The disease characteristics of different strains of scrapie in Sinc congenic mouse lines: implications for the nature of the agent and host control of pathogenesis

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Introduction

Scrapie, a transmissible neurological disease of sheep and goats, is the best understood member of a group of closely similar diseases which includes Creutzfeldt-Jakob disease and Gerstmann-Sträussler syndrome in humans and bovine spongiform encephalopathy. Scrapie has been studied extensively in a range of laboratory models in mice and hamsters. Many distinct strains of scrapie have been identified in mice, differing in their incubation period characteristics, pathology, clinical features and physicochemical properties (Dickinson & Meikle, 1971; Dickinson & Fraser, 1977; Dickinson & Outram, 1988; Bruce & Dickinson, 1987; Fraser, 1976; Bruce et al., 1976; Carp et al., 1984; Kimberlin et al., 1983). Scrapie strain variation has also been observed in other experimental species such as goats (Pattison & Millson, 1961), sheep (Foster & Dickinson, 1988) and hamsters (Kimberlin & Walker, 1978 a; Kimberlin et al., 1989).

The incubation period of the disease in mice is long, ranging from 4 months to over 2 years for the various models, but is remarkably predictable. Its length depends on both the strain of scrapie agent and genetic factors in the host. The major gene controlling incubation period in mice is the Sinc gene (scrapie incubation), two alleles of which have been identified (s7 and p7) (Dickinson et al., 1968; Dickinson & Meikle, 1971; Dickinson & Fraser, 1979; Dickinson & Outram, 1988). Other genes also modify the incubation period to some extent but their effects are small compared with that of Sinc (Outram, 1976; Kingsbury et al., 1983; Bruce & Dickinson, 1985; Carp & Callahan, 1986; Carlson et al., 1988; Race et al., 1990); the Sinc gene closely regulates the incubation period for all scrapie strains so far tested (Dickinson & Outram, 1979, 1988). However, scrapie strains differ in which of the two Sinc homozygotes has the shorter incubation period and also in the apparent type of dominance shown by the two alleles (Dickinson & Meikle, 1971; Dickinson & Outram, 1979). It is not yet known how the Sinc gene exerts these effects but it is clear that its gene product plays a key role in the pathogenesis of the disease.

The Sinc gene was first recognized about 25 years ago...
in a random-bred mouse colony at the Moredun Institute, Edinburgh, U.K. (Dickinson & Mackay, 1964; Dickinson et al., 1968). Selective inbreeding from this colony on the basis of scrapie incubation period resulted in the production of the VM mouse strain, which is homozygous for the p7 allele of Sinc. Almost all other laboratory mouse strains tested have been shown to carry the s7 allele of Sinc (Dickinson & Mackay, 1964; Outram, 1976; Kingsbury et al., 1983; Carp & Callahan, 1986; Carp et al., 1987) [recently some authors have referred to the Sinc gene by the unofficial designation Prn-i; see Mouse Newsletter, July 1987, no. 78, p. 3]. The present paper describes the production of congenic mouse lines which differ only in the vicinity of the Sinc locus. This has been achieved by introducing the s7 allele into a VM background, selecting on the basis of the incubation period of the disease produced by a particular scrapie strain, ME7.

Strains of scrapie differ in the pattern of their incubation periods in the three Sinc genotypes of mouse. They also differ markedly in the severity and distribution of vacuolar degeneration they produce in the brain. This has led to an independent method of strain discrimination in which vacuolar degeneration is scored in a number of specified brain areas to construct a 'lesion profile' (Fraser & Dickinson, 1968; Fraser, 1976). The availability of Sinc congenic lines has made it possible to minimize the minor effects of other genes on incubation periods and lesion profiles. In this report we describe the biological properties of seven scrapie strains in the Sinc congenic lines.

**Methods**

*Mouse strains.* Two fully inbred strains were used in the production of the VM-Sinc<sup>p7</sup> strain. These were VM/Dk (referred to as VM in this paper), which is homozygous for the p7 allele of Sinc, and C57BL/FaBtDk (referred to as C57BL), which is homozygous for the s7 allele. Both of these mouse strains are of the H-<sup>2</sup> major histocompatibility haplotype. The VM strain was at the 21st inbred generation at the start of the selective breeding procedure, described in detail in Results. The characteristics of the VM-Sinc<sup>p7</sup> congenic strain were later compared with those of VM, C57BL and the F<sub>1</sub> crosses, VM × VM-Sinc<sup>p7</sup> and VM × C57BL. Male and female mice were used in approximately equal numbers.

*Scrapie strains.* Seven scrapie strains were used, ME7, 22A, 22C, 22L, 79A, 87V and 139A (Table 1). These strains have been isolated and on a scale of 0 to 3 in three white matter areas. For presentation to the 5 scale to make them visually comparable with grey matter scores. Centrifuged at 500 g for 10 min. Aliquots were stored at −20 °C and thawed and reground before injection. Mice were injected either intracerebrally (i.c.) or intraperitoneally (i.p.) with 0.02 ml of inoculum when they were 3 to 8 weeks old.

*Incubation period measurement.* Injected mice were coded and scored weekly for neurological signs of scrapie. They were killed at a defined clinical endpoint, when they were in extremis or when they had exhibited severe clinical signs for 3 consecutive weeks (Dickinson et al., 1968). After decoding, incubation periods were calculated as the interval between injection and this defined endpoint. This method of incubation period measurement has been shown to give highly repeatable results for a wide range of combinations of scrapie strain and mouse strain.

**Histopathological procedures.** Mice were sacrificed by cervical fracture and their brains were immersion-fixed in 10% formal saline. Sections (6 μm) at four standard coronal levels were stained with haematoxylin and eosin. Vacuolar degeneration was scored from coded sections on a scale of 0 to 5 in nine defined grey matter areas of brain, and on a scale of 0 to 3 in three white matter areas. For presentation purposes, white matter scores were plotted over the same range as the 0 to 5 scale to make them visually comparable with grey matter scores. After decoding, 'lesion profiles' were constructed from the average score in each area, as previously described (Fraser & Dickinson, 1968; Fraser, 1976).

**Results**

*Production of Sinc congenic lines*

To produce Sinc congenic lines, the s7 allele from the C57BL donor strain was introduced into VM by serially backcrossing Sinc<sup>p7</sup> heterozygotes to the VM(Sinc<sup>p7</sup>)

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**Table 1. Details of the seven scrapie strains used in this study**

<table>
<thead>
<tr>
<th>Scrapie strain</th>
<th>Origin*</th>
<th>Mouse strain used for serial passage</th>
</tr>
</thead>
<tbody>
<tr>
<td>ME7†</td>
<td>Suffolk sheep, natural scrapie</td>
<td>C57BL</td>
</tr>
<tr>
<td>22C†</td>
<td>Cheviot sheep, SSBP/1 experimental scrapie</td>
<td>C57BL</td>
</tr>
<tr>
<td>22L</td>
<td>Cheviot sheep, SSBP/1 experimental scrapie</td>
<td>C57BL</td>
</tr>
<tr>
<td>79A†</td>
<td>Goat, 'drowsy' experimental scrapie</td>
<td>C57BL</td>
</tr>
<tr>
<td>139A</td>
<td>'Chandler' mouse isolate originally from 'drowsy' goat scrapie</td>
<td>C57BL</td>
</tr>
<tr>
<td>22A†</td>
<td>Cheviot sheep, SSBP/1 experimental scrapie</td>
<td>VM</td>
</tr>
<tr>
<td>87V†</td>
<td>Cheviot × Border Leicester sheep, natural scrapie</td>
<td>VM</td>
</tr>
</tbody>
</table>

* Animal that the scrapie strain was isolated from and the type of scrapie that it had (Dickinson, 1976).
† Strains previously cloned by between two and four sequential passages at limiting dilution for infectivity (Dickinson & Outram, 1983).
Fig. 1. Breeding strategy used for the production of the VM-Sinc<sup>s7</sup> congenic line, showing the Sinc<sub>gen</sub> genotype of mice at each generation. Genotypes of individual mice in generations marked with an asterisk were determined by crossing them with VM mice (Sinc<sup>pT</sup>) and testing their progeny with ME7 scrapie. Genotypes of individual mice in the generation marked † were determined by crossing them with Sinc<sup>s7</sup> mice and testing their progeny with ME7 scrapie.

parental strain (Fig. 1). Mice carrying the s7 allele were selected on the basis of the incubation periods seen after i.c. infection with the ME7 strain of scrapie. This was possible because there are large differences between the incubation periods in the three Sinc<sub>gen</sub> genotypes with this scrapie strain; for example, in a previous experiment in which mice were injected i.c. with 1% ME7 inoculum, the mean incubation periods ± S.E.M., based on 12 to 16 animals, were 171 ± 2 days for C57BL(Sinc<sup>s7</sup>), 328 ± 4 days for VM(Sinc<sup>pT</sup>) and 251 ± 2 days for the F<sub>1</sub> cross, VM × C57BL(Sinc<sup>pT</sup>).

At each backcross generation, approximately 30 mice of as yet unknown genotype (either Sinc<sup>s7</sup> or Sinc<sup>pT</sup>) were mated with VM(Sinc<sup>pT</sup>) mice. The first ten offspring of each mating were injected i.c. with ME7 scrapie after weaning. Sinc<sup>pT</sup> parents were clearly identified from the bimodal distribution of incubation periods in this first set of offspring (e.g. Fig. 2). The subsequent progeny of Sinc<sup>s7</sup> × VM matings were selected for the next backcrossing to VM. At the 19th generation (i.e. the 18th backcross generation), genotypes of individual mice were determined as above. Nine Sinc<sup>pT</sup> mice were identified, six females and three males, and these were mated amongst themselves to produce progeny representing all three Sinc<sub>gen</sub> genotypes. The individual genotypes of these 20th generation mice were in turn determined by mating them

Fig. 2. Distribution of ME7 i.c. incubation periods in the first set of progeny of matings between VM and 26 mice from the 14th generation. (a) The offspring of 14 Sinc<sup>s7p7</sup> parents were either Sinc<sup>s7</sup> or Sinc<sup>pT</sup> and showed a bimodal distribution of incubation periods. Subsequent offspring of these matings were selected for the next backcrossing to VM. (b) The offspring of 12 Sinc<sup>pT</sup> parents were all Sinc<sup>pT</sup> and showed a unimodal distribution of incubation periods.

Fig. 3. Distribution of ME7 i.c. incubation periods in the progeny of matings between inbred Sinc<sup>s7</sup> mice and 15 mice from the 20th generation. (a) The offspring of three Sinc<sup>s7</sup> parents were all Sinc<sup>s7</sup> and showed a unimodal distribution of incubation periods. Of the three Sinc<sup>s7</sup> parents identified, two were mated with each other to form the basis of the VM-Sinc<sup>s7</sup> line. (b) The offspring of 11 Sinc<sup>s7p7</sup> parents were either Sinc<sup>s7</sup> or Sinc<sup>pT</sup> and showed a bimodal distribution of incubation periods. (c) The offspring of one Sinc<sup>pT</sup> parent were all Sinc<sup>s7p7</sup> and showed a unimodal distribution of incubation periods.

with inbred Sinc<sup>s7</sup> mice and testing their offspring with ME7 scrapie (Fig. 3). In this way we identified three Sinc<sup>s7</sup> mice in the 20th generation, two of which were
mated with each other to form the basis of the VM-Sinc<sup>s7</sup> line; the line was subsequently maintained by full-sibling mating. At no stage during the whole selective breeding procedure were mice which were incubating scrapie used for breeding purposes.

**Disease characteristics of scrapie strains in Sinc congenic lines**

(i) **Incubation periods**

Previous experiments have shown that each of the seven scrapie strains used, injected i.c. as 1 ~ brain inocula under standard experimental conditions, has a characteristic pattern of incubation periods in C57BL, VM and VM × C57BL mice (Table 2). In a later set of experiments, VM-Sinc<sup>s7</sup>, C57BL, VM, VM × C57BL and VM × VM-Sinc<sup>s7</sup> mice were injected i.c. or i.p. with these scrapie strains under the same standard conditions but using 1 ~o inocula prepared from different 'donor' mouse brains. With this dose all mice develop clinical scrapie except Sinc<sup>~7</sup> and Sinc<sup>s7p7</sup> mice injected with 87V scrapie (see below). The incubation periods observed in these experiments are listed in Table 2, together with previous results in VM, C57BL and VM × C57BL mice. For ease of comparison between groups, the data in Table 2 are also presented graphically in Fig. 4. The i.c. incubation periods in C57BL, VM and VM × C57BL mice were closely similar to those in the previous experiments, illustrating the high degree of repeatability of incubation period measurement. For each scrapie strain the incubation period in VM-Sinc<sup>s7</sup> was closest to that in C57BL mice. However, there were clear differences between these two mouse strains and between the two F<sub>1</sub> crosses, VM × VM-Sinc<sup>s7</sup> and VM × C57BL, particularly with the 79A, 139A and 22A scrapie strains. These differences were within the range previously reported for unrelated mouse strains of the same Sinc genotype (Outram, 1976; Kingsbury et al., 1983; Bruce & Dickinson, 1985; Carp & Callahan, 1986). This adds to existing evidence that genes other than Sinc can make some contribution to the differences seen between non-congenic mouse strains. The availability of VM-Sinc<sup>s7</sup> congenic mice makes it possible to study the effect of the Sinc gene, minimizing the complication of effects of these other genes.

The results shown in Table 2 and Fig. 4 confirm that the Sinc gene has an overwhelming influence on the timing of events in scrapie pathogenesis. The largest effect was seen with the 87V strain of scrapie, which had an incubation period of 290 + 4 days in i.c. infected Sinc<sup>p7</sup> mice but did not produce clinical disease within the lifespan of mice of other Sinc genotypes. However, two of seven VM-Sinc<sup>s7</sup> mice and one of seven C57BL mice surviving longer than 700 days after injection with 87V had characteristic scrapie brain lesions when killed.

### Table 2. Mean incubation period for different combinations of scrapie strain and mouse strain or cross

<table>
<thead>
<tr>
<th>Route of infection</th>
<th>Scapie strain</th>
<th>VM</th>
<th>C57BL</th>
<th>VM × C57BL</th>
<th>VM-Sinc&lt;sup&gt;s7&lt;/sup&gt;</th>
<th>VM × VM-Sinc&lt;sup&gt;s7&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>I.c. (previous data)</td>
<td>ME7</td>
<td>328 ± 4(14)*</td>
<td>171 ± 2(16)</td>
<td>251 ± 2(12)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>22C</td>
<td>459 ± 3(11)</td>
<td>182 ± 1(18)</td>
<td>269 ± 4(16)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>22L</td>
<td>208 ± 1(16)</td>
<td>148 ± 1(17)</td>
<td>189 ± 1(17)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>79A</td>
<td>301 ± 6(10)</td>
<td>158 ± 2(14)</td>
<td>280 ± 4(24)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>139A</td>
<td>201 ± 3(20)</td>
<td>155 ± 1(17)</td>
<td>249 ± 3(19)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>22A</td>
<td>203 ± 3(16)</td>
<td>466 ± 4(23)</td>
<td>587 ± 7(18)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>87V</td>
<td>290 ± 3(15)</td>
<td>&gt; 700(20)†</td>
<td>&gt; 700(27)†</td>
<td></td>
<td></td>
</tr>
<tr>
<td>I.c.</td>
<td>ME7</td>
<td>346 ± 3(84)</td>
<td>168 ± 2(19)</td>
<td>252 ± 3(19)</td>
<td>178 ± 1(84)</td>
<td>259 ± 4(19)</td>
</tr>
<tr>
<td></td>
<td>22C</td>
<td>447 ± 9(7)</td>
<td>170 ± 1(6)</td>
<td>269 ± 3(6)</td>
<td>163 ± 1(20)</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>22L</td>
<td>210 ± 3(11)</td>
<td>157 ± 1(11)</td>
<td>192 ± 2(8)</td>
<td>147 ± 1(16)</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>79A</td>
<td>309 ± 4(16)</td>
<td>161 ± 2(10)</td>
<td>276 ± 5(11)</td>
<td>153 ± 2(29)</td>
<td>230 ± 1(6)</td>
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<tr>
<td></td>
<td>139A</td>
<td>208 ± 2(12)</td>
<td>166 ± 2(20)</td>
<td>270 ± 4(9)</td>
<td>137 ± 2(12)</td>
<td>224 ± 4(12)</td>
</tr>
<tr>
<td></td>
<td>22A</td>
<td>199 ± 3(39)</td>
<td>474 ± 7(14)</td>
<td>567 ± 7(9)</td>
<td>441 ± 2(84)</td>
<td>504 ± 5(18)</td>
</tr>
<tr>
<td></td>
<td>87V</td>
<td>290 ± 4(5)</td>
<td>&gt; 700(7)†</td>
<td>ND</td>
<td>&gt; 700(7)†</td>
<td>ND</td>
</tr>
<tr>
<td>I.p.</td>
<td>ME7</td>
<td>524 ± 0(2)</td>
<td>275 ± 3(19)</td>
<td>408 ± 6(17)</td>
<td>310 ± 3(32)</td>
<td>429 ± 7(6)</td>
</tr>
<tr>
<td></td>
<td>22C</td>
<td>603 ± 5(9)</td>
<td>235 ± 3(15)</td>
<td>362 ± 17(4)</td>
<td>248 ± 2(27)</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>22L</td>
<td>431 ± 9(11)</td>
<td>234 ± 7(11)</td>
<td>312 ± 7(16)</td>
<td>260 ± 4(16)</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>79A</td>
<td>464 ± 7(14)</td>
<td>224 ± 5(12)</td>
<td>348 ± 4(12)</td>
<td>211 ± 3(14)</td>
<td>320 ± 5(3)</td>
</tr>
</tbody>
</table>

* Figures in parentheses show number of mice in each group tested.
† No mice in these groups developed clinical disease within their lifespan; the numbers of mice surviving longer than 700 days post-injection are shown.
‡ ND, Not done.
Scrapie strains in Sinc congenic mice

Fig. 4. Graphical representation of the incubation period data in Table 2. (a) Mean incubation periods in previous experiments in which VM, C57BL and VM x C57BL mice were injected i.c. with 1% inocula. (b) Mean incubation periods in later experiments in which VM-Sinc<sup>7</sup> and VM x VM-Sinc<sup>7</sup> mice were also included; mice were again injected i.c. with 1% inocula. (c) Mean incubation periods in the same mouse genotypes injected i.p. with 1% inocula. For each scrapie strain the same inoculum was used in (b) and (c), but that used in (a) was from a different mouse source. Incubation periods are shown in VM (△), VM-Sinc<sup>7</sup> (○), VM × VM-Sinc<sup>7</sup> (■), C57BL (◇) and VM × C57BL (□) mice. Note that mice of the same Sinc genotype are represented by the same shape of symbol and that the VM and VM-Sinc<sup>7</sup> congenic lines and the cross between them are shown as solid symbols. Where symbols are missing, the particular combination of scrapie strain and mouse genotype was not tested, except in the case of 87V with which the projected incubation period was longer than lifespan. For standard errors and numbers of mice/group, see Table 2; groups of less than six mice are shown in parentheses.

Fig. 5. Lesion profiles for six strains of scrapie injected i.c. into VM-Sinc<sup>7</sup> (——), VM (—-) and C57BL (···) mice (n = eight to 25 mice/group). Mice were injected with (a) ME7, (b) 22C, (c) 22L, (d) 79A, (e) 139A and (f) 22A. Vacuolar degeneration was scored in nine grey matter and three white matter areas of brain (Fraser & Dickinson, 1968; Fraser, 1976). The grey matter areas are: 1, dorsal medulla; 2, cerebellar cortex; 3, superior colliculus; 4, hypothalamus; 5, medial thalamus; 6, hippocampus; 7, septum; 8, medial cerebral cortex at the level of the thalamus; 9, medial cerebral cortex at the level of the septum. The white matter areas are 1*, cerebellar white matter; 2*, white matter of the mesencephalic tegmentum; 3*, pyramidal tract.

in senility. Other work (M. E. Bruce, unpublished results) suggests that all of these Sinc<sup>7</sup> mice were incubating scrapie but that the projected incubation period was longer than their lifespan.

For each scrapie strain tested the ranking of incubation periods in the three Sinc genotypes was the same whether the comparison was made in congenic or non-congenic mice. Scrapie strains differed in their absolute incubation periods in any single mouse genotype, in the ranking of incubation periods in the two Sinc homozygotes and in the apparent dominance shown between the two alleles in the F<sub>1</sub> heterozygote. Thus, the incubation period was shorter in Sinc<sup>7</sup> mice than in Sinc<sup>p7</sup> mice for strains ME7, 22C, 22L, 79A and 139A but the reverse was true for 22A and 87V. With strains ME7, 22C, 22L and 79A the incubation period in the heterozygote lay between those of the two parental strains, whereas with 22A and 139A it was beyond the parental range. As expected from previous studies (Kimberlin & Walker, 1978b), incubation periods following i.p. infection were approximately 50% longer than those following i.c. infection with the same inocula. This is mainly because the agent replicates in the periphery before entering the central nervous system (CNS) after an i.p. injection, whereas infection is established directly in the brain by an i.c. injection (Kimberlin & Walker, 1979). Despite this difference in pathogenesis, the ranking of incubation periods in the three Sinc genotypes was the same regardless of route of infection.
(ii) Lesion profiles

The lesion profiles in i.c. infected C57BL and VM mice were as expected from previous studies (Fig. 5). Although they tended to share the same general features, the profiles in these two mouse strains differed from each other in some respects, particularly with the 22A strain of scrapie. The profiles in VM-Sinc^7^ mice were broadly similar to those in C57BL and VM mice but did not consistently correspond to either; for example, the VM-Sinc^7^ grey matter profile was similar to the VM profile for 79A and 139A but more closely resembled the C57BL profile for 22C and ME7. As previously demonstrated (Fraser, 1976; Kimberlin et al., 1987), profiles were generally lower for the i.p than for the i.c. route but showed the same general shape (data not shown). Therefore, the distribution of vacuolar degeneration in the brain, as represented by the lesion profile, depended primarily on the strain of scrapie but was also influenced to a lesser extent by the Sinc gene and other unspecified mouse genes.

Discussion

The present study demonstrates that, without any doubt, there are distinct strains of scrapie which have their own characteristic and reproducible properties. This confirms the conclusions of a large number of previous studies in non-congenic mice, in which about 20 scrapie strains have been identified using the same strain-typing methods (Dickinson & Fraser, 1977; Dickinson & Outram, 1988). This scrapie strain diversity is not simply imposed by the mouse strain used for passage as numerous different strains with stable properties have been isolated in the same mouse genotype; for example, five of the strains used in the present study were maintained by passage in C57BL mice. We have also confirmed in congenic mice that the Sinc gene controls the incubation period for all scrapie strains tested. These results highlight two central questions concerning scrapie and related diseases. Firstly, what is the nature of the causative agent? And, secondly, what is the mechanism of the host control of pathogenesis?

Much of the research on scrapie in recent years has centred on a host-coded protein, PrP (Bolton et al., 1982; McKinley et al., 1983). Relatively protease-resistant forms of this protein accumulate in the brain during scrapie pathogenesis and aggregate into scrapie-associated fibrils (SAF) and amyloid (Merz et al., 1981; Diringer et al., 1983; Hope et al., 1986; DeArmond et al., 1987; McBride et al., 1988; Bruce et al., 1989). Infectivity in tissue extracts has been shown to copurify with SAF, at least to some extent (McKinley et al., 1983; Diringer et al., 1983; Somerville et al., 1986), but no scrapie-specific nucleic acids have yet been identified, even in the relevant protein fractions (Oesch et al., 1988). This failure to detect scrapie-specific nucleic acids and the resistance of scrapie to treatments which would usually inactivate nucleic acids have prompted the ‘prion’ hypothesis, that the replicating infectious agent is composed only of a protein, PrP, and is devoid of nucleic acid (Prusiner, 1982).

The major obstacle to believers in the protein-only model is the existence of many strains of scrapie, which carry information independent of the host. We have shown elsewhere that scrapie strains can be copassaged as mixtures and still retain their separate identities (Dickinson et al., 1986) and that certain strains predictably give rise to mutants, even when passaged in a single host genotype (Bruce & Dickinson, 1987). Although it is possible to elaborate theoretical models in which proteins encode some form of strain specificity (Bolton & Bendheim, 1988; Wills, 1989), it is difficult to account for these aspects of strain variation on a protein-only basis; there is also no direct experimental evidence for replicating proteins. The simplest explanation remains that the scrapie informational molecule is an as yet undetected nucleic acid which is protected by its close association with host tissue components, as detailed in the ‘virino’ hypothesis (Dickinson & Outram, 1983, 1988). PrP (or abnormal PrP) could well be a host component in such a model.

On the other hand, there is growing evidence that PrP is involved in the host’s control of pathogenesis, as well as being pathologically modified in the course of the disease. Using restriction fragment length polymorphism analysis and nucleotide sequencing a close linkage has been demonstrated previously between the gene encoding PrP and the Sinc gene (Carlson et al., 1986; Westaway et al., 1987; Hunter et al., 1987). Our Sinc congenic lines have already been compared using the XbaI and TagI endonucleases, which detect polymorphisms in the non-coding or flanking regions of the PrP gene; VM-Sinc^7^ mice were found to resemble C57BL mice but differ from VM mice in their restriction pattern (Hunter et al., 1987). This confirms the close linkage of the PrP gene and the Sinc gene, a linkage which has been maintained through the 18 successive backcrosses carried out to produce the congenic lines; either the PrP gene lies within the stretch of DNA transferred with the Sinc gene or PrP is in fact the Sinc gene product.

In linkage studies in other laboratories, a few possible individual recombinants have been identified in crosses between non-congenic mouse strains in which incubation period and PrP did not segregate together (Carlson et al., 1988; Race et al., 1990). However, there is doubt as to whether these really were recombinants, firstly because of possible effects of genes other than Sinc on incubation period in these non-congenic strains. Second-
ly, in both studies an uncloned scrapie isolate was used, possibly containing a mixture of strains which would produce confusing incubation period results. Recently, a more definitive study has strengthened the possibility that PrP is indeed the Sinc gene product; the hamster PrP gene, inserted in multiple copies into the Sinc<sup>?</sup> mouse genome, conferred hamster-like incubation period properties on the transgenic mouse line when infected with a hamster-passaged scrapie isolate (Scott et al., 1989).

Two polymorphic sites have been identified within the coding region of the mouse PrP gene, both of which are predicted to result in amino acid substitutions in the protein (Westaway et al., 1987). All of the Sinc<sup>?</sup> mouse strains tested so far are predicted from their gene sequence to have leucine at codon 108 and threonine at codon 189, whereas all Sinc<sup>V</sup> strains are predicted to have phenylalanine at codon 108 and valine at codon 189. If PrP proves to be the Sinc gene product, these differences in the primary structure of the protein could be responsible for the Sinc gene effects on scrapie incubation period and pathology.

The pathogenesis of scrapie is likely to involve a number of stages, including the uptake of infectivity by cells, possibly a processing step before replication commences, actual replication within cells and spread to other cells in which the cycle is repeated (Kimberlin & Walker, 1986, 1988). At least part of the cell-to-cell progression within the CNS has been shown to follow specific neuronal projection pathways (Fraser & Dickinson, 1985; Gregoire et al., 1984). Severe neurological dysfunction, leading to clinical disease and death, has been suggested to occur only when infectivity and damage have reached critical levels in certain 'clinical target areas' which are responsible for vital functions in the animal (Kimberlin & Walker, 1986, 1988).

It is not known which step in the above sequence is regulated by the Sinc gene. However, the effect clearly does not depend on differences in the efficiency of the initial infection as there are only minor differences between estimates of infectivity levels in the same tissue sample, measured by titration in mice of different Sinc genotypes; each combination of scrapie strain and mouse genotype in fact has its own distinct dose–response curve (Dickinson & Outram, 1988). The studies described by Dickinson & Outram (1988) have also shown that the efficiency of infection is not influenced by whether there is a match between the Sinc genotypes of the 'donor' and 'recipient' mice; there are also no great differences between Sinc genotypes in the titre of infectivity in brain in the terminal phase of the disease, showing that mice of different genotypes are not simply becoming ill at different stages in the course of infection. It follows that the Sinc gene exerts its effect either on the rate of cell-to-cell spread of infection or on the rate of replication. As discussed above, it is probable that the Sinc gene encodes PrP, which has the characteristics of a cell surface glycoprotein and could therefore be some type of receptor (Bazan et al., 1987). This might suggest that the Sinc gene effect involves cell-to-cell spread rather than actual replication.

The interaction of the Sinc gene with the replicable information carried by the agent shows a number of peculiar features. Firstly, considering the length of the incubation periods, the control exerted by Sinc is remarkably precise; secondly, the direction of allelic action differs according to the strain of scrapie. However, perhaps the most intriguing feature of the Sinc gene is that the dominance characteristics of the two alleles also appear to differ according to the scrapie strain. With certain scrapie strains one allele or the other exhibits apparent overdominance, giving incubation periods in the heterozygote which are beyond the range of those in the parents. The fact that this is seen even in Sinc congeneric mice shows that it is not simply a non-specific result of hybrid vigour. Rather, as discussed by Dickinson & Outram (1979), the phenomenon may suggest that the two alleles do not act independently in the heterozygote. This led them to the hypothesis that the molecular structure controlling pathogenesis is a complex of subunits which are contributed by both Sinc alleles. However, the incubation period data can be viewed from a different perspective. In their discussion on the action of the Sinc gene, Dickinson & Outram (1979) also pointed out that when the results for many scrapie strains are taken into account, the incubation period in the heterozygote is almost always longer than that in Sinc<sup>?</sup> mice and there is a high correlation (r = 0.98) between incubation periods in these two genotypes. On the other hand, there is no significant correlation between incubation periods in Sinc<sup>V</sup> mice and either of the other two genotypes. The heterozygote therefore behaves like a 'slower' version of a Sinc<sup>?</sup> mouse, irrespective of the scrapie strain; this raises the question of why the p7 allele has so little positive effect in the heterozygote, even though it can have such a large effect in the p7 homozygote.

Finally, it is clear that host genetic factors are also important in field scrapie and related infections in other species. The occurrence of both natural and experimental sheep scrapie is controlled by the Sip gene (a probable analogue of Sinc), which in sheep may also be linked to the PrP gene (Dickinson & Fraser, 1979; Dickinson & Outram, 1988; Hunter et al., 1989). There is evidence that the allelic effects of Sip, like Sinc, differ according to the strain of scrapie encountered (Foster & Dickinson, 1988). Similar host genetic controls are also likely to operate in the scrapie-like diseases in humans,
Creutzfeldt-Jakob disease and Gerstmann-Sträussler syndrome. Recently it has been shown that the occurrence of these conditions in some families is linked to the inheritance of variants of PrP (Owen et al., 1989; Hsiao et al., 1989; Goldgaber et al., 1989; Doh-ura et al., 1990).

Fundamental studies on the genetics of agent-host interactions in Sinc congenic mice are therefore relevant to the whole family of scrapie-like diseases.

References


Scrapie strains in Sinc congenic mice


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