Are Sinc and the PrP gene congruent? Evidence from PrP gene analysis in Sinc congenic mice

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Scrapie, a transmissible neurodegenerative disorder found naturally in sheep and goats, has been studied extensively in a range of laboratory models in mice. The major gene controlling the incubation period of scrapie in mice is Sinc (Dickinson et al., 1968) which has two alleles, s7 and p7. Almost all strains of mice carry Sinc⁷; those known to be Sinc⁷ include VM/Dk, IM/Dk, MB/Dk, I/LnJ and P/J (Dickinson & Outram, 1988; Carlson et al., 1988).

Sinc congenic mice have been produced by introducing the s7 allele into a VM/Dk mouse (Sinc⁷) background and selecting on the basis of incubation period of disease following inoculation with the ME7 strain of scrapie (Hunter et al., 1987; Bruce et al., 1991). The biological properties of seven different scrapie strains in the Sinc congenic lines have also been established (Bruce et al., 1991).

Scrapie-associated fibrils (SAFs) are structures characteristic of the transmissible spongiform encephalopathies (Merz et al., 1981). SAFs are composed of PrP, a host-coded sialoglycoprotein which becomes partially resistant to proteases in all scrapie-like diseases, unlike normal PrP protein which is protease-sensitive (Oesch et al., 1985; Hope et al., 1986). This property has potential as a pre-mortem (Ikegami et al., 1991) and post-mortem (Farquhar et al., 1990) scrapie diagnostic test.

Restriction fragment length polymorphisms (RFLPs) of the PrP gene are linked to alleles of Prn-i (a synonym of Sinc) (Carlson et al., 1986). I/LnJ (Sinc⁷p⁷) and NZW (Sinc⁷p⁷) mice have also been shown to have differences in the coding region of their PrP genes predicted to result in amino acid differences in the PrP protein sequence (Westaway et al., 1987). PrP linked to Sinc⁷ (PrP-Sinc⁷) has a threoneine codon at position 189 and leucine at 108; PrP linked to Sinc⁷ (PrP-Sinc⁷) has valine and phenylalanine codons, respectively, at these positions. The valine/threonine difference (detectable at the DNA level using BstEII) is present in a wide range of Sinc⁷ mouse strains and absent from the three Sinc⁷ strains tested. (Carlson et al., 1988). This work and recent transgenic mouse studies (Prusiner et al., 1990) have suggested that PrP is a protein product of the Sinc locus, but there have been a few possible individual PrP/Sinc recombinant mice reported (Carlson et al., 1988; Race et al., 1990) and there is also transgenic evidence that the congruence of the two genes has not yet been demonstrated with absolute certainty (Westaway et al., 1991).

Preliminary studies on the Sinc congenic mice (described henceforth as VM and VM-Sinc⁷) have demonstrated that there are also RFLPs of the PrP genes in these mouse strains (Hunter et al., 1987), and in this paper we have extended the analysis to the PrP gene protein-coding region and flanking DNA.

Incubation periods of ME7 and 22A scrapie strains in mouse strains of different Sinc genotypes (VM, VM-Sinc⁷, VM × VM-Sinc⁷ F1, C57BL and C57BL × VM F1) are shown in Table 1. This set of data is new to this paper, but injection methods are as described in Hunter et al. (1987) and Bruce et al. (1991). VM-Sinc⁷ mice have a shorter incubation period with ME7 scrapie than VM mice using either intracerebral (i.c.) or intraperitoneal (i.p.) injection and are therefore similar to C57BL mice,
Table 1. Incubation period of ME7 and 22A scrapie in various mouse strains

<table>
<thead>
<tr>
<th>Scrapie strain*</th>
<th>Route of infection</th>
<th>Mouse strain</th>
<th>Sinc</th>
<th>Incubation period (days)</th>
<th>Number</th>
<th>s.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>ME7 i.c.</td>
<td>VM</td>
<td>p7p7</td>
<td>357</td>
<td>40</td>
<td>21.6</td>
<td></td>
</tr>
<tr>
<td>C57BL</td>
<td></td>
<td>s7s7</td>
<td>167</td>
<td>11</td>
<td>6.4</td>
<td></td>
</tr>
<tr>
<td>VM-Sinc</td>
<td></td>
<td>s7s7</td>
<td>175</td>
<td>40</td>
<td>12.7</td>
<td></td>
</tr>
<tr>
<td>C57BL × VM (F1)</td>
<td></td>
<td>s7p7</td>
<td>250</td>
<td>11</td>
<td>7.3</td>
<td></td>
</tr>
<tr>
<td>VM × VM-Sinc (F1)</td>
<td></td>
<td>s7p7</td>
<td>245</td>
<td>4</td>
<td>16.5</td>
<td></td>
</tr>
<tr>
<td>ME7 i.p.</td>
<td>VM</td>
<td>p7p7</td>
<td>553</td>
<td>9</td>
<td>16.2</td>
<td></td>
</tr>
<tr>
<td>C57BL</td>
<td></td>
<td>s7s7</td>
<td>284</td>
<td>10</td>
<td>10.7</td>
<td></td>
</tr>
<tr>
<td>VM-Sinc</td>
<td></td>
<td>s7s7</td>
<td>312</td>
<td>10</td>
<td>24.4</td>
<td></td>
</tr>
<tr>
<td>C57BL × VM (F1)</td>
<td></td>
<td>s7p7</td>
<td>411</td>
<td>9</td>
<td>23.3</td>
<td></td>
</tr>
<tr>
<td>VM × VM-Sinc (F1)</td>
<td></td>
<td>s7p7</td>
<td>425</td>
<td>6</td>
<td>15.1</td>
<td></td>
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<tr>
<td>22A i.c.</td>
<td>VM</td>
<td>p7p7</td>
<td>197</td>
<td>32</td>
<td>15.9</td>
<td></td>
</tr>
<tr>
<td>C57BL</td>
<td></td>
<td>s7s7</td>
<td>469</td>
<td>9</td>
<td>22.3</td>
<td></td>
</tr>
<tr>
<td>VM-Sinc</td>
<td></td>
<td>s7s7</td>
<td>453</td>
<td>27</td>
<td>21.9</td>
<td></td>
</tr>
<tr>
<td>C57BL × VM (F1)</td>
<td></td>
<td>s7p7</td>
<td>571</td>
<td>8</td>
<td>15.5</td>
<td></td>
</tr>
<tr>
<td>VM × VM-Sinc (F1)</td>
<td></td>
<td>s7p7</td>
<td>490</td>
<td>11</td>
<td>17.9</td>
<td></td>
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<tr>
<td>22A i.p.</td>
<td>VM</td>
<td>p7p7</td>
<td>345</td>
<td>6</td>
<td>21.9</td>
<td></td>
</tr>
<tr>
<td>C57BL</td>
<td></td>
<td>s7s7</td>
<td>660</td>
<td>8</td>
<td>35.8</td>
<td></td>
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<tr>
<td>VM-Sinc</td>
<td></td>
<td>s7s7</td>
<td>ND†</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C57BL × VM (F1)</td>
<td></td>
<td>s7p7</td>
<td>ND</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VM × VM-Sinc (F1)</td>
<td></td>
<td>s7p7</td>
<td>700</td>
<td>1</td>
<td></td>
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</tbody>
</table>

* Dose, 0.02 ml of a 1% homogenate of brain from a mouse terminally affected with ME7 or 22A scrapie. Each set of scrapie inoculations was carried out on the same day (different days for ME7 and 22A).
† ND, Not done.

the original s7 allele donor strain. The congenic mice also show an incubation time phenotype 'reversal' with 22A scrapie in that VM mice have a relatively short incubation period after i.c. injection with this scrapie strain compared with VM-Sinc s7 and C57BL mice. The results with i.p. injection (Table 1), owing to the long incubation periods, are not so easy to obtain. The F1 progeny, VM × VM-Sinc and C57BL × VM (of Sinc genotype s7p7), are intermediate between the two parental mouse strains in incubation period after i.c. and i.p. injection with ME7 scrapie, but have longer incubation periods with 22A scrapie (i.c.) than either parent. This latter phenomenon (overdominance) has been described previously (Dickinson & Meikle, 1971; Bruce et al., 1991).

Comparison of the 22A scrapie incubation period after i.c. injection of C57BL × VM F1 mice (571 days) with that of VM × VM-Sinc s7 F1 mice (490 days) shows an apparently significant difference of approximately 80 days (Table 1). This effect is minor compared with that of the Sinc gene, but demonstrates that genes other than Sinc have a small but significant influence on incubation period. The congenic mice were bred in order to study Sinc gene control of scrapie replication without the complicating effects of other less important genes.

SAF preparations from brains of congenic mice with terminal scrapie were examined by PAGE and silver staining as described by Hope et al. (1986). Fig. 1 shows that the resulting PrP protein, which makes up the SAF, is resistant to proteinase K treatment and that there is
little difference apparent at this level of analysis between the PAGE patterns of the abnormal scrapie-specific PrP proteins from VM and VM-Sinc<sup>7</sup> mice. PAGE patterns of SAF/PrP are independent of Sinc genotype and fall into at least three main groups depending on the infecting strain of scrapie used: ME7-like, 139A-like or 87V-like (Kasczas et al., 1986). The SAF/PrP preparations shown in Fig. 1 were taken from mice affected by the 22L strain of scrapie, which is in the ME7-like protein group.

In a comparison of the PrP gene regions in the Sinc congenic mouse lines, DNA extracted from livers of VM, VM-Sinc<sup>7</sup> and C57BL mice was digested with BstEII. Following Southern blot analysis as in Hunter et al. (1987) using a hamster PrP probe (Robakis et al., 1986; Wu et al., 1987), hybridization to a 15-5 kb fragment in VM mouse DNA and to 5-7 and 3-5 kb fragments in VM-Sinc<sup>7</sup> and C57BL mouse DNAs (Fig. 2a) was detected. The F1 cross mice showed all three fragments (not shown). These results indicate that in the PrP gene region, VM-Sinc<sup>7</sup> mice differ from VM mice in having the two BstEII restriction sites (one of which is in the PrP coding region, see Fig. 3) previously described as markers for Sinc<sup>7</sup> (also known as Prn-F) strains (Westaway et al., 1987), and confirms the position 189 amino acid difference between the two mouse strains. VM-Sinc<sup>7</sup> mice also have the same XbaI (3-8 kb), TaqI (9-6 kb) and EcoRI (4-2 kb) polymorphic fragments as C57BL mice (Hunter et al., 1987; Carlson et al., 1988), which differ from those of VM mice (5-5 kb, 8-1 kb and 9-4 kb respectively).

For DNA sequencing, the PrP gene protein-coding sequences were isolated by polymerase chain reaction (PCR) amplification of the mouse genomic DNA (Saiki et al., 1988) using primers based on the published PrP gene sequences from the NZW and I/Ln inbred mouse lines (Westaway et al., 1987). DNA sequence analysis was performed either by cloning of the PCR-amplified product (VM-Sinc<sup>7</sup> mouse PrP gene) and then sequencing supercoiled plasmid DNA on both strands using a United States Biochemical Sequenase Version 2.0 DNA sequencing kit or by direct sequencing of the PCR-amplified product (VM mouse PrP gene) using a modification of the method of Thein (1990). Various sequencing primers were used, taken from the published sequence (Westaway et al., 1987).

DNA sequence analysis of the PrP gene protein-coding regions from VM and VM-Sinc<sup>7</sup> congenic mice demonstrated that the two sequences were identical to those found by Westaway et al. (1987) in I/Ln (Sinc<sup>7</sup>) and NZW (Sinc<sup>4</sup>) mice, respectively (data not shown). Therefore there are two predicted amino acid residue differences between PrP–Sinc<sup>7</sup> and PrP–Sinc<sup>4</sup> at amino acids 108 and 189 in the Sinc congenic mice. This suggests that the transfer of the Sinc<sup>7</sup> gene from C57BL to VM mice has been accompanied by a transfer of the PrP gene, confirms the close genetic linkage of PrP and Sinc, and lends support to the growing number of studies suggesting that the two genes are congruent.

However, closer examination advises caution for the following reasons. First, the likely common origin of the Sinc<sup>7</sup> mouse strains (Carlson et al., 1989) warns that the apparently significant differences in PrP gene sequence in Sinc<sup>7</sup> and Sinc<sup>4</sup> mice could be the result of a founder effect. Second, VM-Sinc<sup>7</sup> congenic mice were produced by 19 generations of backcrossing to VM mice, the progeny of an original VM × C57BL(Sinc<sup>7</sup>) cross, with selection at each generation on the basis of incubation period with ME7 scrapie. Congenic strains, in general, differ by 200/t centiMorgans (cM) (where t is the number of crosses) around the selected locus (Falconer, 1989). After only 19 generations of backcrossing, the Sinc congenic mice could still differ by as much as 10 cM around the Sinc locus. (The size of the mouse genome is estimated to be 1600 cM (Roderick & Davisson, 1981).) Carlson et al. (1989) have calculated from a small number of possible Sinc/PrP recombinant F2 mice that the two genes could be 4±8 ± 1-3 (s.e.) cM apart: well within this range.

Therefore it was important (despite the theory) to establish directly whether the Sinc congenic lines actually did differ by having sufficient DNA around the PrP gene to accommodate a second linked gene. Longer range restriction mapping was carried out by digesting VM, VM-Sinc<sup>7</sup> and C57BL mouse DNAs with HhaI and...
electrophoresing on 1% agarose gels on the Beckman Transverse Alternating Field Electrophoresis (TAFE) system which is designed to size very large DNA fragments. This was followed with Southern blot analysis using a PrP gene probe as before (Fig. 2b). An apparent polymorphism had been noted with HhaI previously (Hunter et al., 1987), but the fragments were not sized accurately. As HhaI is a methylation-sensitive enzyme, this polymorphism may be a result of methylation rather than sequence differences. Inheritance of methylation patterns has been described previously (Cedar, 1988).

As in Hunter et al. (1987), hybridization of the hamster PrP gene probe to HhaI digestion products was smeared (thought to be due to methylation differences in brain tissue used as a source of DNA), but fragments of approximately 50 kb in VM mouse DNA are clearly smaller than those fragments (approximately 100 kb) in both C57BL and VM-Sinc<sup>S7</sup> mouse DNAs (Fig. 2b). (Undigested DNA made by the method used here is about 300 kb.) This was reproducible in several separate DNA preparations. C57BL and VM-Sinc<sup>S7</sup> mouse DNA was found to be different from that of other Sinc<sup>S7</sup> mouse strains (e.g. RI1/FaDk, VL/Dk, LM/Dk and C3H/LaDk) which have much smaller HhaI fragments (Hunter et al., 1987 and unpublished observations). Double digestions with HhaI and KpnI, HindIII or SstI indicated that all three strains (VM, VM-Sinc<sup>S7</sup> and C57BL) have an unmethylated HhaI site approximately 1.5 kb downstream from the PrP coding sequence (Fig. 3). These results suggest that the transfer of genetic material from C57BL to VM mice, to form the new strain VM-Sinc<sup>S7</sup>, involved at least 100 kb of DNA mostly 5' of (but including) the PrP gene. We do not have information which allows us to comment on the 5' or 3' limits of this transfer, but it is clear that there is room for another Sinc locus product within this region.

Experiments using mice transgenic for PrP gene variants (Prusiner et al., 1990; Hsiao et al., 1990) suggest that the introduced PrP genes influence the incubation period of disease and can even result in spontaneous disease. However, such transgenic studies may be complicated by the introduction of multiple PrP gene copies and have not completely ruled out the possibility of PrP and Sinc being separate genes. Westaway et al. (1991) have reported that transgenic mice with multiple copies of the PrP–Sinc<sup>S7</sup> gene on a Sinc<sup>S7</sup>X<sup>S7</sup> background have a markedly shorter incubation period with experimental scrapie than the non-transgenic mice, instead of the longer incubation period which they expected. Since the transgene was inserted using a 40 kb cosmid clone, they could not rule out the possibility of a second gene lying within the region flanking the PrP gene. There is also the added complication of 'anti-PrP' (Goldgaber, 1991), a putative protein product of the opposite strand of the PrP gene which has a very large open reading frame. The existence of such a protein or mRNA transcript is the subject of some controversy (Manson & Hope, 1991; Hewinson et al., 1991), but if anti-PrP exists in mice it would also differ in sequence in each Sinc congenic strain.

The Sinc congenic mice, produced by a classical genetic breeding programme, lend support to the more recent transgenic studies which suggest (but do not prove) that Sinc and PrP are congruent. Second generation transgenic experiments, such as those reported by Bueler et al. (1992), use homologous recombination to alter only the endogenous PrP gene, thus avoiding complications of multiple gene copies, and may finally answer the question: are PrP and Sinc congruent?

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References


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