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Genetics

Prion isolate specified allotypic interactions between the cellular and scrapie prion proteins in congenic and transgenic mice

(strains/incubation times/protein conformation/brain mapping/amyloid plaques)

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ABSTRACT **Different prion isolates, often referred to as "strains," present an enigma because considerable evidence argues that prions are devoid of nucleic acid. To investigate prion diversity, we inoculated three "strains" of prions into congenic and transgenic mice harboring variable numbers of two different alleles, designated *a* and *b*, of the prion protein (PrP) structural gene, *Prn-p*. The length of the incubation time was inversely related to the number of *Prn-p*^{a} genes in mice inoculated with the Rocky Mountain Laboratory (RML) prion strain. Results with mice lacking this locus (*Prn-p*$^{0/0}$) and transgenic mice argue that long incubation times are not a dominant trait as thought for many years, but rather they are due to reduced levels of the substrate PrP^C-A (cellular isoform of PrP, allotype A) in (*Prn-p*^{a} × *Prn-p*^{b})F$_1$ mice. In contrast, the *Prn-p*^{a} gene extended incubation times in mice inoculated with the 87V and 22A prion strains, whereas the *Prn-p*^{b} gene was permissive. Experiments with the 87V isolate suggest that a genetic locus distinct from *Prn-p* controls deposition of the scrapie isoform of PrP (PrP^{Sc}) and attendant neuropathology. Each prion isolate produced distinguishable patterns of PrP^{Sc} accumulation in brain; of note, the patterns in *Prn-p*^{a} and *Prn-p*^{b} congenic mice inoculated with RML prions were more different than those in congenic *Prn-p*^{b} mice with RML or 22A prions. Our results suggest that scrapie "strain-specific" incubation times can be explained by differences in the relative efficiency of allotypic interactions that lead to conversion of PrP^C into PrP^{Sc}.**

Prions are composed largely, if not entirely, of the scrapie isoform of the prion protein (PrP), PrP^{Sc}, and cause neurodegeneration in both animals and humans (1). The lack of a nucleic acid genome distinguishes prions from other infectious pathogens such as bacteria, fungi, viruses, and viroids. The cellular isoform of PrP, PrP^C, is a host-encoded sialoglycoprotein that is bound to the cell surface by a phosphoinositol glycolipid anchor (2) and is converted into PrP^{Sc} by a posttranslational process (3). Although attempts to identify a chemical modification unique to PrP^{Sc} have been unsuccessful (4), spectroscopic studies demonstrated that PrP^C contains 42% α-helix and 3% β-sheet, while PrP^{Sc} has 43% β-sheet (5, 6).

Prions exhibiting different biological properties were first recognized in goats with scrapie where two different clinical syndromes labeled "hyper" and "drowsy" were manifest (7). Incubation times and the distribution of vacuolar change define distinct isolates or "strains" of prions in mice (8, 9). The interpretation of the results from many of these investigations came into question when it was learned that alleles of the PrP structural gene (*Prn-p*) in the two strains of mice encoded proteins differing at two residues (10). The term "allotype" is used to describe these allelic variants of PrP.

Molecular genetic studies with inbred mice demonstrated the influence of a PrP-linked gene designated *Prn-i* on scrapie incubation time regulation (11). Subsequently, investigations with transgenic (Tg) (12–14) and gene-ablated mice (15, 16) established that the PrP itself can profoundly modify scrapie incubation times and the synthesis of prions. Although it is clear that passage history can be responsible for the prolongation of incubation time when prions are passed between mice expressing different PrP allotypes (17) or between species (13), several scrapie strains show distinct incubation times in the same inbred host (18).

We report here on three prion isolates inoculated into congenic and Tg mice expressing the *Prn-p*^{b} allele. This approach enabled us to extend the analysis of scrapie incubation times beyond the three naturally occurring *Prn-p* genotypes by altering the ratio of the *a* and *b* alleles. Our results suggest that scrapie "strain-specific" incubation times can be explained by differences in the relative efficiency of allotypic interactions that lead to conversion of PrP^C to PrP^{Sc}.

MATERIALS AND METHODS

VM/Dk mice were derived from breeding pairs that were a gift from James Hope (Neuropathogenesis Unit, Agricultural and Food Research Council, Edinburgh). Tg(MoPrP-B)15 mice (MoPrP = mouse PrP) were constructed by microinjection of (C57BL/6J × LT/Sv)F$_2$ fertilized eggs with the cosmid clone cos6.I/LnJ-4 insert spanning the *Prn-p* gene as described (19, 20). Tg(MoPrP-B)15 mice contain three copies of the *Prn-p*^{b} transgene, as determined by phosphor imaging (19). Production of *Prn-p* gene-ablated mice has been described (21). Tg(MoPrP-A) mice were constructed by microinjection of Friend virus B (FVB) fertilized eggs with the open reading frame of wild-type *Prn-p*^{a} inserted into the CosTet vector (22).

Four lines of congenic mice are designated: B6 for C57BL/6J, B6.I-4 for B6.I-*B2m*^{a}, B6.I-1 for B6.I-*Prn-p*^{b}, B6.I-2 for B6.I-*Il-1a*^{d} *Prn-p*^{b} (where Il-1a = interleukin 1α), and B6.I-3 for B6.I-*B2m*^{a} *Prn-p*^{b} (see Fig. 1*A*) (24).

The Rocky Mountain Laboratory (RML) isolate (26) was provided by William Hadlow (RML, Hamilton, MT) in its

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Abbreviations: PrP, prion protein; PrP^C, cellular isoform of PrP; PrP^{Sc}, scrapie isoform of PrP; *Prn-p*, mouse PrP gene, structural locus; *Prn-i*, mouse PrP gene, scrapie incubation time regulation locus; Tg, transgenic; SHaPrP, Syrian hamster PrP; MoPrP, mouse PrP; B6, C57BL/6J; B6.I-4, B6.I-*B2m*^{a}; B6.I-1, B6.I-*Prn-p*^{b}; B6.I-2, B6.I-*Il-1a*^{d} *Prn-p*^{b}; B6.I-3, B6.I-*B2m*^{a} *Prn-p*^{b}; RML, Rocky Mountain Laboratory; IL-1a, interleukin 1α.

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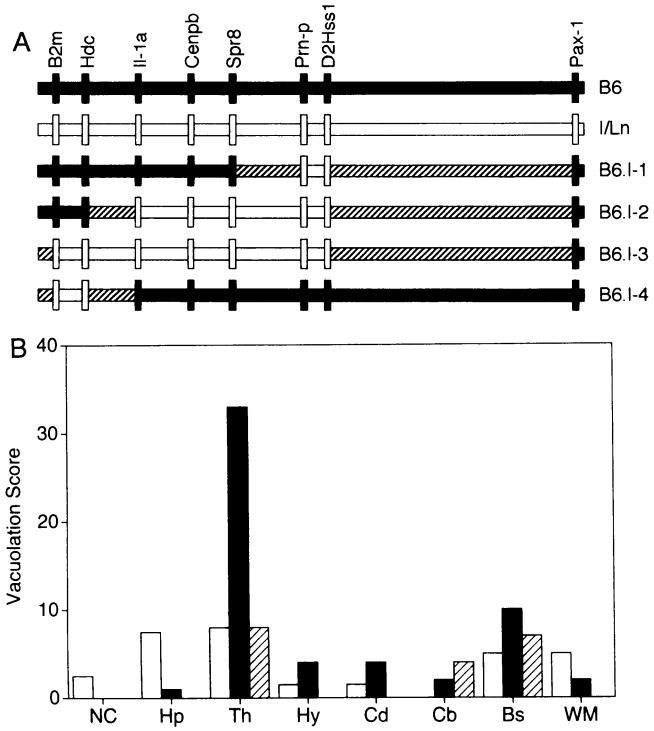

FIG. 1. Congenic mice inoculated with scrapie prions. (*A*) Parental origins of loci on chromosome 2 in B6.I congenic mice. Loci and chromosomal segments derived from the B6 background strain are black, while those from the I/Ln donor are white. Chromosomal segments containing the crossover sites are hatched, but the relative contributions of background and donor mice are not known. These maps are not drawn to scale. In different crosses, the *B2m* (β_2-microglobulin gene) to *Prn-p* interval ranged from 1.4 ± 0.7 (SEM) to 6.25 ± 2.7 centimorgans (cM) (23), and the *Prn-p* to *Pax-1* interval ranged from 4.0 ± 0.9 (24) to 5.0 ± 0.3 cM (25). Intervals in cM between markers closely linked to *Prn-p* in a recent backcross were as follows: *Il-1a*–0.49–*Cenpb* (centromere protein b)–0.44–*Spr8* (G protein receptor)–0.67–*Prn-p*–0.22–*D2Hss1* (anonymous marker) (24). (*B*) Vacuolation in the brains of I/Ln (n = 2, open bars), B6.I-1 (n = 3, filled bars), and B6.I-2 mice (n = 3, hatched bars). Mice were inoculated with 87V prions previously passaged in VM/Dk mice and sacrificed after showing clinical signs of scrapie. Vacuolation scores are described in *Materials and Methods* and are given for the following brain regions: NC, neocortex; Hp, hippocampus; Th, thalamus; Hy, hypothalamus; Cd, caudate nucleus; Cb, cerebellar grey matter; Bs, brainstem; WM, white matter of the cerebellum.

fourth passage in Swiss mice. Scrapie isolates 22A and 87V were given to us by James Hope as frozen brains from VM/Dk mice. Isolates were prepared by homogenizing brains of clinically ill mice in phosphate-buffered saline free of Mg^{2+} and Ca^{2+} to yield a 10% (wt/vol) homogenate; aliquots were frozen and diluted just prior to use. Mice were inoculated intracerebrally and scored for scrapie as described (23).

The genotype for endogenous *Prn-p* and presence or absence of the *Prn-p* transgene was assessed by using restriction fragment length polymorphism analysis (19). Intensity of bands following autoradiography was used to distinguish transgene homozygous from hemizygous mice.

Ten-micrometer-thick cryostat sections were processed for histoblots by using anti-PrP antisera (R073) raised in rabbits (27). Eight-micrometer-thick sections of formalin-fixed and paraffin-embedded brain were stained with hematoxylin/eosin. Areas occupied by vacuoles in a high-power field were scored: 0, no vacuoles; 3, occasional vacuolation; 5, mild; 20, moderate; and 70, severe. Peroxidase immunohistochemistry with antibodies to glial fibrillary acidic protein was used to evaluate reactive astrocytic gliosis.

RESULTS

PrP^C-A Expression Shortens Incubation Times in Mice Inoculated with RML Prions. *Prn-p^a* mice hemizygous for ablation of the PrP gene had an incubation time of 426 ± 18 days (16). Increasing the *Prn-p^a* gene copy number to two reduced the incubation time to 143 ± 4 days, whereas ≈30 copies reduced the time to 50 ± 2 days (Table 1). Although the effect of the *Prn-p^b* allele was not as great as that of *Prn-p^a*, increasing copy number also shortened incubation times. Congenic (B6 × B6.I-1)F_1 mice had an incubation time of 268 ± 4 days, while Tg(MoPrP-B$^{+/0}$)15 crossed with B6.I-1 mice having one copy of *Prn-p^a* and four copies of *Prn-p^b* gave an incubation time of 164 ± 2 days. That *Prn-p^a* is the major determinant of RML incubation times is illustrated by Tg(MoPrP-B$^{+/0}$)15 mice with two copies of *Prn-p^a* and three copies of *Prn-p^b*, where the incubation time was to 115 ± 2 days. Increasing the number of *Prn-p^b* transgenes to >30 copies in the presence of two copies of *Prn-p^a* reduced incubation times to 75 ± 2 days (19).

PrP^C-A Expression Prolongs Incubation Times in Mice Inoculated with 22A Prions. The phenomenon of scrapie incubation times in F_1 hybrids being longer than that of either parent was called "overdominance" (28). In congenic mice inoculated with the 22A isolate, the scrapie incubation time in B6 mice was 405 ± 2 days, in B6.I-1 mice was 194 ± 10 days, and in (B6 × B6.I-1)F_1 mice was 508 ± 14 days (Table 2). In contrast to PrP^C-A, increasing PrP^C-B shortened the incubation time from 395 ± 12 days in hemizygous Tg(MoPrP-B$^{+/0}$)15 mice to 286 ± 15 days in homozygous Tg(MoPrP-B$^{+/+}$)15 mice.

Spongiform degeneration in B6 and B6.I-1 mice inoculated with 22A prions was minimal except for modest vacuolation in the cerebellar white matter and the granule cell layer in B6 but not B6.I-1 mice. Moderately intense vacuolation was found in the hippocampus of (B6 × B6.I-1)F_1 mice and in the brainstem of Tg(MoPrP-B$^{+/0}$)15 mice.

PrP^C-A Expression Confers Resistance to 87V Prions. 87V prions produced scrapie in all 36 B6.I-1, B6.I-2, I/LnJ, and VM/Dk mice that are *Prn-p^b* homozygotes, whereas scrapie was seen in only 1 of 52 mice expressing *Prn-p^a* (Table 3). Unlike the results with other scrapie isolates (Tables 1 and 2), expression of the three-copy *Prn-p^b* transgene array in Tg(MoPrP-B)15 mice did not render the mice susceptible to scrapie.

Neuropathologic examination of B6.I-1, B6.I-2, I/LnJ, and VM/Dk mice inoculated with 87V prions showed numerous PrP amyloid plaques in accord with an earlier report on VM/Dk mice (29). In B6.I-1 mice, intense spongiform degeneration, gliosis, and PrP immunostaining was found in the ventral posterior lateral nucleus of the thalamus, the habenula, and the raphe nuclei of the brainstem (Fig. 1*B*). These same regions showed intense immunoreactivity for PrP^{Sc} on histoblots (Fig. 2 *K* and *L*). Unexpectedly, B6.I-2 and ILn/J mice exhibited only mild vacuolation of the thalamus and brainstem. These findings suggest that a locus near *Prn-p* influences the deposition of PrP^{Sc} and thus vacuolation in the thalamus, the habenula, and raphe nuclei (Fig. 1*A*).

Patterns of PrP^{Sc} in Congenic Mice. RML prions were inoculated into B6 or B6.I-1 mice, and the patterns of PrP^{Sc} accumulation were compared (Fig. 2 *A–F*), revealing that (*i*) PrP^{Sc} was more widely and uniformly distributed in the neocortex and hippocampus of B6 mice (compare *B* and *E* in Fig. 2), (*ii*) PrP^{Sc} was more intense in the granule cell layer of the cerebellum in B6.I-1 mice (compare *F* and *C* in Fig. 2), and (*iii*) PrP^{Sc} accumulation was much greater in the cerebellar white matter (compare *C* and *F* in Fig. 2) and the hypothalamus of B6 mice. These findings argue that the distribution of PrP^{Sc} is influenced by the amino acid sequence of host PrP^C and the prion isolate.

Table 1. MoPrP-A expression is a major determinant of incubation times in mice inoculated with the RML scrapie prions

Mice	*Prn-p* Genotype	Transgenes, no. of copies	Alleles, no. of copies *a*	Alleles, no. of copies *b*	Incubation time,* days ± SEM	*n*
Prn-p$^{0/0}$	*0/0*		0	0	>600	4
Prn-p$^{+/0}$	*a/0*		1	0	426 ± 18	9*
B6.I-1	*b/b*		0	2	360 ± 16	7†
B6.I-2	*b/b*		0	2	379 ± 8	10†
B6.I-3	*b/b*		0	2	404 ± 10	20
(B6 × B6.I-1)F_1	*a/b*		1	1	268 ± 4	7
B6.I-1 × Tg(MoPrP-B$^{0/0}$)15	*a/b*		1	1	255 ± 7	11‡
B6.I-1 × Tg(MoPrP-B$^{0/0}$)15	*a/b*		1	1	274 ± 3	9§
B6.I-1 × Tg(MoPrP-B$^{+/0}$)15	*a/b*	*bbb/0*	1	4	166 ± 2	11‡
B6.I-1 × Tg(MoPrP-B$^{+/0}$)15	*a/b*	*bbb/0*	1	4	162 ± 3	8§
C57BL/6BJ (B6)	*a/a*		2	0	143 ± 4	8
B6.I-4	*a/a*		2	0	144 ± 5	8
Non-Tg(MoPrP-B$^{0/0}$)15	*a/a*		2	0	130 ± 3	10
Tg(MoPrP-B$^{+/0}$)15	*a/a*	*bbb/0*	2	3	115 ± 2	18
Tg(MoPrP-B$^{+/+}$)15	*a/a*	*bbb/bbb*	2	6	111 ± 5	5
Tg(MoPrP-B$^{+/0}$)94	*a/a*	>30*b*	2	>30	75 ± 2	15¶
Tg(MoPrP-A$^{+/0}$)B4053	*a/a*	>30*a*	>30	0	50 ± 2	16

n, Number of mice inoculated and developing scrapie.
*Data from ref. 16.
†Data from ref. 24.
‡The homozygous Tg(MoPrP-B$^{+/+}$)15 mice were maintained as a distinct subline selected for transgene homozygosity two generations removed from the (B6 × LT/Sv)F_2 founder. Hemizygous Tg(MoPrP-B$^{+/0}$)15 mice were produced by crossing the Tg(MoPrP-B$^{+/+}$)15 line with B6 mice.
§Tg(MoPrP-B$^{+/0}$)15 mice were maintained by repeated backcrossing to B6 mice.
¶Data from ref. 19.

Although the general features of PrP^{Sc} accumulation in the brains of B6.I-1 mice were similar with RML or 22A prions, clear differences were observed, such as more intense PrP^{Sc} in the molecular layer of the dentate gyrus with RML, whereas the hypothalamus contained more PrP^{Sc} with 22A prions (Fig. 2 *D–I*). The full thickness of the neocortex exhibited PrP^{Sc} immunostaining with 22A prions, but only the inner layers stained with RML prions—reminiscent of the differences between Sc237 and 139H scrapie in Syrian hamsters (30). More PrP^{Sc} was found in the corpus callosum and in the white matter tracts coursing through the caudate nucleus with RML than with 22A prions (compare *D* and *G* in Fig. 2). With both RML and 22A prions, there was intense PrP^{Sc} staining in the granule cell layer of the cerebellum as well as the absence of PrP amyloid plaques.

PrP^{Sc} deposition in B6.I-1 mice inoculated with 87V prions was markedly different from that with 22A and RML prions (Fig. 2 *D–L*) and was most intense in the thalamus, particularly in the ventral posterior lateral nucleus (Fig. 2*K*), in the habenula (Fig. 2*K*), and in the locus coeruleus and raphe nuclei of the brainstem (Fig. 2*L*). Little or no PrP^{Sc} accumulated in the neocortex, the hippocampus, or the hypothalamus. Relatively weak PrP^{Sc} signals were distributed diffusely in the brainstem tegmentum, inferior colliculi, and amygdala. Multiple intense, punctate signals were located beneath the corpus callosum overlying the hippocampus (Fig. 2*K*), in the caudate nucleus, and in the cerebellum. Histological sections stained with the periodic acid/Schiff reagent method or anti-PrP antibodies indicated that the punctate PrP^{Sc} signals were kuru-type and primitive PrP amyloid

Table 2. Influence of MoPrP-B transgene expression on incubation times in mice inoculated with 22A scrapie prions

Mice	*Prn-p* Genotype	Transgenes, no. of copies	Alleles, no. of copies *a*	Alleles, no. of copies *b*	Incubation time, days ± SEM	*n*
B6.I-1	*b/b*		0	2	194 ± 10	7
(B6 × B6.I-1)F_1	*a/b*		1	1	508 ± 14	7
B6	*a/a*		2	0	405 ± 2	8
Non-Tg(MoPrP-B$^{0/0}$)15	*a/a*		2	0	378 ± 8	3*
Tg(MoPrP-B$^{+/0}$)15	*a/a*	*bbb/0*	2	3	318 ± 14	15*
Tg(MoPrP-B$^{+/0}$)15	*a/a*	*bbb/0*	2	3	395 ± 12	6†
Tg(MoPrP-B$^{+/+}$)15	*a/a*	*bbb/bbb*	2	6	266 ± 1	6*
Tg(MoPrP-B$^{+/+}$)15	*a/a*	*bbb/bbb*	2	6	286 ± 15	5†

n, Number of mice inoculated and developing scrapie.
*The homozygous Tg(MoPrP-B$^{+/+}$)15 mice were maintained as a distinct subline selected for transgene homozygosity two generations removed from the (B6 × LT/Sv)F_2 founder. Hemizygous Tg(MoPrP-B$^{+/0}$)15 mice were produced by crossing the Tg(MoPrP-B$^{+/+}$)15 line with B6 mice.
†Tg(MoPrP-B$^{+/0}$)15 mice were maintained by repeated backcrossing to B6 mice.

Table 3. Expression of MoPrP-A confers resistance to 87V scrapie prions

Mice	*Prn-p* Genotype	Transgenes, no. of copies	Alleles, no. of copies *a*	*b*	Incubation time, days ± SEM	n/n_0
VM/Dk	b/b		0	2	279 ± 3	7/7
I/LnJ	b/b		0	2	244 ± 8	13/13
B6.I-1	b/b		0	2	306 ± 8	7/7
B6.I-2	b/b		0	2	288 ± 8	10/10
(B6 × B6.I-1)F_1	a/b		1	1	>600	0/8
B6	a/a		2	0	>600	0/8
NZW/LacJ	a/a		2	0	700	1/31*
Tg(MoPrP-$B^{+/0}$)15	a/a	bbb/0	2	3	>722	0/15

n, Number of mice developing scrapie; n_0, number of mice originally inoculated.

*Two mice died without confirming diagnosis of scrapie at 313 and 547 days. The experiment was terminated at 875 days. Neuropathologic examination showed mild spongiform degeneration and infrequent amyloid plaques confirming the clinical diagnosis of scrapie in this mouse.

plaques. Much of the intense PrP^{Sc} signal in the raphe nuclei and locus coeruleus of the brainstem (Fig. 2*L*) seemed to be perivascular PrP plaques.

DISCUSSION

The hierarchy of scrapie incubation times ranging from 426 days to 50 days in mice expressing *Prn-p*^{a} demonstrates the profound influence of copy number of this allele on the rate of RML prion propagation (Table 1), in accord with earlier studies on Tg(SHaPrP) mice (SHaPrP = Syrian hamster PrP) (13). It seems likely that reduced levels of PrP^C-A in both *Prn-p*$^{+/0}$ and (*Prn-p*^{a} × *Prn-p*^{b})F_1 mice are responsible for prolonged incubation times rather than an inhibitory effect of PrP^C-B in the F_1 mice. This interpretation is consistent with studies of Tg(MoPrP-B) mice that have shorter incubation times than non-Tg mice (19). More importantly, Tg(MoPrP-$B^{+/0}$)15/*Prn-p*^{a} × *Prn-p*^{b} heterozygous mice had longer incubation times than Tg(MoPrP-$B^{+/0}$)15/*Prn-p*^{a} homozygous mice (Table 1). These results argue that long incubation times are not a dominant trait as thought for many years (8, 9), but rather they are due to reduced levels of the substrate PrP^C-A in (*Prn-p*^{a} × *Prn-p*^{b})F_1 mice.

PrP^C-A Inhibits the Conversion of PrP^C-B to PrP^{Sc}. For both the 22A and 87V isolates, PrP^C-A appears to inhibit the formation of PrP^{Sc} from PrP^C-B. This postulate is consistent with studies of Tg(SHaPrP)81/*Prn-p*$^{0/0}$ mice which have shorter incubation times than Tg(SHaPrP)81/*Prn-p*$^{a/a}$ mice inoculated with Sc237 Syrian hamster prions (15, 16) as well as results from investigations with Tg(SHaPrP) mice inoculated with RML prions (13). These findings provide an alternate explanation for the phenomenon of overdominance,

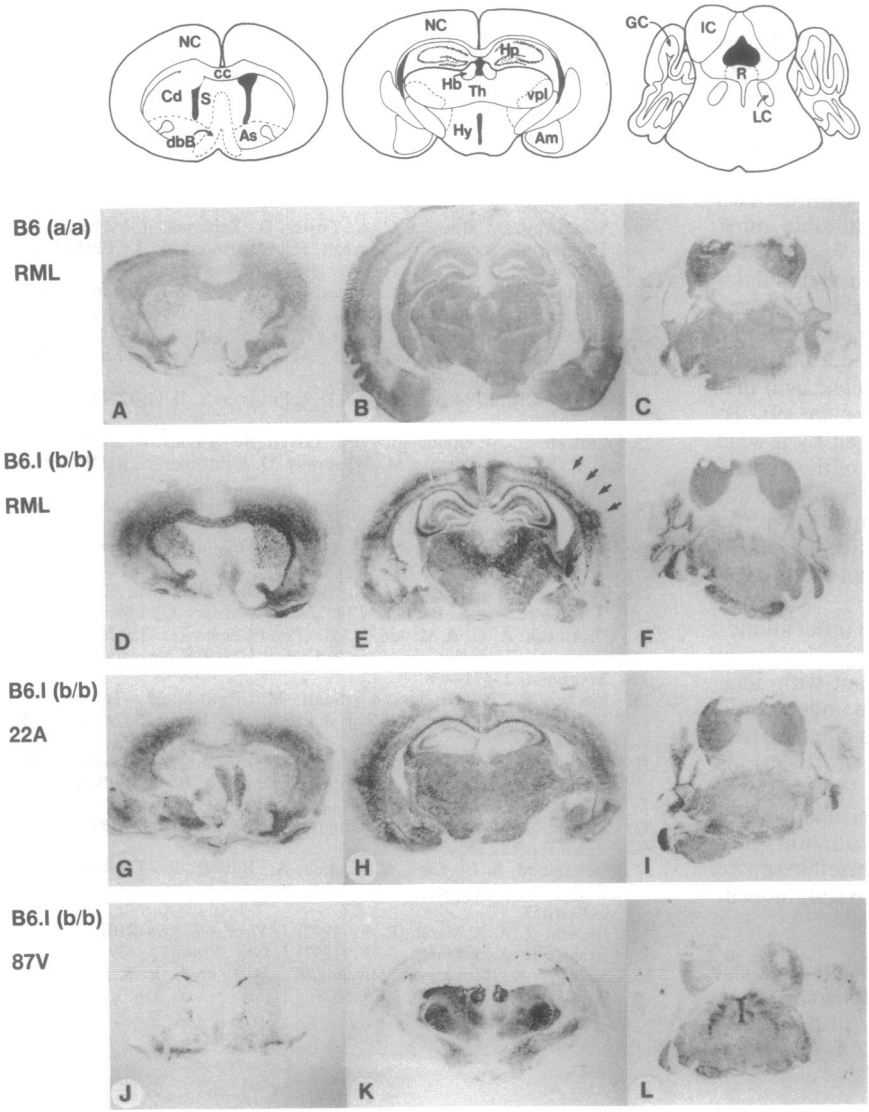

FIG. 2. Histoblots showing the PrP^{Sc} in coronal sections of mouse brain. Sections were cut at three levels: caudate and septal nuclei (*Left*), hippocampus and thalamus (*Middle*), and inferior colliculus (*Right*) B6 (*A–C*) and B6.I-1 (*D–F*) mice were inoculated with RML prions and sacrificed after showing clinical signs of scrapie. 22A (*G–I*) and 87V (*J–L*) prions were inoculated into B6.I-1 mice. Arrows in *E* indicate the outer surface of the cerebral cortex. Am, amygdala; As, accumbens septi; cc, corpus callosum; dbB, diagonal band of Broca; GC, granule cell layer of the cerebellar cortex; Hb, habenula; IC, inferior colliculus; LC, locus coeruleus; R, raphe nuclei of the brainstem; S, septal nuclei; vpl, ventral posterior lateral nucleus of the thalamus. For other abbreviations, see Fig. 1.

which previously had been attributed to dimers of the *Prn-i* gene product (31). Whether the relative paucity of individuals heterozygous for the methionine/valine polymorphism at codon 129 of the human PrP gene in spontaneous and iatrogenic Creutzfeldt–Jakob disease (CJD) (32) is due to an inhibition of allotypic restricted interactions between PrP^{Sc} and PrP^{C} remains to be established.

Prion Isolate and *Prn-p* Gene-Specified PrP^{Sc} Patterns. Earlier studies indicated that the pattern of PrP^{Sc} accumulation in the central nervous system of Syrian hamsters and Tg(SHaPrP) mice is characteristic for a particular strain (30, 33). To extend those findings, we determined the patterns of PrP^{Sc} accumulation for three isolates in B6.I-1 mice (Fig. 2). The pattern of PrP^{Sc} deposition for 87V prions was distinct from those seen with RML and 22A prions, while the differences between RML and 22A prions were relatively minor. In fact, the differences in regional PrP^{Sc} deposition were much greater for RML prions in B6 mice compared with those in B6.I-1 mice than the differences between RML and 22A prions in B6.I-1 mice. This finding indicates that the *Prn-p* genotype as well as strain properties influence the patterns of PrP^{Sc} deposition.

From the results of earlier studies, we hypothesized that a different set of cells might propagate each prion isolate. Whether different covalent modifications of asparagine-linked carbohydrates function to target PrP^{Sc} to a particular set of cells remains to be established (30). Alternatively, distinct prion isolates might reflect different PrP^{Sc} conformers that act as templates for the folding of *de novo* synthesized PrP^{Sc} molecules during prion "replication." Although this proposal is rather unorthodox, it is consistent with observations on Tg mice that express SHaPrP (13) or chimeric Mo/SHaPrP (14) and with the work reported here. How many different conformations PrP^{Sc} can adopt is unknown, but spectroscopic studies clearly show that the conformations of PrP^{Sc} and PrP^{C} are quite distinct (5). Of note, two different isolates from mink dying of transmissible mink encephalopathy exhibit different sensitivities of PrP^{Sc} to proteolytic digestion, supporting the suggestion that isolate-specific information may be carried by PrP^{Sc} (34, 35).

Does Prion Diversity Demand a Second Component? Some investigators have argued that scrapie is caused by a virus-like particle that contains a scrapie-specific nucleic acid that encodes the information expressed by each isolate (9). To date, no such polynucleotide has been identified by a wide variety of techniques, including measurements of the nucleic acids in purified preparations (1, 36). While our results do not establish that PrP^{Sc} is the sole functional component of the prion, they do suggest a model whereby prion strains specify incubation times without invoking an additional macromolecule that "encodes information."

The foregoing results as well as findings from other studies argue that the most likely explanation for prion strains lies in the structure of PrP^{Sc}. The difficulties attendant with deciphering the molecular basis of prion strains are underscored by our initial interpretation of the abbreviated incubation times in Tg(PrP-B)94 mice inoculated with RML prions (Table 1). We described those findings as "paradoxical shortening of scrapie incubation times" (19) because we and others had believed for many years that long incubation times are dominant traits (8). In view of the data reported here, we now realize that these findings were not paradoxical; indeed, they result from increased PrP gene dosage.

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