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Ablation of the prion protein (PrP) gene in mice prevents scrapie and facilitates production of anti-PrP antibodies

(immune tolerance/gene targetting/prion diseases/gene therapy/antisense prion protein)

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ABSTRACT **Mice, homozygous for prion protein (PrP) gene ablation (Prn-p$^{0/0}$), develop normally and remain well >500 days after inoculation with murine scrapie prions. In contrast, wild-type mice developed scrapie <165 days after inoculation and most Prn-p$^{0/+}$ mice, heterozygous for disruption of the PrP gene, exhibited signs of central nervous system dysfunction between 400 and 465 days after inoculation. *In situ* immunoblots showed widespread deposition of scrapie PrP (PrP^{Sc}) in the brains of both wild-type Prn-p$^{+/+}$ and Prn-p$^{0/+}$ mice, while neither cellular PrP (PrP^{C}) nor PrP^{Sc} was detected in the brains of Prn-p$^{0/0}$ mice. In contrast to Prn-p$^{+/+}$ and Prn-p$^{0/+}$ mice, Prn-p$^{0/0}$ mice failed to propagate prion infectivity as measured by bioassays. Syrian hamster (SHa) PrP transgenes rendered Prn-p$^{0/0}$ mice susceptible to prions containing SHaPrP^{Sc}. Immunization of Prn-p$^{0/0}$ mice with purified, infectious mouse or SHa prions dispersed in Freund's adjuvant produced antisera that bound mouse, SHa, and human PrP on Western blots. Presumably, the lack of PrP^{C} expression in Prn-p$^{0/0}$ mice prevents them from becoming tolerant to the immunogen. The resistance of Prn-p$^{0/0}$ mice to developing scrapie after inoculation with murine prions supports the hypothesis that PrP^{Sc} is essential for both transmission and pathogenesis of the prion diseases.**

Scrapie was the first prion disease to be transmitted to laboratory rodents (1) and hence is the most widely studied of these transmissible neurodegenerative diseases. Scrapie is a naturally occurring disease of sheep and goats (2). Some investigators have contended that scrapie is an infectious disorder (3), while others have argued that it is a genetic disease (4). Studies of the human prion diseases, which are manifest as infectious, genetic, or sporadic disorders, argue that both views of natural scrapie are likely to have merit (5, 6).

The prion protein (PrP) was discovered by progressively enriching fractions for scrapie infectivity first from mouse (Mo) brain and later from Syrian hamster (SHa) (7–9). N-terminal sequencing of the protease-resistant protein, later designated PrP 27–30, allowed synthesis of an isocoding mixture of oligonucleotides (10) that were used to screen cDNA libraries prepared from hamster and mouse brains (11, 12). The PrP gene was found to be a chromosomal gene and the levels of PrP mRNA were unchanged throughout the course of scrapie infection (11). This finding led to the identification of cellular PrP (PrP^{C}) from which scrapie PrP (PrP^{Sc}) (and PrP 27–30) are produced during scrapie.

The central role of PrP^{Sc} in the transmission and pathogenesis of prion diseases was established over the past decade by a wide variety of experimental approaches (6, 13). These studies have also demonstrated that PrP^{Sc} is an essential, and possibly the only, component of the infectious prion particle (14).

With the development of gene targeting technology, it became feasible to disrupt the MoPrP gene (*Prn-p*) by creating a construct in which ≈75% of the open reading frame was replaced by an aminoglucoside phosphotransferase gene (15). Unexpectedly, ablation of both alleles of the single copy PrP gene (Prn-p$^{0/0}$) has had no deleterious effects on the development, behavior, or life-span of the mice.

Since earlier studies with transgenic (Tg) mice had shown that the incubation time is inversely related to the level of PrP transgene expression (16–19), the availability of Prn-p$^{0/+}$ and Prn-p$^{0/0}$ mice created opportunities to extend those findings and to test again the hypothesis that PrP^{Sc} is an essential component of the infectious prion. Those earlier investigations also indicated that the relative levels of SHaPrP^{C} and MoPrP^{C} influenced incubation times. Thus, we inoculated Prn-p$^{0/+}$ and Prn-p$^{0/0}$ mice with Mo and SHa prions and compared the incubation times to those in wild-type Prn-p$^{+/+}$ and Tg(SHaPrP) mice. We also examined the patterns of PrP^{Sc} accumulation in the mice and measured the titers of infectious prions produced. Our results are similar to those reported by others using the same line of Prn-p$^{0/0}$ mice (20).

The production of anti-PrP antibodies (Abs) in rabbits required large amounts of purified SHaPrP 27–30 (21, 22) as did the subsequent production of anti-PrP monoclonal antibodies (mAbs) in mice (23, 24) but these mAbs recognize only a few epitopes on SHaPrP and human PrP and none on MoPrP (25, 26). These anti-PrP mAbs react equally well with denatured PrP^{Sc} and nondenatured PrP^{C} (27). Since Prn-p$^{0/0}$ mice should not be tolerant to MoPrP^{C}, we immunized these animals to produce anti-PrP Abs.

MATERIALS AND METHODS

All chemicals were of the highest grades commercially available. SDS, acrylamide, and protein standards were obtained from Bio-Rad, guanidinium salts and Sarkosyl were from Fluka, and urea was from Schwarz/Mann.

Mice were inoculated with prions derived from the Chandler scrapie isolate (1), which was designated RML, after repeated passage in Swiss CD-1 mice obtained from Charles River Breeding Laboratories. Homogenates were prepared from the brains of clinically ill CD-1 mice by dispersion in 0.32 M sucrose (10%, wt/vol).

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Abbreviations: PrP, prion protein; PrP^{C}, cellular PrP; PrP^{Sc}, scrapie PrP; PrP 27–30, protease-resistant fragment of PrP^{Sc}; Tg, transgenic; SHa, Syrian hamster; Ab, antibody; mAb, monoclonal antibody; Prn-p$^{0/0}$, both PrP alleles ablated; Prn-p$^{0/+}$, one PrP allele ablated.

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Scrapie prion isolates designated Sc237 and 139H were passaged in random-bred Syrian hamsters (Lak:LVG) obtained from Charles River Breeding Laboratories. Sc237 prions were derived from an inoculum provided by Richard Marsh (University of Wisconsin, Madison) (28) and are similar to 263K prions (29). 139H prions were obtained from Richard Kimberlin and Richard Carp (Institute for Basic Research, Staten Island, NY) (30).

Mice were anesthetized with ether and inoculated intracerebrally with 30 μl of 10% (wt/vol) brain homogenates using a 27-gauge disposable hypodermic needle inserted into the right parietal lobe. Criteria for diagnosis of scrapie in mice have been described (31). After inoculation, the mice were examined for neurologic dysfunction three times per week. Prion titers in brain homogenates were calculated from curves relating titers to incubation times (32).

A molecular clone containing the third exon of the *Prn-p* gene was modified by removing 183 codons of PrP and substituting the neomycin phosphotransferase gene. This modified PrP gene was introduced into ES cells derived from agouti 129 mice (33) and recombinants were selected (15). Chimeric offspring were mated to C57BL6 mice; those heterozygous (Prn-$p^{0/+}$) for the PrP gene ablation were mated to each other. Homozygous (Prn-$p^{0/0}$) offspring were found to develop normally (15) and remain healthy for >600 days.

Screening for Prn-$p^{0/0}$ and Prn-$p^{0/+}$ mice was accomplished by PCR using three primers: RK1 (TCAGCCTAAATACTGGGCAC), RK2 (GCCTAGACCACGAGAAATGC), and RK3 (GCATCAGCCATGATGGATAC). The 5′ primer RK1 and the 3′ primer RK2 are located outside the MoPrP open reading frame; they create an 880-bp fragment. Amplification of the *Neo* gene was achieved with RK1 and the 3′ primer RK3 producing a 730-bp fragment. DNA was extracted from an amputated piece of tail and amplified by PCR. The PCR mixture contained 200 μM each dNTP, 0.2 μM RK1, 0.2 μM RK2, 0.2 μM RK3, 1 unit of *Taq* DNA polymerase (Perkin–Elmer), 50 mM KCl, 20 mM Tris·HCl (pH 8.4), and 2.5 mM $MgCl_2$ in 25 μl. A Geneamp 9600 (Applied Biosystems) was programmed for one cycle at 94°C, 30 s followed by 42 cycles: 94°C, 15 s for denaturation; 62°C, 15 s for annealing; and 72°C, 45 s for polynucleotide extension. Since RK1 and RK2 are specific for MoPrP, foreign transgenes such as SHaPrP were not amplified by PCR with these primers. SHaPrP transgenes were detected as described (16).

Cryostat sections of brain were digested with proteinase K to eliminate PrP^{C} prior to denaturation of PrP^{Sc} with guanidinium thiocyanate to enhance its antigenicity (34). These *in situ* immunoblots were stained with anti-PrP polyclonal rabbit antiserum (R073) (27).

Purified prion rods were prepared from the brains of clinically ill CD-1 mice inoculated with RML prions or Syrian hamsters with Sc237 prions (35). Prion rods were recovered from sucrose gradient fractions by diluting the 50% sucrose 2:1 with distilled H_2O followed by centrifugation at 100,000 × *g* for 6 h at 4°C. The pellet was washed in an equal volume of distilled H_2O and after centrifugation at 100,000 × *g* for 6 h at 4°C the rods were resuspended at 1 mg/ml in Ca/Mg-free phosphate-buffered saline (PBS) containing 0.2% Sarkosyl. The major protein in both preparations was PrP 27–30 as judged by silver staining after SDS/PAGE. Protein was determined by a bicinchoninic acid dye binding with bovine serum albumin used as the standard (Pierce). Prn-$p^{0/0}$ mice were injected intraperitoneally with 30 μg of prion rods emulsified in complete Freund's adjuvant. Mice were given booster injections at 2-week intervals with incomplete Freund's adjuvant containing first 30 μg and then 15 μg of rods. After the second injection, mice were bled from the tail; antisera were stored at −20°C.

Rodent and human brain tissues were disrupted in Mg/Ca-free PBS by passage through a 20-gauge needle 5 times and followed by passage through a 22-gauge needle 10 times. The 10% (wt/vol) homogenate was centrifuged at 1600 × *g* for 5 min at 4°C. Typically, 500 μg of supernatant protein as measured by dye binding was present in 30–50 μl. The protein was diluted to a final concentration of 1 mg/ml in Mg/Ca-free PBS containing 0.2% Sarkosyl. Samples were mixed with an equal volume of 2× SDS/PAGE buffer without 2-mercaptoethanol and boiled for 5 min before SDS/PAGE (36). Immunoblotting was performed as described (37) except for the use of primary mouse antiserum diluted 1:1000.

RESULTS

Scrapie Incubation Times. Homogenates of Mo(RML) prions containing 0.2% Sarkosyl were heated at 80°C for 20 min and irradiated with 230 kJ of irradiation per m^2 at 254 nm (38) to eliminate any spurious pathogens such as the Casitas murine retrovirus that produces spongiform degeneration (39, 40) but no PrP^{Sc} (D.G., R. Jaenisch, and S.B.P., unpublished data). Bioassay of this inoculum in CD-1 mice gave an incubation time for onset of illness of 151 ± 2.0 days (mean ± SEM; n = 18) compared to mice with an untreated inoculum of 146 ± 1.3 (mean ± SEM; n = 18), indicating similar titers of $\approx 10^6$ ID_{50} units/ml (32).

Prn-$p^{+/+}$ mice as well as PrP gene ablated Prn-$p^{0/+}$ and Prn-$p^{0/0}$ mice were inoculated with Mo(RML) prions that had been both heated and irradiated. Both non-Tg littermates and CD-1 Swiss mice expressing wild-type MoPrP-A had incubation times of <165 days (Fig. 1). Prn-$p^{0/+}$ mice, heterozygous for ablation of the PrP gene, had prolonged incubation times ranging between 400 and 465 days. In contrast, Prn-$p^{0/0}$ mice have remained resistant to scrapie prions for >500 days.

Patterns of PrP^{Sc} Accumulation in Brain. Prn-$p^{+/+}$ mice were inoculated with Mo(RML) prions and sacrificed after developing signs of neurologic dysfunction; PrP^{Sc} was found by *in situ* immunoblotting in most brain regions except the neocortex, hippocampus, and hypothalamus (Fig. 2*A*). The histopathology, including spongiform degeneration and reactive astrocytic gliosis, was most intense in the brainstem and thalamus where PrP^{Sc} accumulated; little or no pathology was found in the hippocampus or neocortex. Asymptomatic Prn-$p^{0/0}$ mice were sacrificed 500 days after inoculation but, as expected, neither PrP^{Sc} nor histopathology was found (Fig. 2*B*). The absence of histopathology in Prn-$p^{0/0}$ mice

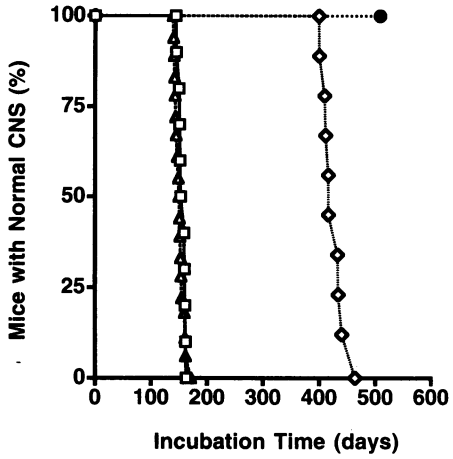

FIG. 1. Incubation times in PrP gene ablated Prn-$p^{0/+}$ and Prn-$p^{0/0}$ mice as well as wild-type Prn-$p^{+/+}$ and CD-1 mice inoculated with Mo(RML) prions. The RML prions were heated and irradiated at 254 nm prior to intracerebral inoculation into CD-1 Swiss mice (open triangles), Prn-$p^{+/+}$ mice (open squares), Prn-$p^{0/+}$ mice (open diamonds), or Prn-$p^{0/0}$ mice (solid circle). CNS, central nervous system.

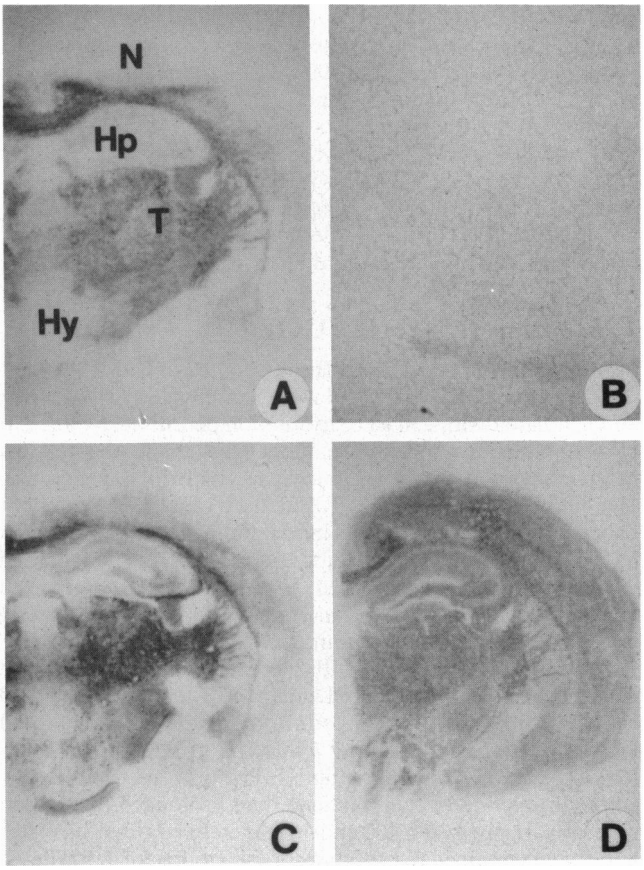

FIG. 2. Patterns of PrP^{Sc} accumulation in brains of Prn-$p^{+/+}$, Prn-$p^{0/+}$, and Prn-$p^{0/0}$ mice sacrificed at various times after inoculation with RML prions. PrP^{Sc} accumulation was measured by the histoblotting technique using anti-PrP antiserum (34). (*A*) Prn-$p^{+/+}$ mouse exhibited clinical signs of scrapie and was sacrificed at 160 days. (*B*) Prn-$p^{0/0}$ mouse with no clinical signs of neurologic dysfunction was sacrificed at 500 days. (*C*) Prn-$p^{0/+}$ mouse sacrificed 330 days after inoculation. (*D*) Prn-$p^{0/+}$ mouse with signs of neurologic dysfunction was sacrificed at 450 days. Hp, hippocampus; Hy, hypothalamus; N, neocortex; T, thalamus. (×10.)

sacrificed ≈400 days after inoculation with RML prions has been reported (20).

Prn-$p^{0/+}$ mice were sacrificed 330 days after inoculation with RML mouse prions prior to developing neurologic signs; in their brains PrP^{Sc} deposits were found throughout the cerebral hemispheres and brainstem but the PrP^{Sc} in the hippocampus and neocortex were relatively low (Fig. 2*C*). After developing signs of neurologic dysfunction, Prn-$p^{0/+}$ mice were sacrificed at 450 days after inoculation and the patterns of PrP^{Sc} accumulation were measured (Fig. 2*D*). At this time, PrP^{Sc} deposits as well as spongiform degeneration and reactive astrocytic gliosis appeared widely distributed throughout the brain. Additional studies are needed to determine the mechanism responsible for the differences in PrP^{Sc} accumulation between Prn-$p^{+/+}$ and Prn-$p^{0/+}$ mice.

Table 1. Prion titers in brains of Prn-$p^{0/0}$ and Prn-$p^{0/+}$ mice

Mouse	Time of sacrifice after inoculation with RML scrapie prions		
	5 days	60 days	120 days
Prn-$p^{+/+}$	<1	3.9 ± 0.4	6.4 ± 0.3
	<1	4.8 ± 0.3	7.1 ± 0.1
	<1	4.6 ± 0.2	6.6 ± 0.2
Prn-$p^{0/+}$	<1	<1	5.1 ± 0.2
	0.6 ± 0.7	<1	5.2 ± 0.6
	1.2 ± 0.1*	3.4 ± 0.2	2.8 ± 0.1
Prn-$p^{0/0}$	<1†	<1	<1
	<1‡	<1	<1
		<1	

Results are expressed as log of scrapie prion titers in ID_{50} units/ml (±SE). Titers are for 10% (wt/vol) brain homogenates. Log titers of <1 reflect no signs of central nervous system dysfunction in CD-1 mice for >250 days after inoculation except as noted.

*Three of nine mice developed scrapie between 208 and 210 days after inoculation.

†Two of nine mice developed scrapie between 208 and 225 days after inoculation.

‡Two of ten mice developed scrapie between 208 and 225 days after inoculation.

Prion Titers in Brain. Bioassays in CD-1 mice were performed on brain extracts collected from Prn-$p^{+/+}$, Prn-$p^{0/+}$, Prn-$p^{0/0}$ mice sacrificed 5, 60, and 120 days after inoculation. No prions were detected in brain extracts from Prn-$p^{0/0}$ mice at 60 and 120 days after inoculation (Table 1). In contrast, Prn-$p^{+/+}$ mice propagated prions to high titers as reported (20, 41, 42). Prn-$p^{0/+}$ mice had intermediate titers of prions at these times.

Incubation Times with Hamster Prions. PrP gene ablated mice, Tg(SHaPrP) mice, and crosses between them were inoculated with Mo(RML), SHa(Sc237), and SHa(139H) prions (Table 2). Prn-$p^{+/+}$, Prn-$p^{0/+}$, and Prn-$p^{0/0}$ mice were resistant to both the Sc237 and 139H isolates from Syrian hamsters although more prolonged observation is required for the Prn-$p^{+/+}$ mice inoculated with 139H. The CD-1 mice were more susceptible to 139H than Sc237 prions as reported (43). Incubation times of ≈50 days were found with the Tg(SHaPrP$^{+/+}$)7 and Tg(SHaPrP$^{+/0}$)7 mice inoculated with either isolate of SHa prions. Interestingly, incubation times of ≈50 days were also recorded in Tg(SHaPrP$^{+/+}$)81 and

Table 2. Scrapie incubation times in Tg(SHaPrP) and Prn-$p^{0/0}$ mice inoculated with mouse or Syrian hamster prions

Mice	Prion inocula								
	Mo(RML)			SHa(Sc237)			SHa(139H)		
	Illness*	Death*	n†	Illness*	Death*	n†	Illness*	Death*	n†
Prn-$p^{+/+}$	156 ± 5.6	169 ± 13	10	>525		9	>175		9
CD-1	151 ± 2.0	165 ± 3.4	18	>500		9	512 ± 10	530 ± 13	9
Prn-$p^{+/0}$	426 ± 18	430 ± 21	9	>525		10	>450		9
Prn-$p^{0/0}$	>510		8	>450		10	>450		8
Tg(SHaPrP$^{+/0}$)7	174 ± 17	181 ± 16	25	48 ± 1.0	51 ± 1.0	26	40 ± 3.0	42 ± 3.0	11
Tg(SHaPrP$^{+/+}$)7				49 ± 1.5	50 ± 1.6	7	40 ± 0	42 ± 0	6
Tg(SHaPrP$^{+/0}$)81	194 ± 3.5	200 ± 3.2	20	75 ± 1.1	75 ± 1.1	22	112 ± 9.5	117 ± 8.0	26
Tg(SHaPrP$^{+/+}$)81	198 ± 5.4	201 ± 5.0	12	57 ± 2.2	60 ± 2.9	10	58 ± 1.1	58 ± 1.1	10
Tg(SHaPrP$^{+/0}$)81/Prn-$p^{0/0}$	>150		10	54 ± 1.1	56 ± 1.8	9			

*Incubation time in days (means ± SE).

†Number of animals with scrapie or well after a specified period of time. In some experiments, fewer animals were used to calculate the death times because a fraction of the ill animals were sacrificed for neuropathological studies.

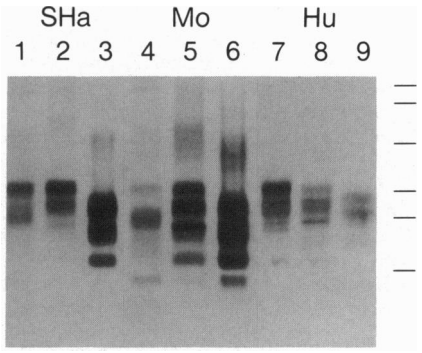

FIG. 3. Western immunoblots demonstrating anti-PrP Abs produced in Prn-$p^{0/0}$ mice after immunization with Mo prions. Lanes: 1–3, Syrian hamster brain; 4–6, CD-1 mouse; 7–9, human; 1, 4, and 7, normal homogenates; 2 and 5, scrapie-infected homogenates; 3 and 6, purified scrapie prions containing PrP 27–30; 8, homogenate from a patient dying of sporadic Creutzfeldt–Jakob disease (CJD); 9, purified CJD prions containing PrP 27–30. Molecular size values for proteins are denoted by horizontal lines and were determined by using prestained markers: phosphorylase b, 106 kDa; bovine serum albumin, 80 kDa; ovalbumin, 45 kDa; carbonic anhydrase, 32.5 kDa; soybean trypsin inhibitor, 27.5 kDa; lysozyme, 18.5 kDa.

Tg(SHaPrP$^{+/0}$)81/Prn-$p^{0/0}$ mice inoculated with SHa(Sc237) prions. Incubation times of ≈150 days after inoculation with Mo(RML) prions in Prn-$p^{+/+}$ and CD-1 Swiss mice with two PrP alleles contrast to longer times in Tg(SHaPrP) mice expressing high levels of transgene $SHaPrP^C$ (17) and in Prn-$p^{0/+}$ mice expressing low levels of $MoPrP^C$ (15).

Production of Anti-PrP Abs. We immunized Prn-$p^{0/0}$ mice with Mo and SHa prions dispersed in Freund's adjuvant. In our initial study, 1 of 2 Prn-$p^{0/0}$ mice immunized with Mo prions and 0 of 2 mice immunized with SHa prions produced anti-PrP Abs. In a second study, 10 of 10 mice immunized with Mo prions and 10 of 10 mice immunized with SHa prions produced anti-PrP Abs. Anti-PrP Abs in sera were detected by Western blotting. The anti-PrP Abs reacted with Mo, SHa, and human PrP (Fig. 3). The anti-PrP antisera had titers exceeding 1:5000.

DISCUSSION

Although there has been a remarkable convergence of experimental results contending that PrP^{Sc} is essential for transmission and pathogenesis of prion diseases (6), this conclusion continues to be challenged by some investigators (44–48). The inoculation of Prn-$p^{0/0}$ mice with scrapie prions provided yet another opportunity to test the hypothesis that PrP^{Sc} is neither required for development of disease nor necessary for multiplication of scrapie infectivity.

If Prn-$p^{0/0}$ mice inoculated with Mo prions had developed disease or propagated infectivity, then it could be argued that PrP^{Sc} is unrelated to the transmission as well as the pathogenesis of disease or that Mo prions stimulate the conversion of another cellular protein into a pathological isoform through an autocatalytic process. Since Prn-$p^{0/0}$ mice are resistant to scrapie and fail to propagate scrapie infectivity as presented here and elsewhere (20), we conclude that the PrP is necessary for both disease pathogenesis and prion propagation.

Increased SHaPrP transgene expression shortened incubation times for homologous SHa prions and extended them for heterologous Mo prions (16, 17, 19). Crosses between Tg(SHaPrP$^{+/+}$)81 and Prn-$p^{0/0}$ mice inoculated with SHa prions (Table 2) lend further support to the hypothesis that competition between endogenous $MoPrP^C$ and transgene PrP^C for inoculated PrP^{Sc} modulate incubation times. The Tg(SHaPrP$^{+/0}$)81/Prn-$p^{0/0}$ mice heterozygous for the SHaPrP transgene array had incubation times of ≈50 days compared to Tg(SHaPrP$^{+/0}$)81/Prn-$p^{+/+}$ mice with times of ≈75 days. Interestingly, Tg(SHaPrP$^{+/+}$)81/Prn-$p^{+/+}$ mice also had ≈50-day incubation times. To date, we have encountered difficulty in producing Tg(SHaPrP$^{+/+}$)81/Prn-$p^{0/0}$.

Whether conformation-dependent mAbs that discriminate between PrP^{Sc} and PrP^C can be produced from Prn-$p^{0/0}$ mice producing anti-MoPrP Abs (Fig. 3) remains to be determined but such mAbs would be invaluable. Spectroscopic data show that PrP^C has a high α-helical content and virtually no β-sheet (37), supporting a four-helix bundle model for PrP^C (49). Since PrP^{Sc} has a high β-sheet content, it is likely that conversion of PrP^C into PrP^{Sc} involves the unfolding of α-helical domains and their refolding into β-sheets (37).

Since ablation of both alleles of the PrP gene does not seem to be deleterious to mice, scrapie cannot be considered a disease of PrP^C inhibition (15). Rather, considerable evidence argues that the accumulation of PrP^{Sc} is responsible for the central nervous system dysfunction that attends scrapie (43, 50–52). In accord with these findings is the slow deposition of PrP^{Sc} in Prn-$p^{0/+}$ mouse brains, which is accompanied by a prolongation of the incubation time (Figs. 1 and 2). The results presented here and by others (20) with Prn-$p^{0/0}$ mice suggest therapeutics for prion diseases. Gene-targeted domestic animals such as sheep and cattle could be produced with ablated PrP genes if this does not prove deleterious to the health of the animals. Such sheep would be protected from scrapie and the cattle would be protected from bovine spongiform encephalopathy (53). Alternatively, antisense PrP genes or oligonucleotides should reduce PrP mRNA levels and thus diminish the production of PrP^{Sc}. Whether such therapy might be effective in humans who present early in the clinical course of a prion disease is unknown, but it could be administered to patients without symptoms who are at risk for inherited prion diseases.

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