The Genomic Identity of Different Strains of Mouse Scrapie Is Expressed in Hamsters and Preserved on Reisolation in Mice

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SUMMARY

263K is the most widely used strain of agent in scrapie research because it produces very short incubation periods in golden hamsters and exceptionally high infectivity titres in clinically affected brain. 263K is also remarkable in having a very low pathogenicity for mice. Evidence is presented that 263K originated as a mutant that was strongly selected on passage in hamsters. Seven new passage lines have been established in hamsters using well characterized strains of mouse scrapie representing the 'drowsy goat' and SSBP/1 families of scrapie strains, and one natural scrapie source. Considerable differences between scrapie strains were found in hamsters using incubation period criteria alone. There was evidence that the parent strain of 263K might be 79V or a strain like it in the 'drowsy goat' family. Four of the hamster passage lines were established from scrapie strains that had been cloned in mice. Reisolates in mice were compared with original strains. By the criteria used, two of the reisolates were the same as the original strains. Two others were mutants with incubation periods longer than those of their parental strains but the mutants were different from one another. It is concluded that passage between mice and hamsters can select mutants that would otherwise be lost but there is also clear evidence that the genotypic identity of some scrapie strains is preserved on passage between different host species. These findings are important in the search for the putative nucleic acid genome of the scrapie agent.

INTRODUCTION

High doses of 263K scrapie injected intracerebrally (i.c.) into golden hamsters can produce clinical disease in as little as 60 days after infection, about half the incubation period of the fastest model of scrapie in mice. In addition, clinically affected brain contains nearly 10 times the infectivity (about \(10^{10}\) i.c. ID\(_{50}\) units/g) found in any other scrapie model (Kimberlin & Walker, 1977, 1986, 1989). Attempts to purify 263K infectivity from brain led to the discovery of the glycoprotein PrP (Prusiner et al., 1982) and to the development of rapid methods of purifying scrapie-associated fibrils (SAF) which are a pathologically aggregated form of PrP (Diringer et al., 1983). Further studies in hamsters have revealed the structure and sequence of the PrP gene (Oesch et al., 1985; Basler et al., 1986) and shown that scrapie infection induces a subtle modification of normal PrP so that it accumulates in brain and acquires the ability to form fibrils (Hope et al., 1986). The copurification of modified PrP (SAF) and infectivity (Diringer et al., 1983; McKinley et al., 1983) prompted suggestions that PrP might also be either the 'infectious scrapie protein' embodied in the prion hypothesis, or the host protein that, according to the virino hypothesis, enables the scrapie-specific genome to be infectious (see Kimberlin & Hope, 1987).

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Evidence for a host-independent scrapie genome comes from the wide range of scrapie strains that have been identified in mice (see Dickinson, 1976; Outram, 1976) and from the evidence for mutation (Bruce & Dickinson, 1987; Kimberlin et al., 1987a). We think it likely that the scrapie genome is a very small 'regulatory' nucleic acid which may not code for protein (hence the need for a 'protective' host-coded protein such as PrP). A major criterion for recognizing candidate genomes (nucleic acid or some other macromolecule) is that there should be sequence differences according to the strain of agent. Further studies based on the use of 263K in hamsters would be facilitated if different hamster scrapie models were available. We have therefore investigated scrapie strain variation in hamsters after transmission of several well characterized strains of mouse-passaged scrapie.

A wide range of mouse scrapie strains was selected for transmission to hamsters. The ME7 strain was originally derived from natural Suffolk sheep scrapie (Zlotnik & Rennie, 1963). The strains 22A, 22C and 22L are three of the four scrapie strains in the SSBP/1 family which were isolated in mice from the passage line of the SSBP/1 scrapie source in Cheviot sheep (Dickinson, 1976). A subpassage line (see Fig. 1) of SSBP/1 Cheviot scrapie in goats (Pattison & Millson, 1961; Dickinson, 1976), gave rise to the 'drowsy goat' family of strains (79A, 79V, 139A) after transmission to mice. The fourth member of the 'drowsy goat' family is 263K. Its passage history differs from all the above strains in that, after mice, serial passages were made in rats and then in hamsters in which 263K was identified (Kimberlin & Walker, 1977). Even then it was not clearly identified until after the fourth serial passage in hamsters, by which stage, incubation periods after i.c. injection had steadily decreased from over 300 days at first pass to a stable value of 60 to 70 days. Simultaneously, pathogenicity for mice was lost suggesting that at least two strains of scrapie were present at the early hamster passages, only one of which was readily pathogenic for mice. The other, 263K, had an extremely low pathogenicity for mice (of the Sinc genotype s7s7) but was strongly selected in hamsters because of its very short incubation period (Kimberlin & Walker, 1978).

The low pathogenicity of 263K for mice is interesting given that it came from a passage line that started in mice (Fig. 1). How did 263K arise? The first part of this study suggests that 263K is a mutant that was selected in hamsters. The incubation period properties of 263K were then compared with those of seven new hamster passage lines, of which three were derived from each of the SSBP/1 and 'drowsy goat' families. There was striking evidence for scrapie strain differences in hamsters (see also Kimberlin et al., 1987a; Buyukmihci et al., 1987). Finally reisolations in mice from four of the hamster passage lines showed both the phenotypic stability of some strains and the mutation of others when passaged between species. The studies of 139A scrapie presented here have been described in detail in a previous paper on the transmission of scrapie between mice, rats and hamsters (Kimberlin et al., 1987a).

**METHODS**

_Sources of scrapie strains._ The scrapie strains ME7, 22A, 22C, 22L, 79A and 79V were supplied either cloned or uncloned (see Fig. 1) by Dr A. G. Dickinson. The 139A strain was derived from the first successful transmission of scrapie to mice (Chandler, 1961). The cloning of 139A, the origin and cloning of 263K, and the source of isolate 302K have been described (Kimberlin & Walker, 1978, 1986; Kimberlin et al., 1987a).

_Passage experiments._ Considerable care was taken to avoid cross-contamination between scrapie strains (Kimberlin et al., 1987a). All scrapie inocula were prepared from brain pools stored at −20 °C before use. With the exception of the attempted blind passage of 263K in mice (Fig. 2b), brains were collected from animals with unambiguous clinical signs of scrapie. All experiments used uncentrifuged 1% brain homogenates in normal saline injected i.c. into golden hamsters (30 µl) or mice (30 µl). Limiting dilutions of brain homogenate were used in two cloning experiments shown in Fig. 2(a). All animals were outbred females injected as young adults. They were caged singly (hamsters) or in groups of six to eight (mice). Cages were coded to reduce observer bias when assessing the clinical signs of scrapie. Incubation periods were calculated to the time of the first appearance of definite and consistent signs. IM/Dk mice have the Sinc genotype p7p7; Compton White (CW) and C57BL mice are both Sinc7.

_Nomenclature._ This study used one uncharacterized isolate (302K) in mice and one scrapie strain (263K) identified in hamsters. All the other scrapie strains were originally defined by their properties in mice. When these strains were transmitted to hamsters (Fig. 3), their original designation was preserved by a prefix of three numerals.
Scrapie strains in hamsters and mice

Fig. 1. SSBP/1 and drowsy goat families of scrapie strains (bold type) used in this study. More detailed passage histories are shown in Fig. 10.2 in Dickinson (1976) and in Fig. 1 of Kimberlin & Walker (1978). All passages were by i.c. injection of brain homogenates from clinically affected animals and cloning was done by serial passage at limiting dilution. Mice were homozygous at the Sinc gene locus for either the s7 allele or the p7 allele.

(e.g. 139) or two numerals and a letter (e.g. 22C), and the suffix '-H' denotes the new host species (e.g. 139-H, 22C-H). Reisolation in mice is indicated by an additional suffix '/M' (e.g. 139-H/M, 22C-H/M). This nomenclature is used operationally and does not necessarily mean that a permanent change in agent strain has occurred on transmission to a different host (see Results and Discussion).

RESULTS AND DISCUSSION

263K is a mutant strain selected in hamsters

In our original studies, 263K scrapie only emerged as the major strain in hamsters after the fourth serial passage. It was identified by its stable incubation period of 60 to 70 days (under standard conditions of i.c. infection) and by its extremely low pathogenicity in Sinc s~ mice (no clinical cases at first passage during an observation period of about 2 years). However, transmission to mice was readily demonstrated at hamster passages 2 to 4 and we have now studied three of these subpassage lines in CW mice [the origin of one is described in Kimberlin & Walker, 1979; the other two (designated 431K and 302K) are described in Kimberlin & Walker, 1978]. Each subline was passaged three times in CW mice to see what effect this had on the appearance of 263K when passaged back into hamsters. Similar results were obtained from all three experiments, one of which is shown in Fig. 2(a).

There was a substantial species barrier effect (see Kimberlin et al., 1987a) when isolate 302K in the mouse subline was transmitted back to hamsters; incubation periods at first passage (186 ± 16 days) were much longer than at subsequent passage (Fig. 2a). Scrapie from this first hamster passage was as readily transmissible to mice (302 ± 4 days) as the initial hamster-to-mouse passage had been (316 ± 7 days). However, transmission to mice was not achieved (within an observation period of 600 days) after the third and fourth passages in hamsters when incubation periods were typical of cloned 263K (Fig. 2a).
Fig. 2. (a) Effect of the passage conditions of isolate 302K in mice (CW) on the subsequent isolation of 263K scrapie in hamsters (GH). All passages were made by i.c. injection of 1% homogenates of clinically affected brain pools except for the cloning in hamsters (at successive limiting dilutions of $10^{-6}$, $10^{-7}$, $10^{-7}$ and $10^{-5}$) and in mice (at limiting dilutions of $10^{-7}$, $10^{-7}$, $10^{-7}$ and $10^{-5}$). The data are either mean incubation periods (days) ± S.E.M. for (n) animals in groups with 100% scrapie cases or the minimum observation period (e.g. > 600 days) of groups with no scrapie cases. Dotted boxes indicate the first passage in mice and hamsters and the dotted arrows show the attempted back-passages to mice. Solid boxes identify the three passage lines in hamsters as 263K, almost certainly 263K ('263K') and possibly 79A-H (79A-H). (b) Attempts to isolate 263K in hamsters after serial blind passage in groups of four CW mice at intervals (days) corresponding to the first three passages of isolate 302K in CW mice. None of the hamsters developed scrapie within an observation period of 410 days.

This experiment shows that three passages in mice did not prevent the subsequent isolation of 263K in hamsters. Neither did cloning at the first passage back in hamsters followed by cloning at the next three hamster passages (Fig. 2a). However, four additional passages in CW mice at high dilution led to the isolation of a quite different scrapie strain in hamsters with a stable incubation period nearly three times that of 263K (Fig. 2a). The new strain resembles 79A-H described in the next section (Fig. 3) and its isolation from the same subpassage line in mice as 263K is compelling evidence for scrapie strain variation in hamsters. This experiment also suggests that 263K is a mutant derived from a parental strain which was present in the mouse subpassage line but removed by cloning in CW mice.
Scrapie strains in hamsters and mice

Fig. 3. Passage lines of 263K scrapie and of seven different mouse scrapie strains in golden hamsters (GH). All passages were made by i.c. injection of 1% homogenates of clinically affected brains and all injected animals developed scrapie. The data are mean incubation periods (days) ± s.e.m. for (n) animals. Dotted boxes indicate the first passage in GH. Solid boxes show the designation used for each scrapie strain in hamsters, as described in Methods.

The only alternative explanations are that 263K can replicate 'silently' in mice without causing disease or that it was passaged passively (without replication) through mice in sufficient quantity to infect hamsters. Both possibilities are excluded by a parallel experiment (Fig. 2b) in which cloned 263K was blind passaged in mice at time intervals corresponding to the three mouse passages of isolate 302K shown in Fig. 2(a). Each mouse in the first blind passage was injected with 1.9 x 10^6 ID_{50} i.c. hamster units of cloned 263K. The theoretical dilution of original 263K inoculum after each blind passage (assuming 100% retention of inoculum in brain) was 7.5 x 10^{-4}, 5.6 x 10^{-7} and 7 x 10^{-10}, respectively. As expected from these calculations, some original 263K inoculum persisted in brain during the first mouse passage and produced scrapie cases in six out of the eight hamsters injected (the incubation period was 157 ± 2 days). However, no scrapie cases occurred in hamsters injected with mouse brain from the second or third blind passages and observed for 410 days (Fig. 2b). And none of the mice developed scrapie.

It is concluded that 263K itself could not have been present in the mouse subpassage line shown in Fig. 2(a). This line must have contained two strains of scrapie, but only one of them was the parent strain of 263K. The parental strain must have been present in clinically affected mouse brain at a lower effective concentration than the other strain because serial cloning in CW mice removed it. The fact that the two other subpassage lines in mice also yielded 263K in hamsters (not presented in detail) suggests a high mutation rate in the parental strain. Relatively high mutation rates have also been suggested in studies of transmissible mink encephalopathy (a disease similar to scrapie) in Chinese hamsters (Kimberlin et al., 1986) and of scrapie in mice (Bruce & Dickinson, 1987; Kimberlin et al., 1987a). This could be taken as evidence that the scrapie genome is RNA rather than DNA.
Comparisons with other scrapie strains in hamsters

Three scrapie strains have been isolated in mice from the 'drowsy goat' passage line (Dickinson, 1976; Outram, 1976). It seemed possible that one of these might be the parent strain of 263K and passage lines of all three were established in hamsters. All showed a marked species barrier effect at first passage in hamsters but incubation periods became completely stable at the second or third passage (Fig. 3). One of these lines, designated 79V-H (see methods) had stable incubation periods identical to those of 263K suggesting that 79V (or a second strain present in the original uncloned 79V in mice) is the parent strain of 263K. The other two passage lines in hamsters (designated 139-H and 79A-H) were clearly different from 263K and 79V-H. The incubation periods produced by 79A-H were slightly longer than those of 139-H (Fig. 3; and see Kimberlin et al., 1987a) but very similar to those found in hamsters after isolate 302K had been cloned in mice (Fig. 2a).

We have studied three of the mouse passage scrapie strains belonging to the SSBP/1 family (Fig. 1). The resulting passage lines in hamsters were similar to one another but none of them resembled 263K (Fig. 3). Neither did the hamster passage line obtained from ME7 scrapie whose origin was different from that of the 'drowsy goat' and SSBP/1 families (see Introduction). The ME7-H passage line had incubation periods much longer than occurred with any of the others shown in Fig. 3 but there were similarities with a hamster passage line derived by Zlotnik & Rennie (1965) from the same natural scrapie source.

In conclusion, Fig. 3 provides convincing evidence for scrapie agent strain variation in hamsters; notably, there were twofold differences in incubation period between 263K and 139-H and between 139-H and ME7-H. It is important that this strain variation in hamsters was not random but reflected (at least to some extent) differences both within and between families of source strains. This suggests that some degree of genotypic identity is preserved on transmitting scrapie from mice to hamsters. However, because strain typing depends on phenotypic properties (incubation period, brain pathology) it is difficult to compare strains in different host species. We have therefore made reisolates in mice from four of the hamster passage lines shown in Fig. 3, and compared these with the original mouse passage strains.

Reisolations of hamster-passaged scrapie in mice

Despite the very large species barrier effect when 22A-H was reisolated in IM mice, the incubation periods were stable at the second (22A-H/M2) and third (22A-H/M3) passages and very similar to those of 22A at the equivalent number of continuous mouse passages (Fig. 4). The severity of vacuolation was measured in nine grey matter areas of brain at the clinical stage of 22A-H/M2 and M3; the 'vacuolation profiles' (see Fraser, 1976) were identical to those of 22A (Fig. 5 a). By these criteria, 22A-H/M and 22A are the same suggesting that 22A had maintained its genotypic identity on passage from mice to hamsters and back to mice. The extended incubation periods seen only at the first passage in hamsters (Fig. 3) and again at the first reisolation passage in mice (Fig. 4) are further examples of the 'donor species effect' described by Kimberlin et al. (1987a).

The same conclusions can be drawn from the studies of ME7-H. The incubation periods of ME7-H/M2 and M3 were the same as for mouse-passaged ME7 (Fig. 4). Incubation periods were also similar at the first reisolation pass in C57BL mice irrespective of whether this was made after the first hamster passage (212 ± 2 days), the second passage (204 ± 2 days) or the third (224 ± 2; Fig. 4). These values are within the dose-incubation end point of ME7 scrapie in Sinc s7 mice (Kimberlin et al., 1987b) suggesting that, in this case, the 'donor species effect' reduces the effective dose injected into mice. It is concluded that ME7 scrapie, like 22A, was probably passaged unchanged through hamsters.

We have previously shown that 139A remains unchanged after three serial passages in rats but, in contrast, passage through hamsters (139-H) led to the reisolation of a mutant strain (139-H/M) with double the incubation period of 139A in mice (Kimberlin et al., 1987a). A similar kind of result occurred with 22C-H/M. This reisolate differed from 22C in having a much longer incubation period (Fig. 4). In addition, the 'vacuolation profile' (at the clinical stage of 22C-H/M2 and M3) was statistically different from that of 22C in four out of the nine grey matter
### Scrapie strains in hamsters and mice

**Fig. 4.** Passage of three cloned strains of mouse scrapie through hamsters (GH: shown in more detail in Fig. 3) followed by reisolation in IM or C57BL mice. All passages were made by i.c. injection of 1% homogenates of clinically affected brains and all injected animals developed scrapie. The data are mean incubation periods (days) ± S.E.M. for (n) animals. Dotted boxed indicate the first reisolation passage in mice. The bottom line indicates the similarity (=) or difference (#) between each reisolate and the original strain maintained in continuous mouse passage (see Methods for explanation of scrapie strain nomenclature).

<table>
<thead>
<tr>
<th>Strain</th>
<th>Initial Cloning</th>
<th>Passages in GH</th>
<th>Passages in IM or C57</th>
<th>Incubation Periods (days) ± S.E.M.</th>
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<tbody>
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<td>IM (15) 174 ± 1</td>
<td>GH (5) 146 ± 2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>IM (15) 167 ± 2</td>
<td>C57 (15) 156 ± 1</td>
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<td></td>
<td></td>
<td></td>
<td>IM (15) 177 ± 1</td>
<td>C57 (13) 153 ± 1</td>
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<td></td>
<td></td>
<td>IM (16) 177 ± 1</td>
<td>C57 (9) 402 ± 2</td>
</tr>
<tr>
<td>22C</td>
<td>Cloned</td>
<td>2 passes</td>
<td>IM (15) 167 ± 2</td>
<td>GH (4) 145 ± 1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>C57 (15) 156 ± 1</td>
<td>C57 (10) 393 ± 1</td>
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<td>C57 (16) 130 ± 2</td>
<td>C57 (16) 135 ± 2</td>
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<tr>
<td>ME7</td>
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<td>2 passes</td>
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<td>GH (5) 263 ± 1</td>
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<td>C57 (15) 137 ± 1</td>
<td>C57 (14) 137 ± 1</td>
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<td></td>
<td></td>
<td></td>
<td>C57 (16) 130 ± 2</td>
<td>C57 (16) 135 ± 2</td>
</tr>
</tbody>
</table>

- **22A = 22A-H/M**
- **22C ≠ 22C-H/M**
- **ME7 = ME7-H/M**

Fig. 4. Lesion profiles of grey matter vacuolation in nine anatomically defined areas of brain as described in Kimberlin et al. (1987a). Profiles are based on the last two passages of each line (when incubation periods were similar) as shown in Fig. 4. Each point is the mean score (arbitrary units) ± S.E.M. of 15 to 24 brains. (a) Profile of 22A (solid line) was similar to that of 22A-H/M (broken line). (b) Profile of 22C (solid line) was significantly different from that of 22C-H/M (broken line) in four brain areas: upward arrowheads, \( P < 0.01 \); downward arrowheads, \( P < 0.001 \).

areas of brain (Fig. 5b). The 2.5-fold longer incubation period of 22C-H/M means that it could not have been present in the original cloned 22C and must, therefore, be a mutant (or recombinant?). The mutant probably arose in hamsters because incubation periods became stable immediately after the first passage in mice (Fig. 4) with no evidence of strain selection over three or four passages as occurred with 139-H/M in mice (Kimberlin et al., 1987a) or 263K in hamsters (Kimberlin & Walker, 1977).
In conclusion, we now have data on four different, cloned scrapie strains which were passaged through hamsters and reisolated in mice. Two of the reisolates (139-H/M and 22C-H/M) were mutants but it is important to note that they were different mutants with respective incubation periods in mice of about 200 days (Kimberlin et al., 1987a) and 400 days (Fig. 4). In other words, the mutants were different because the parental strains were different. The other two reisolates (22A-H/M and ME7-H/M) were indistinguishable from the original strains. These studies show that a high degree of genotypic identity is preserved when cloned strains of mouse scrapie are serially passaged through hamsters and reisolated in mice. Any worthwhile hypothesis on the nature of the scrapie agent must be able to account for this fact.

The animal experiments described in this paper were carried out at the AFRC Institute for Animal Health laboratory at Compton, Newbury, Berkshire.

REFERENCES


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