposed to Gdn-HCl to denature PrP^{Sc} and thereby enhance its immunoreactivity. With 139H prions, PrP^{Sc} was distributed more diffusely in the gray and white matter than with Sc237. For example, PrP^{Sc} was uniformly distributed in all layers in the neocortex with 139H prions, whereas it was mostly confined to layers 4–6 in those infected with Sc237 (Fig. 4). Immunostaining of the caudate nucleus was intense in 139H-inoculated hamsters, but it was absent with Sc237 (data not shown). These results demonstrate that vacuolation, reactive astrocytic gliosis, and PrP^{Sc} colocalize in both 139H and Sc237 scrapie. Therefore, while the distribution of PrP^{Sc} and the accompanying neuropathologic changes are different for the two scrapie prion isolates, the relationship between PrP^{Sc} deposition and neuropathologic lesions remained constant.

Kinetics and patterns of PrP^{Sc} accumulation in Syrian hamsters

To extend observations on the patterns of PrP^{Sc} accumulation, the kinetics of PrP^{Sc} deposition in the brains of Syrian hamsters were compared for Sc237 and 139H prions. Seven to 14 days after unilateral intrathalamic inoculation with Sc237 prions, PrP^{Sc} became detectable at the inoculation site (Fig. 5A). Spread of PrP^{Sc} from this region occurred by at least two routes. Spread to the neocortex appeared to be by axonal transport, as the most intense immunostaining for PrP^{Sc} 65 days postinoculation occurred in neocortical layers 4 and 6, the main termination sites of thalamocortical projections (Fig. 4; Fig. 5A: 65 days). Spread of PrP^{Sc} to the contralateral thalamus and the septum, which was detectable by 21 and 28 days, respectively, was probably not by axonal transport because there are few, if any, direct neuroanatomical interconnections between the two lateral halves of the thalamus or between the thalamus and the septum. Histoblots showed that the spread of PrP^{Sc} in the thalamus was not by a continuous, radially directed process starting from the initial site of PrP^{Sc} accumulation but, rather, by an apparently discontinuous process in which PrP^{Sc} was found within identical nuclei in the contralateral thalamus (Fig. 5A, 21, 28, 35 days). By 35 days, PrP^{Sc} was distributed symmetrically in the thalamus within homologous nuclei. This pattern of accumulation suggests that PrP^{Sc} released from specific populations of neurons into the extracellular space has a propensity to target to homologous contralateral neurons. That PrP^{Sc} can be carried in the extracellular space from the unilaterally inoculated thalamus to the same contra-

Figure 4. Colocalization of neuropathological changes and PrP^{Sc} in the brains of Syrian hamsters with clinical signs of scrapie after inoculation with either Sc237 (70 days postinoculation) or 139H prions (168 days postinoculation). (*Left panels*) Vacuolation assessed on brain sections stained with hematoxylin and eosin (H & E). (*Middle panels*) Reactive astrocytic gliosis demonstrated by immunohistochemistry with antibodies to glial fibrillary acidic protein (GFAP). (*Right panels*) Location of PrP^{Sc} revealed by histoblots of coronal brain sections at the level of the thalamus and hippocampus. The lettered black and white dots in the histoblots indicate the approximate locations in the cerebral cortex of the photomicrographs in the *left* and *middle* panels. Note the difference in magnification: Bar on histoblot, 1 mm; bar on histologic sections, 50 μ m. With Sc237 prions, little or no PrP^{Sc}, vacuolation or reactive gliosis was present in the outer layers of the cerebral cortex (A), whereas all were present in the inner half (B), consistent with an earlier immunohistochemical study (DeArmond et al. 1987). In contrast with 139H prions, PrP^{Sc} accumulated in all layers of the neocortex (C,D), as did vacuolation and reactive astrocytic gliosis. Increased cellularity in regions with vacuolation, particularly with 139H, is due in part to astrocyte proliferation and infiltration by microglial cells.

lateral structures was supported by the fate of unilateral injections of India ink (50 μ l of a 1 : 10 dilution). The ink was distributed rapidly and symmetrically to those subpial, subependymal, and subcallosal regions where PrP amyloid plaques occur (data not shown).

Specific targeting of PrP^{Sc} to distant neurons was also evident in the septum. In this case, PrP^{Sc} appeared to be transported by the cerebrospinal fluid (CSF). As early as 7 days postinoculation, PrP^{Sc} was detected in the walls of the lateral ventricles, which form the lateral surface of the septum and the medial surface of the caudate nucleus (Fig. 5A). While a portion of this PrP^{Sc} could have been derived from the Sc237 inoculum, the majority appears to originate from newly formed PrP^{Sc} in the thalamus because the thickness and intensity of immunostaining at the ventricular wall increased over the next 3 weeks. By 28 days, PrP^{Sc} was detectable in the medial septal nucleus and the diagonal band of Broca. By day 35, the medial septal region was strongly immunopositive for PrP^{Sc}, and by day 70, PrP^{Sc} was present in both the medial and lateral septal regions. These results argue

that some of the newly formed PrP^{Sc} in the thalamus is released into the ventricles and that the septum and basal forebrain region (diagonal band of Broca) are specifically targeted. Infection of the septum by the CSF appears to be unique to this structure because the caudate nucleus, whose walls also contained PrP^{Sc} by 7–14 days, did not begin to accumulate PrP^{Sc} until after 63 days (Jendroska et al. 1991). Specific targeting of the medial septal and diagonal band of Broca neurons by de novo-synthesized PrP^{Sc} supports the hypothesis that particular populations of neurons specify the behavior of PrP^{Sc}.

Syrian hamsters inoculated in the thalamus with 139H prions have scrapie incubation times of $\sim$ 165 days, and the kinetics of PrP^{Sc} accumulation were correspondingly slower (Fig. 5B). PrP^{Sc} was barely detectable in the thalamus at 40 days, indicating a significant delay in its appearance in contrast to hamsters inoculated with Sc237. There was little or no accumulation of PrP^{Sc} in the walls of the ventricles and no evidence for spread of prions through CSF routes with 139H prions. While PrP^{Sc} accumulated in the septum late in 139H scrapie, it

Figure 5. Kinetics of PrP^{Sc} accumulation in Syrian hamster brains after inoculation with either Sc237 or 139H scrapie prions. Cryostat coronal sections through the hippocampus–thalamus and the septum–caudate nucleus were taken from Syrian hamster brains at 7, 21, 28, 35, 50, and 65 days after inoculation with Sc237 prions (A) or at 40, 80, 120, and 170 days after inoculation with 139H prions (B). The prion inoculum contained $\sim 10^7$ ID₅₀ units, and 50 μ l was inoculated intracerebrally. Histoblots were stained for PrP^{Sc} with the PrP antiserum R073. (C) Diagrams identify major anatomical structures seen in coronal sections: (NC) neocortex; (Hp) hippocampus; (Th) thalamus; (Hy) hypothalamus; (S) septum; (Cd) caudate; (dbB) diagonal band of Broca.

was absent in the early phase of the incubation period. The relative concentrations of PrP^{Sc} from 80 to 160 days, based on intensity of immunostaining, indicated a substantially slower rate of accumulation of 139H PrP^{Sc} compared with Sc237 PrP^{Sc}. When clinical signs first appeared, more brain regions had accumulated PrP^{Sc} by 165 days in hamsters inoculated with 139H prions than those inoculated with Sc237 at 75 days.

Kinetics and patterns of PrP^{Sc} accumulation in transgenic mice

Interpretation of the results of Sc237 and 139H prions in Syrian hamsters with respect to the kinetics and patterns of PrP^{Sc} accumulation (Figs. 4 and 5) is complicated because scrapie incubation times were so markedly different (Table 4). To circumvent this problem, we examined the kinetics of PrP accumulation in Tg(SHaPrP)⁷ mice because scrapie incubation times were ~ 50 days with both 139H and Sc237 prions (Fig. 3B).

The differences in PrP^{Sc} kinetics and neuroanatomic distribution were even more striking in Tg(SHaPrP)⁷ mice (Fig. 6). At ~ 49 days, PrP^{Sc} was found in most thalamic nuclei with 139H prions, whereas it was confined to the lateral–ventral tier of thalamic nuclei with Sc237 prions (Figs. 6 and 7). Also, by day 49, high concentrations of PrP^{Sc} were found in Ammon’s horn of the hippocampus and in the neocortex with 139H, whereas little or no PrP^{Sc} was detected in either structure with Sc237 prions. There were also striking differences in the distribution of PrP^{Sc} in the brain stem, particularly in the reticular formation of the midbrain, pons and medulla, but also in the vestibular nuclei and the deep cerebellar nuclei (Fig. 8). An intense PrP^{Sc} accumulation in the corpus callosum was found with 139H prions while little or none was found with Sc237 prions. Other white matter tracts were less immunopositive with 139H.

The patterns of PrP^{Sc} accumulation with Sc237 and 139H prions were similar in two respects. First, PrP^{Sc} accumulation in Tg(SHaPrP)⁷ mice did not begin at the

Figure 6. Kinetics of PrP^{Sc} accumulation in the brains of transgenic mice expressing SHaPrP genes after inoculation with either Sc237 or 139H scrapie prions. Histoblots of coronal sections through the hippocampus and thalamus taken from Tg(SHaPrP)7 mouse brains at 21, 35, 49, and 56 days after inoculation with Sc237 or 139H prions. The prion inoculum contained $\sim 10^7$ ID_{50} units, and 30 μl was inoculated intracerebrally. Histoblots were developed with the PrP antiserum R073. The diagram (lower left) identifies anatomical structures seen in coronal sections: (Am) Amygdala; (CC) corpus callosum; (Hb) habenula; (Hp) hippocampus; (Hy) hypothalamus; (LTh) lateral-ventral tier of thalamic nuclei; (MTh) medial thalamic group of nuclei; (NC) neocortex.

site of inoculation in the thalamus as it did in Syrian hamsters inoculated with Sc237 prions. Rather, it first appeared in the form of extracellular plaque-like accumulations beneath the corpus callosum at 14–21 days. This finding suggests that the rate of release of newly formed PrP^{Sc} from murine neurons in the thalamus, assuming synthesis there, was greater than the rate of retention. Second, a small number of brain regions in Tg(SHaPrP)7 mice accumulated PrP^{Sc} with both Sc237 and 139H, specifically the locus coeruleus, brain-stem raphe nuclei, habenula, hypothalamus, and lateral-ventral thalamus (Figs. 7 and 8).

Discussion

Although a converging body of evidence argues that infectious prions are devoid of foreign nucleic acids (Meyer et al. 1991; Prusiner 1991), the mechanism responsible for prion diversity remains enigmatic. The studies reported here offer a novel mechanism that might account for the diversity of prions in the absence of a scrapie-specific polynucleotide.

Although most studies of prion diversity have been

performed with inbred mice, we chose to study two distinct isolates from Syrian hamsters with scrapie for the following reasons: (1) Incubation times of Sc237 and 139H prions are the shortest for any pair of distinct isolates (Kimberlin et al. 1987a); (2) the molecular properties of Sc237 prions and PrP^{Sc} are the most extensively characterized (Gabizon and Prusiner 1990); (3) other hamster species have closely related but distinct PrP genes (Lowenstein et al. 1990); and (4) transgenic mice expressing SHaPrP are well characterized with respect to Sc237 prions (Scott et al. 1989; Prusiner et al. 1990).

Molecular properties of scrapie isolates

PrP^{Sc} is the only component of the infectious prion particle identified to date. Copurification of Sc237 prion infectivity and PrP^{Sc} (or PrP 27–30) is well established (Prusiner et al. 1982a, 1983; Gabizon et al. 1988). Purification protocols similar to those used for Sc237 prions were employed for 139H prions, resulting in fractions highly enriched for PrP^{Sc} . Our finding that PrP 27–30 molecules of Sc237 and 139H prions were indistinguishable by silver staining and immunoblotting after SDS-

Figure 7. Distribution of PrP^{Sc} in Tg(SHaPrP)7 mice 49 days after inoculation with Sc237 or 139H prions in histoblots of horizontal sections of the brain through the thalamus. (Cb) Cerebellum; (Cd) caudate nucleus; (Hb) habenula; (Hp) hippocampus; (IC) inferior colliculus; (Lg) lateral geniculate nucleus; (Mb) midbrain; (NC) neocortex; (S) septum; (SC) superior colliculus; (Th) thalamus. The asterisk (*) marks the region of the corpus callosum in the horizontal sections. The level of the plane of section is indicated in the sagittal brain diagram.

PAGE is in accord with earlier studies showing similar Western blotting patterns for various mouse and hamster isolates of scrapie prions (Kascsak et al. 1985, 1986). Digestion of PrP 27–30 by endoproteinase Lys C followed by reverse-phase high-performance liquid chromatography (HPLC) failed to show any differences between the PrP peptides prepared from Syrian hamster brains inoculated with either Sc237 or 139H prions, as assessed by liquid secondary ion and electrospray mass spectrometry (R. Hecker, M.A. Baldwin, N. Stahl, S. Hall, A.L. Burlingame, and S.B. Prusiner, in prep.). Similarly, studies on the structure of the glycoinositol phospholipid (GPI) anchor of PrP^{Sc} failed to reveal any differences between the covalent structures of the GPI glycans from the two isolates (Stahl et al. 1992).

Electron microscopy was used to evaluate the ultrastructure of prion rods in purified fractions isolated from brains of Syrian hamsters inoculated with either Sc237 or 139H prions. Although prion rods were formed from both isolates under similar conditions (McKinley et al. 1991), the 139H rods appeared to be thicker and longer than those commonly found in Sc237 preparations (M. McKinley and R. Hecker, unpubl.). The parallel subfilaments of the 139H rods are reminiscent of rods found in purified fractions from human brains of patients dying of Creutzfeldt-Jakob disease (CJD) (Bockman et al. 1985).

These straight subfilaments contrast sharply with the helically wound subfilaments of scrapie-associated fibrils found in extracts of scrapie and CJD brains (Merz et al. 1981, 1983). Additional studies are needed to determine the structural basis of the apparent differences in the morphology of the prion rods in the Sc237 and 139H prion preparations.

Replication of distinct isolates of Syrian hamster prions

The first distinct isolates of scrapie prions exhibiting distinguishable phenotypes were found in goats based on their clinical signs and were designated either "drowsy" or "scratching" (Gordon and Pattison 1957; Pattison and Millson 1961; Pattison 1966). Later, prion isolates, or strains, were studied in mice on the basis of incubation time measurements and neuropathologic changes (Dickinson et al. 1968; Dickinson and Meikle 1971; Bruce and Dickinson 1987; Carp and Callahan 1991). Although the use of *Prn-p^a* and *Prn-p^b* strains of mice unknowingly complicated the study of distinct scrapie isolates (Westaway et al. 1987; Carlson et al. 1989), the existence of a limited number of isolates with distinct properties lesion on both incubation times and neuropathologic lesion profiles seems firmly established (Bruce et al. 1991).

Figure 8. Histoblots of PrP^{Sc} in transverse sections of the rostral pons and medulla 49 days after inoculation with Sc237 or 139H prions in Tg(SHaPrP)7 mice. Structures that accumulate PrP^{Sc} common to both prion isolates include the nucleus of locus coeruleus and raphe nuclei. Clinical signs of scrapie were the same with both inocula.

Our results with Sc237 prions inoculated into Syrian hamsters at increasing intervals after inoculation with 139H prions (Table 3) contrast with those reported by Dickinson and colleagues. They reported that inoculation of mice with a long incubation time isolate prevented replication of a short incubation time isolate that was inoculated later (Dickinson and Fraser 1972; Dickinson et al. 1975; Kimberlin and Walker 1985). For Sc237 and 139H prions, the lack of competition between these two isolates (Table 3) is consistent with the hypothesis that they are synthesized in different cells. Studies with Tg(SHaPrP) mice indicate that the replication of SHa prions is independent of Mo prions (Table 1), also raising the possibility of the two prions being replicated in the same or different populations of CNS cells. It will be important to determine whether murine prion isolates that are thought to compete with each other, replicate in the same cells or different ones.

Fidelity of prion replication

Investigations with transgenic mice contend that prions multiply through a mechanism where PrP^C/PrP^{Sc} complexes feature as replication intermediates (Prusiner et al. 1990). Although recent studies with hsp60 and p53 proteins (Cheng et al. 1990; Milner and Medcalf 1991) provide interesting precedents for the hypothesis that the conversion of PrP^C to PrP^{Sc} involves a conformational change, explaining multiple, stable scrapie isolates in terms of multiple, stable conformations of PrP^{Sc} remains unprecedented. If the conformation of PrP^{Sc} determines the properties of the Sc237 and 139H prions,

then passage into Chinese or Armenian hamsters must initiate the synthesis of different CHaPrP^{Sc} or Armenian hamster PrP^{Sc} (AHaPrP^{Sc}) molecules for each isolate (Table 4). In Chinese and Armenian hamsters with PrP gene sequences that differ from that of the Syrian hamster at 7 and 8 codons, respectively (Lowenstein et al. 1990), 139H produces incubation times that are either shorter or similar to those observed with Sc237 (Table 5). Upon repassage into Syrian hamsters, the Sc237 or 139H CHaPrP^{Sc} and AHaPrP^{Sc} molecules presumably direct the synthesis of Sc237 or 139H SHaPrP^{Sc} molecules, respectively. These observations argue that heritable features can be perpetuated by transfer between PrP^{Sc} molecules in spite of changes in PrP sequence among these three hamster species. Under these experimental conditions, the properties of the Sc237 and 139H isolates seem to be independent of the primary structure of PrP. However, passage of prions between species with larger differences in PrP primary structures such as those that distinguish MoPrP from SHaPrP seem to result in the frequent emergence of new isolates (Kimberlin et al. 1987b, 1989).

Distinct prion isolates produce different patterns of PrP^{Sc} accumulation

Although the concentration of PrP^{Sc} and prion titers in brain homogenates was similar for the Sc237 and 139H isolates, the kinetics and patterns of PrP^{Sc} accumulation for the two isolates were different (Figs. 4 and 5). The kinetics of PrP^{Sc} accumulation in Syrian hamster brains inoculated with Sc237 prions, as determined by serial

histoblots (Fig. 5A), were similar to those measured by quantitative Western blots of homogenates of dissected regions of brain (Jendroska et al. 1991). The movement of prions from one brain region to another, as measured by PrP^{Sc} accumulation, seems to have occurred primarily by axonal transport because PrP^{Sc} spread sequentially from one neuroanatomically connected region to another. However, some patterns of PrP^{Sc} accumulation suggest that diffusion through the extracellular space, as well as the CSF, may feature in the spread of prions within the CNS.

If regional differences in PrP^{Sc} accumulation are found to represent cell-specific replication of prions, these observations might explain the biological basis of prion diversity and would provide a mechanism through which multiple distinct isolates could replicate in a single host. While there were many more differences than similarities in the sites of PrP^{Sc} accumulation for the two prion isolates, those regions in which prion isolates induced similar levels of PrP^{Sc} require explanation. First, synthesis of PrP^{Sc} could occur in the same cells, but this would seemingly limit the diversity of prions. Second, PrP^{Sc} synthesis might proceed in different but adjacent cells in the same region. Third, the formation of PrP^{Sc} might occur in different cells outside the region followed by transport to it.

That vacuolation correlates with the sites of PrP^{Sc} accumulation (Fig. 4) is of interest with respect to many early studies on different prion isolates in mice. Murine scrapie isolates were characterized extensively by scoring the degree of neuropil vacuolation in different mouse brain regions (Fraser and Dickinson 1968, 1973). Those results, together with the correlative neuropathology and PrP^{Sc} accumulation patterns (Fig. 4) reported here, raise the possibility that each prion isolate may have a distinct pattern of PrP^{Sc} accumulation in brain.

Using immunohistochemical procedures (DeArmond et al. 1987), other investigators found that the intensity of regional PrP staining increased with scrapie and was specific for individual prion strains (Bruce et al. 1989). Although their conclusions are similar to ours in some respects, the immunostaining procedures that they used were not capable of distinguishing among several possibilities including increased PrP^C synthesis, diminished PrP^C degradation, and PrP^{Sc} accumulation. The histoblot technique permits assignment of the immunostaining signal to PrP^{Sc} (Taraboulos et al. 1992).

New approaches to prion diversity

Many molecular models have been offered to explain the diversity and heritable properties of prions. Despite the lack of physical, chemical, and biological evidence for a scrapie-specific nucleic acid, the remote scenario that such a molecule exists remains a formal possibility to explain the distinct isolates of prions. The hypothesis that a given scrapie isolate can catalyze the synthesis of more of itself in the absence of a nucleic acid is rather unorthodox. In some respects, this hypothesis is reminiscent of early theories concerning the diversity and

synthesis of antibodies (Burnet and Fenner 1949). Such theories now appear to be largely, if not entirely, incorrect because DNA rearrangements and somatic mutations seem to account for the diverse set of antibody molecules generated by mammals (Tonegawa 1983). Attempts to demonstrate DNA rearrangements, as well as alternative splicing and RNA editing of PrP transcripts, have been unsuccessful (Oesch et al. 1985; Basler et al. 1986). Studies of PrP^{Sc} prepared from Sc237 prions have established that the amino acid sequence of PrP^{Sc} is identical to that predicted from the PrP gene sequence (N. Stahl, M. Baldwin, and S.B. Prusiner, in prep.).

A second hypothesis suggests that PrP^{Sc} alone can cause scrapie with the properties of Sc237 but that the 139H isolate contains an accessory RNA molecule that modulates the properties of this prion (Weissmann 1991). Such accessory RNAs are hypothesized to be of cellular origin and thus would not be detected by subtractive hybridization and differential cloning studies. A third hypothesis considers the possibility that scrapie isolates may have their origin in a non-PrP molecule that purifies with PrP^{Sc} but is not a nucleic acid and, as yet, is undetected. A fourth possibility is that different chemical or conformational modifications of PrP^{Sc} are responsible for the particular biological properties exhibited by scrapie prion isolates. Whether a particular isolate of infectious prions is composed of a uniform population of PrP^{Sc} molecules or oligomers containing heterogeneously modified PrP^{Sc} molecules remains to be determined.

The numerous sugar chain structures found in Asn-linked complex type oligosaccharides of PrP^{Sc} (Endo et al. 1989; Prusiner 1989) make them candidates to regulate the apparent cell-specific targeting of prions. Asn-linked oligosaccharides seem likely to possess sufficient variation to account for the diversity of prion isolates that have been recorded. Many studies have shown that oligosaccharides as well as glycoproteins can bind to the surface of cells and act as ligands in cell-cell adhesion, modulators of neurite outgrowth, mediators of viral infection, and regulators of some hormonal activities (Wiley and Skehel 1987; Brandley et al. 1990; Doherty et al. 1991; Key and Akeson 1991; Takeuchi and Kobata 1991). However, studies with scrapie-infected cultured cells have shown that PrP^{Sc} can be formed in the presence of tunicamycin and from recombinant PrP vectors with open reading frames (ORFs) mutated at the recognition sites for Asn-linked glycosylation (Taraboulos et al. 1990b). Whether unglycosylated PrP^{Sc} can transmit disease and initiate production of more PrP^{Sc} molecules is unknown. While the structures of the Asn-linked sugar chains of PrP^{Sc} isolated from Syrian hamsters inoculated with Sc237 prions have been defined (Endo et al. 1989; Haraguchi et al. 1989), we have no knowledge of those linked to PrP^{Sc} of 139H prions. Although lectin-binding studies have failed to reveal any differences in PrP^{Sc} oligosaccharides among three Mo prion isolates (Somerville and Ritchie 1990), such studies can provide only limited information about the structure of the sugar chains. It is unlikely that the glycans of the GPI anchors

attached to PrP^{Sc} from Sc237 and 139H prions are isolate specific. Both GPI anchors have indistinguishable sugar chains, including sialic acid (Stahl et al. 1992), but many of the specific chemical bonds linking these sugars are unknown. A third possibility, as noted above, is that the diversity of prion strains might reside in the tertiary structure of PrP^{Sc}. Studies on two isolates of transmissible mink encephalopathy (TME) prions in hamsters support this hypothesis. One isolate with a 75-day incubation period shows PrP^{Sc} protease resistance similar to that exhibited by Sc237, whereas the isolate with a 160-day incubation period is considerably less protease resistant in contrast to 139H scrapie prions (Bessen and Marsh 1992).

Determining the limits of prion diversity and the mechanism by which "heritable" information is transferred during the replication of prions is of considerable interest. The results presented here offer a new view of prion replication in which prion diversity may be perpetuated by cell-specific replication. Studies with transgenic mice present convincing evidence that PrP^{Sc} directs the synthesis of nascent PrP^{Sc} molecules (Prusiner et al. 1990), and the results of studies reported here support that hypothesis (Tables 1 and 2). If the replication of prions in specific cell types proves to be responsible for the properties of distinct isolates, then the cell-specific modification of PrP^{Sc} must direct PrP^{Sc} molecules to enter similar cells in the inoculated recipient for the isolate to "breed true." This hypothesis argues that cell-specific tropism generates a unique set of biological properties that are characteristic of an individual isolate.

Materials and methods

Passage history and derivation hamster scrapie isolates

After multiple passages in random-bred Syrian golden hamsters (Marsh and Kimberlin 1975), the scrapie agent was passaged in an Lak/LHC inbred Syrian hamster and the brain was given to us by Dr. Richard Marsh (Prusiner et al. 1980). Upon three subsequent passages in Lak/LVG hamsters, the pool of prions was enlarged and the fourth passage pool was designated Sc237 (Scott et al. 1989). Repeated passage of the scrapie agent in Lak/LVG hamsters at limiting dilution produced the cloned isolate 263K (Kimberlin and Walker 1978). Many studies have been performed with Sc237 and 263K prions; they seem to exhibit similar properties in Syrian hamsters (Marsh and Hanson 1977; Prusiner et al. 1982b). After >20 passages of the Chandler isolate in mice, an isolate designated 139A was obtained (Dickinson 1976). Passage of mouse 139A prions in Lak/LVG Syrian golden hamsters produced the 139H isolate (Kimberlin et al. 1987b). After six passages in Syrian hamsters, 139H prions were provided to us by Drs. Richard Kimberlin and Richard Carp.

Determination of scrapie incubation periods and prion bioassays

Lak/LVG random-bred Syrian hamsters (Charles River Breeding Laboratories, Inc., Wilmington, MA) and inbred Armenian hamsters and Chinese hamsters (Cytogen, West Roxbury, MA) were inoculated with brain extracts as described previously (Prusiner et al. 1982a). Clinical diagnosis of scrapie in Syrian hamsters inoculated with $\sim 10^7$ ID₅₀ units of Sc237 prions has been de-

scribed (Prusiner et al. 1982b). In Syrian hamsters inoculated with $\sim 10^7$ ID₅₀ units of 139H, the first evidence of altered behavior appeared after 100–125 days. The hamsters exhibited agitation, increased aggressiveness, and frequent fighting with each other. Subsequently, neurologic dysfunction and weight gain became evident. By ~ 160 days, the hamsters exhibited signs of neurologic impairment manifest by truncal ataxia and bradykinesia. We used these signs to define the onset of illness for incubation time measurements. As clinical illness progressed, hamsters showed increasing difficulty righting themselves from a supine position. Syrian hamsters inoculated with 139H prions were obese compared with uninoculated controls. At the onset of illness, the inoculated hamsters weighed 233 ± 3 grams ($n = 31$), and age-matched uninoculated controls weighed 145 ± 5 grams ($n = 16$). Hamsters inoculated with Sc237 prions weighed 143 ± 5 grams ($n = 8$) at the onset of illness. Tg(SHaPrP) mice were constructed and produced at the University of California, San Francisco. Inoculation of these animals and determination of incubation times have been described (Scott et al. 1989; Prusiner et al. 1990).

End-point titrations of the scrapie agent were performed by intracerebral inoculation of six weanling female hamsters at 10-fold dilutions ranging from 10^{-1} to 10^{-12} , as described (Prusiner et al. 1982a). Hamsters inoculated with dilutions at 10^{-1} to 10^{-5} of 139H prions were housed three per cage. Because hamsters seem to grow more aggressive as they get older and fighting is more frequent resulting in non-scrapie-related deaths (Prusiner 1987), we housed hamsters receiving dilutions at 10^{-6} to 10^{-12} individually. Prion titers (ID₅₀/ml) were calculated by use of the method of Spearman and Kärber (Dougherty 1964).

Scrapie prion purification and measurement of PrP

Large-scale purification of Sc237 prions was performed with a protocol employing discontinuous sucrose gradient centrifugation in a zonal rotor (Prusiner et al. 1983). Purification of 139H prions was performed on a smaller scale, typically with 100–200 brains from Syrian hamsters sacrificed ~ 150 days after intracerebral inoculation with $\sim 10^7$ ID₅₀ units by use of a protocol described previously (Prusiner et al. 1982a). Fraction P4 was either subjected to sucrose gradient centrifugation (Prusiner et al. 1982a) or to ultrafiltration. For ultrafiltration, the fraction P4 was adjusted to a protein concentration of 5–10 mg/ml in 20 mM Tris-OAc (pH 8.3), 1 mM EDTA, 1 mM DTT, and 0.2% Sarkosyl. Before starting the ultrafiltration, fraction P4 was adjusted to 2% Triton X-100 and 0.8% SDS and incubated at room temperature for 15 min. The buffer for the ultrafiltration was 20 mM Tris-OAc (pH 8.3) containing 2% Sarkosyl. The ultrafiltration was carried out in a 10-ml Omega cell (Filtron, North Boro, MA) with an Omega membrane (exclusion limit, 300 kD). After 16 hr, ~ 150 ml of flowthrough accumulated. The retentate containing the prion rods was precipitated with 10 volumes of cold ethanol (-20°C), or used directly.

The quantitation of PrP from brain homogenates was determined by a modified enzyme-linked immunosorbent assay (ELISA) as described in Prusiner et al. (1990), except that the 10% brain homogenate was prepared in 0.32 M sucrose. All samples were measured in triplicate.

Neuropathologic studies and histoblots

Brain tissue was immersion or perfusion fixed in 10% buffered formalin or periodate/lysine/0.5% paraformaldehyde fixative (DeArmond et al. 1987). In all cases, the brain tissue was embedded in paraffin, and 8- μm -thick histological sections were

prepared. The sections were then treated with proteinase K (5 µg/ml) for 10 min at room temperature followed by three rinses with PBS. Each section was treated with 3 M guanidinium thiocyanate for 10 min because this denaturation step was found to greatly enhance immunoreactivity of proteinase K-resistant PrP on Western transblots (Serban et al. 1990; Taraboulos et al. 1990a).

Cryostat sections (8 µm thick) on glass slides were blotted onto nitrocellulose membranes, as described previously (Taraboulos et al. 1992). To determine the precise neuroanatomical localization of PrP^{Sc} accumulation in histoblots, adjacent serial sections were mounted on glass slides and stained with hematoxylin and eosin.

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