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Three Hamster Species with Different Scrapie Incubation Times and Neuropathological Features Encode Distinct Prion Proteins

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Given the critical role of the prion protein (PrP) in the transmission and pathogenesis of experimental scrapie, we investigated the PrP gene and its protein products in three hamster species, Chinese (CHa), Armenian (AHa), and Syrian (SHa), each of which were found to have distinctive scrapie incubation times. Passaging studies demonstrated that the host species, and not the source of scrapie prions, determined the incubation time for each species, and histochemical studies of hamsters with clinical signs of scrapie revealed characteristic patterns of neuropathology. Northern (RNA) analysis showed the size of PrP mRNA from CHa, AHa, and SHa hamsters to be 2.5, 2.4, and 2.1 kilobases, respectively. Immunoblotting demonstrated that the PrP isoforms were of similar size (33 to 35 kilodaltons); however, the monoclonal antibody 13A5 raised against SHa PrP did not react with the CHa or AHa PrP molecules. Comparison of the three predicted amino acid sequences revealed that each is distinct. Furthermore, differences within the PrP open reading frame that uniquely distinguish the three hamster species are within a hydrophilic segment of 11 amino acids that includes polymorphisms linked to scrapie incubation times in inbred mice and an inherited prion disease of humans. Single polymorphisms in this region correlate with the presence or absence of amyloid plaques for a given hamster species or mouse inbred strain. Our findings demonstrate distinctive molecular, pathological, and clinical characteristics of scrapie in three related species and are consistent with the hypothesis that molecular properties of the host PrP play a pivotal role in determining the incubation time and neuropathological features of scrapie.

A cardinal feature of prion diseases is a prolonged incubation period during which the host shows no sign of dysfunction (29, 59). Early studies of different breeds of sheep exposed to experimental scrapie demonstrated that the genetic background of the host influences the incubation time (31). This was followed by transmission genetic studies of inbred mice, which yielded a number of candidate incubation time genes, including *Sinc* (24), *Pid-1* (43), and *Prn-i* (13).

Enrichment of Syrian golden hamster (SHa) brain fractions for scrapie infectivity led to the discovery of the prion protein (PrP) (8, 60). Determination of the N-terminal sequence of PrP (62) permitted identification of a cognate cDNA clone (55). The isolation of a PrP cDNA made possible a new approach to the genetics of scrapie and other prion diseases. The *Prn-i* gene was found to be tightly linked, if not identical, to the PrP gene (13) (*Prn-p*) located on chromosome 2 of the mouse (70). *Prn-p* encodes both a normal cellular protein (PrP^{C}) and a molecule, PrP^{Sc}, identified as a major and necessary component of the etiological infectious particle or prion. Molecular genetic analysis has provided compelling support for the concept that the gene encoding PrP exerts control on the incubation time of prion diseases. First, Westaway et al. (76) have shown that the *Prn-p* allele of short-incubation mice (designated *Prn-p*^{a}) encodes a PrP that differs by two amino acids from that of long-incubation mice (*Prn-p*^{b}). Second, Hsiao et al. (38) have identified a single amino acid substitution within the human PrP open reading frame (ORF) which is tightly linked to an ataxic form of Gerstmann-Sträussler syndrome (GSS), an inherited and experimentally transmissible encephalopathy. Third, mice expressing SHa PrP transgenes exhibit incubation times, scrapie infectivity, and amyloid plaques characteristic of Syrian hamsters (66a).

Given the importance of the PrP gene in the pathogenesis of prion diseases, we decided to investigate further the characteristics of the PrP gene and scrapie infection in various species of hamsters. Hamsters have been used extensively in prion research because of their susceptibility to experimental passage of various prion diseases including scrapie, transmissible mink encephalopathy, Creutzfeldt-Jakob disease, and GSS as well as the replication of scrapie prions to high titers in their brains. The first transmission of scrapie to hamsters was described by Zlotnik and Rennie (80), who found that SHa inoculated originally with the ME7 isolate of mouse-passaged scrapie agent (10) exhibited an incubation time of about 4 months after three SHa passages. Marsh and Kimberlin (49) and Kimberlin and Walker (42) later discovered that successive passages of the Chandler mouse scrapie isolate (16) in SHa resulted in an incubation time of approximately 60 days, about one-half of the minimum incubation time observed in mice. Furthermore, the titers of scrapie infectivity in the SHa brain were significantly higher than those found in mouse. These observations were exploited to develop an efficient incubation time assay with the SHa (61) and to obtain large quantities of SHa-derived scrapie prions for biochemical studies (60, 64). In contrast to the SHa, the Chinese hamster (CHa), which was first shown to be susceptible to rat-derived scrapie by Chandler and Turfrey (17), has a significantly longer scrapie incubation time. As reported here, we confirmed this relatively prolonged scrapie incubation time in the CHa and

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found that the Armenian hamster (AHa) has an incubation time that lies between those of the SHa and CHa.

On the basis of the recent discoveries that polymorphisms within the PrP gene may account for differences in scrapie incubation times in mice or the presence of a prion disease in humans and of the pleiotropic effects of SHa PrP transgenes, we reasoned that the disparate incubation times and other features of scrapie observed in these hamster species may be due to sequence differences in the hamster PrP gene. To investigate further the molecular basis of these wide variations in incubation times between related species, we characterized the PrP gene, mRNA, and prion proteins of the CHa and AHa and carried out passaging experiments and neuropathological studies of scrapie-sick animals. Our results showed that the three hamster species have distinct differences in their PrP genes and protein products and in the neuropathology seen late in the course of scrapie. Most importantly, the polymorphisms within the PrP ORF that distinguish the three hamster species are all within a hydrophilic domain of 11 amino acids that includes one of the polymorphisms linked to the incubation time gene in mice (76) and the amino acid substitution linked to GSS in humans (38). This observation provides further evidence that molecular properties of PrP play an integral role in modulating the pathogenesis of scrapie.

MATERIALS AND METHODS

Source and passage of scrapie prions. A hamster-adapted isolate of the scrapie agent was provided by R. Marsh (49). LVG/LAK random-bred SHa (Charles River Breeding Laboratories, Inc., Wilmington, Mass.) were used for passaging the isolate, subsequently designated SHa Sc237, prior to these studies and in the passages in this study. Inbred CHa and AHa (Cytogen, West Roxbury, Mass.) were inoculated with brain homogenates from clinically ill SHa as previously described (61, 63). Weanling CHa and AHa hamsters received 10^7 50% infective dose units in 50 μl through a 27-gauge needle inserted into the parietal region of the left cerebral hemisphere to a depth of approximately 2 mm. Clinically ill CHa and AHa were sacrificed, and brain inocula were repassaged into CHa, AHa, and SHa weanlings as shown in Table 1.

Histochemistry and immunocytochemistry. Upon sacrifice of animals, brains were immersion fixed in 10% Formalin. Thick sections (10 μm) were cut from paraffin-embedded blocks of tissue and were stained with hematoxylin and eosin, the periodic acid-Schiff histochemical stain, or immunoperoxidase stain for PrP (23).

Preparation of DNA and RNA. High-molecular-weight brain DNA from uninfected hamsters was prepared with proteinase K (32) as described previously (55, 58). Total RNA was isolated from the brains of uninfected hamsters by either the guanidinium–hot-phenol method (28) or the guanidinium-LiCl method (15). Poly(A)$^+$ RNA was recovered by one cycle of oligo(dT) chromatography (1).

Molecular cloning of CHa PrP gene. High-molecular-weight DNA from CHa brain was partially digested with *Sau*3A by the methods described by Kaiser and Murray (40). A 16- to 24-kilobase (kb) size fraction was electroeluted after preparative agarose gel electrophoresis with an Elu-Trap (Schleicher & Schuell, Inc., Keene, N.H.), extracted with phenol, and precipitated with ethanol. Various amounts of these fractions were then ligated to *Bam*HI-cleaved λEMBL3 arms (Stratagene, San Diego, Calif.) in a total volume of 5 μl at 14°C in a reaction buffer containing 100 mM Tris hydrochloride (pH 7.8 at 25°C), 10 mM $MgCl_2$, 20 mM β-mercaptoethanol, 1 mM ATP, and a final activity of T4 DNA ligase (International Biotechnologies, Inc., New Haven, Conn.) of 200 U/ml. The ligation products were packaged in vitro directly (33, 37) (Gigapack Plus; Stratagene). The packaging reactions were quenched by the addition of 500 μl of bacteriophage dilution buffer (1% $NaKPO_4$ [pH 7.0], 0.05% NaCl, 1 mM $MgSO_4$, 0.1 mM $CaCl_2$, 0.001% gelatin), titered, and plated on *Escherichia coli* K802 (78) at a density of 25,000 phage per 150-mm petri dish. Hybridization to plaques in situ was based on the method of Benton and Davis (7). A total of three positive clones were isolated. Two of these (CHO7 and CH18) were restriction mapped by Southern analysis of digests with and without *Sal*I together with *Hin*dIII, *Eco*RI, *Bgl*II, *Bam*HI, or *Bst*YII. Based on these results, an *Eco*RI digestion of CH18 yielded a 4.3-kb insert containing the putative exon 2, which was then subcloned into pUC18 (79).

Amplification and cloning of AHa PrP gene. The methods for gene amplification from genomic DNA by using the polymerase chain reaction (PCR) were adapted from Saiki et al. (66). One 28-nucleotide and one 27-nucleotide primer were used for the PCRs (5′-CGGGATCCTTGTTCTTCAT TTTGCAGA-3′ 5′-CCAAGCTTGTACAAGCAGGGAGGC TTCC-3′). These were designed to allow polymerization into the PrP ORF from either the immediate 5′- or 3′-flanking regions, based on the known flanking region sequences of the CHa (see Fig. 4A), SHa (3), and mouse (76) PrP gene. Adjacent to a 20-nucleotide annealing segment, the oligonucleotides encoded a restriction site (*Hin*dIII or *Bam*HI) that was later used to facilitate subcloning. PCR reactions of 100 μl contained the following: 600 ng of AHa brain DNA, 50 mM KCl, 10 mM Tris hydrochloride (pH 8.4 at 25°C), 2.5 mM $MgCl_2$, 1 μM each primer, 200 μM each dATP, dCTP, dTTP, and dGTP, gelatin at 200 μg/ml, and 2 U of *Taq* DNA polymerase (Cetus Corp, Emeryville, Calif.). The samples were overlaid with 100 μl of mineral oil and amplified for 35 cycles with a programmable heat block (Perkin-Elmer-Cetus Instruments). Reaction conditions for the first five cycles were 94°C for 1 min, 50°C for 1 min, and 72°C for 2.5 min and were the same for the next 30 cycles except the annealing temperature was raised from 50 to 55°C. The reaction product was electrophoresed on a preparative agarose gel, and a band corresponding to the putative amplified ORF (calculated size, 818 base pairs) was excised and isolated with Gene-Clean (Bio 101 Inc., La Jolla, Calif.). This was then digested with *Hin*dIII and *Bam*HI and directly cloned into a Bluescribe vector (Stratagene) (48). The presence of the AHa PrP ORF in the clones was confirmed by Southern blot analysis.

DNA sequencing. Supercoiled plasmid DNA was prepared as described by Hattori and Sakaki (35). For sequencing the CHa ORF, a single clone (CH18) was used, whereas for the AHa ORF, 30 individual clones derived from the PCR product were pooled (see Results). The plasmid DNA was alkali denatured by the protocol of Chen and Seeburg (18) and supercoil sequenced by using Sequenase, [^{35}S]dATP, dideoxynucleotides (with dITP instead of dGTP), and the protocol suggested by the supplier (United States Biochemical Corp., Cleveland, Ohio). Reaction products were electrophoresed on standard sequencing gels. Oligonucleotides originally used for sequencing the hamster PrP cDNA were used; some of these had single-base-pair mismatches with the AHa and CHa clones, but this had no adverse effects. Other new oligonucleotides were prepared to complete the sequencing strategies (see Fig. 3).

TABLE 1. Scrapie incubation times in three hamster species

Passage[a] (donor → recipient)	n[b]	Incubation time[c]	
		Clinical symptoms	Death
SHa → SHa	13	69 ± 2	79 ± 5
SHa → AHa	4	174 ± 2	194 ± 4
SHa → CHa	4	344 ± 13	358 ± 31
AHa → AHa	16	125 ± 9	148 ± 12
AHa → SHa	14	113 ± 2	129 ± 4
AHa → CHa	11	314 ± 23	351 ± 36
CHa → CHa	15	272 ± 12	307 ± 23
CHa → SHa	16	121 ± 8	133 ± 6
CHa → AHa	5	181 ± 9	187 ± 9

[a] The initial passage from SHa into AHa or CHa used the scrapie isolate SHa Sc237. Brain inocula from clinically ill AHa were then passaged into CHa and SHa, and brain inocula from clinically ill CHa were passaged into AHa and SHa. For homologous passages, the incubation times after at least two subsequent passages are shown.

[b] Number of hamsters for given passage.

[c] Incubation times are given as mean days ± SE from inoculation of weanlings to clinical symptoms or death.

Preparation of oligonucleotides. A total of 10 oligonucleotides were synthesized on an Applied Biosystems 380B DNA synthesizer by C. Mirenda. The oligonucleotides for DNA sequencing of the coding strand were as follows (5′ to 3′): TGATGTTGGCCTCTGCA, TGGTGGCTACATGCTGG, GAGAACTTCACGGAGAC, GTAAAACGACGGCCAGT, and GTGGCACCCACAATCAGTGG. The anticoding strand oligonucleotides (5′ to 3′) were as follows: GCTTCTTG CAGAGGCCAACATCA, GGCCCCTGCCGCAGCAGCGC, TCCACTGGCCGGTAATACAC, AACAGCTATGACCA TG, and ACATCTGCTCCACCACGCGC.

Oligonucleotides were purified by preparative electrophoresis on 15% (30:1 acrylamide-bisacrylamide) gels. The oligonucleotides were visualized by UV shadowing (50) and eluted by soaking the excised gel slices overnight in double-distilled water. For DNA sequencing, the recovered oligonucleotides were used directly. For use in PCR reactions, oligonucleotides were further purified by reverse-phase chromatography on Sep-Pak columns (Waters Associates, Inc., Boston, Mass.).

Southern blot analyses. DNA (5 or 10 μg) was digested with restriction endonucleases under conditions recommended by the suppliers, and samples were electrophoresed on agarose gels and transferred to nitrocellulose (0.45-μm pore size) (69). Two methods were subsequently used to identify restriction products complementary to probes. For analyses of exon 2, restriction fragments were labeled to a specific activity exceeding 10^9 cpm/μg by using [^{32}P]dCTP (3,000 Ci/mmol) (Amersham Corp., Arlington Heights, Ill.) and the random priming method of Taylor et al. (71) as modified by Feinberg and Vogelstein (27). Hybridization and posthybridization conditions were as described by Basler et al. (3) (method A), using heparin in a formamide hybridization buffer (68), except that posthybridization washes were performed at 55°C rather than 65°C. For analysis of exon 1, a *Bam*HI-*Hin*dIII restriction fragment encompassing the SHa exon 1 (3) was biotinylated with Photoprobe Biotin (Vector Laboratories, Burlingame, Calif.). The labeling and recovery of the restriction fragment, posthybridization conditions, and reactions to visualize signals were as described by the supplier. Prehybridization and hybridization conditions were as above.

Northern (RNA) blot analyses. RNA samples (10 μg) were ethanol precipitated, washed once with absolute ethanol, and dissolved in 50% formamide–2.2 M formaldehyde. After the addition of 0.2 volume of 10% Ficoll and 1% (wt/vol) bromophenol blue, the samples were electrophoresed through a 1% agarose gel. The running buffer was 20 mM [3-(*N*-morpholino)]-propane sulfonic acid (MOPS) (pH 7.0)–1 mM EDTA–2.2 M formaldehyde. After electrophoresis, the gel was soaked in 20× saline sodium citrate (SSC) (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate), the RNA was transferred to nitrocellulose (72), and the filters were prehybridized and hybridized essentially as described by Shank et al. (67). The *Sau*I to *Taq*I fragment representing primarily the ORF of the pHaPrPcDNA-1 insert (55, 77) was radiolabeled as described above for Southern blot analyses. Hybridizations were performed at 42°C in 3× SSC–50% formamide–0.05 M HEPES (*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid) (pH 7.4)–0.2 mg of salmon sperm DNA per ml–0.15 mg of yeast RNA per ml–0.02% polyvinyl pyrrolidone. Filters were washed in 0.1× SSC–0.1% sodium dodecyl sulfate at 60°C and autoradiographed at −70°C with Cronex intensifying screens and Kodak XAR-5 film.

Immunoblot (Western) analyses. Protein from normal and scrapie-sick CHa, AHa, and SHa brains was obtained by preparing a 10% brain homogenate of brain samples stored at −70°C. Samples (20 μl) of these homogenates added to 20 μl of electrophoresis sample buffer (100 mM Tris chloride [pH 8.9 at 25°C], 2% sodium dodecyl sulfate, 5% 2-mercaptoethanol, 10% glycerol, and 0.002% bromophenol blue) were heated at 100°C for 1 to 2 min. For proteinase K treatment, 50 μl of the brain homogenate was first incubated with 50 μg of proteinase K (Beckman Instruments, Inc., Palo Alto, Calif.) per ml for 30 min at 37°C. The reaction was quenched with 5 mM phenylmethylsulfonyl fluoride, and a 20-μl sample of this product was then prepared as described above. The proteins were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (10% monomer) by the method of Laemmli (45). Transfer of proteins from polyacrylamide gels to nitrocellulose was performed overnight at 5 V/cm with 0.1% sodium dodecyl sulfate added to the transfer buffer (74).

Rabbit PrP antisera and monoclonal antibody 13A5 were

TABLE 2. Neuropathological findings in scrapie-infected hamsters[a]

Species	Thalamus		Hippocampus			White matter		Caudate		Amyloid plaques
	Vacuoles	Gliosis	Vacuoles	Cell loss	Gliosis	Vacuoles	Gliosis	Vacuoles	Gliosis	
CHa	+++ (coarse)	++	+ (fine)	0	++	0	+	+ (coarse)	+	+ (mature)
AHa	++ (coarse)	++	++ (fine)	0	++	++ (coarse)	++	++ (coarse [w.m.])	+	−
SHa	++	++	++	+	++	0	0	0	0	+++ (primitive)
SHa neonate	++ (fine)	+++	++ (fine)	+++	+++	0	+	+ (fine)	+	−

[a] Coarse, 20 to 40 μm; fine, 5 to 20 μm; w.m.; white matter. 0, none; +, mild; ++, moderate; +++, severe.

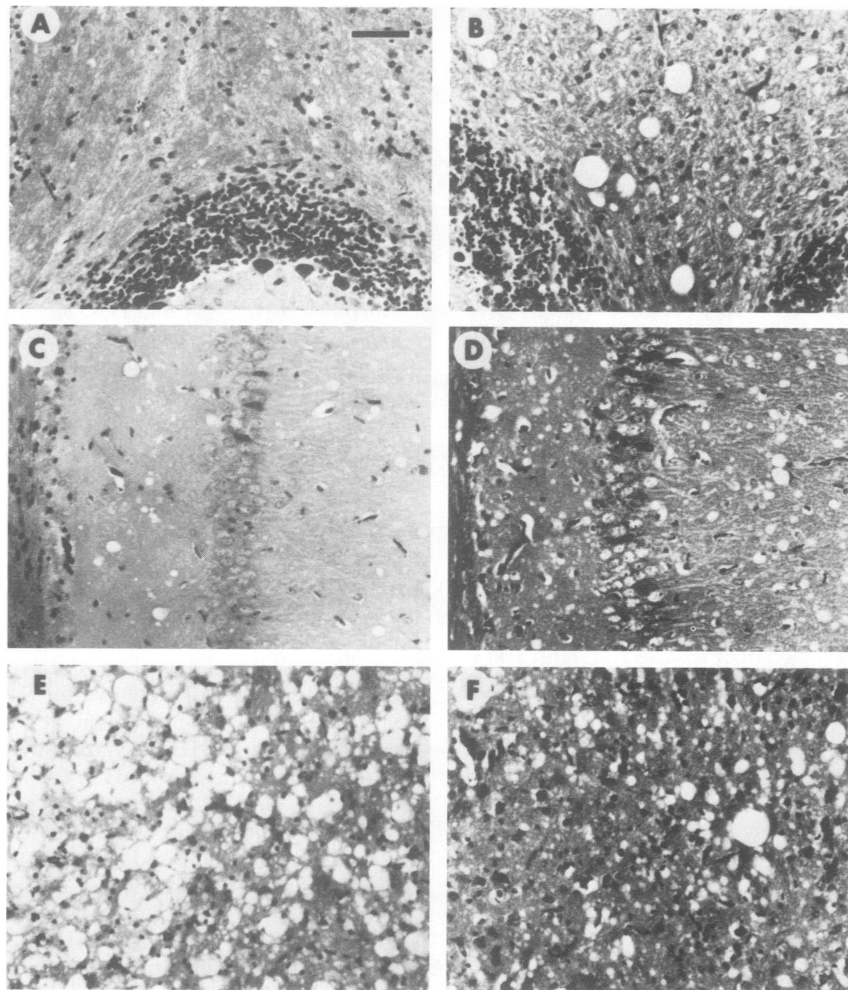

FIG. 1. Neuropathological findings in scrapie-sick AHa and CHa. White matter vacuolization is characteristic of the AHa but not the CHa. (A) CHa cerebellar white matter. (B) AHa cerebellar white matter. Gray matter vacuolization is also distinctive. There are far fewer vacuoles in the hippocampus of the CHa (C) versus AHa (D). In contrast, vacuoles are larger and more numerous in the thalamus of the CHa (E) versus AHa (F). The bar represents 100 μm and applies to all panels. Hematoxylin and eosin.

produced as described previously (2, 6). PrP monoclonal antibody 3F4 was provided by R. Kascsak (41).

Lanes containing molecular weight markers were excised from the nitrocellulose blots and stained with amido black. The blots were then blocked for 30 min in 5% (wt/vol) nonfat dry milk in 10 mM Tris hydrochloride (pH 8.2 at 25°C)–200 mM NaCl–0.05% Tween 20. Immunoreactive proteins were detected by incubation with diluted rabbit primary antiserum or monoclonal antibody (1 to 3 μg/ml) in the same buffer for 8 to 12 h and then with alkaline phosphatase-conjugated secondary antibody for 1 to 2 h and developed with Nitro Blue Tetrazolium and 5-bromo-4-chloro-3-indolyl phosphate as suggested by the supplier (Promega Biotec, Madison, Wis.).

RESULTS

Heterologous and homologous passage of scrapie prions in hamsters. Table 1 shows the results of homologous and heterologous passages of the scrapie isolate SHa Sc237 through the three hamster species. When the passage was performed from a donor to a recipient of the same species (i.e., homologous passage), the incubation times, i.e., from inoculation to the onset of clinical symptoms, averaged 69 days (standard error [SE] of 2 days) for the SHa, 125 days (SE of 9 days) for the AHa, and 272 days (SE of 12 days) for the CHa. Each hamster species has a distinct incubation time which remained unchanged upon subsequent passage in the homologous host. The only exception to this was an initial passage through SHa, which had a relatively prolonged incubation time of 111 days (SE of 5 days). However, the next passage from these animals into SHa gave an incubation time of 73 days (SE of 14 days). Whether this was a result of a technical error or a biologic phenomenon remains unexplained.

Also shown in Table 1 are the incubation times when passages were made between different hamster species (heterologous passages). In all cases, the incubation time for the recipient species was substantially longer after these heterologous passages than the homologous passages described above. For example, when brain homogenates from SHa (SHa Sc237) were passaged into AHa, the incubation time was 174 days (SE of 2 days), or approximately 40% longer than the incubation time for homologous passages between AHa. Likewise, passage of prions from CHa to AHa resulted

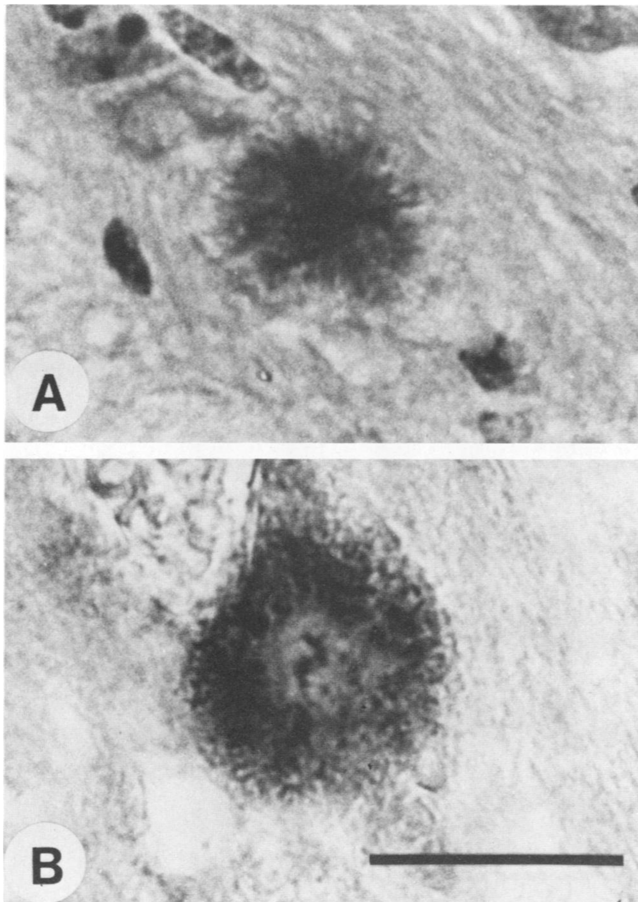

FIG. 2. Amyloid plaques found in CHa. (A) Periodic acid-Schiff histochemical stain. (B) Immunoperoxidase stain for the prion protein. Plaques in panels A and B are from different animals. The bar is 25 μm for both panels.

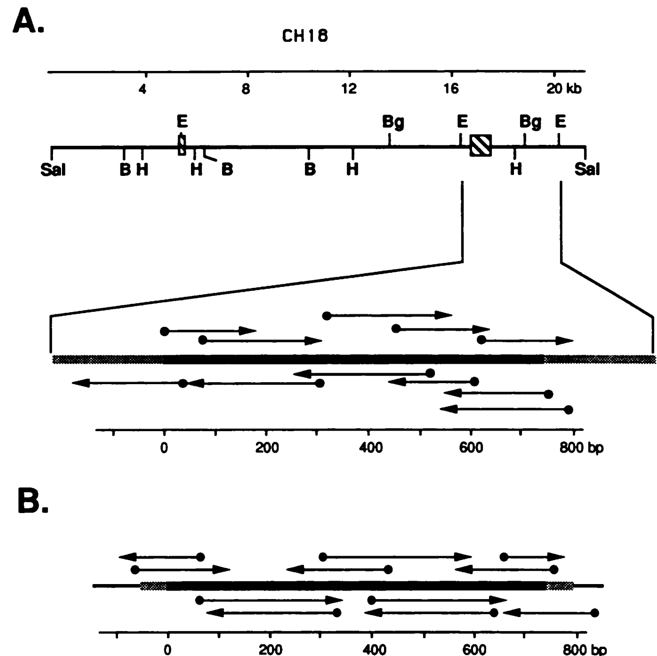

FIG. 3. Restriction map of the CHa PrP-CH18 clone insert and sequencing strategy for the CHa and AHa PrP ORF. (A) The 22-kb insert recovered from a CHa genomic library is shown. The approximate locations of exons 1 and 2 are shown by the hatched boxes; the size of exon 1 is by inference only to what is known of the SHa PrP gene structure. Restriction sites: Sal, *Sal*I; B, *Bam*HI; H, *Hin*dIII; E, *Eco*RI; Bg, *Bgl*II. The 4.3-kb *Eco*RI subclone containing the ORF is depicted (not to scale), with the extent of exon 2 denoted by the solid black line. Oligonucleotide primers used for sequencing are indicated by filled circles; the extent and direction of sequencing from each site are indicated by an arrow. (B) The sequencing strategy for the PCR-amplified AHa ORF is depicted in a similar fashion. bp, Base pairs.

in substantial prolongation of the incubation time to 181 days (SE of 9 days).

Comparative neuropathology. The neuropathology of two scrapie-sick CHa and two AHa from both the first and second passages of the above-described passaging experiments was studied. The degree of vacuolization was assessed in hematoxylin- and eosin-stained sections, and reactive astrocytic gliosis was assessed by immunohistochemistry with glial fibrillary acidic protein antibodies. PrP immunohistochemistry and periodic acid-Schiff histochemistry were used to identify amyloid plaques.

Several generalities can be made regarding the degree and distribution of spongiform degeneration, reactive astrocytic gliosis, and PrP amyloid plaque formation in AHa and CHa compared with that observed for SHa as previously described (23). The histopathology was distinct for each species (Table 2). In AHa, vacuolization and reactive astrocytic gliosis were seen throughout the white matter (Fig. 1); this pattern was not seen in CHa or SHa, but it is a characteristic of scrapie in NZW and I/Ln mice (13). The vacuolization found in CHa and AHa was more coarse (20- to 40-μm-diameter vacuoles) than in SHa (5 to 20 μm). Furthermore, the intensity and regional distribution of lesions were different for each strain. Within the hippocampus, there was little or no nerve cell loss in the CHa and fine vacuolization in both the AHa and SHa, while in the thalamus, the most intense vacuolization was found in the CHa followed by the AHa and then the SHa (Fig. 1). Finally, an occasional mature kuru-type PrP immunopositive plaque was present in the region of the hippocampal stratum oriens in the CHa (Fig. 2), while no amyloid plaques were observed in the AHa. This is in contrast to the numerous primitive PrP amyloid plaques found in scrapie-infected SHa brains when the hamsters were inoculated as weanlings (23).

Isolation and sequencing of CHa genomic clone. The CHa PrP gene was cloned from a CHa genomic library as described in the Materials and Methods. A restriction map of one of the positive isolates is depicted in Fig. 3 and shows that the Cha PrP gene has an organization similar to that previously described for the SHa PrP gene; i.e., there are regions homologous to both exon 1 and exon 2 of the SHa and an intron of approximately 11 kb. A 4.3-kb *Eco*RI fragment containing the putative exon 2 was subcloned into a pUC plasmid vector and subsequently sequenced by the supercoil method with oligonucleotide primers as shown in Fig. 3 (18).

Amplification and sequencing of AHa PrP ORF. The PCR was used to amplify the AHa ORF from genomic DNA as described in Materials and Methods. To avoid potential artifacts introduced by the PCR (26, 54, 73), we pooled and sequenced 30 clones using the strategy depicted in Fig. 3 and found no obvious ambiguity in the sequencing data.

Nucleotide sequence of PrP gene from CHa and AHa. The exon 2 nucleotide sequences obtained from the CHa clone

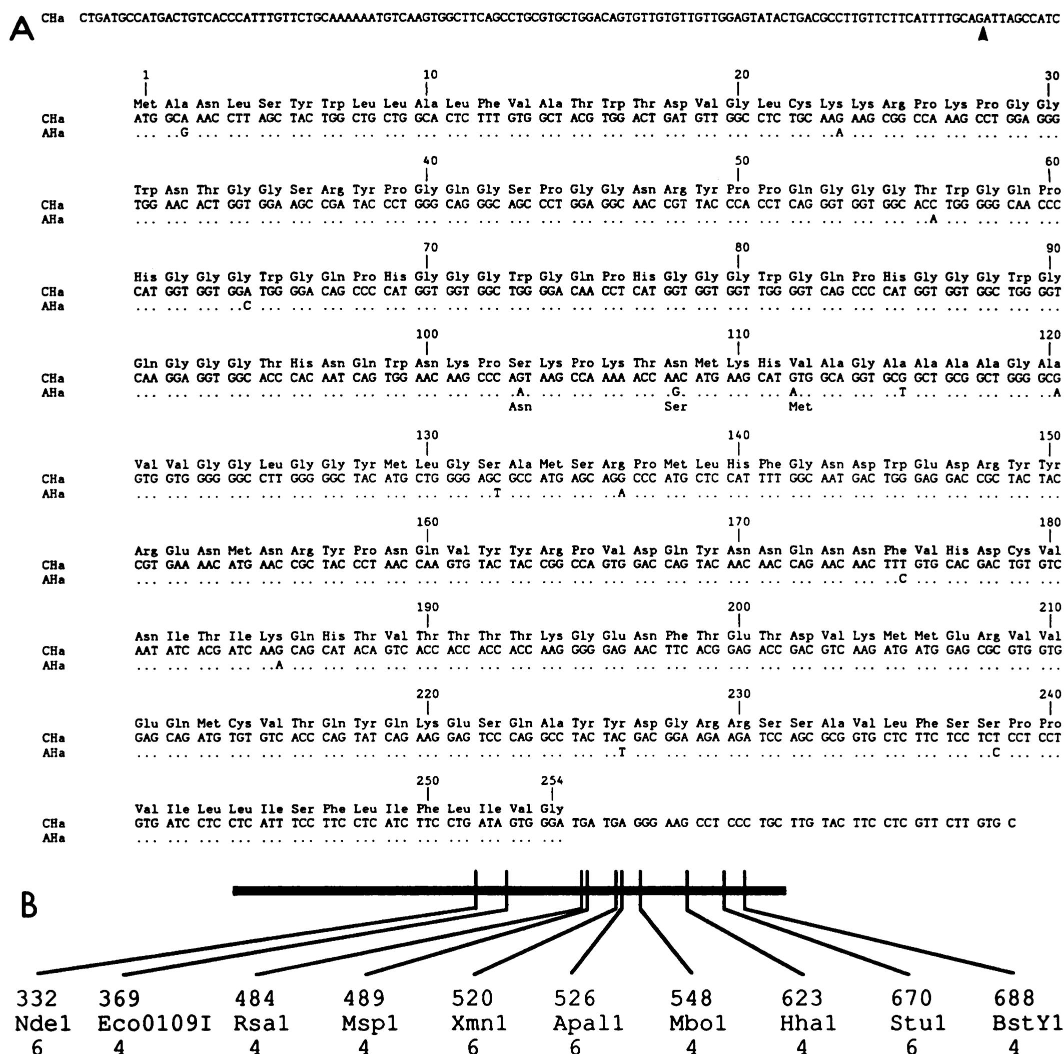

FIG. 4. (A) PrP ORF sequences from CHa and AHa. The nucleotide sequence spanning the PrP ORF of the CHa is shown in its entirety, with an arrow between nucleotides −11 and −10 denoting a putative 3′ splice site. The lower sequence depicts the AHa ORF; dots indicate sequence identity with the CHa ORF. (B) Restriction map of the AHa ORF showing the 10 enzymes used to test the accuracy of the primary nucleotide sequence data. Listed above each enzyme are the nucleotide positions 5′ to the restriction site, and below are the number of nucleotides that determine the restriction site.

and the pooled AHa clones are presented in Fig. 4A. Sequences of the flanking regions were not available for the AHa since only the ORF was amplified. For the CHa, nucleotides −126 to −14 show little homology to the SHa sequence (3), whereas nucleotides at positions −14 to −1 differ only at position −8. This segment contains an AG consensus dinucleotide at positions −10 and −11 that represents a putative 3′ splice site. Interestingly, there is more homology of the 5′-flanking region between the CHa and NZW or I/Ln mouse (76) than with the SHa, since only five differences are seen between positions −36 and −1.

The PrP ORFs for the CHa and AHa extend from positions +1 to +762 and show extensive homology with one another and other known PrP exon 2 sequences. There are 15 nucleotide differences between the CHa and AHa, 35 nucleotide differences between the CHa and SHa, and 35 nucleotide differences between the AHa and SHa. Among the three species, the most highly conserved regions are a 67-nucleotide segment spanning positions +241 to +307 and a 68-nucleotide segment from +418 to +485. Three amino acid substitutions distinguish the CHa from AHa: serine (CHa) to asparagine (AHa) at codon 103, asparagine (CHa) to serine (AHa) at codon 108, and valine (CHa) to methionine (AHa) at codon 112 (Fig. 4A). Compared with the SHa, there are seven amino acid changes between the CHa and SHa and eight between the AHa and SHa. To assess the accuracy of the amplification sequencing strategy used to determine the AHa PrP sequence, we tested amplified AHa PrP ORF derived from genomic DNA for the presence of 10 restriction sites predicted by our sequencing data. Digestion with each of the 10 enzymes (accounting for a total of 48 base pairs or 6.3% of the ORF) yielded restriction fragments of the expected size (Fig. 4B).

Unique characteristics of PrP mRNA and protein. To explore other potential species differences related to the PrP gene, we also compared the PrP mRNAs and protein from the CHa, AHa, and SHa. Northern analysis of brain mRNAs showed a hybridization fragment of 2.5 kb for the CHa, 2.4 kb for the AHa, and 2.1 kb for the SHa (data not shown), and the steady-state level, as deduced from RNA loadings nor-

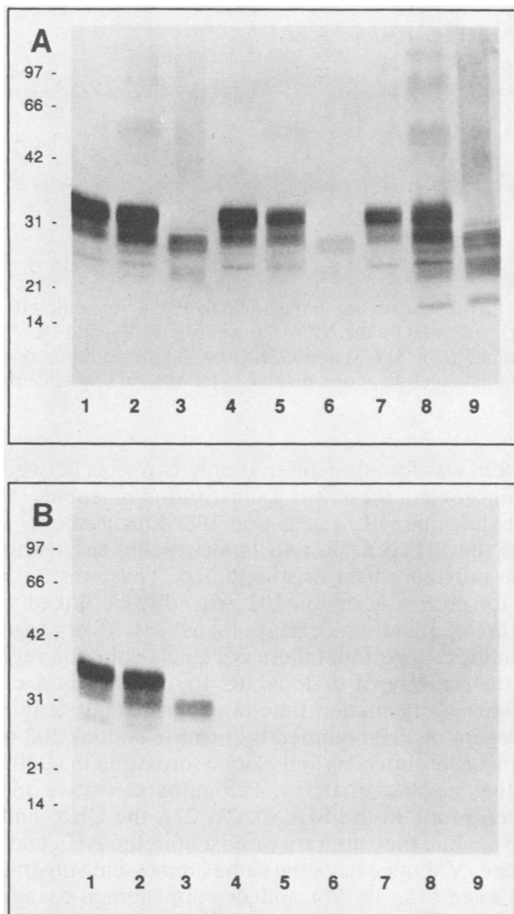

FIG. 5. Western blot analysis of PrP proteins from normal and scrapie-infected CHa, AHa, and SHa. Both blots were loaded with identical samples (20-μl samples from 10% brain homogenates). Proteins from uninfected hamsters were analyzed directly, while samples from scrapie-infected hamsters were either treated with proteinase K (+PK) or untreated (−PK). Blots were incubated with immune serum from a rabbit immunized with PrP27-30 (A) or with the monoclonal antibody 13A5 (B) (2, 6). Lanes: 1, normal SHa; 2, scrapie SHa (−PK); 3, scrapie SHa (+PK); 4, normal CHa; 5, scrapie CHa (−PK); 6, scrapie CHa (+PK); 7, normal AHa; 8, scrapie AHa (−PK); 9, scrapie AHa (+PK).

malized by weight, appeared to be slightly greater in the CHa than in the other two species. Positions of migration for molecular size standards are given in kilodaltons on the ordinate.

Protein derived from both normal and scrapie-infected hamster brains was analyzed by immunoblotting with either polyclonal antiserum or monoclonal antibodies raised against the SHa 27- to 30-kDa PrP (Fig. 5). The polyclonal antiserum R073 reacted with the PrP protein from all three species and revealed equivalent sizes and intensities of the 33- to 35-kilodalton protein in the normal and scrapie-infected brains. Additionally, proteinase K digestion of scrapie-infected brain produced the characteristic 27- to 30-kilodalton band indicative of partially hydrolyzed PrP^{Sc} (51). A similar immunoblot with the monoclonal antibody 13A5 reacted only with protein from the SHa (Fig. 5). Furthermore, a different monoclonal antibody raised against the SHa 27- to 30-kDa PrP by Kascsak et al. (41), denoted 3F4, was found to recognize SHa and AHa but not CHa PrP (data not shown). Taken together, these findings indicate that each of the hamster species has PrP proteins with distinctive antigenic sites.

DISCUSSION

The determinants of incubation time in scrapie infection have emerged as a major area of interest in prion research. Amino acid substitutions in the PrP gene of mice (*Prn-p*^{b}) with exceptionally long incubation times (13) and in the PrP genes of patients with GSS (38) have underscored the role of PrP in the pathogenesis of both animal and human prion disorders. Prolonged incubation times are one of the most unique and intriguing characteristics of the prion diseases. In humans, for example, the delay between exposure to prions and the onset of symptoms of kuru may span decades (29), and in patients with GSS, a genetic form of the human prion diseases, the symptoms may fail to appear until the fifth decade or longer (9). Thus, the pathophysiology of prions includes a molecular clock that operates on a time scale quite dissimilar to other known nervous system infections. This clock may share features with other slowly progressive, degenerative diseases (59). It is also possible that efforts to prolong the incubation time will constitute a viable therapy for the prion diseases; i.e., if the incubation time can be altered to exceed the expected life span of the organism.

Host determinants of scrapie incubation time in hamsters. Our passaging experiments demonstrated a wide variation in incubation times among the three hamster species, despite the common origin of the scrapie agent used in these studies. The passaging studies also highlight two previous observations concerning the incubation times in experimental scrapie. First, the passage of the scrapie isolate SHa Sc237 into the AHa or CHa and subsequent passages into heterologous species resulted in incubation times far in excess of the relatively fixed incubation period that is observed in these animals after successive homologous passages. This is in keeping with the concept of a species barrier introduced by Pattison (57). Second, the studies suggest that it is the host species which is a major determinant of the incubation time. Thus, despite the common origin of the scrapie agent, successive passages in homologous hamster species resulted in a return of the incubation time to the base line for the given species. These results are consistent with recent passaging studies in allogeneic and syngeneic mice that implicate genes within the *Prn* complex in the passage behavior of prion isolates (14).

Characteristic neuropathology for each hamster species. The pathological hallmarks of experimental scrapie include neuronal vacuolation with degeneration and astrocytic gliosis (5). Amyloid plaques, which are immunoreactive with PrP antiserum and have structural similarities with kuru plaques, are seen in certain animals (5, 6). The distributions of these changes within the central nervous system are variable. For example, the regions predominantly affected in the mouse are the dorsal and medial cortex, thalamus, and white matter, with a lesser involvement of the hippocampus and hypothalamus (5). In the SHa, the thalamus and hippocampus are severely affected, while the white matter is usually not involved (21, 23).

The neuropathological changes observed in the CHa and AHa constitute yet another pattern of pathology in experimental scrapie (Table 2). In comparison to the SHa, the CHa and AHa have much larger vacuoles, less involvement of the hippocampus, and more involvement of the caudate. The AHa is further distinguished by significant vacuolization and gliosis in the white matter and no PrP-immunopositive

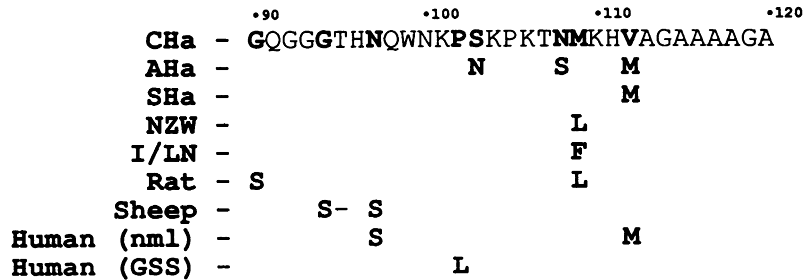

FIG. 6. Highly polymorphic region of the PrP protein. Further comparisons of the region from amino acid 90 to 120. The predicted amino acid sequences of the three hamster species are shown in rows 1 to 3 (3; this report), followed by the NZW mouse (row 4) (76) and I/Ln mouse (row 5) (76), the rat (row 6) (47), the sheep (row 7) (30), and the normal (nml) human (row 8) (44) and GSS (row 9) (38) proteins. A mouse polymorphism at codon 108 (76) is shown here at position 109 because of an *N*-terminal deletion of one residue in the mouse sequence relative to the CHa prototype.

plaques. These new observations suggest that the neuropathological changes in scrapie-sick animals are not a consequence of a fixed pattern of spread of inoculated scrapie prions that is solely governed by the incubation time. Indeed, there is no obvious correlation between the regional distribution of the neuropathology and the incubation time.

Unique PrP-coding sequences for each hamster species. The PrP gene has been shown to be highly conserved in a number of divergent species of mammals (77), and it is not surprising to find substantial homology between the CHa and AHa PrP genes reported here and the other PrP genes which have been fully sequenced. The overall structure of the CHa gene is similar to that of the SHa. There is an uninterrupted coding exon flanked on the 5′ side by a consensus splice acceptor site and separated from a region homologous with the SHa promoter by a putative intron of approximately 11 kb. Although we have not yet determined the overall structure of the AHa PrP gene, it is likely that the exon structure outside the ORF varies between all three species, given the differences in sizes of the mRNA. These differences in structure and the potentially distinctive roles they may play in the control of PrP expression are unexplored. An alteration in PrP gene expression has not been considered a likely mechanism for the synthesis of PrP^{Sc}, since the steady-state levels of SHa PrP mRNA appear to be unchanged during scrapie infection (55). However, expression of PrP mRNA is developmentally regulated (52, 53), and recent work suggests a possible relationship between PrP mRNA expression and incubation time in neonates (51).

Host genotype and prion isolate behavior. The species barrier (Table 1) and host-specific determination of neuropathology (Fig. 1 and 2) imply a profound impact of host genotype upon prion isolate behavior. Genetic studies in mice detailed above reveal a PrP-linked gene or the PrP gene itself as the critical determinant host locus. Accordingly, transgenes derived from SHa cosmid clones remove a species barrier for passage of SHa prions into mice (66a).

The identification of distinct prion protein sequences for three hamster species in this study raises the possibility that variations in PrP primary structure affect species barriers, incubation times, and the neuropathology of scrapie infection in hamsters (75). Regarding this possibility, it is interesting that only one cluster of polymorphisms, at codons 103, 108, and 112, serves to distinguish each species from the other two. This is in the same region where unique amino acid substitutions have been identified in studies of the PrP gene in mice and humans (Fig. 6). For example, when the PrP genes from NZW and I/Ln mice were cloned and sequenced (76), the predicted amino acid sequence of the PrP protein was found to differ at only two sites between the two strains; one of these was a substitution of leucine (NZW) for phenylalanine (I/Ln) at codon 108. Furthermore, in the study of the PrP gene in two human pedigrees with GSS, only one polymorphism was identified. This substitution of leucine for proline at codon 102 proved to be linked with a LOD score of 3.26 to the disease locus (38). Taken together, these findings suggest that there is a single common region of PrP in the vicinity of codons 102 to 112 that is a critical determinant of incubation time in experimental scrapie.

The region of PrP bounded by or near codons 102 to 112 may control cerebral amyloid plaque formation in addition to modulating incubation times. PrP-immunopositive plaques have been found in the SHa (6, 21, 22), the CHa, and VM mice (65), while they are rare or absent in the AHa and most other mice. VM mice have the same amino acid substitutions as I/Ln mice (12, 39, 76) and develop numerous amyloid plaques after inoculation with the 87V isolate of the scrapie agent (11). Comparison of the predicted PrP amino acid sequences from these five rodents again demonstrates only one region in the protein, codons 102 to 109, in which amino acid substitutions might explain the presence or absence of plaques. It is interesting that this segment of the prion protein is relatively hydrophilic and is immediately adjacent to a strongly hydrophobic region that is thought to span the cell membrane and includes a stop-transfer sequence (4). Furthermore, this region has been shown to be a critical determinant of protein topology in cell-free translation systems (36; C. S. Yost, D. C. Lopez, S. B. Prusiner, R. M. Myers, and V. R. Lingappa, Nature (London, in press). Nonetheless, the possibility that other regions of the PrP protein can influence these attributes of experimental scrapie remains open. Recent genetic studies of GSS and familial Creutzfeldt-Jakob disease have revealed polymorphisms at codons 117 and 200, as well as a 45-amino-acid residue insert at codon 52, associated with the disease (20, 25, 29a, 56).

Differences in the amino acid sequences of the three hamster species are also likely to account for the singular recognition of the monoclonal antibody 13A5 for PrP protein from the SHa (2), since this same specificity is seen in deglycosylated proteins (34) and in recombinant SHa protein produced in *E. coli* (B. Oesch, unpublished data). This antibody did not react with human and mouse PrP (2; D. Serban, unpublished data). When the predicted amino acid sequences from all five organisms are compared, only a methionine at codon 138 uniquely distinguishes the SHa sequence, suggesting that the antigenic determinant for 13A5 includes this amino acid. It also appears that the monoclonal antibody 3F4 (41) recognizes an antigenic site present on the

PrP protein of SHa and AHa but not CHa. Similar comparisons of the sequence data predict that the 3F4 antigenic determinant encompasses codon 112.

The interpretation of the data at hand must be considered in light of the genetic variation that exists between the three hamster species. The CHa and AHa are classified under the genus *Cricetulus*, while the SHa is in the genus *Mesocricetus* (19), and there are distinct morphological characteristics of the chromosomes from all three species (46). Furthermore, only the CHa and AHa are inbred. These differences preclude the use of classical genetic studies to localize further the gene(s) influencing scrapie incubation time in hamsters, as has been done in mice. Based on the findings reported here and the success of using transgenic mice to analyze the role of the SHa PrP gene in scrapie, transgenic mice harboring CHa or AHa PrP genes should advance our knowledge of prion structure, scrapie incubation time control, and the molecular mechanisms of amyloid deposition.

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LITERATURE CITED

1. **Aviv, H., and P. Leder.** 1972. Purification of biologically active globin messenger RNA by chromatography on oligothymidylic acid cellulose. Proc. Natl. Acad. Sci. USA **69:**1408–1412.
2. **Barry, R. A., and S. B. Prusiner.** 1986. Monoclonal antibodies to the cellular and scrapie prion proteins. J. Infect. Dis. **154:** 518–521.
3. **Basler, K., B. Oesch, M. Scott, D. Westaway, M. Wälchli, D. F. Groth, M. P. McKinley, S. B. Prusiner, and C. Weissmann.** 1986. Scrapie and cellular PrP isoforms are encoded by the same chromosomal gene. Cell **46:**417–428.
4. **Bazan, J. F., R. J. Fletterick, M. P. McKinley, and S. B. Prusiner.** 1987. Predicted secondary structure and membrane topology of the scrapie prion protein. Protein Eng. **1:**125–135.
5. **Beck, E., and P. M. Daniel.** 1987. Neuropathology of slow transmissible encephalopathies, p. 331–385. *In* S. B. Prusiner and M. P. McKinley (ed.), Prions - novel infectious pathogens causing scrapie and Creutzfeldt-Jakob disease. Academic Press, Inc., Orlando, Fla.
6. **Bendheim, P. E., R. A. Barry, S. J. DeArmond, D. P. Stites, and S. B. Prusiner.** 1984. Antibodies to a scrapie prion protein. Nature (London) **310:**418–421.
7. **Benton, W. D., and R. W. Davis.** 1977. Screening λ-gt recombinant clones by hybridization *in situ*. Science **196:**100–102.
8. **Bolton, D. C., M. P. McKinley, and S. B. Prusiner.** 1982. Identification of a protein that purifies with the scrapie prion. Science **218:**1309–1311.
9. **Brown, P., F. Cathala, P. Castaigne, and D. C. Gajdusek.** 1986. Creutzfeldt-Jakob disease: clinical analysis of a consecutive series of 230 neuropathologically verified cases. Ann. Neurol. **20:**597–602.
10. **Bruce, M. E., and A. G. Dickinson.** 1986. Biological evidence that the scrapie agent has an independent genome. J. Gen. Virol. **68:**79–89.
11. **Bruce, M. E., and H. Fraser.** 1975. Amyloid plaques in the brains of mice infected with scrapie: morphological variation and staining properties. Neuropathol. Appl. Neurobiol. **1:**189–202.
12. **Carlson, G. A., P. A. Goodman, M. Lovett, B. A. Taylor, S. T. Marshall, M. Peterson-Torchia, D. Westaway, and S. B. Prusiner.** 1988. Genetics and polymorphism of the mouse prion gene complex: the control of scrapie incubation time. Mol. Cell. Biol. **8:**5528–5540.
13. **Carlson, G. A., D. T. Kingsbury, P. A. Goodman, S. Coleman, S. T. Marshall, S. J. DeArmond, D. Westaway, and S. B. Prusiner.** 1986. Linkage of prion protein and scrapie incubation time genes. Cell **46:**503–511.
14. **Carlson, G. A., D. Westaway, S. J. DeArmond, M. Peterson-Torchia, and S. B. Prusiner.** 1989. Primary structure of prion protein may modify scrapie isolate properties. Proc. Natl. Acad. Sci. USA **86:**7475–7479.
15. **Cathala, G., J. F. Savouret, B. Mendez, B. L. West, M. Karin, J. A. Martial, and J. D. Baxter.** 1983. A method for isolation of intact, translationally active ribonucleic acid. DNA **2:**329–335.
16. **Chandler, R. L.** 1963. Experimental scrapie in the mouse. Res. Vet. Sci. **4:**276–285.
17. **Chandler, R., and B. Turfrey.** 1972. Inoculation of voles, Chinese hamsters, gerbils and guinea-pigs with scrapie brain material. Res. Vet. Sci. **13:**219–224.
18. **Chen, E. Y., and P. H. Seeburg.** 1985. Supercoil sequencing: a fast and simple method for sequencing plasmid DNA. DNA **4:**165–170.
19. **Clark, J.** 1987. The golden or Syrian hamster, p. 3–7. *In* G. Van Hoosier and C. McPherson (ed.), Laboratory hamsters. Academic Press, Inc., Orlando, Fla.
20. **Collinge, J., A. E. Harding, F. Owen, M. Poulter, R. Lofthouse, A. M. Boughey, T. Shah, and T. J. Crow.** 1989. Diagnosis of Gerstmann-Sträussler syndrome in familial dementia with prion protein gene analysis. Lancet **ii:**15–17.
21. **DeArmond, S. J., H. A. Kretzschmar, M. P. McKinley, and S. B. Prusiner.** 1987. Molecular pathology of prion diseases, p. 387–414. *In* S. B. Prusiner and M. P. McKinley (ed.), Prions - novel infectious pathogens causing scrapie and Creutzfeldt-Jakob disease. Academic Press, Inc., Orlando, Fla.
22. **DeArmond, S. J., M. P. McKinley, R. A. Barry, M. B. Braunfeld, J. R. McColloch, and S. B. Prusiner.** 1985. Identification of prion amyloid filaments in scrapie-infected brain. Cell **41:**221–235.
23. **DeArmond, S. J., W. C. Mobley, D. L. DeMott, R. A. Barry, J. H. Beckstead, and S. B. Prusiner.** 1987. Changes in the localization of brain prion proteins during scrapie infection. Neurology **37:**1271–1280.
24. **Dickinson, A. G., V. M. Meikle, and H. Fraser.** 1968. Identification of a gene which controls the incubation period of some strains of scrapie agent in mice. J. Comp. Pathol. **78:**293–299.
25. **Doh-ura, K., J. Tateishi, H. Sasaki, T. Kitamoto, and Y. Sakaki.** 1989. Pro→Leu change at position 102 of prion protein is the most common but not the sole mutation related to Gerstmann-Sträussler syndrome. Biochem. Biophys. Res. Commun. **163:** 974–979.
26. **Dunning, A. M., T. Philippa, and S. E. Humphries.** 1988. Errors in the polymerase chain reaction. Nucleic Acids Res. **16:**10393.
27. **Feinberg, A. P., and B. Vogelstein.** 1983. A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. Anal. Biochem. **132:**6–13.
28. **Feramisco, J. R., D. M. Helfman, J. E. Smart, K. Burridge, and G. P. Thomas.** 1982. Coexistence of vinculin-like protein of higher molecular weight in smooth muscle. J. Biol. Chem. **257:**11024–11031.
29. **Gajdusek, D. C.** 1977. Unconventional viruses and the origin and disappearance of kuru. Science **197:**943–960.

29a. **Goldgaber, D., L. G. Goldfarb, P. Brown, D. M. Asher, W. T. Brown, S. Lin, J. W. Teener, S. M. Feinstone, R. Rubinstein, J. W. Boellard, and D. C. Gajdusek.** 1989. Mutations in familial Cruetzfeldt-Jakob disease and Gerstmann-Straüssler-Scheinker's syndrome. Exp. Neurol. **106:**204–206.

30. **Goldman, W., N. Hunter, G. Multhaup, J. M. Salbalm, J. D. Foster, K. T. Beyreuther, and J. Hope.** 1988. The PrP gene in natural scrapie. Alzheimer Dis. Assoc. Disorders **2:**330.
31. **Gordon, W. S.** 1966. Review of work on scrapie at Compton, England, 1952–1964, p. 19–36. *In* Report of scrapie seminar.

ARS 91-53. U.S. Department of Agriculture, Washington, D.C.
32. **Gross-Bellard, M., P. Oudet, and P. Chambon.** 1973. Isolation of high molecular weight DNA from mammalian cells. Eur. J. Biochem. **36:**32–38.
33. **Grosveld, F. G., M. H. H. Dahl, E. de Boer, and R. A. Flavell.** 1981. Isolation of β-globin-related genes from a human cosmid library. Gene **13:**227–237.
34. **Haraguchi, T., S. Fisher, S. Olofsson, T. Endo, D. Groth, A. Tarentino, D. Teplow, L. Hood, A. Burlingame, E. Lycke, A. Kobata, and S. B. Prusiner.** 1989. Asparagine-linked glycosylation of the scrapie and cellular prion proteins. Arch. Biochem. Biophys. **274:**1–13.
35. **Hattori, M., and Y. Sakaki.** 1986. Dideoxy sequencing method using denatured plasmid templates. Anal. Biochem. **152:**232–238.
36. **Hay, B., R. A. Barry, I. Lieberburg, S. B. Prusiner, and V. R. Lingappa.** 1987. Biogenesis and transmembrane orientation of the cellular isoform of the scrapie prion protein. Mol. Cell. Biol. **7:**914–920.
37. **Hohn, B., and K. Murray.** 1977. *In vitro* packaging of recombinant DNA. Proc. Natl. Acad. Sci. USA **74:**3259–3263.
38. **Hsiao, K., H. F. Baker, T. J. Crow, M. Poulter, F. Owen, J. D. Terwilliger, D. Westaway, J. Ott, and S. B. Prusiner.** 1989. Linkage of a prion protein missense variant to Gerstmann-Sträussler syndrome. Nature (London) **338:**342–345.
39. **Hunter, N., J. Hope, I. McConnell, and A. G. Dickinson.** 1987. Linkage of the scrapie-associated fibril protein (PrP) gene and *Sinc* using congenic mice and restriction fragment length polymorphism analysis. J. Gen. Virol. **68:**2711–2716.
40. **Kaiser, K., and N. E. Murray.** 1985. The use of phage lambda replacement vectors in the construction of representative genomic DNA libraries, p. 1–47. *In* D. M. Glover (ed.), DNA cloning, vol. 1. IRL Press, Ltd., Oxford.
41. **Kascsak, R. J., R. Rubenstein, P. A. Merz, M. Tonna-DeMasi, R. Fersko, R. I. Carp, H. M. Wisniewski, and H. Diringer.** 1987. Mouse polyclonal and monoclonal antibody to scrapie-associated fibril proteins. J. Virol. **61:**3688–3693.
42. **Kimberlin, R., and C. Walker.** 1977. Characteristics of a short incubation model of scrapie in the golden hamster. J. Gen. Virol. **34:**295–304.
43. **Kingsbury, D. T., K. C. Kasper, D. P. Stites, J. D. Watson, R. N. Hogan, and S. B. Prusiner.** 1983. Genetic control of scrapie and Creutzfeldt-Jakob disease in mice. J. Immunol. **131:**491–496.
44. **Kretzschmar, H. A., L. E. Stowring, D. Westaway, W. H. Stubblebine, S. B. Prusiner, and S. J. DeArmond.** 1986. Molecular cloning of a human prion protein cDNA. DNA **5:**315–324.
45. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227:**680–685.
46. **Lavappa, K. S., and G. Yerganian.** 1970. Spermatogonial and meiotic chromosomes of the Armenian hamster, *Cricetulus migratorius*. Exp. Cell Res. **61:**159–172.
47. **Liao, Y.-C., Z. Tokes, E. Lim, A. Lackey, C. H. Woo, J. D. Button, and G. A. Clawson.** 1987. Cloning of rat "prion-related protein" cDNA. Lab. Invest. **57:**370–374.
48. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
49. **Marsh, R. F., and R. H. Kimberlin.** 1975. Comparison of scrapie and transmissible mink encephalopathy in hamsters. II. Clinical signs, pathology and pathogenesis. J. Infect. Dis. **131:**104–110.
50. **Maxam, A. M., and W. Gilbert.** 1980. Sequencing end-labeled DNA with base-specific chemical cleavages. Methods Enzymol. **65:**499–560.
51. **McKinley, M. P., D. C. Bolton, and S. B. Prusiner.** 1983. A protease-resistant protein is a structural component of the scrapie prion. Cell **35:**57–62.
52. **McKinley, M. P., B. Hay, V. R. Lingappa, I. Lieberburg, and S. B. Prusiner.** 1987. Developmental expression of prion protein gene in brain. Dev. Biol. **121:**105–110.
53. **Mobley, W. C., R. L. Neve, S. B. Prusiner, and M. P. McKinley.** 1988. Nerve growth factor increases mRNA levels for the prion protein and the beta-amyloid protein precursor in developing hamster brain. Proc. Natl. Acad. Sci. USA **85:**9811–9815.
54. **Newton, C. R., N. Kalsheker, A. Graham, S. Powell, A. Gammack, J. Riley, and A. F. Markham.** 1988. Diagnosis of α_1-anti-trypsin deficiency by enzymatic amplification of human genomic DNA and direct sequencing of polymerase chain reaction products. Nucleic Acids Res. **16:**8233–8243.
55. **Oesch, B., D. Westaway, M. Wälchli, M. P. McKinley, S. B. Kent, R. Aebersold, R. A. Barry, P. Tempst, D. B. Teplow, L. E. Hood, S. B. Prusiner, and C. Weissmann.** 1985. A cellular gene encodes scrapie PrP 27-30 protein. Cell **40:**735–746.
56. **Owen, F., M. Poulter, R. Lofthouse, J. Collinge, T. J. Crow, D. Risby, H. F. Baker, R. M. Ridley, K. Hsiao, and S. B. Prusiner.** 1989. Insertion in prion protein gene in familial Creutzfeldt-Jakob disease. Lancet **i:**51–52.
57. **Pattison, I. H.** 1965. Experiments with scrapie with special reference to the nature of the agent and the pathology of the disease, p. 249–257. *In* D. C. Gajdusek, C. J. Gibbs, Jr., and M. P. Alpers (ed.), Slow, latent and temperate virus infections. NINDB monograph 2. U.S. Government Printing Office, Washington, D.C.
58. **Payne, G. S., S. A. Courtneidge, L. B. Crittenden, A. M. Fadly, J. M. Bishop, and H. E. Varmus.** 1981. Analysis of avian leukosis virus DNA and RNA in bursal tumors: viral gene expression is not required for maintenance of the tumor state. Cell **23:**311–322.
59. **Prusiner, S. B.** 1987. Prions and neurodegenerative diseases. N. Engl. J. Med. **317:**1571–1581.
60. **Prusiner, S. B., D. C. Bolton, D. F. Groth, K. A. Bowman, S. P. Cochran, and M. P. McKinley.** 1982. Further purification and characterization of scrapie prions. Biochemistry **21:**6942–6950.
61. **Prusiner, S. B., S. P. Cochran, D. F. Groth, D. E. Downey, K. A. Bowman, and H. M. Martinez.** 1982. Measurement of the scrapie agent using an incubation time interval assay. Ann. Neurol. **11:**353–358.
62. **Prusiner, S. B., D. F. Groth, D. C. Bolton, S. B. Kent, and L. E. Hood.** 1984. Purification and structural studies of a major scrapie prion protein. Cell **38:**127–134.
63. **Prusiner, S. B., D. F. Groth, S. P. Cochran, F. R. Masiarz, M. P. McKinley, and H. M. Martinez.** 1980. Molecular properties, partial purification, and assay by incubation period measurements of the hamster scrapie agent. Biochemistry **19:**4883–4891.
64. **Prusiner, S. B., M. P. McKinley, K. A. Bowman, D. C. Bolton, P. E. Bendheim, D. F. Groth, and G. G. Glenner.** 1983. Scrapie prions aggregate to form amyloid-like birefringent rods. Cell **35:**349–358.
65. **Roberts, G. W., R. Lofthouse, R. Brown, T. J. Crow, R. A. Barry, and S. B. Prusiner.** 1986. Prion-protein immunoreactivity in human transmissible dementias. N. Engl. J. Med. **315:**1231–1233.
66. **Saiki, R. K., D. H. Gelfand, S. Stoffel, S. J. Scharf, R. Higuchi, G. T. Horn, K. B. Mullis, and H. A. Erlich.** 1988. Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. Science **239:**487–491.
66a. **Scott, M., D. Foster, C. Mirenda, D. Serban, F. Coufal, M. Wälchli, M. Torchia, D. Groth, G. Carlson, S. J. DeArmond, D. Westaway, and S. B. Prusiner.** 1989. Transgenic mice expressing hamster prion protein produce species-specific scrapie infectivity and amyloid plaques. Cell **59:**847–857.
67. **Shank, P. R., S. H. Hughes, H. J. Kung, J. E. Majors, N. Quintrell, R. V. Guntaka, J. M. Bishop, and H. E. Varmus.** 1978. Mapping integrated avian sarcoma virus DNA: termini of linear DNA bear 300 nucleotides present once or twice in two species of circular DNA. Cell **15:**1383–1395.
68. **Singh, L., and K. N. Jones.** 1984. The use of heparin as a simple cost-effective means of controlling background in nucleic acid hybridization procedures. Nucleic Acids Res. **12:**5627–5638.
69. **Southern, E. M.** 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. **98:**503–517.
70. **Sparkes, R. S., M. Simon, V. H. Cohn, R. E. K. Fournier, J. Lem, I. Klisak, C. Heinzman, C. Blatt, M. Lucero, T. Mohandas, S. J. DeArmond, D. Westaway, S. B. Prusiner, and L. P. Weiner.** 1986. Assignment of the human and mouse prion protein genes

to homologous chromosomes. Proc. Natl. Acad. Sci. USA **83**:7358–7362.

71. **Taylor, J. M., R. Illmensee, and J. Summers.** 1976. Efficient transcription of RNA into DNA by avian sarcoma virus polymerase. Biochim. Biophys. Acta **442**:324–330.
72. **Thomas, P. S.** 1980. Hybridization of denatured RNA and small DNA fragments to nitrocellulose. Proc. Natl. Acad. Sci. USA **77**:5201–5205.
73. **Tindall, K. R., and T. A. Kunkel.** 1988. Fidelity of DNA synthesis by the *Thermus aquaticus* DNA polymerase. Biochemistry **27**:6008–6013.
74. **Towbin, H., T. Staehelin, and J. Gordon.** 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc. Natl. Acad. Sci. USA **76**:4350–4354.
75. **Westaway, D., G. A. Carlson, and S. B. Prusiner.** 1989. Unraveling prion diseases through molecular genetics. Trends Neurosci. **12**:221–227.
76. **Westaway, D., P. A. Goodman, C. A. Mirenda, M. P. McKinley, G. A. Carlson, and S. B. Prusiner.** 1987. Distinct prion proteins in short and long scrapie incubation period mice. Cell **51**: 651–662.
77. **Westaway, D., and S. B. Prusiner.** 1986. Conservation of the cellular gene encoding the scrapie prion protein. Nucleic Acids Res. **14**:2035–2044.
78. **Wood, W.** 1966. Host specificity of DNA produced by *Escherichia coli*: bacterial mutations affecting the restriction and modification of DNA. J. Mol. Biol. **16**:118–133.
79. **Yanisch-Perron, C., J. Vieira, and J. Messing.** 1985. Improved M13 phage cloning vectors and host strains: nucleotide sequences of the M13mp18 and pUC19 vectors. Gene **33**:103–119.
80. **Zlotnik, I., and J. C. Rennie.** 1965. Experimental transmission of mouse passaged scrapie to goats, sheep, rats and hamsters. J. Comp. Pathol. **75**:147–157.