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Genetics

Assignment of the human and mouse prion protein genes to homologous chromosomes

(scrapie/Creutzfeldt–Jakob disease/slow infections/dementia/gene mapping)

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ABSTRACT **Purified preparations of scrapie prions contain one major macromolecule, designated prion protein (PrP). Genes encoding PrP are found in normal animals and humans but not within the infectious particles. The PrP gene was assigned to human chromosome 20 and the corresponding mouse chromosome 2 using somatic cell hybrids. *In situ* hybridization studies mapped the human PrP gene to band 20p12→pter. Our results should lead to studies of genetic loci syntenic with the PrP gene, which may play a role in the pathogenesis of prion diseases or other degenerative neurologic disorders.**

Scrapie and Creutzfeldt–Jakob disease (CJD) are transmissible, degenerative neurologic disorders of animals and humans, respectively (1). Both diseases are caused by unusual infectious agents called prions (2). The most well-studied prions are those isolated from scrapie-infected hamster brains. To date, the only abundant macromolecule identified in purified preparations of hamster scrapie prions is a protein designated prion protein (PrP) 27–30 (3, 4). cDNA cloning studies have shown that PrP 27–30 is encoded by a cellular gene and not by a nucleic acid molecule within the infectious prion (5). In fact, all attempts to demonstrate a scrapie-specific nucleic acid within the infectious particle have been unsuccessful.

PrP 27–30 is derived from a larger protein, PrP 33–35^{Sc} (5, 6). Normal and scrapie-infected hamster brain cells contain an isoform of PrP 33–35^{Sc}, which has been designated PrP 33–35^{C} (6, 7). Since the scrapie and cellular PrP isoforms are encoded by the same chromosomal gene, it is likely that the differences in their molecular properties result from posttranslational events (40).

PrP proteins are conserved; a portion of a PrP cDNA from Swiss mice exhibits 90% sequence homology with PrP cDNA from hamsters (8). A human PrP cDNA was also cloned and sequenced; similarly, ≈90% homology between the human and hamster PrP genes was observed (9). Recent studies in mice have linked the gene that encodes the prion protein (*Prn-p*) to another gene that controls the length of the scrapie incubation period (*Prn-i*) (10). In the studies reported here, we mapped the *Prn-p* structural gene to mouse chromosome 2. The human PrP gene (PRNP) was found on chromosome 20 and mapped to band 20p12→pter.

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MATERIALS AND METHODS

Mapping the Mouse PrP Gene. Microcell hybrid clones retaining various mouse chromosomes were used in these studies. The isolation and characterization of these clones have been described in detail elsewhere (11–13). ABm-30 and -31 are hamster–mouse microcell hybrids, whereas F(11)G, F(11)O, F(2.8)D, F(8)D, and FB(2.8)D are rat–mouse microcell hybrids. FF4-3a and A1-9v are derivatives of the rat × mouse hybrids FF4-3 and FF1-9, respectively (14).

DNA was isolated from parental (DBA and C57BL mouse DNA; rat and hamster) and hybrid cells. Approximately 5 μg of each DNA sample was digested with *Eco*RI. DNA fragments were separated by agarose gel electrophoresis and transferred to GeneScreen*Plus* filters by the method of Southern (15).

Hamster PrP cDNA insert was isolated from pHaPrP-cDNA-1 and labeled with [α-^{32}P]dCTP by nick-translation. Hybridization on filters was carried out at 37°C for 24 hr on a rotating platform. Filters were washed with 0.3 M NaCl/30 mM sodium citrate/0.2% $NaDodSO_4$ at 65°C for 2 hr and 30 mM NaCl/3 mM sodium citrate/0.2% $NaDodSO_4$ at 25°C for 1 hr and then wrapped in clear plastic and placed on Kodak XAR-5 x-ray film with intensifying screens for 1–5 days at −70°C.

Mapping the Human PrP Gene. Somatic cell hybrids were derived from the fusion of thymidine kinase-deficient mouse cells (B82, GMO347A) and normal human male fibroblasts (IMR91), both obtained from the Mutant Cell Repository (Camden, NJ). The cells were fused in a mixed monolayer using a 50% solution of PEG (M_r 1000) in a balanced salt solution (16). Following a 24-hr culture period, the cells were added to multiple independent culture dishes containing hypoxanthine/aminopterin/thymidine medium (17, 18) and ouabain (19). Multiple independent hybrid clones were isolated and a preliminary cytogenetic analysis was done on 10 Q-banded photographed chromosome metaphases. Sixteen hybrid clones were selected from an initial set of 40 clones, based on growth characteristics, human chromosome content, and a lack of detectable human chromosome rearrangements. As expected with this type of procedure, the hybrid clones contained different human chromosome content. The

Abbreviations: PrP, prion protein; *Prn*, mouse gene complex; *Prn-p*, mouse prion protein structural gene; *Prn-i*, mouse scrapie prion incubation time gene; PRNP, human prion protein structural gene; RFLP, restriction fragment length polymorphism; CJD, Creutzfeldt–Jakob disease; GSS, Gerstmann–Sträussler syndrome; ORF, open reading frame.

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clones were then grown in multiple dishes and pooled, and cell pellets were prepared for DNA extraction. From the same pooled cells of each clone, an analysis of chromosome content was made on a minimum of 30 Q-banded photographed metaphases per hybrid clone. Because these mouse–human hybrids do not retain human chromosome 9, DNA from a Chinese hamster–human hybrid clone selectively retaining 9pter→9q34 by virtue of an X/9 translocation (20) was also analyzed.

DNA from the parental cell lines and somatic cell hybrid clones was purified from isolated nuclei (20) by incubation in 10 μM EDTA, pH 8/0.2% $NaDodSO_4$/600 μg of proteinase K (Sigma) per ml at 37°C for 24 hr followed by phenol extraction and ethanol precipitation.

A hamster PrP cDNA subclone derived primarily from the open reading frame (ORF) was used to detect the presence or absence of the human PRNP gene (21). The enzymes were obtained from Bethesda Research Laboratories and radiolabeled nucleotides were obtained from Amersham. The probe was radiolabeled with ^{32}P to a specific activity of $\approx 1 \times 10^9$ cpm/μg by oligolabeling (22). Genomic DNAs from the parental cell lines and the hybrid clones were digested with the restriction endonuclease *Hin*dIII (8 units/μg). Approximately 10 μg of DNA from each sample was electrophoresed through a 1.2% agarose gel in TAE (40 mM Tris acetate/1 mM EDTA, pH 7.4) buffer and transferred by blotting to nylon (Schleicher & Schuell) by the method of Southern (15). Hybridization was performed in 1% bovine serum albumin/0.5 M sodium phosphate, pH 7.0/1 mM EDTA/7% $NaDodSO_4$/250 μg of denatured salmon sperm DNA per ml for 16 hr at 65°C with shaking. The filter was then washed twice, first in 0.3 M NaCl/30 mM sodium citrate/0.1% $NaDodSO_4$ and then in 15 mM NaCl/1.5 mM sodium citrate/0.1% $NaDodSO_4$, each time for 20 min at 65°C. The filter was dried briefly and exposed to Kodak XAR-5 x-ray film.

***In Situ* Hybridization of the Human PrP Gene.** The *in situ* hybridization studies used the hamster PrP cDNA subclone probe labeled with ^3H-labeled deoxynucleotides to a specific activity of $\approx 5 \times 10^7$ cpm/μg. *In situ* hybridization to normal chromosomes followed the method of Harper and Saunders (23) as modified by Zabel *et al.* (24). Slides were exposed for 7 days and silver grains on or touching chromosomes were scored.

RESULTS

Hybrids retaining substantial numbers of mouse chromosomes were screened for retention of murine *Prn-p* gene sequences. The rat–mouse hybrids FF43a, A1-9v, F(11)G, and F(11)O, which collectively retain 14 (of 20) different mouse chromosomes (Table 1), were found to contain only rat-specific PrP sequences. In contrast, the hamster–mouse hybrids ABm-30 and ABm-31 were found to contain hamster- and mouse-specific PrP DNA fragments. The only murine chromosome present in both hamster–mouse hybrid clones but absent from the rat–mouse hybrids is mouse chromosome 2. To confirm that mouse chromosome 2 carries the murine *Prn-p* gene, monochromosomal microcell hybrids were used.

Microcell hybrid F(2.8)D is a rat–mouse hybrid that contains a Robertsonian translocation (centric fusion) between mouse chromosomes 2 and 8. No other murine chromosomes have been retained in this clonal line (Table 1). The RB(2.8) translocation was maintained in the hybrid population by direct selection for the chromosome 8-encoded $APRT^+$ phenotype (25). F(2.8)D contains rat- and mouse-specific PrP gene sequences; FB(2.8)D is a multiclonal population of $APRT^-$ cells derived from F(2.8)D by back-selection in medium containing 2,6-diaminopurine. The FB(2.8)D population had uniformly segregated the mouse Rb(2.8) translocation chromosome (Table 1) and no longer retains murine *Prn-p* gene sequences. To confirm that *Prn-p* segregates with chromosome 2, DNA from hybrid clone F(8)D was examined. This monochromosomal microcell hybrid, which retains only mouse chromosome 8, does not contain *Prn-p*. From these studies, we conclude that *Prn-p* can be assigned to mouse chromosome 2.

Southern blots of mouse and human DNA as well as DNA derived from 17 mouse–human hybrid clones were probed with a PrP cDNA derived primarily from the hamster PrP gene ORF (21). A 14-kilobase human band was found in three of four hybrid DNAs restricted with *Hin*dIII. Table 2 shows that chromosome 20 is the only human chromosome that shows no discordancies between the presence or absence of the chromosome and the hybridizing human band, whereas all of the other chromosomes had four or more discordancies. Analysis of a single Chinese hamster–human hybrid clone selectively retaining most of human chromosome 9 was negative for the presence of the PrP sequence. These results allow the assignment of the gene locus PRNP to human chromosome 20.

Fig. 1 *Upper* shows the histogram of the *in situ* hybridization studies, which confirms the assignment of the PRNP to chromosome 20. Fig. 1 *Lower* shows the distribution of the grains on chromosome 20 in more detail and maps PRNP to 20p12→pter.

DISCUSSION

Although the complete molecular structure of the scrapie agent or prion remains to be established, considerable evidence indicates that PrP 27–30 is required for, and inseparable from, scrapie infectivity. Moreover, recent studies with

Table 1. Segregation of the PrP gene with mouse chromosomes in hamster–mouse and rat–mouse microcell hybrids

Hybrid clone	Mouse chromosome retained (fraction of cells)																				No. of cells*	PrP†
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	X		
FF4-3a	—	—	—	—	—	—	—	0.47	0.47	0.33	—	0.47	0.40	0.53	0.67	0.27	—	0.60	0.40	0.67	15	−
A1-9v	—	—	—	—	—	—	0.58	0.58	0.95	—	—	—	—	—	—	—	—	—	—	—	19	−
F(11)G	—	—	—	—	—	0.66	—	—	—	—	0.86‡	—	0.71	0.79	0.83	—	—	—	—	—	24	−
F(11)O	—	—	—	—	—	—	—	—	—	—	0.80‡	—	0.74	—	0.50	—	—	—	—	—	24	−
ABm-30	—	0.90‡	—	—	—	0.56	0.38	—	0.46	0.31	—	0.51	—	—	0.31	0.51	—	—	—	—	39	+
ABm-31	—	0.95‡	—	—	—	0.40	0.51	—	0.59	—	—	0.65	—	0.43	0.27	—	0.73	—	—	—	37	+
F(2.8)D	—	0.93‡	—	—	—	—	—	0.93‡	—	—	—	—	—	—	—	—	—	—	—	—	21	+
FB(2.8)D	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	24	−
F(8)D	—	—	—	—	—	—	—	1.00‡	—	—	—	—	—	—	—	—	—	—	—	—	19	−

*Number of cells karotyped.
†Homologous sequences.
‡Chromosome selectively retained.

Table 2. Segregation of the PrP gene with human chromosomes in mouse–human cell hybrids

Hybrid clone	PrP*	Human chromosome†																							
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	X	Y
84-2	+	+	+	−	+	+	+	+	+	−	−	+	+	−	+	+	+	+	+	+	+	−	+	−	−
84-3	+	+	+	+	+	−	+	+	+	−	+	−	−	+	+	+	−	+	+	+	+	−	−	−	−
84-4	+	+	+	+	+	−	+	+	+	−	+	−	−	+	+	+	−	+	+	+	+	−	−	−	−
84-7	+	−	−	+	+	−	+	+	+	−	−	+	+	−	+	−	+	+	−	−	+	−	−	−	−
84-13	+	−	−	+	−	−	+	−	−	−	+	−	+	+	−	+	−	+	+	−	+	+	−	−	−
84-20	+	−	(+)	+	+	+	−	+	+	−	−	(+)	(+)	+	+	−	−	+	+	−	+	+	+	(+)	−
84-21	+	−	−	−	−	−	−	+	−	−	+	−	+	−	+	−	−	+	−	−	+	+	−	−	(+)
84-26	+	+	−	+	+	+	+	+	+	−	+	−	+	+	+	+	+	+	+	+	+	+	+	−	+
84-27	+	−	+	−	−	+	+	−	+	−	−	+	+	−	−	+	−	+	+	−	+	+	−	−	−
84-30	+	−	−	+	+	(+)	+	−	−	−	+	−	(+)	+	+	−	−	+	+	−	(+)	+	+	−	−
84-34	+	−	−	−	+	−	+	+	+	−	+	+	−	−	+	−	−	+	−	(+)	+	−	+	−	−
84-37	+	−	−	−	+	+	+	+	(+)	−	−	+	+	−	+	−	−	+	−	−	+	+	+	−	−
84-38	+	+	−	+	+	(+)	+	+	+	−	+	−	+	+	+	+	−	+	+	+	(+)	−	+	−	−
84-5	−	−	+	+	−	+	+	−	+	−	−	−	−	(+)	+	+	−	+	−	+	−	+	+	+	+
84-25	−	−	−	−	+	+	+	−	+	−	+	+	(+)	−	−	−	−	+	−	−	−	+	−	−	−
84-35	−	−	−	+	+	−	+	+	+	−	−	−	+	−	+	+	−	+	+	−	−	−	−	−	−
84-39	−	−	−	+	−	−	−	+	+	−	−	−	−	−	−	+	−	+	−	−	−	−	−	−	−
No. of discordant hybrids		8	9	8	5	8	5	5	7	13	6	8	5	7	4	9	10	4	5	8	0	8	7	13	12

*+ indicates presence of the PrP sequences in the hybrid clone determined by the presence of the human band; − indicates absence of the gene.
†+ indicates presence of the human chromosome in >30% of metaphases analyzed; (+) indicates presence of the chromosome in 10–30% of metaphases analyzed; − indicates absence of the human chromosome.

inbred mice have shown that the gene (*Prn-p*) encoding the prion protein is linked to a locus (*Prn-i*) controlling the scrapie incubation time (10). Whether *Prn-p* and *Prn-i* are identical or separate genes remains to be established. Most strains of inbred mice have been shown to have scrapie incubation periods ranging from 100 to 150 days after intracerebral inoculation with 10^6 ID_{50} units of murine prions. However, using the same dosage of inoculum, I/LnJ mice have incubation periods ranging from 200 to 300 days (26). Using hamster and mouse PrP cDNA ORF probes (21), a restriction fragment length polymorphism (RFLP) between short-incubation inbred strains and I/LnJ long-incubation mice was found. This RFLP was designated *Prn-p*^{a} in short-incubation period mice and *Prn-p*^{b} in long-incubation period I/LnJ mice. In genetic crosses, the *Prn-p*^{b} RFLP was found to segregate with long scrapie incubation periods (10).

In view of the findings described above, it is perhaps not surprising that attempts to map the *Prn-p* gene using RFLPs in DNA derived from recombinant inbred (RI) strains of mice were unsuccessful. Genomic DNA from RI strains A/Jax × C57BL and C57BL × DBA digested with 22 different restriction enzymes (*Eco*RI, *Eco*RV, *Hin*dIII, *Msp* I, *Pst* I, *Bam*HI, *Xba* I, *Kpn* I, *Pvu* II; *Taq* I, *Sst* I, *Bgl* I, *Bgl* II, *Xma* I, *Cla* I, *Hae* II, *Acc* I, *Hde* I, *Sph* I, *Stu* I, *Mvi* I, *Nru* I) failed to exhibit polymorphisms. C57BL and DBA mice have relatively short scrapie incubation periods ranging between 100 and 150 days (26) and show the *Prn-p*^{a} RFLP (10). (No data on the scrapie incubation times or *Prn-p* RFLP in A/Jax mice are available.) The use of somatic cell hybrids for gene mapping circumvented this problem as the parasexual mapping approach depends upon inter- rather than intraspecific polymorphisms.

The experiments reported here demonstrate the PrP cDNA hybridizes to a unique sequence (*Prn-p*) in the murine genome, a sequence that resides on mouse chromosome 2. This assignment is based on the concordant presence of a murine-specific RFLP mouse chromosome 2 containing microcell hybrids (ABm-30 and -31) and its absence in clones containing a variety of other mouse chromosomes (Table 1). The assignment was confirmed by showing the presence of PrP homologous sequences in a monochromosomal microcell hybrid retaining only mouse chromosomes 2 and 8 [as a Rb(2.8) translocation] and their absence in back-selectants that had segregated Rb(2.8) or in hybrid cells retaining mouse chromosome 8 alone.

The somatic cell hybrid (Table 2) and the *in situ* hybridization studies (Fig. 1 *Upper*) map the PrP gene (PRNP) to human chromosome 20. The *in situ* hybridization studies regionally map PRNP to band 20p12→pter. At the present time, only a few other genes have been assigned to the short arm of chromosome 20 (27): inosine triphosphatase; a chromosome fragile site to p11.23; and two anonymous DNA segments (D20S5 to p12; D20S6 to p). Therefore, the PrP cDNA probe should be useful for further mapping and linkage studies of the short arm of chromosome 20.

There is an indication that parts of human chromosome 20 and mouse chromosome 2 are homologous (27). More specifically, the mouse equivalent of the inosine triphosphatase gene (on human 20p) is found on mouse chromosome 2 (28), as are adenosine deaminase (29–32) and Src I (33, 34). Thus, the results of our comparative mapping studies of the PrP gene in mice and humans provide evidence for a syntenic group containing the genes for inosine triphosphatase, adenosine deaminase, Src, and PrP.

The studies reported here suggest that attempts to link genes on chromosome 20 with the development of human degenerative neurologic diseases may be useful. Of interest are studies on human families with two prion diseases, CJD and Gerstmann–Sträussler syndrome (GSS) (35, 36). In addition, families in which multiple cases of CJD and Alzheimer disease are found in the same kindred should be investigated (37). GSS and CJD generally occur during the fifth and sixth decades of life, respectively, whereas Alzheimer disease exhibits an age-dependent incidence beginning with the seventh decade of life (35, 36). It will be of interest to learn whether or not there exists a human gene homologous to mouse *Prn-i* that controls the scrapie incubation times (10).

Although some investigators continue to suggest that the causative agents or prions of scrapie, CJD, and GSS are viruses (38, 39), the results presented here and elsewhere argue against that contention. The invariance of restriction sites flanking the *Prn-p* gene and the homologous mapping of

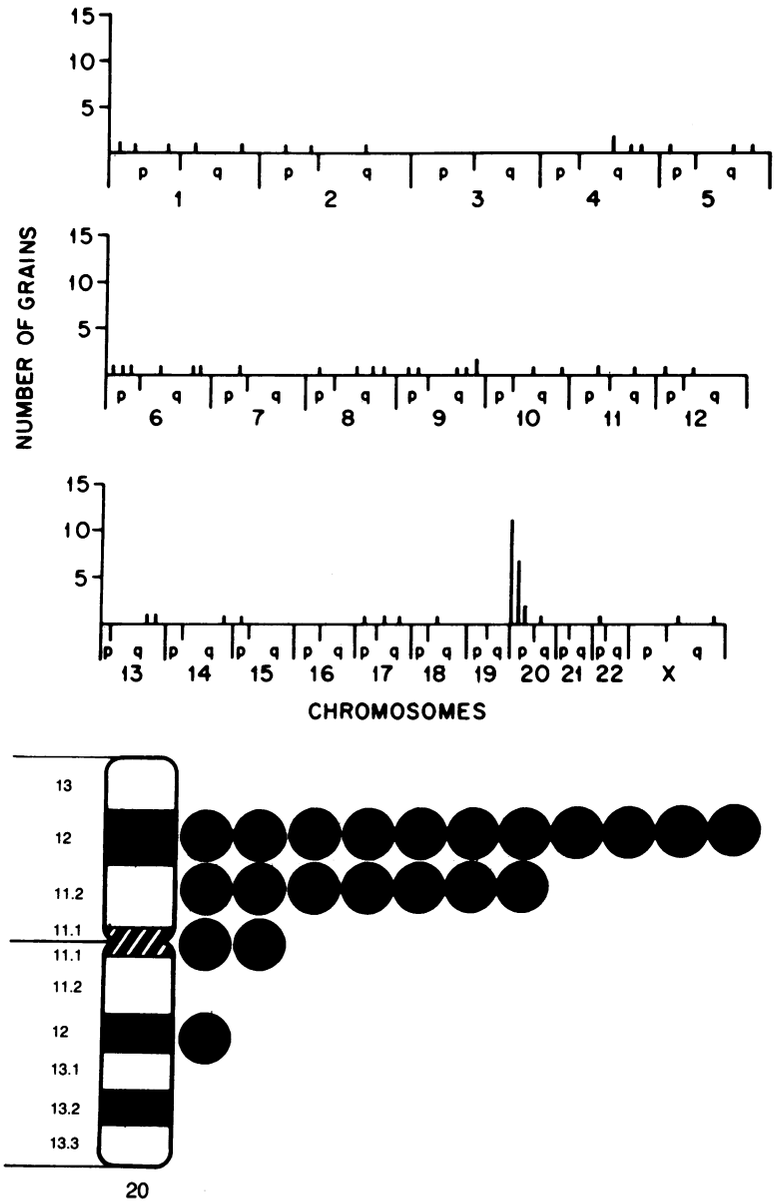

FIG. 1. (*Upper*) *In situ* distribution of silver grains on all of the human chromosomes. The major hybridization is over the short arm of chromosome 20. (*Lower*) *In situ* distribution of silver grains on human chromosome 20. The major concentration of grains is on p12→pter, indicating this is the site of the prion gene. These results are from 60 cells examined.

PrP genes in mice and humans are inconsistent with random chromosomal insertion of a viral sequence. Furthermore, the occurrence of PrP DNA sequences in humans, mice, hamsters, rats, goats, sheep, dogs, cats, and possibly invertebrates (5, 21) would demand that a putative scrapie virus have an extremely broad host range. Instead, the homologous chromosome localization of PrP genes as well as the sequence conservation of these genes argue that a cellular gene encoding a PrP protein existed in a common ancestor of mice and humans—i.e., prior to the speciation of mammals.

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