Linkage of the Scrapie-associated Fibril Protein (PrP) Gene and Sinc Using Congenic Mice and Restriction Fragment Length Polymorphism Analysis

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SUMMARY

Sinc, with two alleles p7 and s7, is the major gene determining the incubation period of all strains of scrapie in mice. The major protein (PrP) of scrapie-associated fibrils is encoded by a cellular gene and we have used a cDNA copy of the hamster PrP mRNA to carry out restriction fragment length polymorphism (RFLP) analysis of different inbred mouse strains including VM(Sinc p7) and VM(Sinc s7) congenic mice. In VM(Sinc p7) mice, a 5.5 kb XbaI fragment hybridized to the PrP cDNA sequence whereas VM(Sinc s7) congenic mice had a 3.8 kb XbaI fragment. The VM x VM(Sinc s7) congenic F1 mice had both the 5.5 kb and the 3.8 kb fragments. The Sinc s7 donor mouse strain, C57BL, had the 3.8 kb fragment suggesting that the Sinc gene and the gene coding for PrP are linked, and could even be the same gene. Other Sinc p7 inbred mice (IM and MB) had the 5.5 kb fragment but so too did some Sinc s7 strains (RIII and VL), implying that the XbaI site polymorphism is not functionally involved in the difference between the two Sinc alleles. We have mapped the polymorphic XbaI site to the 3' flanking region of the PrP gene. TaqI and HhaI were also found to show polymorphisms in the inbred mouse strains studied. The apparent RFLP with HhaI may be a result of differences in methylation rather than in sequence.

Scrapie is an infectious neurodegenerative disorder found naturally in sheep and goats but usually studied in mice and hamsters. Scrapie-associated fibrils (SAF) are structures characteristic of the group of unconventional slow infections which includes scrapie and Creutzfeldt-Jakob disease of man (Merz et al., 1984).

The major protein of hamster SAF is a glycoprotein (PrP) with a molecular weight of 33000 to 35000 (Oesch et al., 1985; Hope et al., 1986). This protein is encoded by a single host gene (Basler et al., 1986), but in the uninfected animal does not form SAF. Although no scrapie-specific nucleic acid has yet been identified, the existence of at least 20 strains of scrapie agent (Dickinson et al., 1984) and the detection of mutation (Bruce & Dickinson, 1987; Kimberlin et al., 1987) indicate that it has a genome independent of the host.

Several genes influence scrapie incubation period. The 'PID-l' gene (which is linked to the H-2D locus in mice; Kingsbury et al., 1983) has relatively small effects and may act at the initial stage of infection but not during incubation itself. In contrast, the Sinc gene (Dickinson et al., 1968) acts throughout pathogenesis and has a major effect on the incubation period of all scrapie strains in mice. Most inbred mouse strains carry Sinc s7 and have a relatively short incubation period for the ME7 group of scrapie strains. Until recently, VM and lines derived from this stock were the only known source of the Sinc p7 allele which determines a longer incubation period for the ME7 group strains relative to their incubation period in Sinc s7 mice. However, members of the 22A group of scrapie strains have a longer incubation period in s7 than in p7 mice and this is shown in Table 1 for the mouse strains used in this study.

Kingsbury and colleagues have discovered (Kingsbury et al., 1983) that I mice ('1/LnJ') have a
Table 1. Incubation period of scrapie strains ME7 and 22A injected intracerebrally into various mouse strains*

<table>
<thead>
<tr>
<th>Mouse strain</th>
<th>Sinc</th>
<th>Haplotype</th>
<th>ME7</th>
<th>22A</th>
</tr>
</thead>
<tbody>
<tr>
<td>VM/Dk</td>
<td>p7p7</td>
<td>H-2b</td>
<td>355 ± 3</td>
<td>200 ± 2</td>
</tr>
<tr>
<td>VM(Sinc(^{p7}))/Dk congenics</td>
<td>s7s7</td>
<td>H-2b</td>
<td>170 ± 2</td>
<td>444 ± 4</td>
</tr>
<tr>
<td>VM × VM(Sinc(^{p7})) congenics F(_{1})</td>
<td>p7s7</td>
<td>H-2b</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>C57BL/FaDk</td>
<td>s7s7</td>
<td>H-2b</td>
<td>168 ± 3</td>
<td>470 ± 4</td>
</tr>
<tr>
<td>VM × C57BL F(_{1})</td>
<td>p7s7</td>
<td>H-2b</td>
<td>250 ± 4</td>
<td>560 ± 8</td>
</tr>
<tr>
<td>VL/Dk</td>
<td>s7s7</td>
<td>H-2b</td>
<td>165 ± 3</td>
<td>449 ± 6</td>
</tr>
<tr>
<td>RIII/FaDk</td>
<td>s7s7</td>
<td>H-2a</td>
<td>163 ± 1</td>
<td>430 ± 8</td>
</tr>
<tr>
<td>IM/Dk</td>
<td>p7p7</td>
<td>H-2b</td>
<td>342 ± 3</td>
<td>210 ± 4</td>
</tr>
<tr>
<td>MB/Dk</td>
<td>p7p7</td>
<td>H-2m</td>
<td>326 ± 3</td>
<td>-</td>
</tr>
</tbody>
</table>

* Dose was 0·02 ml of the low speed supernatant of 1 % homogenate of terminally affected brain. Results are expressed as days ± s.e.m. † Data not yet available.

markedly different incubation period with a given ME7 group scrapie strain than NZW and several other mouse strains. Cross-breeding studies with I and NZW mice demonstrated the control of scrapie incubation period by a mouse gene which was provisionally called Prn-i (Carlson et al., 1986). Carp et al. (1987) have shown using classical genetical tests for allelism and several strains of scrapie agent that the gene in I mice controlling incubation period is Sinc and that I mice carry Sinc\(^{p7}\).

Carlson and colleagues (1986) recently described a restriction fragment length polymorphism (RFLP) linked to the Sinc gene (which they were designating Prn-i). The RFLP was found using XbaI restriction endonuclease and a nucleic acid probe specific for the coding region of PrP gene (now provisionally called Prn-p). In other words they discovered a linkage between Sinc and the gene coding for SAF protein.

We have been able to confirm this finding using the congenic mouse strains VM(Sinc\(^{p7}\)) and VM(Sinc\(^{s7}\)). VM(Sinc\(^{p7}\)) congenic mice were produced by 19 generations of backcrossing to VM mice the progeny of an original VM × C57BL(Sinc\(^{s7}\)) cross, with selection at each generation on the basis of scrapie incubation times. Incubation periods for the scrapie strains ME7 and 22A in the mouse strains used in this study are shown in Table 1 and throughout this paper the term VM refers to Sinc\(^{p7}\) unless otherwise stated.

High molecular weight DNA was extracted from the livers of normal, uninfected mice using a modification of the method of Blin & Stafford (1976), and subjected to Southern analysis (Maniatis et al., 1982) using as a probe pEA974, a cDNA clone made to hamster PrP mRNA (Robakis et al., 1986; Wu et al., 1987). The mouse DNA (15 μg per lane) was digested with XbaI, electrophoresed through a 1 % agarose gel and blotted onto a nitrocellulose membrane in 20 × SSPE (20 × SSPE is 3·6 M-NaCl, 200 mM-NaH\(_{2}\)PO\(_{4}\), 22 mM-EDTA, pH 7·4). For hybridization to pEA974, membranes were incubated for at least 4 h at 42 °C in the following solution: 5 × SSPE, 5 × Denhardt’s solution, 50 % deionized formamide, 0·1 % SDS and 100 μg/ml sheared fish milt DNA. Hybridization was in the same solution for 18 h at 42 °C with the addition of the insert fragment of pEA974 (8 ng/ml), isolated from an agarose gel after digestion with EcoRI and HindIII and labelled to 10\(^{9}\) c.p.s. per μg with \(^{32}\)P using the random primer method (Feinberg & Vogelstein, 1984). Membranes were then washed repeatedly in 1 × SSPE, 0·1 % SDS at 65 °C and exposed to Kodak XAR-5 film at - 70 °C with a Lightning Plus intensifier screen for 2 days.

The hamster PrP probe hybridized to a 5·5 kb XbaI fragment in VM DNA and a 3·8 kb fragment in VM(Sinc\(^{p7}\)) congenics (Fig. 1a). These results are similar to those previously described (Carlson et al., 1986) except that we used congenic mice which differ at the Sinc locus and in regions closely linked to it. VM × VM(Sinc\(^{p7}\)) congenic F\(_{1}\) mice had both the 5·5 kb and
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Zig. 1. Southern analysis of normal mouse DNA restricted with (a) XbaI and (b) TaqI. The mouse strains in both (a) and (b) were: lane 1, RIII; lane 2, VM(Sinc s7) congenics; lane 3, VM × VM(Sinc s7) congenics F1; lane 4, VM; lane 5, VM × C57BL F1; lane 6, C57BL; lane 7, IM; lane 8, VL; lane 9, MB.

Fig. 1. Southern analysis of normal mouse DNA restricted with (a) XbaI and (b) TaqI. The mouse strains in both (a) and (b) were: lane 1, RIII; lane 2, VM(Sinc s7) congenics; lane 3, VM × VM(Sinc s7) congenics F1; lane 4, VM; lane 5, VM × C57BL F1; lane 6, C57BL; lane 7, IM; lane 8, VL; lane 9, MB.

the 3.8 kb fragments. The mouse strain (C57BL) which donated the s7 allele to the congenics also had the 3.8 kb fragment and other Sinc s7 inbred mice (IM and MB which were derived from VM crosses) had the 5.5 kb XbaI fragment. No such RFLP has been shown with several other enzymes (BamHI, PvuII, HindIII, EcoRI, PstI, KpnI, SstI). On this evidence the two genes PrP and Sinc appear to be closely linked and could even be the same gene.

However the 5.5 kb fragment is not a marker for the p7 allele because the two unrelated mouse strains RIII and VL, both homozygous for Sinc s7, showed the 5.5 kb XbaI fragment. Any possibility that this fragment was produced by a polymorphism different to that found in VM mice is made even less likely by the results of double digestion experiments. Mouse DNA was digested with pairs of restriction enzymes and hybridized to pEA974 as before. RIII, VL and VM DNA gave the same pattern of hybridization with the following enzyme pairs: XbaI with EcoRI, HindIII, SstI or PstI; SstI with EcoRI, HindIII, PstI or KpnI; PstI with HindIII or EcoRI; HindIII with EcoRI (data not shown). It may be that in RIII and VL mice (Sinc s7 but
Fig. 2. Southern analysis of normal mouse DNA restricted with *HhaI*. Lanes 1 to 5, liver DNA; lanes 6 to 8, brain DNA. The mouse strains were: lane 1, VM(Sinc*7) congenics; lane 2, VM × VM(Sinc*7) congenics F1; lane 3, VM; lane 4, RIII; lane 5, VL; lane 6, VM(Sinc*7) congenics; lane 7, VM; lane 8, RIII.

Fig. 3. Physical map of the region coding for PrP in the mouse genome. The boxed area indicates the region of transcribed sequence and the translated section is shown in black. *XbaI polymorphic site.

with the 5.5 kb *XbaI fragment) there has been a crossover between Sinc and the PrP gene. However it is also possible that the differences have arisen as a result of independent mutations. Cloning and sequencing of the genes and flanking regions from each mouse strain or large scale family studies would be needed to distinguish between these possibilities.

In a search for other RFLPs, it was found that pEA974 hybridized to a 9.6 kb *TaqI fragment in the s7 mouse lines studied [RIII, VM(Sinc*7) congenics, C57BL, VL] and to an 8-1 kb *TaqI fragment in the p7 lines (VM, IM, MB). VM × VM(Sinc*7) congenics F1 and VM × C57BL F1 had both (9.6 and 8.1 kb) fragments (Fig. 1b).

Digestion with *HhaI* and Southern analysis using pEA974 as a probe also showed differences among the mouse strains (Fig. 2). *HhaI* recognizes the sequence GCGC in DNA but does not cleave it when the internal cytosine residue is methylated. The doublet CpG is rare in vertebrate DNA but, when it does occur, the cytosine is usually (60 to 90%) methylated (Bird, 1986). However, CpG is both abundant and unmethylated in the ‘HTF islands’ which occur at the 5’ end of ‘housekeeping’ genes. There is evidence that this type of gene is inactive if the islands are methylated although it is unclear whether methylation is the primary inactivating factor. Methylation of CpG in non-island regions shows no such obvious correlation with inactivity (Bird, 1986). The promoter region of the hamster PrP gene contains clusters of CpG and resembles the promoter of a housekeeping gene (Basler et al., 1986).

After digestion with *HhaI*, fragments in mouse liver DNA hybridizing to pEA974 (Fig. 2) were > 30 kb with VM(Sinc*7) congenics (and with C57BL; not shown), 20 kb with VM (and with IM and MB, not shown), and 20 kb and 9.4 kb with RIII and VL. DNA was also prepared from uninfected mouse brain using the same method as before and digested with *HhaI*. VM and VM(Sinc*7) congenic brain DNA gave essentially the same hybridization patterns with pEA974 as did liver DNA (Fig. 2); however, with RIII brain DNA the 20 kb fragment showed up much more strongly than in liver and the 9-4 kb fragment very much more faintly. This finding was confirmed with other RIII brain and liver DNA preparations. Because of the methylation sensitivity of *HhaI* these results probably indicate differences in methylation patterns rather than in DNA sequence, and that in RIII mice the methylation pattern around the PrP gene in brain differs from that in the liver. However levels of PrP mRNA are high in mouse brain and undetectable by Northern analysis in RML outbred mouse liver (Chesebro et al., 1985). Although the levels of PrP mRNA have not yet been determined in RIII liver, it is unlikely that in this case expression of the PrP gene is correlated with a lower level of DNA methylation. This
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is simply another connection between Sinc and PrP although its functional significance and its relationship to the action of the Sinc gene require further investigation.

We have constructed a provisional map of the mouse PrP gene region using restriction enzyme data (Fig. 3). In doing so, we have assigned the direction of transcription by comparison with the sequence of RML outbred mouse PrP cDNA (Locht et al., 1986). We have also assumed that the mouse PrP gene structure is similar to that of the hamster PrP gene (Basler et al., 1986) in having only one very large intron. The boxed region in Fig. 3 represents the second exon of the gene. On this basis the polymorphic Xbal site was located in the 3' flanking region of the PrP gene. The TaqI sites have not yet been mapped.

We conclude that the mice used in this study could be divided into three groups on the basis of hybridization to the hamster PrP cDNA (pEA974): (i) Sinc<sup>+</sup> with a 5.5 kb Xbal fragment, an 8.1 kb TaqI fragment and highly methylated HhaI sites in liver and brain DNA (VM, IM, MB); (ii) Sinc<sup>+</sup> with a 3.8 kb XbaI fragment, a 9.6 kb TaqI fragment and very highly methylated HhaI sites in liver and brain DNA [VM(Sinc<sup>+</sup>) congenics, C57BL]; (iii) Sinc<sup>+</sup> with a 5.5 kb XbaI fragment, a 9.6 kb TaqI fragment and a lower level of methylation at HhaI sites in liver than in brain DNA (RIII and VL).

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REFERENCES


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