MOLECULAR AND CELLULAR BIOLOGY, Dec. 1988, p. 5528–5540
0270-7306/88/125528-13$02.00/0

Vol. 8, No. 12

Genetics and Polymorphism of the Mouse Prion Gene Complex: Control of Scrapie Incubation Time

GEORGE A. CARLSON[1]†* PATRICIA A. GOODMAN,[2] MICHAEL LOVETT,[3] BENJAMIN A. TAYLOR,[1] SUSAN T. MARSHALL,[1] MARILYN PETERSON-TORCHIA,[2] DAVID WESTAWAY,[2] AND STANLEY B. PRUSINER[2,4]

The Jackson Laboratory, Bar Harbor, Maine 04609,[1] and Departments of Neurology,[2] Pediatrics,[3] and Biochemistry and Biophysics,[4] University of California, San Francisco, California 94143

Received 6 June 1988/Accepted 13 September 1988

The mouse prion protein (PrP) gene (*Prn-p*), which encodes the only macromolecule that has been identified in scrapie prions, is tightly linked or identical to a gene (*Prn-i*) that controls the duration of the scrapie incubation period in mice. Constellations of restriction fragment length polymorphisms distinguish haplotypes *a* to *f* of *Prn-p*. The *Prn-p*^{b} allele encodes a PrP that differs in sequence from those encoded by the other haplotypes and, in inbred mouse strains, correlates with long scrapie incubation time (Westaway et al., Cell 51: 651–662, 1987). In segregating crosses of mice, we identified rare individuals with a divergent scrapie incubation time phenotype and *Prn-p* genotype, but progeny testing to demonstrate meiotic recombination was not possible because scrapie is a lethal disease. Crosses involving the *a*, *d*, and *e* haplotypes demonstrated that genes unlinked to *Prn-p* could modulate scrapie incubation time and that there were only two alleles of *Prn-i* among the mouse strains tested. All inbred strains of mice that had the *Prn*^{b} haplotype were probably direct descendants of the I/LnJ progenitors. We established the linkage relationship between the prion gene complex (*Prn*) and other chromosome 2 genes; the gene order, proximal to distal, is *B2m–Il-1a–Prn–Itp–A*. Recombination suppression in the *B2m–Prn-p* interval occurred during the crosses involved in transferring the I/LnJ *Prn*^{b} complex into a C57BL/6J background. Transmission ratio distortion by *Prn*^{a}/*Prn*^{b} heterozygous males was also observed in the same crosses. These phenomena, together with the founder effect, would favor apparent linkage disequilibrium between *Prn-p* and *Prn-i*. Therefore, transmission genetics may underestimate the number of genes in *Prn*.

Scrapie is a neurodegenerative disease that occurs naturally in sheep and goats; it can also be transmitted to rodents. Although scrapie resembles a virus infection in its transmissibility and replication, the infectious agent possesses highly unusual properties, and no scrapie-specific nucleic acid has been detected (for reviews see references 19 and 50). These unusual properties prompted the proposal that scrapie pathogens be called "prions" (49) to distinguish them from viruses and viroids.

Biochemical fractionation of scrapie-infected hamster brains, aimed solely at enriching for infectivity, led to the identification of PrP 27-30, a scrapie isoform of prion protein (6, 51). PrP 27-30 was subsequently shown to be derived by limited proteolysis from a larger protein designated PrP^{Sc}, which is encoded by a chromosomal gene and not by a putative nucleic acid within the prion (2, 44). Although many biochemical (6, 44, 51), genetic (2, 7, 65), and pathological (4, 5, 36, 52) results argue persuasively that PrP^{Sc} is a component of the infectious scrapie particle, only recently have immunoaffinity chromatography and neutralization data been obtained that establish that this is indeed the case (22a). Dispersion of prions into detergent-lipid-protein complexes permitted purification of scrapie infectivity by a PrP monoclonal antibody affinity column as well as neutralization with polyclonal PrP 27-30 antibodies. These findings delineate an important feature which distinguishes prions from viruses: PrP^{Sc} is a necessary, and possibly the only, component of infectious scrapie prions and it is encoded by a chromosomal gene. PrP^{Sc} and its cellular isoform (PrP^C) are both encoded by the same single-copy host gene (2, 44); differences between PrP^{Sc} and PrP^C seem to arise from a posttranslational event.

Genetic analysis of natural and experimental scrapie infections in sheep established that host genes exert a profound influence on disease susceptibility and course (14, 24, 47). Following transmission of scrapie to mice (10), Dickinson and MacKay (15) identified a partially inbred strain of mice that had a prolonged scrapie incubation period. The VM and related IM mouse strains were used to demonstrate that a single autosomal gene, designated *Sinc*, profoundly influences scrapie incubation time (17). Unfortunately, distribution of VM and IM mice was highly restricted and the chromosomal location of *Sinc* was unknown.

Linkage between the prion incubation period phenotype (*Prn-i*) and the prion protein gene (*Prn-p*) was discovered (7) by analyzing the genes responsible for the exceptionally long scrapie incubation time of I/LnJ mice (35). Crosses between I/LnJ and a short-incubation-time strain (NZW/LacJ) were established, and incubation time phenotypes in (NZW/LacJ × I/LnJ)F_1 × NZW/LacJ backcross mice segregated into two groups, one with an incubation time of 130 ± 1 (SE) days and the other with one of 195 ± 2 (SE) days (7). An *Xba*I restriction fragment length polymorphism (RFLP) distinguished the NZW prion protein gene allele (*Prn-p*^{a}) from the I/LnJ allele (*Prn-p*^{b}); this polymorphism segregated with incubation time phenotype in 65 of 66 mice. We cannot be certain about a single mouse that was *Prn-p* heterozygous and had a 144-day scrapie incubation time; whether this animal was a true recombinant between *Prn-i* and *Prn-p*

* Corresponding author.
† Present address: McLaughlin Research Institute, 1625 3rd Avenue North, Great Falls, MT 59401.

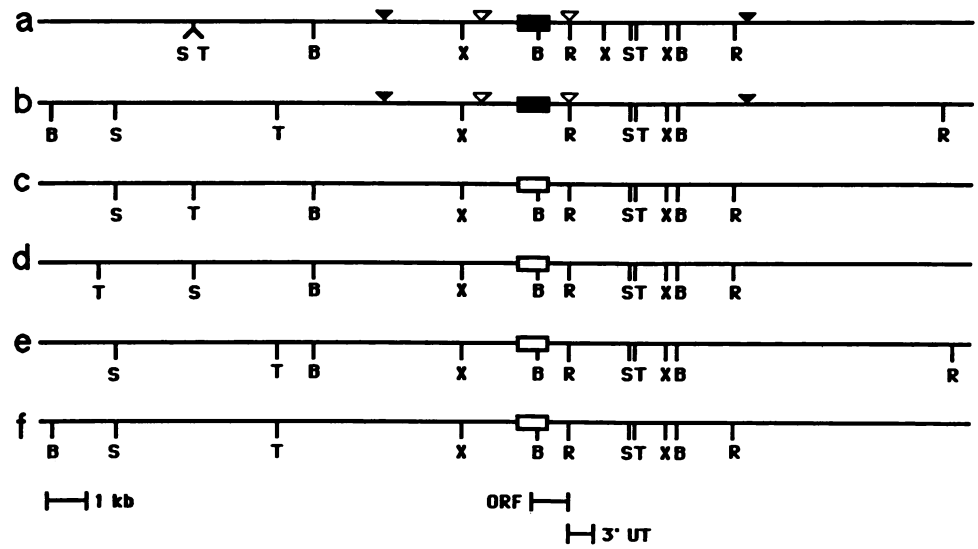


FIG. 1. Positions of polymorphic sites which define six haplotypes of the prion protein gene (*Prn-p*). The rectangles represent the second exon of *Prn-p*, which includes the entire ORF; they are shaded for the *a* and *b* haplotypes to indicate that the complete nucleotide sequences of these two alleles have been determined. *Eco*RI (open triangles) and *Bam*HI (solid triangles) are clones described previously (Westaway et al. [65]). Restriction enzyme site abbreviations: B, *Bst*EII; R, *Eco*RI; S, *Sac*I; T, *Taq*I; X, *Xba*I. The two probes used to generate the map are indicated as ORF and 3'UT (see text). Restriction site positions were assigned by single and double digests of genomic DNA and of the *Bam*HI clones; additional sites for the enzymes shown may exist within the 21-kb domain illustrated.

remains uncertain because scrapie is a lethal disease and its bioassay precludes progeny testing.

A significant problem in scrapie genetics is to distinguish between the single-locus and two-loci models for the prion gene complex. The predicted sequences of the NZW (PrP-A) and I/LnJ (PrP-B) proteins differ at codons 108 (NZW → I/Ln, Leu → Phe) and 189 (Thr → Val), compatible with the possibility that PrP itself is the regulator of scrapie incubation time (65). PrP mRNA and prion protein levels are similar in NZW and I/LnJ mice; therefore, if *Prn-p* and *Prn-i* are congruent, it is likely that one or both of the differences in primary protein sequence would be responsible for the incubation period phenotype. Had the protein sequences encoded by the a and b alleles of *Prn-p* been identical, it would have been most probable that a distinct locus controlled the duration of the scrapie incubation period.

Recent reports (32, 33) suggested that VM and IM mice have the *Prn-p*^{b} allele, as judged from the *Xba*I RFLP. We obtained a sample of IM DNA and found that these mice were indeed *Prn-p*^{b} (65). The independent occurrence of mutations at codons 108 and 189 which distinguish the b and a *Prn-p* alleles and concomitant long scrapie incubation period would favor direct control of scrapie susceptibility by *Prn-p*. Conversely, a common origin for the *Prn-p*^{b} allele would leave open the possibility that the prion incubation time gene was effectively in linkage disequilibrium in strains with the *Prn*^{b} haplotype.

MATERIALS AND METHODS

Mice. All segregating crosses of mice were produced in our research colony at the Jackson Laboratory, and the tail tip (~1 cm) was removed from each individual as a source of DNA. In crosses, the female parent is listed first. Inbred strains were either produced in the research colonies or obtained from Animal Resources at the Jackson Laboratory. Scrapie incubation time studies were performed at the University of California, San Francisco.

DNA. DNA from the tail tip was prepared as described previously (7). Genomic DNA samples from some of the inbred strains used in these studies were obtained from the DNA Resource of the Jackson Laboratory. Tissue samples from IM mice were kindly provided by Colin Masters, and high-molecular-weight DNA was prepared from brain tissue (29, 48).

Scrapie prions. The Chandler murine scrapie isolate (10) in its fourth passage in Swiss mice was kindly provided by William Hadlow. This isolate was passaged two additional times in Swiss mice, and the brains of clinically ill animals were homogenized in 0.8 M sucrose to yield a 10% (wt/vol) homogenate. The homogenates were diluted 10-fold immediately before inoculation.

Determination of scrapie incubation periods. Mice were inoculated intracerebrally with 30 μl of Chandler scrapie isolate prepared as described above. Inoculation was performed with a 27-gauge disposable hypodermic needle, which was inserted into the right parietal lobe. Criteria for diagnosis have been detailed previously (7). Beginning 10 weeks after inoculation, the mice were examined for neurological dysfunction every 3 days; incubation period is the time from inoculation to the time that signs were first observed, followed by consecutive positive diagnoses or death. Diagnosis was performed by trained personnel, and the genotypes of individual mice from segregating crosses were not known by the readers. When mice whose death was clearly imminent were identified, their brains were taken for histological confirmation of the diagnosis of scrapie.

Hybridization probes. (i) *Prn-p*. Three *Prn-p* cDNA probes were used. Both a 2-kilobase (kb) insert from a hamster PrP cDNA clone (44) and a 900-base-pair (bp) *Eco*RI fragment from subclone 13 of a mouse cDNA (11), kindly provided by Bruce Chesebro, were used as probes hybridizing to the open reading frame (ORF) of *Prn-p* (Fig. 1). The 3' untranslated (UT) portion of the gene was identified with subclone 9, provided by Dr. Chesebro, containing an 800-bp *Eco*RI

fragment (Fig. 1). These and all other DNA fragments for use as hybridization probes were labeled with [α-^{32}P]dCTP (3,000 Ci/mmol; Amersham) with random oligonucleotides (dN_6; Pharmacia) as primers (20) and the Klenow fragment of DNA polymerase I (61).

Oligonucleotide probes were synthesized on a Systec synthesizer by Leslie A. Johnston and Babette Gwynn and were purified by preparative electrophoresis followed by reverse-phase chromatography on SEP-PAK columns (Waters Associates, Boston). A probe specific for the 5'UT region of *Prn-p* cDNA (11) was produced by synthesizing two 40-mers that overlapped at their 3' ends by eight nucleotides and radiolabeling with [α-^{32}P]dCTP and [α-^{32}P]dATP (Amersham) and the Klenow fragment to fill in the complementary nucleotides as described previously (34, 65). The antisense 40-mer used to synthesize this oligonucleotide probe initiated primer extension specifically with mouse but not with hamster brain RNA, consistent with the species-specific differences in the two 5'UT regions (65). The oligonucleotide also produced weaker signals with spleen RNA than brain RNA, consistent with the known abundance of prion protein mRNA in the two tissues (44).

Allele-specific oligonucleotides complementary to the I/LnJ *Prn-p*^{b} sequences encoding phenylalanine (underlined) at codon 108 (5'-CCAACTTCAAGCATGTGGC), to the NZW *Prn-p*^{a} sequence encoding leucine at codon 108 (5'-CCAACCTCAAGCATGTGGC), and to the I/LnJ sequences encoding a valine at codon 189 (5'-CACACGGTCGTCAC CACCACC) were labeled with [γ-^{32}P]ATP (>5,000 Ci/mmol; Amersham) with T4 polynucleotide kinase (Pharmacia).

(ii) *B2m*. The 600-bp fragment from a genomic mouse *B2m* clone (46) encompassing the large exon of *B2m* was isolated following digestion with *Sac*I and *Kpn*I. The two alleles of *B2m* are distinguished by the presence of a *Bgl*I site within the ORF of the *b* allele.

(iii) *Il-1a*. A 2-kb *Bam*HI insert of plasmid pIL-1 1301, which contains a murine interleukin-1 alpha cDNA, was used (39). This plasmid was kindly provided by Peter Lomedico, Hoffmann-La Roche Inc., and purified insert was graciously supplied by Robert Evans. This probe was used to detect *Taq*I and *Msp*I RFLPs in the murine *Il-1a* gene as previously described (13) and an *Xba*I RFLP that was scored by the presence of an additional 1.0-kb fragment. Based on RFLPs detected with these three enzymes, 11 haplotypes of *Il-1a*, designated *a* through *k*, were distinguished among the mouse strains tested.

(iv) Marker for the agouti locus. The pRI subclone of genomic mouse DNA was described previously (40) as a molecular marker for different agouti locus alleles. The pRI subclone contains a 1.1-kb single-copy mouse DNA *Eco*RI fragment that hybridizes to a 5.4-kb *Hin*dIII fragment from *a/a* mice and a 2.6-kb *Hin*dIII fragment from *A/A* mice. At a 95% confidence limit, the restriction site polymorphism detected with this probe is 0 to 3.79 centimorgans (cM) away from the agouti locus itself.

Southern analysis. Following restriction endonuclease digestion according to the manufacturer's specifications, 5 μg (for cDNA or genomic probes and 5'UT oligoprobe) or 30 μg (for allele-specific oligonucleotides) of DNA per lane was electrophoresed in agarose gels. Southern transfer and covalent binding to positively charged nylon (Zetabind, AMF-CUNO) were achieved by overnight blotting with 0.4 N NaOH. The filters were briefly prehybridized and then hybridized with heat-denatured cDNA or 5'UT probe overnight in 7% sodium dodecyl sulfate (SDS)–0.5 M $NaPO_4$ buffer (pH 7.2)–1 mM EDTA at 65°C. The final wash was done in 1% SDS–40 mM phosphate buffer–1 mM EDTA at 65°C for the plasmid inserts and 50°C for the 5'UT probe.

Stringent hybridization and wash conditions for the allele-specific oligonucleotides were determined as described previously (59), and these probes failed to hybridize to sequences with a single-base-pair mismatch, as shown previously (65). In 0.25 M phosphate buffer–7% SDS, 1 mM EDTA–5× Denhardt solution (1× = 0.2 g each of Ficoll, bovine serum albumin, and polyvinyl pyrolidone per liter), the hybridization temperature was 58°C for the codon 108 oligonucleotides and 64°C for the codon 189 probes; inclusion of oligonucleotides (18 to 25-mers) specific for unrelated sequences (kindly provided by Jim Stone) at 1 μg/ml greatly reduced nonspecific background spotting. The final wash was performed at hybridization temperature in 2× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate)–1% SDS. For reprobing, filters were erased by having boiling 0.1× SSC–0.1% SDS poured over them.

ITPase isozymes. Differential migration in an electric field was used to distinguish the A and B isozymes of ITPase as described previously (60).

Statistical analysis. Scrapie incubation time data were analyzed by standard statistical tests for normalcy of distribution and by analysis of variance and Student's *t* test for significance of distribution between genotypes with the Statworks statistical package for the Macintosh (Data Metrics, Inc).

Recombination frequencies and standard errors for backcross analysis were determined as described in Green et al. (28), and recombination frequencies in F_2 crosses were determined with a program devised by Earl Green (27) to perform maximum-likelihood analysis with Fisher's tables. The χ^2 and exact tests to determine the statistical significance of recombination suppression and segregation distortion were used as described by Bailey (1).

RESULTS

Polymorphism of mouse prion protein genes. Restriction site polymorphism mapping of the mouse *Prn-p* gene was performed to identify markers for linkage analysis. In a previous study, we classified mice as having either the *a* (3.6-kb fragment) or *b* (5.2-kb fragment) allele of *Prn-p* with a single restriction enzyme, *Xba*I (7). In addition to the I/LnJ mouse strain, P/J, RIIIS/J, and MA/MyJ mice had the 5.2-kb *Xba*I fragment, but their scrapie incubation times were unknown. Concurrent with determining scrapie susceptibility in "*Prn-p*^{b}" mice, a more detailed analysis of *Prn-p* polymorphisms and a more extensive strain survey were performed.

Fifty-five inbred mouse strains were typed for *Prn-p* RFLPs detected with *Bam*HI, *Bst*EII, *Eco*RI, *Sac*I, *Taq*I, and *Xba*I. As shown in Table 1, six constellations of RFLPs were detected, defining haplotypes of the prion protein gene. Mice with the *b*, *c*, *d*, *e*, and *f* haplotypes had the 5.2-kb *Xba*I *Prn-p* fragment initially used to define the *b* haplotype. The positions of polymorphic restriction sites for the six haplotypes are illustrated in Fig. 1. These data also showed that there were no gross structural differences among the haplotypes of *Prn-p*.

Although the overall structure of the different alleles was similar, polymorphic differences flanking the ORF were seen with several restriction enzymes. Physical mapping positioned the first exon containing most of the 5'UT sequence as lying between 11.5 and 23.5 kb upstream of the exon 2

TABLE 1. Restriction fragments that distinguish haplotypes of the prion protein gene

Haplotype	Fragment length (kbp)					
	ORF and 3'UT probes					*Bam*HI (5'UT probe)
	*Bst*EII	*Xba*I[a]	*Taq*I	*Sac*I	*Eco*RI	
a	3.5, 5.7	3.6	11.0	10.9	4.2	10.9
b	15.7	5.2	9.1	12.9	9.4	12.2
c	3.5, 5.7	5.2	11.0	12.9	4.2	12.2
d	3.5, 5.7	5.2	13.5	10.9	4.2	10.9
e	3.5, 5.7	5.2	9.1	12.9	9.7	10.9
f	3.5, 12.2	5.2	9.1	12.9	4.2	12.2

[a] Initially described as a polymorphism of 5.5 or 3.8 kb; more accurate sizing was established by analysis of the *Bam*HI molecular clones.

splice acceptor site (C. Mirenda and D. Westaway, unpublished results). A *Bam*HI restriction site polymorphism detected by using the oligonucleotide probe for the 5'UT region distinguished the *a*, *d*, and *e* haplotypes of *Prn-p* (10.9-kb fragment) from the *b*, *c*, and *f* alleles (12.2 kb). All but one of the other polymorphisms appeared to be randomly distributed around exon 2 with no evidence of clustering. *Bst*EII digestion revealed two polymorphic sites, one within the large intron and the other within the ORF itself. This is the only restriction enzyme polymorphism identified within the ORF to date and corresponds to the Thr to Val transition at codon 189 (65).

***Prn-p* polymorphisms and scrapie incubation time.** The strain distribution pattern for the six haplotypes is shown in Table 2. Most (45 of 55) of the mouse strains had the *a* haplotype; 6 of the remaining 10 had the *b* haplotype; and each of the other haplotypes was represented by a single mouse strain. Note that RIIIS/J (*Prn-p*^{c}) and MA/MyJ (*Prn-p*^{d}) mice were previously classified as *Prn-p*^{b} on the basis of the *Xba*I polymorphism (7). Scrapie incubation periods following inoculation with the Chandler or ME7 scrapie isolate are known for 19 of these inbred strains.

The prolonged interval between inoculation of scrapie prions and onset of illness is a cardinal feature of scrapie, and this interval is called the incubation period. The classic signs used for diagnosis of murine scrapie include generalized tremor, ataxia, difficulty coming upright from a supine position, and tail rigidity. We should emphasize, however, that these distinctive signs of neurological dysfunction do not always precede death from scrapie. I/LnJ mice are particularly difficult to diagnose; clinical signs in these mice are often limited to a dull, glazed expression, lack of usual motor activity, and a "hunched-up," stilted gait. The interval between inoculation and death was also recorded, and data for mice that died without obvious signs of illness are included.

The most striking aspect of the data summarized in Table 2 is that all three inbred strains of mice with incubation times in excess of 200 days were *Prn-p*^{b}. Mice with the *Prn-p*^{a} haplotype had shown incubation times (ranging from 105 to 158 days), as did RIIIS/J mice, which have the *c* prion protein allele. MA/MyJ (*Prn-p*^{d}) mice had an incubation period of 170 ± 0 (standard error [SE], $n = 6$) days. CAST/Ei and MOLF/Ei are inbred strains derived from *Mus castaneus* and *M. molossinus*, and they had incubation times of 172 ± 6 ($n = 5$) and 164 ± 1 ($n = 7$) days, respectively.

Two previously untyped mouse strains, IS/Cam and JE/Le, were identified as having the *b* haplotype of *Prn-p*. As can be seen in Table 1, the absence of the *Bst*EII site within the ORF was unique to the *Prn-p*^{b} haplotype. The I/LnJ DNA sequence revealed that the *Bst*EII site was absent because two nucleotides at codon 189 were different, causing substitution of a valine for the threonine at this position in NZW/LacJ mice. The only other sequence difference in the ORF between NZW and I/LnJ mice was at codon 108, where the NZW gene encoded a leucine and the I/LnJ gene encoded a phenylalanine. Using synthetic oligonucleotide probes encompassing codons 108 and 189 and hybridization conditions to detect single-base-pair mismatches, we found that the long-incubation-time strains IM and P/J, as well as BDP/J, mice (incubation time not yet known) had the same DNA sequences around codons 108 and 189 as I/LnJ mice (65). As shown in Fig. 2, genomic DNAs from JE/Le and IS/Cam mice also hybridized specifically to oligoprobes for the I/LnJ sequences in these regions. The 19-bp sequences including codon 108 in the *c*, *d*, *e*, and *f* *Prn-p* haplotypes were identical to those of NZW/LacJ. The scrapie incubation times of BDP/J, JE/Le, and IS/Cam mice will be of interest.

Evidence for only two alleles of *Prn-i*. To determine whether the strains with the *d* and *e* haplotypes carried a *Prn-p*-linked prion incubation time allele that was distinct from that in *Prn-p*^{a} strains, incubation and death times in *Prn-p*-typed backcross animals were determined. As shown

TABLE 2. Scrapie incubation time relative to distribution of *Prn-p* haplotypes

Haplotype	Mouse strain(s)		Incubation time,[a] days (range or mean ± SE)
	Unknown scrapie incubation time	Known scrapie incubation time	
Prn-p^a	A/J, ABP/Le, AEJ/GnLe, AU/SsJ, BNT/Le, BUB/BnJ, CE/J, C57BL/10Sn, C57L/J, C58/J, DA/HuSn, DBA/1J, DW/J, FL/1Re, Fs/Ei, HP/ EiTy, HRS/J, LP/J, LT/SvEi, PERA/Ei, PL/J, RSV/Le, SEA/GnJ, SEC/1ReJ, SF/CamEi, SK/CamEi, ST/bJ, V/Le, WB/ReJ, WC/ReJ, YBR/Ei, 129/J	AKR/J, BALB/cJ, CBA/J, C3H/HeB/FeJ, C57BL/6J, DBA/2J, LG/J, NZB/BINJ, NZW/LacJ, SJL/J, SM/J, SWR/J	105–158
Prn-p^b	BDP/J, JE/Le, IS/CamEi	I/LnJ	255 ± 14
		P/J	295 ± 9*
		IM (VM)	300 ± 3
Prn-p^c		RIIIS/J	136 ± 3
Prn-p^d		MA/MyJ	170 ± 0
Prn-p^e		CAST/Ei	172 ± 6
Prn-p^f		MOLF/Ei	164 ± 1*

[a] Incubation times are the period from inoculation of scrapie prions to diagnosis of neurological dysfunction. The scrapie incubation times for individual strains are listed in Dickinson and MacKay (15), Kingsbury et al. (35), or Westaway et al. (65) or for the first time in this paper (*).

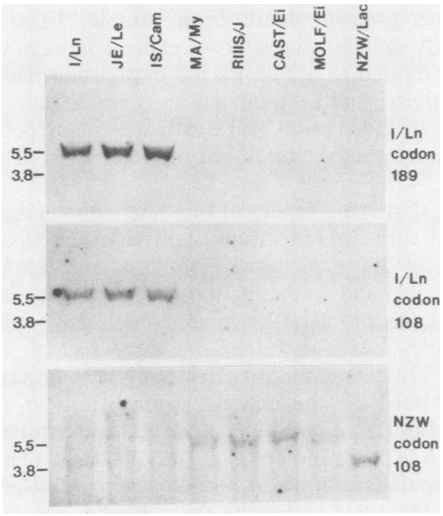


FIG. 2. Codons 108 and 189 in IS/Cam and JE/Le mice are identical to those of other *Prn-p*^{b} mice. Southern blot of electrophoresed, *Xba*I-digested DNA from (left to right) I/LnJ, JE/Le, IS/Cam, MA/MyJ, RIIIS/J, CAST/Ei, MOLF/Ei, and NZW/LacJ mice. Lines marked 5.5 and 3.8 indicate the 5.2-kb (*b*, *c*, *d*, *e*, and *f*) and 3.6-kb (*a*) *Prn-p* fragments, respectively. The top panel shows an autoradiogram of the filter hybridized to the 21-bp oligonucleotide specific for I/LnJ codon 189. The annealed radioactive probe was removed from the filter by heat denaturation and dilution (erased) and reprobed with the 19-bp probe specific for I/LnJ codon 108 (middle panel), and then erased and reprobed with the 19-bp probe specific for NZW codon 108 (bottom panel).

in Table 3, this was of particular interest because incubation periods and survival times in certain F_1 hybrids exceeded those of both parents and approached or exceeded 200 days. For example, *Prn-p*^{a}/*Prn-p*^{d} (B6 × MA/MyJ)F_1 mice had an incubation time of 204 ± 5 days (SE, n = 4) and *Prn-p*^{a}/*Prn-p*^{e} (NZW × CAST)F_1 mice had one of 182 ± 4 days (n = 13).

Figure 3 shows incubation times and death for three different backcrosses. In B6 × (B6 × I/LnJ)F_1 backcross mice, long incubation time cosegregated with *Prn-p*^{b}. In contrast, no linkage of incubation times and the *d* haplotype was observed, with a random distribution of the *a* and *d* alleles in mice with different incubation periods. This indicates that genes unlinked to *Prn-p* are responsible for the differences in incubation times in this cross and that MA/MyJ mice do not differ phenotypically from *Prn*^{a} strains at *Prn-i*. Some clustering of *Prn-p*^{e}/*Prn-p*^{a} mice towards longer incubation times (but not death) was evident in the CAST/Ei backcross mice, but two of the mice with the shortest incubation periods were also *Prn-p* heterozygous. It is unlikely that a gene encoding novel scrapie incubation time phenotype is linked to *Prn-p*^{e}.

TABLE 3. Scrapie incubation times of inbred and F_1 hybrid mice with different *Prn* haplotypes

Mouse strain	*Prn* haplotype	No. of mice	Mean time (days) ± SE to:	
			Onset of illness	Death
NZW/LacJ	*a*	20	113 ± 2	120 ± 2
B6	*a*	21	128 ± 2	143 ± 4
I/LnJ	*b*	11	314 ± 14	322 ± 15
RIIIS/J	*c*	4	135 ± 5	141 ± 3
MA/MyJ	*d*	6	170 ± 0	190 ± 5
CAST/Ei	*e*	5	172 ± 6	216[a]
(NZW × I/Ln)F_1	*a/b*	24	223 ± 3	234 ± 2
(B6 × RIIIS/J)F_1	*a/c*	5	157 ± 2	162 ± 2
(B6 × MA/MyJ)F_1	*a/d*	4	204 ± 5	215 ± 3
(NZW × CAST)F_1	*a/e*	13	182 ± 4	203 ± 6

[a] Only one mouse died of scrapie; the other four were sacrificed at 175 days.

Mapping the prion gene complex. Previous work with somatic cell hybrids localized the mouse *Prn-p* gene to chromosome 2 and the homologous human gene, PRNP, to chromosome 20p (56). Linkage with the agouti (*A*) locus was established by using the same (NZW × I/LnJ)F_1 × NZW/LacJ backcross mice that were used to establish the linkage between *Prn-p* and the prion incubation time gene *Prn-i* (7). The agouti locus controls the relative amounts and distribution of yellow and black pigment in the hairs of the coat; the recessive nonagouti allele (*a*) causes a lack of the yellow banding in the hairs that typifies the wild-type agouti allele. NZW mice are *A/A* and I/Ln mice are *a/a*, so offspring of the backcross to NZW could not be typed by coat color phenotype. Instead, we used a molecular marker that is very closely linked to the agouti locus (40). DNAs from 48 (NZW × I/Ln)F_1 × NZW *Prn-p*-typed backcross mice were analyzed for this agouti-linked marker, and 7 recombinants were found, indicating a map distance of 14.6 ± 5.1 (SE) cM between the two markers. The results from this backcross are represented in Fig. 4, map IV. Included in this population was the single *Prn-p*^{a}/*Prn-p*^{b} heterozygous mouse that had a short scrapie incubation time (144 days) (7); this animal was also heterozygous for the agouti-linked marker. These data did not indicate whether *Prn-p* was proximal or distal to agouti.

That *Prn-p* was located proximal to the agouti-linked marker was demonstrated in a larger population (102 animals) of (NZW × I/LnJ)F_2 mice. The interleukin-1 alpha locus (*Il-1a*) is proximal from agouti, and its position relative to other chromosome 2 markers, deduced by recombinant inbred strain analysis, is illustrated in Fig. 4, map I (13). RFLPs distinguish haplotypes of *Il-1a* (13; unpublished results); I/Ln has the *d* haplotype and NZW has the *i* haplotype. It is of interest that five of the *Prn-p*^{b} strains that were typed had the *Il-1a*^{d} haplotype. The results from the (NZW × I/LnJ)F_2 cross are summarized in Table 4, and the gene order that required postulating the minimum number of double recombinants was *Il-1a*–4.0 ± 1.5 cM–*Prn-p*–10.9 ± 2.3 cM–*A*, with map distances determined by maximum-likelihood analysis (Fig. 5). The linkage map derived from this F_2 cross is illustrated in Fig. 4, map V.

The position of *Prn-p* relative to the agouti and the beta-2-microglobulin loci (*B2m*) was established by using (B6 × MA/MyJ)F_1 × B6 backcross mice. The genotype of B6 is *B2m*^{b}*Prn-p*^{a}*a* and that of MA/MyJ is *B2m*^{a}*Prn-p*^{d}*A*. The *B2m* and *Prn-p* genotypes of the backcross mice were determined by Southern analysis (7, 46), and the agouti genotype was assessed by the coat color phenotype. Five recombinants between *B2m* and *Prn-p* and 13 recombinants between *Prn-p* and *A* were found among 80 mice in this cross. Therefore, *Prn-p* was 6.25 ± 2.7 (SE) cM distal from *B2m* and 16.25 ± 4.1 cM proximal from agouti (Fig. 4, map III). By inference, these results placed the prion gene complex in the same region of chromosome 2 as the ITPase gene (*Itp*).

The location of *Itp* relative to *Prn-p* and *A* was determined in another cross. F_1 hybrids of CAST/Ei (*Prn-p*^{e}*Itp*^{a}*A*) with a linkage-testing strain (*Prn-p*^{a}*Itp*^{b}*a*) were crossed to another strain that was *Prn-p*^{a}*Itp*^{b}*a*. Fifty-four offspring were typed for the three markers. Differential electrophoretic migration

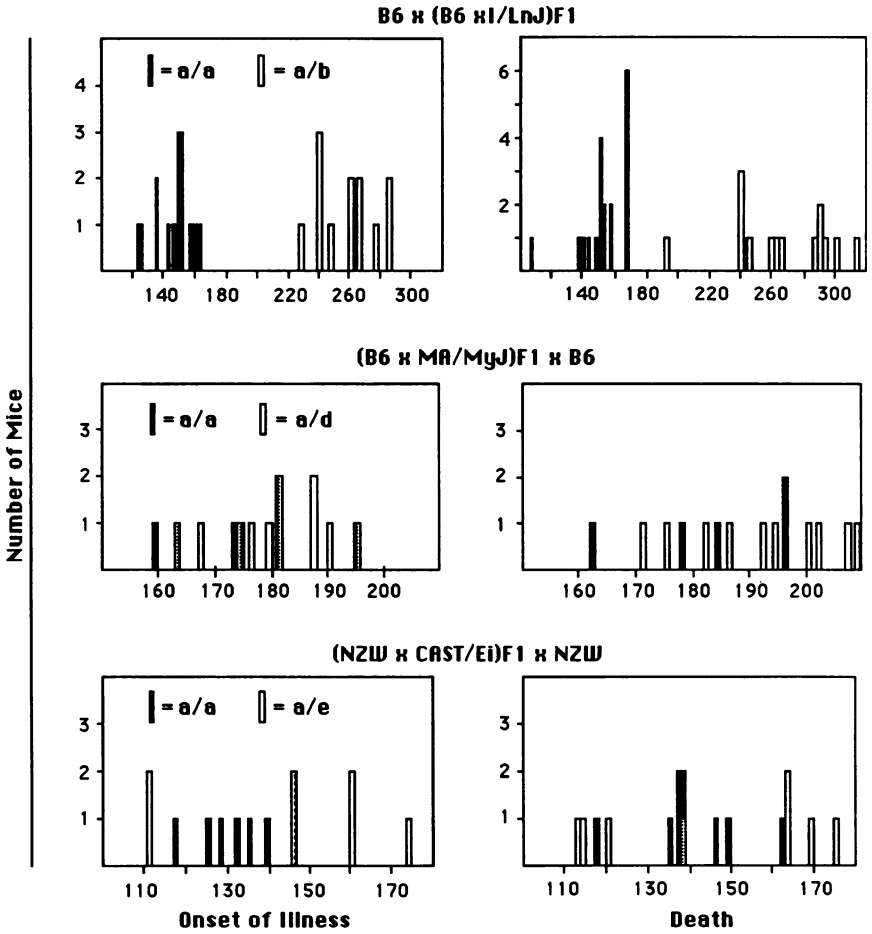


FIG. 3. Non-*Prn* genes can modulate prion incubation time. All mice were inoculated with the Chandler isolate of scrapie prions, and time to onset of illness and death (in days) was recorded. The crosses are indicated in the figure, as are the prion protein genotypes for each individual (open bars, *Prn-p* heterozygous; solid bars, *Prn-p*^{a} homozygous). Note that the scale differs for each cross.

of ITPase was used to score *Itp* genotype (60); the agouti genotype was determined by coat color, and the *Xba*I RFLP was used to distinguish the *a* and *e* haplotypes of *Prn-p*. The map distances obtained were: between *Prn-p* and *Itp*, 1.85 ± 1.83 (SE) cM (1 of 54 mice); between *Itp* and *A*, 9.2 ± 3.9 cM (5 of 54); and between *Prn-p* and *A*, 1.11 ± 4.3 cM (6 of 54). The linkage map derived from this cross is illustrated in Fig. 4, map VI. The human ITP and PRNP genes both map to chromosome 20p, indicating that the chromosome portions containing these two genes may be homologous in mouse and human.

Recombination suppression and segregation distortion in a cross involving the I/LnJ *Prn*^{b} haplotype. The construction of strains congenic for the *Prn* chromosomal region is in progress. The isolation of *Prn*^{b} in the B6 genetic background began by crossing B6 and I/LnJ and backcrossing the F_1 hybrids to B6. The offspring were typed for *Prn-p* and *Prn-p*^{a}/*Prn-p*^{b} heterozygous individuals were again backcrossed to B6. Typing and backcrossing to B6 will repeat for a minimum of 10 generations, when heterozygotes were intercrossed, selected for donor type homozygosity, and maintained as an inbred line. This will result in a B6.1-*Prn*^{b} congenic strain that differs from B6 only in chromosomal regions adjacent to the selected gene (55). At this writing, the eighth backcross to B6 is in progress.

During the production of this line, the mice were typed for *B2m* to determine the recombination frequency between this locus and *Prn-p*. B6 is *B2m*^{b}, and I/Ln is *B2m*^{a}. Four recombinants out of a total of 280 mice were found, giving a separation between *B2m* and *Prn-p* of only 1.4 ± 0.7 (SE) cM (Table 5) compared with 6.25 ± 2.7 cM in the (B6 × MA/MyJ)F_1 × B6 backcross. With an exact test used to allow for the small numbers of recombinants, the probability that this difference in map distance was due to chance was less than 10^{-5}. DNAs from 141 incipient congenic mice, including the 4 *B2m–Prn-p* recombinants, were also typed for *II-1a* (B6 is *II-1a*^{b}). Three of the four *B2m–Prn-p* recombinants were crossovers between *B2m* and *II-1a*, and the other was a crossover between *II-1a* and *Prn-p*; no additional *II-1a–Prn-p* recombinants were found in the incipient congenic mice. Comparative maps in Fig. 4 illustrate the linkage data from this cross and four other crosses.

A second striking feature of the data in Table 5 is the distortion of the normal 50:50 *Prn-p*^{a}–*Prn-p*^{b} ratio when the male was the heterozygous parent. Of a total of 162 offspring of *Prn-p*-heterozygous males backcrossed to B6, 106 were *Prn-p*^{a} homozygous and 56 were *Prn-p*^{a}/*Prn-p*^{b} heterozygous. This differed significantly ($P < 0.001$) from the expected distribution. This distorted segregation ratio, first observed in the first backcross, persisted in the seventh backcross. In contrast, heterozygous females transmitted the *a* and *b* alleles in the expected ratio (58 *Prn-p*^{a}-homozygous offspring and 59 heterozygous offspring). We do not know whether the segregation distortion reflects de-

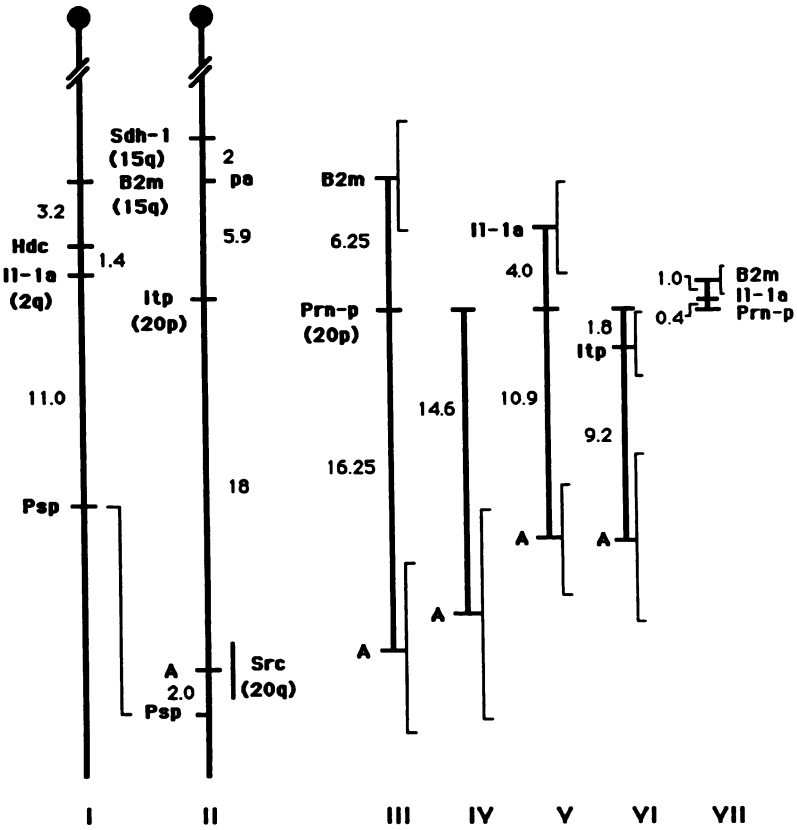


FIG. 4. Chromosome 2 linkage maps for *Prn-p* defined by different crosses. Maps I and II are from published results of others. Map I represents the location of *Il-1a* in relation to *B2m*, *Hdc*, and *Psp* deduced from analysis of recombinant inbred strains (D'Eustachio et al. [13]). Map II shows the results of a (PL/J × B6-*pa*)F_1 × B6-*pa* backcross (n = 85) that localized *Itp* in relation to *pa* and *A* (Taylor et al. [60]). Maps III, IV, V, VI, and VII position *Prn-p* relative to *B2m*, *Il-1a*, *Itp*, and *A*, respectively, and were derived from data presented in this paper. The SEs of the map distances in centimorgans (indicated between two markers) are shown by the thin brackets. The crosses were as follows: III, (B6 × MA/MyJ)F_1 × B6 (see text); IV, (NZW × I/LnJ)F_1 × NZW/LacJ; V, (NZW × I/LnJ)F_2 (Table 4); VI, (*Prn-p*^{a}*Itp*^{b}*a*/*Prn-p*^{c}*Itp*^{a}*A*)F_1 × *Prn-p*^{a}*Itp*^{b}*a* mice (see text); VII, backcrosses involved in transferring the I/LnJ *Prn-p*^{b} allele into a B6 background (Table 5). Gene symbols: *Hdc*, histidine decarboxylase; *Il-1a*, interleukin-1 alpha; *Psp*, parotid secretory protein; *Sdh*-1, sorbitol dehydrogenase-1; *Itp*, ITPase; *A*, agouti; *Src*, proto-oncogene *src*; *B2m*, beta-2-microglobulin; *Prn-p*, prion protein. The chromosomal assignment for the homologous human gene (if known) is indicated in parentheses below the murine gene symbol.

creased viability of *Prn-p*^{a}/*Prn-p*^{b} heterozygous embryos or *Prn-p*^{b} sperm are at a disadvantage compared with *Prn-p*^{a}-bearing sperm. The mean litter size of B6 mice impregnated by heterozygous males was 7.8 ± 2.9 pups (50 litters), compared with 6.6 ± 3.6 (47 litters) pups born to heterozygous females. It may be noteworthy that I/LnJ females are exceptionally poor breeders, and beginning with the fifth backcross, *Prn-p*^{a}/*Prn-p*^{b} heterozygous female breeders showed poor productivity, with several litters of mice born dead or stunted. For this reason, subsequent backcross generations included both heterozygous males and females; it would be premature, however, to conclude that *Prn-p* or linked genes influence female reproductive performance. Similarly, it should be emphasized that *Prn-p* itself is not

TABLE 4. Genotypes for the prion protein gene (*Prn-p*), an agouti (*A*)-linked marker, and *Il-1a* in (NZW/LacJ × I/LnJ)F_2 mice[a]

Prn-p genotype	No. of mice					
	Agouti genotype			*Il-1a* genotype		
	A/A	*A/a*	*a/a*	*i/i*	*i/d*	*d/d*
a/a	18	7	0	25	0	0
a/b	4	45	4	3	46	3
b/b	1	4	19	0	2	23

[a] NZW/LacJ is *IL-1a*^{i} *Prn-p*^{a} *A*, and I/LnJ is *Il-1*^{d} *Prn-p*^{b}*a*.

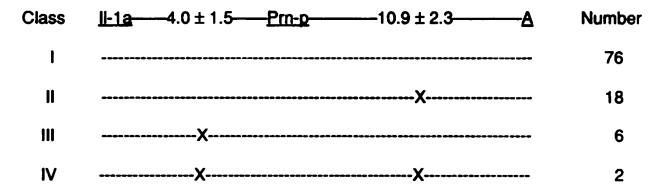


FIG. 5. Offspring in classes with indicated gene order deduced from data in Table 4. Because recombination may occur in either parent, the classes represent phenotypes. Class I, nonrecombinant phenotype—may include recombinant chromosomes from both parents; class II, single *Prn-p*–*A* recombinant phenotype; class III, single *Il-1a*–*Prn-p* recombinant phenotype; class IV, double recombinant phenotype—includes single chromosome with two crossovers or two chromosomes with single crossovers. Map distances (in centimorgans) were calculated by maximum-likelihood analysis as described by Green et al. (28). The gene order given required postulating the least number of phenotypic double recombinants (class IV). The number of mice in each class is shown.

TABLE 5. Recombination suppression and segregation distortion during construction of B6.I *Prn-p*^{b} congenic strain by backcrossing *Prn-p*^{a} *B2m*^{b}/*Prn-p*^{b} *B2m*^{a} mice to B6 (*Prn-p*^{a} *B2m*^{b})[a]

Heterozygous parent	Genotype of gamete		Sex of progeny	No. of progeny derived from each gamete genotype in backcross							Total no. of progeny
	Prn-p	*B2m*		1	2	3	4	5	5	7	
Female	*a*	*b*	F	—[b]	7	9	—	6	2	6	30
	b	*a*	F	—	7	4	—	6	3	7	27
	a	*a*	F	—	0	0	—	0	0	0	0
	b	*b*	F	—	0	0	—	1	0	0	1
	a	*b*	M	—	6	7	—	7	1	7	28
	b	*a*	M	—	4	12	—	5	5	5	31
	a	*a*	M	—	0	0	—	0	0	0	0
	b	*b*	M	—	0	0	—	0	0	1	1
Male	*a*	*b*	F	19	—	—	15	—	1	16	51
	b	*a*	F	11	—	—	9	—	4	7	31
	a	*a*	F	0	—	—	0	—	0	0	0
	b	*b*	F	0	—	—	0	—	0	0	0
	a	*b*	M	26	—	—	12	—	3	13	54
	b	*a*	M	11	—	—	8	—	2	3	24
	a	*a*	M	0	—	—	1	—	0	0	1
	b	*b*	M	0	—	—	1	—	0	0	1

[a] Recombinant fraction was 4 of 280 (1.4 ± 0.7 cM) versus recombinant fraction of 5 of 80 (6.25 ± 2.7 cM) in (B6 × MA/MyJ)F_1 × B6 backcross (Table 4).
[b] —, No matings established.

necessarily responsible for either the recombination suppression or the segregation distortion.

Prion incubation times in an F_2 cross. (NZW/LacJ × I/LnJ)F_2 mice were inoculated with the Chandler isolate of scrapie prions to determine whether the long-incubation-time allele *Prn-i*^{l} behaved as a fully dominant gene or as a codominant gene in prolonging scrapie incubation period. These mice were also typed for the agouti-linked marker and for *II-1a* and their data were included in the linkage data shown in Table 4 and Fig. 4, map V. Incubation periods for 62 and death times for 90 F_2 mice are illustrated in Fig. 6. The discrepancy between the number of mice positive for scrapie signs and the number that died emphasizes the difficulties of scrapie diagnosis in some mice. Even including the mice whose incubation times fell outside the normal distribution, the mean incubation times grouped according to *Prn-p* genotype differed significantly ($P < 0.001$); the mean (±SE) incubation time for *Prn-p*^{a} homozygotes was 141.7 ± 10.7 days ($n = 17$), that for *a/b* heterozygotes was 210.5 ± 4.3 days ($n = 33$), and that for *b* homozygous mice was 274.75 ± 14.1 days ($n = 12$). It is interesting that the span of incubation times seen in *Prn-p*^{b} homozygous F_2 mice was large, ranging from 193 to 359 days. A similar broad range of incubation times was also seen in inbred I/LnJ mice that were inoculated with the Chandler scrapie isolate (7). This effect may be particular to the scrapie isolate used in our studies; Carp and his colleagues (8; personal communication) found little variation in incubation times in I/LnJ mice inoculated with other scrapie isolates.

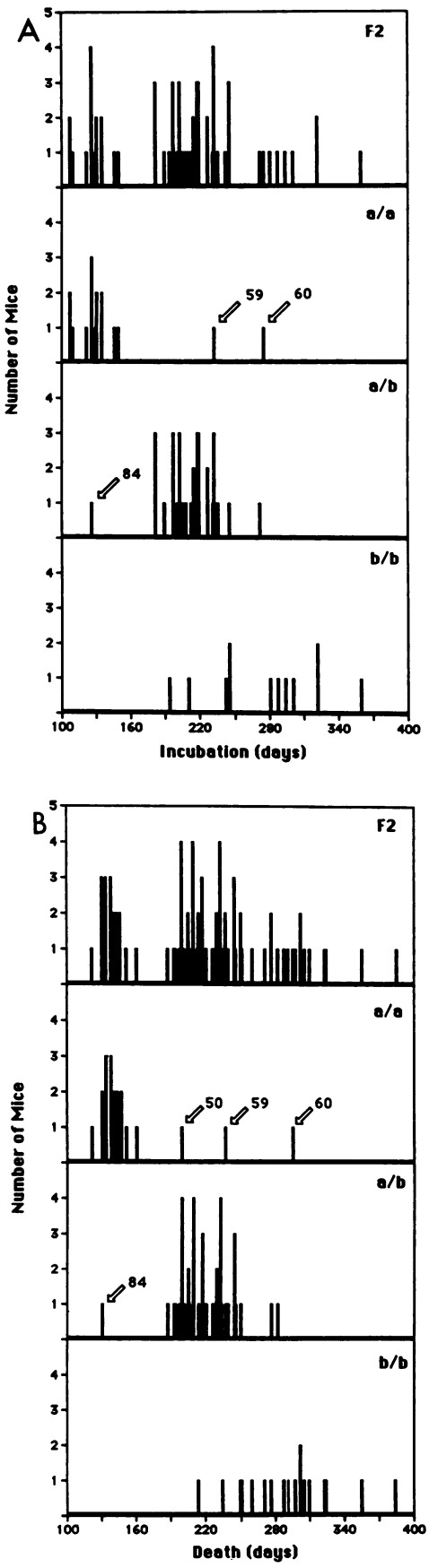
Three of the 62 F_2 mice diagnosed had incubation times that were discordant with the *Prn-p* genotype; an additional discordant mouse was indicated in the death time data (4 of 90). This could indicate that recombination had occurred between the prion incubation time gene and prion protein gene. Two *Prn-p*^{a} homozygous mice (no. 59 and 60) had scrapie incubation times of 232 (died at 236 days) and 275 (died at 295 days) days, both values lying significantly outside the normal range for *Prn-p*^{a}. These two mice were recombinants between *Prn-p* and the agouti-linked marker. A single *Prn-p* heterozygous animal (no. 84, which was also heterozygous for the agouti-linked marker) had an incubation time of 139 days, dying 2 days later. An undiagnosed *Prn-p*^{a} homozygote (no. 50) that was also *A* homozygous died 198 days after inoculation. None of the deviant mice were recombinants between *II-1a* and *Prn-p*, suggesting that if *Prn-i* is a distinct locus, it lies distal from *Prn-p*. The possibility that recombination occurred between the 3′-flanking *Xba*I polymorphic site used as the marker for *Prn-p* typing and the *Prn-p* ORF was eliminated by retyping the DNA from the putative recombinants with *Bst*EII, which distinguishes the codon 189 polymorphism.

By maximum-likelihood analysis of the F_2 mice, *Prn-i* and *Prn-p* could be 4.8 ± 1.3 (SE) cM apart. Unfortunately, the nature of the scrapie incubation time assay precluded progeny testing. Mechanisms other than recombination could account for the discordance between scrapie incubation time phenotype and *Prn-p* genotype.

DISCUSSION

The mechanism by which the prion gene complex exerts such a powerful influence on scrapie incubation period is unknown. Central to understanding the process is determining whether control of scrapie incubation time is a pleiotropic effect of *Prn-p* or whether a distinct locus is responsible. Although the *Prn-p*^{a} and *Prn-p*^{b} alleles encode distinct proteins and this finding argues for the congruency of *Prn-i* and *Prn-p* (65), the data from an F_2 cross presented here emphasize that *Prn-i* and *Prn-p* could be separate genes. We have tried to avoid nomenclature and terminology that prejudge the configuration of *Prn*.

The *Prn*^{b} haplotype. Using a battery of restriction enzymes, we defined six haplotypes for the prion protein gene. All but one of the polymorphic restriction sites were found in sequences flanking the ORF of *Prn-p*, and none of these flanking polymorphisms individually correlated with scrapie incubation period. The overall organization of *Prn-p* appeared to be similar in all mice, but the *Prn*^{b} haplotype was unique in two respects. First, the predicted PrP-B protein had a phenylalanine at position 108 and a valine at position 189, while the PrP proteins encoded by the other five haplotypes should have a leucine and a threonine at these



positions. Second, the only strains of mice (I/LnJ, P/J, and IM/Dk and VM/Dk sublines) that are known to have exceptionally long scrapie incubation times following inoculation with the Chandler or ME7 scrapie isolate are *Prn-p*^{b} as assessed both by shared flanking restriction site polymorphisms and by shared coding sequences in the *Prn-p* ORF (Table 2, Fig. 1). This is certainly compatible with the single-gene hypothesis for the prion gene complex, but the sharing of flanking polymorphisms might also indicate a common origin for all mice with the *Prn*^{b} haplotype, suggesting that large stretches of DNA around *Prn-p* could also be shared by these strains. That the extent of the region of chromosome 2 that is shared by strains with the *Prn*^{b} haplotype might be quite large is suggested by the finding that five of the six *Prn-p*^{b} strains had the *d* haplotype of *II-1a*. IM mice shared the *Taq*I and *Xba*I RFLPs with *II-1a*^{d} mice, but had a different-sized *Msp*I fragment; a limited amount of DNA from this strain prevented us from definitively assigning a haplotype to IM. Of the 34 inbred strains that have been typed for *II-1a*, only one other, DBA/2J, had the *d* haplotype.

Origin of *Prn*^{b} mice. Based on the similar responses of I/LnJ, IM, and (I/LnJ × IM)F_1 mice to a variety of scrapie isolates, Carp and his colleagues (8) suggested that the long incubation time of I/LnJ mice was due to an independent mutation to *Sinc*^{p7} because they knew of no relationship between the *Sinc*^{p7} VM and IM mouse strains, which were derived in Edinburgh, and I/LnJ. If this were the case, it would provide strong evidence for direct control of scrapie incubation time by *Prn-p*; however, an independent origin for the Edinburgh strains is not likely. A postulated lineage showing the relatedness of *Prn-p*^{b} mice is illustrated in Fig. 7.

I/LnJ mice derive from Strong's I strain, which he produced from a group of unpedigreed mice obtained in 1926. BDP/J was derived from a cross by Gates that included a pink-eyed mouse provided by Strong shortly thereafter (57); I/LnJ mice carry the pink-eyed mutation. I/LnJ and BDP/J are among the oldest inbred strains. The P/J strain was extracted following an outcross of BDP/J by Snell in Bar Harbor. Dr. Snell maintained many mutations in stocks of mice rather than isolating them in inbred strains and used these stocks to extract linkage data and made the mice freely available to other investigators. The IS/Cam strain was inbred by Margaret Wallace in Cambridge by crossing a *Mus musculus praetextus* caught in an Israeli port with a female from a composite laboratory stock carrying *bt/bt*, *m/m*, *b/b*, and *a/a*. In a recent conversation, Dr. Wallace indicated that the laboratory mouse used in the initial cross was provided by Dr. Snell in the early 1940s. She also noted that Snell had provided stocks of mice to the Moredun Institute in Edinburgh prior to the arrival of Dickinson, who used randombred stocks from the Moredun Institute to derive his *Sinc*^{p7} VM and IM strains (15). The jerker (*je*) mutation was described in 1941 (30), and stocks of mice carrying this mutation were used in linkage analysis both in Bar Harbor

FIG. 6. Individuals discordant in scrapie incubation time phenotype and *Prn-p* genotype among (NZW × I/LnJ)F_2 mice. Time to onset (A) and death (B) following inoculation with the Chandler isolate of scrapie prions are shown. The top panel (labeled F_2) represent data for the total population; the lower three panels show data for mice of each of the *Prn-p* genotypes *a/a*, *a/b*, and *b/b*. Arrows indicate mice whose incubation or death times fell significantly outside the normal distribution for their *Prn-p* genotype. Numbers adjacent to arrows identify specific mice.

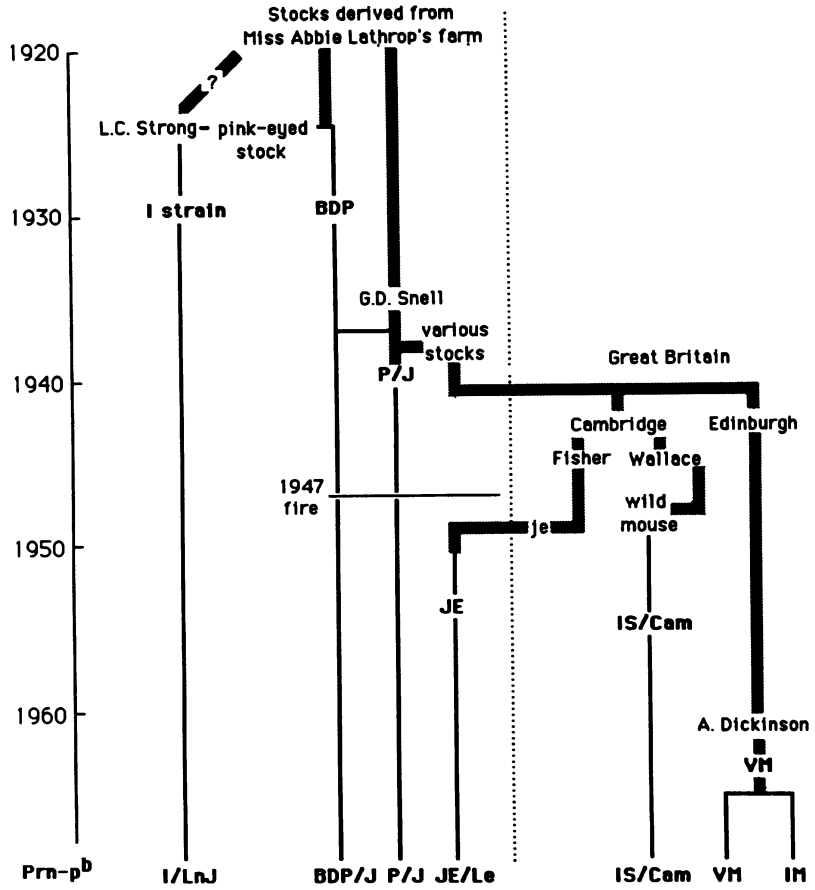


FIG. 7. Common origin of *Prn-p*^{b} mice. A probable genealogy for mouse strains with the *Prn*^{b} haplotype is shown. The *Prn*^{b} haplotype was present in Strong's original colony, from which he derived the I strain. The six other mouse strains with the *b* haplotype can be traced back to pink-eyed stocks of mice provided to other investigators by Dr. Strong. The thick gray lines indicate multiple stocks or inbred strains of mice without the *Prn*^{b} haplotype; the thick gray lines overlaying a thin black line represent non-inbred stocks of mice in which the *b* haplotype was segregating. Thin solid lines represent inbred strains. The dotted line indicates separation between colonies in the United States and Great Britain; approximate dates are on the left.

and in the Department of Genetics in Cambridge (22). A stock of mice carrying *je* from the Department of Genetics at Cambridge was sent to Snell by R. A. Fisher following the destruction of the Jackson Laboratory by fire in 1947. Snell maintained these mice as a closed colony with only the occasional outcross to B6 (*Prn-p*^{a}); the mice were transferred to Lane in 1968 and inbred as JE/Le. The most economical interpretation for the shared *Prn-p*^{b} haplotype of I/LnJ, BDP/J, P/J, IS/Cam, IM, VM, and JE/Le mice is that they share a common ancestor in Strong's colony. VM and IM mice were the last of these to be inbred, compatible with their having a *II-1a* haplotype distinct from that of the other *Prn*^{l} strains.

Only two alleles of *Prn-i* have been found. A common origin and subsequent inbreeding following separation would increase the probability that genes closely linked to *Prn-p* are also shared by I/LnJ, BDP/J, P/J, JE/Le, IS/Cam, and IM mice. The sharing of multiple RFLPs and the close relatedness of mice with the *Prn-p*^{b} allele also suggest that *Prn-i* may be identical to the *Sinc* locus discovered more than 20 years ago (17).

Although flanking polymorphic restriction sites define six haplotypes of *Prn-p*, there seem to be only two phenotypes for the prion incubation time gene among the mouse strains that we tested. Most inbred strains of mice are closely related, as indicated by analysis of mitochondrial DNA (21) and by being derived from the mouse colony maintained by Abbie Lathrop (43, 57). With the exception of MA/MyJ (*Prn-p*^{d}), all standard inbred strains whose ancestry has been traced to Miss Lathrop's colony are *Prn-p*^{a}. However, many strains of independent origin, such as PERA/Ei (wild derived), PL/J, WB/ReJ, WC/ReJ, ST/bJ, CE/J, NZB, and NZW/LacJ, have also been typed as *Prn-p*^{a}.

The RIII strain originated in Paris, and the RIII/Dm subline is *Prn-p*^{a}. However, the two sublines known to have short scrapie incubation times, RIIIS/J (which is the result of a cross involving RIII/Dm, RIII/AnJ, and SEC/1Re) and the RIII/Fa subline (low incidence of mammary tumors) used by Dickinson and MacKay (15) are *Prn-p*^{c}. RIIIS/J was initially classified as *Prn-p*^{b} based on its *Xba*I RFLP (7); the data presented here clarify the erroneous conclusion of Hope and Kimberlin (32) that RIII/Fa mice represented a discordance between *Prn-p* genotype and scrapie incubation time.

Recombination suppression. The gene order on chromosome 2 determined with the various crosses illustrated in Fig. 4 is *B2m–II-1a–Prn-p–Itp–A*. The linkage of *Prn-p* and *Itp* suggests that a region of mouse chromosome 2 is homologous with a region on human chromosome 20p, on which both the ITP and PRNP genes are located (56). In most crosses, the map distances we obtained were in good agree-

ment with the composite mouse linkage map (12) and with the recombination frequency between pallid (*pa*) and *A*, which has been assessed often due to the convenience of coat color markers (9, 38). Pallid and *B2m* are distinct, closely linked loci, but the gene order and recombination frequency have not been determined directly (26).

In contrast, recombination frequency between *B2m* and *Prn-p* differed significantly between *Prn-p*^{a}/*Prn-p*^{d} × *Prn-p*^{a} backcross mice (5 of 80, or 6.25 cM) and offspring of the *Prn-p*^{a}/*Prn-p*^{b} × *Prn-p*^{a} and *Prn-p*^{a} × *Prn-p*^{a}/*Prn-p*^{b} crosses involved in the construction of *Prn-p* congenic strains (4 of 280, or 1.43 cM). Recombination between *Prn-p* and *II-1a* was also much less frequent in the incipient congenics than in the (NZW × I/Ln)F_2 cross. Differences in recombination frequency between the same genes in crosses involving different laboratory strains are not uncommon, but in most cases the mechanisms responsible are unknown. Pairing of a normal chromosome with one containing a deletion or inversion is known to suppress meiotic recombination in mammals (53, 64). In G-banded chromosome spreads of (NZW × I/LnJ)F_1 and (B6 × I/LnJ)F_1 cells, the two chromosome 2's were indistinguishable (L. Washburn and G. Carlson, unpublished results); however, inversions with profound suppressive effects on recombination, such as the *t* complex, are not visible microscopically (53, 63, 66). In general, homologous pairing facilitates recombination, but recombination frequency clearly is not directly proportional to the number of bases separating two genes (37, 58). The reason for reduced recombination frequency in the B6 × I/LnJ cross is not known, but the low recombination frequency obtained during production of *Prn-p* congenic strains could be an overestimate of the recombination frequency in (B6 × I/LnJ)F_1 mice. Homology of regions of chromosome 2 flanking the donor segment including the *Prn-p* gene should increase with repeated backcrossing, and no recombinants were seen among the first 123 mice that were typed.

Segregation distortion. A second unusual finding noted during the production of the B6.I congenic line was segregation distortion. Nearly twice as many *Prn-p*^{a} homozygous mice (n = 106) as *Prn-p*^{a}/*Prn-p*^{b} heterozygotes (n = 56) were born to B6 dams impregnated by *Prn-p* heterozygous sires. The reason for this distortion is not known, and it also is not clear whether the effect is restricted to this particular cross.

There are at least three possibilities to explain this segregation distortion. First, survival of *Prn-p* heterozygous embryos might be compromised. The fact that the mean litter size of B6 mice impregnated by heterozygous males was not different from the published mean litter size of inbred B6 mice (31) argues against this interpretation, but data from our own research colony for direct comparison are not available. A second possibility is that *Prn-p*^{a} and *Prn-p*^{b} sperm are produced in equal numbers but that *Prn-p*^{b} sperm are at a disadvantage in fertilization. A third possibility is that production of viable sperm might be distorted.

Profound recombination suppression-and-transmission ratio distortion by male heterozygotes is associated with the murine *t* complex, which is located on chromosome 17 and includes the *H-2* complex (54). The recombination suppression is due to inversions in gene order on *t* chromosomes spanning as much as 12 cM of the wild-type chromosome and causing up to a 250-fold reduction in recombination frequency (42, 53, 54). Transmission distortion is thought to be due to distorter and responder loci within the *t* complex, similar to the distorter system in *Drosophila* (3, 41, 62, 67). Whether a comparison between the *t* complex and the region of chromosome 2 encompassing *B2m* and *Prn* has any physical basis awaits the results of further analysis. We should also emphasize that there is no evidence that the *Prn* complex itself plays any role in either the recombination suppression or segregation distortion; even fully congenic strains carry a differential chromosomal segment encompassing (on average) 20 cM. Recombination suppression and segregation distortion are mechanisms that would favor linkage disequilibrium between *Prn-p* and neighboring loci.

Genetic dominance in prion incubation times. The long-scrapie-incubation-time *Prn-i*^{l} allele is not fully dominant, as shown by inoculation of (NZW/LacJ × I/LnJ)F_2 mice with scrapie prions (Fig. 6). Although there was overlap between the incubation periods of *Prn-p*^{a}/*Prn-p*^{b} heterozygous and *Prn-p*^{b} homozygous mice, mean incubation times differed significantly between the two groups. In addition, the large range of incubation times in *b*/*b* homozygous mice (from 193 to 359 days) was not seen in heterozygotes. Therefore, although *Prn-i*^{l} has a dramatic effect in prolonging scrapie incubation time, the short *Prn-i*^{n} allele is also expressed in heterozygous mice. *Sinc*, which is linked to *Prn-p* and possibly identical with *Prn-i*, is also expressed codominantly (16–18).

Discordance between incubation time phenotype and *Prn-p* genotype. In both the initial study demonstrating linkage between the prion protein and prion incubation time genes (7) and the current study, we defined mice whose scrapie incubation times were discordant with their prion protein genotypes. One obvious interpretation is recombination between *Prn-i* and *Prn-p*. We are reluctant to conclude that the occurrence of deviant mice indicates that *Prn-i* and *Prn-p* are distinct loci without progeny testing to demonstrate that the recombinant phenotype is stably transmitted.

The divergence of scrapie incubation time phenotype from *Prn-p* genotype may be due to convergence of other genes segregating in the backcross and F_2 mice. For example, previous work has shown that sex, an *H-2D*-linked gene (*Pid-1*), and non-*Prn* background genes can influence prion incubation period in mice (7, 35, 45). In Fig. 3, incubation times ranged from 159 to 194 days in (B6 × MA/MyJ)F_1 × B6 mice and from 111 to 174 days in (NZW × CAST)F_1 × NZW mice; in both cases, genes unlinked to *Prn-p* were responsible for these spreads in incubation time.

It would be just as imprudent to conclude that *Prn-i* and *Prn-p* are distinct loci as it would be to claim that control of the length of prion incubation time is a pleiotropic effect of *Prn-p*. Experiments, including construction of transgeneic mice and a recombinant capture scheme, to distinguish between the two alternatives are in progress.

***Prn* offers new approaches to neurodegenerative diseases.** One of the major problems facing investigators studying most neurodegenerative diseases is the lack of animal models that recapitulate all features of these disorders (23). Although Alzheimer's disease is not transmissible (25) and its amyloid does not contain detectable amounts of PrP (36, 52), biochemical processes similar to those in prion diseases may be involved. Posttranslational modification of a normal cellular protein to an abnormal polymerizing isoform may be a process common to several degenerative diseases, and initiation of the pathological cascade could be caused by defects in the protein gene itself or in "incubation time" loci, as well as by environmental insults. An exogenous infectious source for initiation of pathology may be restricted to those diseases characterized by accumulation of PrP^{Sc}. Mapping of *Prn* to a region of murine chromosome 2 which contains a member of the immunoglobulin supergene family (*B2m*) and which exhibits recombination suppression as well as segre-

gation distortion is of considerable interest. Future studies of *Prn* must include defining the number of genes in this complex as well as elucidating the function of its only known product, PrP^C. Whether studies on *Prn* in mice will lead to the development of more suitable animal models for human neurodegenerative diseases other than those caused by prions remains uncertain.

ACKNOWLEDGMENTS

This work was supported by Public Health Service grants NS14069, NS22786, and GM37821 from the National Institutes of Health and by gifts from the Sherman Fairchild Foundation and RJR/Nabisco.

We thank Priscilla Lane and George D. Snell at the Jackson Laboratory and Margaret Wallace at the Department of Genetics in Cambridge for invaluable assistance in tracing the ancestry of mice with the *b* allele of the prion protein gene. We thank Monika Wälchli, Carol Mirenda, Sherry Kelly-Delcourt, and Kathy McGraw for expert technical assistance. We gratefully acknowledge the important contributions of the animal technicians Ramon Augusto, Vinton Bacon, Phillip Davis, Marcella Pierce, and Dennis Rapp in diagnosing scrapie-infected mice. We also are grateful to Margaret Green at the Jackson Laboratory for her interest and encouragement.

ADDENDUM IN PROOF

Recent results (L.D. Siracusa, N. Copeland, and N. Jenkins, personal communication) indicate that the positions of *Psp*, *Src*, and *A* illustrated in Fig. 4, map II (based on references 12 and 60) are incorrect. The correct gene order, proximal to distal, is *Psp–A–Src*.

LITERATURE CITED

1. **Bailey, N. T. J.** 1981. Statistical methods in biology, 2nd ed. John Wiley & Sons, New York.
2. **Basler, K., B. Oesch, M. Scott, D. Westaway, M. Wächli, D. Groth, M. P. McKinley, S. B. Prusiner, and C. Weissman.** 1986. Scrapie and cellular PrP isoforms are encoded by the same chromosomal gene. Cell **46**:417–428.
3. **Bennett, D., A. K. Altyon, and K. Artzt.** 1983. Genetic analysis of transmission ratio distortion by *t*-haplotypes in the mouse. Genet. Res. (Cambridge) **41**:29–45.
4. **Bockman, J. M., D. T. Kingsbury, M. P. McKinley, P. E. Bendheim, and S. B. Prusiner.** 1985. Creutzfeldt-Jakob disease prion proteins in human brains. N. Engl. J. Med. **312**:73–78.
5. **Bockman, J. M., S. B. Prusiner, J. Tateishi, and D. T. Kingsbury.** 1987. Immunoblotting of Creutzfeldt-Jakob disease prion proteins: host species-specific epitopes. Ann. Neurol. **20**:204–208.
6. **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.
7. **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.
8. **Carp, R. I., R. C. Moretz, M. Natelli, and A. G. Dickinson.** 1987. Genetic control of scrapie: incubation period and plaque formation in I mice. J. Gen. Virol. **68**:401–407.
9. **Carter, T. C., and R. J. S. Phillips.** 1954. Ragged, a semidominant coat texture mutant in the house mouse. J. Hered. **45**:151–154.
10. **Chandler, R. L.** 1961. Encephalopathy in mice produced by inoculation with scrapie brain material. Lancet **i**:1378–1379.
11. **Chesebro, B., R. Race, K. Wehrly, J. Nishio, M. Bloom, D. Lechner, S. Bergstrom, K. Robbins, L. Mayer, J. M. Keith, C. Garon, and A. Haase.** 1985. Identification of scrapie prion protein-specific mRNA in scrapie-infected and uninfected brain. Nature (London) **315**:331–333.
12. **Davisson, M., and T. Roderick.** 1987. Linkage map of the mouse, p. 430. *In* S. O'Brien (ed.), Genetic maps 1987: a compilation of linkage and restriction maps of genetically studied organisms, vol. 4. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
13. **D'Eustachio, P. D., S. Jadidi, R. C. Fuhlbrigge, P. W. Gray, and D. D. Chaplin.** 1987. Interleukin-1 α and β genes: linkage on chromosome 2 in the mouse. Immunogenetics **26**:339–343.
14. **Dickinson, A. G., and H. G. Fraser.** 1979. An assessment of the genetics of scrapie in sheep and mice, p. 367–386. *In* S. B. Prusiner and W. J. Hadlow (ed.), Slow transmissible diseases of the nervous system, vol. 1. Academic Press, New York.
15. **Dickinson, A. G., and J. M. K. MacKay.** 1964. Genetical control of the incubation period in mice of the neurological disease, scrapie. Heredity **19**:279–288.
16. **Dickinson, A. G., and V. M. Meikle.** 1971. Host-genotype and agent effects in scrapie incubation: change in allelic interaction with different strains of agent. Mol. Gen. Genet. **112**:73–79.
17. **Dickinson, A. G., V. M. H. Meikle, and H. G. 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.
18. **Dickinson, A. G., and G. W. Outram.** 1979. The scrapie replication site hypothesis and its implications for pathogenesis, p. 13–31. *In* S. B. Prusiner and W. J. Hadlow (ed.), Slow transmissible diseases of the nervous system, vol. 2. Academic Press, New York.
19. **Diener, T. O.** 1987. PrP and the nature of the scrapie agent. Cell **49**:719–721.
20. **Feinberg, A. P., and B. Vogelstein.** 1983. A technique for radiolabeling DNA restriction fragments to high specific activity. Anal. Biochem. **132**:6–13.
21. **Ferris, S. D., R. D. Sage, and A. C. Wilson.** 1982. Evidence from mtDNA sequences that common laboratory strains of inbred mice are descended from a single female. Nature (London) **295**: 163–165.
22. **Fisher, R. A., and G. D. Snell.** 1948. A twelfth linkage group of the house mouse. Heredity **2**:271–273.

22a. **Gabizon, R., M. P. McKinley, D. F. Groth, and S. B. Prusiner.** 1988. Immunoaffinity purification and neutralization of scrapie prion infectivity. Proc. Natl. Acad. Sci. USA **85**:6617–6621.

23. **Glenner, G. G.** 1988. Alzheimer's disease: its proteins and genes. Cell **52**:307–308.
24. **Gordon, W. S.** 1964. Advances in veterinary research. Vet. Res. **58**:516–520.
25. **Goudsmit, J., C. H. Morrow, D. M. Asher, R. T. Yanagihara, C. L. Masters, C. J. Gibbs, and D. C. Gajdusek.** 1980. Evidence for and against the transmissibility of Alzheimer's disease. Neurology **30**:945–950.
26. **Graff, R. J., D. Martin-Morgan, and M. E. Kurtz.** 1987. Multiplicity of chromosome 2 histocompatibility genes: new loci, H-44 and H-45. Immunogenetics **26**:111–114.
27. **Green, E. L.** 1985. Tables and a computer program for analyzing linkage date. Mouse Newslett. **73**:20.
28. **Green, M. C.** 1981. Gene mapping, p. 105–117. *In* H. L. Foster, J. D. Small, and J. D. Fox (ed.), The mouse in biomedical research, vol. 1. Academic Press, New York.
29. **Gross-Bellard, M., P. Oudet, and P. Chambon.** 1973. Isolation of high molecular weight DNA from mammalian cell. Eur. J. Biochem. **36**:32–38.
30. **Grüneberg, H., J. B. Burnett, and G. D. Snell.** 1941. The origin of jerker, a new gene mutation in the house mouse, and linkage studies made with it. Proc. Natl. Acad. Sci. USA **27**:562–565.
31. **Heiniger, H.-J., and J. J. Dory.** 1980. Handbook on genetically standardized jax mice, 3rd ed. The Jackson Laboratory, Bar Harbor, Maine.
32. **Hope, J., and R. H. Kimberlin.** 1987. The molecular biology of scrapie: the last two years. Trends Neurol. Sci. **10**:149–151.
33. **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.
34. **Johnsson, A., C.-H. Heldin, B. Westermark, T. F. Deuel, J. S.**

Huang, P. H. Seeburg, A. Gray, A. Ullrich, G. Scrace, P. Stroobant, and M. D. Waterfield. 1984. The *c-sis* gene encodes a precursor of the B chain of platelet-derived growth factor. EMBO J. **3**:921–928.

35. **Kingsbury, D. T., K. C. Kasper, D. P. Stites, J. C. Watson, R. N. Hogan, and S. B. Prusiner.** 1983. Genetic control of scrapie and Creutzfeldt-Jakob disease in mice. J. Immunol. **131**:491–496.
36. **Kitamoto, T., J. Tateishi, T. Tashima, I. Takeshita, R. A. Barry, S. J. DeArmond, and S. B. Prusiner.** 1986. Amyloid plaques of Creutzfeldt-Jakob disease stain with prion protein antibodies. Ann. Neurol. **20**:204–208.
37. **Kobori, J. A., A. Winoto, J. McNicholas, and L. Hood.** 1984. Molecular characterization of the recombination region of six murine major histocompatibility (MHC) I-region recombinants. J. Mol. Cell. Immunol. **1**:125–137.
38. **Lilly, F.** 1967. The location of histocompatibility-6 in the mouse genome. Transplantation **5**:83–85.
39. **Lomedico, P. T., U. Gubler, C. P. Hellmann, M. Dukovich, J. G. Giri, Y.-C. E. Pan, K. Collier, R. Semionow, A. O. Chua, and S. B. Mizel.** 1984. Cloning and expression of murine interleukin-1 cDNA in *Escherichia coli*. Nature (London) **312**:458–462.
40. **Lovett, M., Z.-Y. Cheng, E. M. Lamela, T. Yokoi, and C. J. Epstein.** 1987. Molecular markers for the agouti coat color locus of the mouse. Genetics **115**:747–754.
41. **Lyon, M. F.** 1984. Transmission ratio distortion in mouse *t*-haplotypes is due to multiple distorter genes acting on a responder locus. Cell **37**:621–628.
42. **Lyon, M. F., and R. J. S. Phillips.** 1959. Crossing over in mice heterozygous for *t*-alleles. Heredity **13**:23–32.
43. **Morse, H. C., III.** 1981. The laboratory mouse—a historical perspective, p. 1–16. *In* H. L. Foster, J. D. Small, and J. D. Fox (ed.), The mouse in biomedical research, vol. 1. Academic Press, New York.
44. **Oesch, B., D. Westaway, M. Wächli, M. P. McKinley, S. B. H. Kent, R. Aebersold, R. A. Barry, P. Tempst, D. B. Teplow, L. Hood, S. B. Prusiner, and C. Weissman.** 1985. A cellular gene encodes scrapie PrP 27-30 protein. Cell **40**:735–746.
45. **Outram, G. W.** 1976. The pathogenesis of scrapie in mice, p. 325–357. *In* R. H. Kimberlin (ed.), Slow virus diseases of animals and man. Elsevier, Amsterdam.
46. **Parnes, J. R., and J. G. Seidman.** 1982. Structure of wild-type and mutant mouse β_2-microglobulin genes. Cell **29**:661–669.
47. **Parry, H. B.** 1983. Scrapie disease in sheep. Academic Press, New York.
48. **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.
49. **Prusiner, S. B.** 1982. Novel proteinaceous infectious particles cause scrapie. Science **216**:134–144.
50. **Prusiner, S. B.** 1987. Prions and neurodegenerative diseases. N. Engl. J. Med. **317**:1571–1581.
51. **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.
52. **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.
53. **Shin, H.-S., L. Flaherty, K. Artzt, D. Bennett, and J. Ravetch.** 1983. Inversion in the *H-2* complex of *t*-haplotypes in mice. Nature (London) **306**:380–383.
54. **Silver, L. M.** 1985. Mouse *t*-haplotypes. Annu. Rev. Genet. **19**: 179–208.
55. **Snell, G. D.** 1948. Methods for the study of histocompatibility genes. J. Genet. **49**:87–108.
56. **Sparkes, R. F., M. Simon, V. H. Cohn, R. E. K. Fournier, J. Lem, I. Klisak, C. Heinzmann, 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.
57. **Staats, J.** 1985. Standardized nomenclature for inbred strains of mice: eighth listing. Cancer Res. **45**:945–977.
58. **Steinmetz, M., K. Minard, S. Horvath, J. McNicholas, J. Frelinger, C. Wake, E. Long, B. Mach, and L. Hood.** 1982. A molecular map of the immune response region of the major histocompatibility complex of the mouse. Nature (London) **300**: 35–42.
59. **Suggs, S. V., T. Hirose, T. Miyake, E. H. Kawashime, M. J. Johnson, K. Itakura, and R. B. Wallace.** 1981. Use of synthetic oligonucleotides for the isolation of specific cloned DNA sequences. ICN-UCLA Symp. Dev. Biol. **23**:683–693.
60. **Taylor, B. A., D. M. Walls, and M. J. Wimsatt.** 1987. Localization of the inosine triphosphatase locus (*Itp*) on chromosome 2 of the mouse. Biochem. Genet. **25**:267–286.
61. **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.
62. **Tokuyasu, K. T., W. J. Peacock, and R. W. Hardy.** 1977. Dynamics of spermiogenesis in *Drosophila melanogaster*. VII. Effects of *segregation distortion (SD)* chromosome. J. Ultrastruct. Res. **58**:96–107.
63. **Tres, L. L., and R. P. Erickson.** 1982. Electron microscopy of *t*-allele synaptonemal complexes discloses no inversions. Nature (London) **299**:752–754.
64. **Wallace, M. E.** 1972. Reduction in recombination due to a deletion in a colony of wild mice. J. Hered. **63**:297–300.
65. **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.
66. **Womack, J. E., and T. H. Roderick.** 1974. *t*-alleles in the mouse are probably not inversions. J. Hered. **65**:308–310.
67. **Yanagisawa, K.** 1965. Studies on the mechanism of abnormal transmission ratios at the *T*-locus in the house mouse. IV. Some morphological studies on the mature sperm in males heterozygous for *t*-alleles. Jpn. J. Genet. **40**:97–104.