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Transmission of Bovine Spongiform Encephalopathy and Scrapie to Mice: Strain Variation and the Species Barrier

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Phil. Trans. R. Soc. Lond. B 1994 **343**, 405-411
doi: 10.1098/rstb.1994.0036

Transmission of bovine spongiform encephalopathy and scrapie to mice: strain variation and the species barrier

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SUMMARY

Transmissions of bovine spongiform encephalopathy (BSE) from seven unrelated cattle sources have given remarkably uniform disease characteristics in mice, differing from over twenty previous and contemporary transmissions of sheep and goat scrapie. Transmissions to mice of spongiform encephalopathy from six species (including sheep and goats) which have been experimentally or naturally infected with BSE have given similar results to direct BSE transmissions from cattle. Therefore the BSE agent has retained its identity when passaged through a range of species and the 'donor' species has little specific influence on disease characteristics in mice, adding to evidence for an agent-specific informational molecule. On transmission of BSE or scrapie to mice the incubation periods are long compared with subsequent mouse-to-mouse passages (the 'species barrier'). Contributing factors include a low efficiency of infection on interspecies transmission, the apparent failure of intracerebrally injected 'foreign' inoculum to establish infection directly in mouse brain and the selection of variant strains of agent which replicate most readily in the new host species.

1. INTRODUCTION

Sheep scrapie has been endemic in the United Kingdom for at least 200 years. Bovine spongiform encephalopathy (BSE), on the other hand, was first recognized only in 1985, but since then over 100 000 cases have been recorded. It is most likely that BSE originally occurred as a result of the dietary exposure of cattle to feed supplements containing rendered scrapie-infected sheep tissues; later in the epidemic the major source of infection has almost certainly been BSE cattle tissues 'recycled' in these supplements (Wilesmith 1991). Recently, novel spongiform encephalopathies have been reported in domestic cats (Wyatt *et al.* 1991) and a number of zoo species: greater kudu, nyala, gemsbok, eland, Arabian oryx, cheetah and puma (Bradley & Matthews 1992). There is a strong suspicion that these animals were also infected from BSE-contaminated feed (Wilesmith 1991).

When scrapie is transmitted experimentally from one species to another, the incubation period of the disease is usually longer than that seen on subsequent passage within the new species and there may be survivors. This relative difficulty of transmitting the disease between species is referred to as the 'species barrier' (Dickinson 1976). There is evidence from experiments in rodents that the species barrier is a complex phenomenon, representing the cumulative effect of a number of different components, including the efficiency of infection, pathogenesis effects and agent strain selection (Kimberlin *et al.* 1987, 1989). It has been suggested that the species barrier depends on the incompatibility of PrP proteins between donor and recipient animals (Prusiner *et al.* 1990).

There are many strains of scrapie and related agents which can be distinguished on the basis of their disease characteristics in experimentally infected animals, in particular in genetically defined mice. The *Sinc* gene in mice (Dickinson *et al.* 1968) exerts a major influence on the incubation period of experimental scrapie, each mouse-passaged scrapie strain producing a characteristic, highly reproducible pattern of incubation periods in the three possible *Sinc* genotypes (Bruce *et al.* 1991; Bruce 1993). The *Sinc* gene almost certainly encodes the PrP protein and the two known alleles of *Sinc*, s7 and p7, are consistently linked with variants of PrP which differ by two amino acids (Westaway *et al.* 1987; Hunter *et al.* 1992); all $Sinc^{s7}$ mouse strains tested so far have leucine at codon 108 and threonine at codon 189, whereas all $Sinc^{p7}$ mouse strains have phenylalanine at codon 108 and valine at codon 189. No other polymorphic sites have been identified within the coding region of the mouse PrP gene.

Scrapie strains also differ in the type, severity and distribution of pathological changes they produce in the brain. In mice a semiquantitative representation of the distribution of vacuolar changes, the 'lesion profile', reliably distinguishes between strains of agent (Fraser & Dickinson 1968; Bruce *et al.* 1991). Studies

Phil. Trans. R. Soc. Lond. B (1994) **343**, 405–411
Printed in Great Britain

Table 1. *Mean incubation periods (days ± s.e.m.) in transmissions of spongiform encephalopathies to mice*

	mouse strain or cross				
	Sinc^{s7}		*Sinc*^{p7}		*Sinc*^{s7p7}
source	RIII	C57BL	VM	IM	C57BL × VM
BSE:					
cow 1	328 ± 3	438 ± 7	471 ± 8	537 ± 7	not tested
cow 2	327 ± 4	407 ± 4	499 ± 8	548 ± 9	743 ± 14
cow 3	316 ± 3	436 ± 6	518 ± 7	561 ± 9	not tested
cow 4	314 ± 3	423 ± 5	514 ± 11	565 ± 8	not tested
cow 5	321 ± 4	444 ± 14	516 ± 9	577 ± 12	745 ± 22
cow 6	319 ± 3	447 ± 11	545 ± 7	576 ± 13	755 ± 18
cow 7	335 ± 7	475 ± 14	545 ± 12	not tested	unfinished
scrapie:					
sheep 1	386 ± 10	404 ± 5	769 ± 16	815 ± 23	610 ± 8
sheep 2	-ive	-ive	-ive	-ive	-ive
sheep 3	612 ± 28	618 ± 27	-ive	unfinished	unfinished
FSE:					
cat 1[a]	348 ± 3	434 ± 12	542 ± 12	573 ± 13	731 ± 23
cat 2	312 ± 4	426 ± 4	457 ± 10	523 ± 10	676 ± 13
cat 3	302 ± 3	405 ± 8	469 ± 12	502 ± 14	692 ± 10
SE:					
kudu[a]	339 ± 5	465 ± 14	536 ± 10	560 ± 12	754 ± 24
nyala[a]	378 ± 8	529 ± 11	548 ± 17	614 ± 11	772 ± 3
experimental BSE:					
sheep	297 ± 3	408 ± 9	446 ± 10	478 ± 9	662 ± 13
goat	308 ± 3	392 ± 8	480 ± 11	512 ± 12	685 ± 14
pig	316 ± 5	433 ± 6	489 ± 8	534 ± 16	717 ± 11

[a] Transmission from formol-fixed tissue.

using isolates which have been passaged many times in rodents have demonstrated that scrapie agents have a strain-specific informational molecule which determines disease characteristics and which can retain its identity on passage through different host species or genotypes (Kimberlin *et al.* 1989; Bruce 1993), but the molecular nature of this informational molecule is unknown.

In the present paper we summarize a series of studies in which scrapie, BSE and novel spongiform encephalopathies in other species have been transmitted to mice, extending a previously reported investigation (Fraser *et al.* 1992). The primary purpose was to explore the epidemiological links between the diseases in different species by comparing their disease characteristics in mice. However, these studies also have important implications when considering the basis of agent strain variation and the species barrier.

2. TRANSMISSION STUDIES

(*a*) *Transmissions from cattle with BSE*

BSE was transmitted to mice from seven dairy cattle sources (six Holstein-Friesians and one Friesian), collected from widely separated geographical locations and at different times during the epidemic. Inocula were prepared from brainstem areas which consistently show pathology in BSE cattle (Wells *et al.* 1992). The experimental design for these and the transmissions from other species was as described by Fraser *et al.* (1992). Panels of four inbred strains of mouse and one cross were challenged: C57BL and RIII (both of the *Sinc*^{s7} genotype), VM and IM (both of the *Sinc*^{p7} genotype) and the F_1 cross between C57BL and VM. Ten per cent brain homogenates were injected into groups of 20–24 mice of each strain by a combination of the intracerebral (i.c.) and intraperitoneal (i.p.) routes. Incubation periods were calculated as the interval between injection and a standard clinical endpoint when the mice were showing clear signs of neurological disease, as described previously (Dickinson *et al.* 1968). Diagnosis was confirmed histopathologically and vacuolar changes were scored in nine grey matter areas of brain to construct a lesion profile (Fraser & Dickinson 1968). Groups of saline-injected control mice, maintained alongside transmission experiments, showed no clinical signs or pathology indicative of scrapie-like disease.

All seven BSE sources transmitted easily to mice, producing disease in 100% of mice in all groups. The results of the seven transmissions were remarkably uniform, suggesting that each cow was infected with the same major strain of agent. It is not possible to make general statements about the strains involved in the epidemic as a whole, based on results from this small sample of cases. However, the consistency of the pathology reported in cattle with BSE (Wells *et al.* 1992) is evidence that a single or a limited number of strains is involved.

Each BSE source produced a closely similar pattern of incubation periods in the five mouse strains (table 1). There were large and reproducible differences

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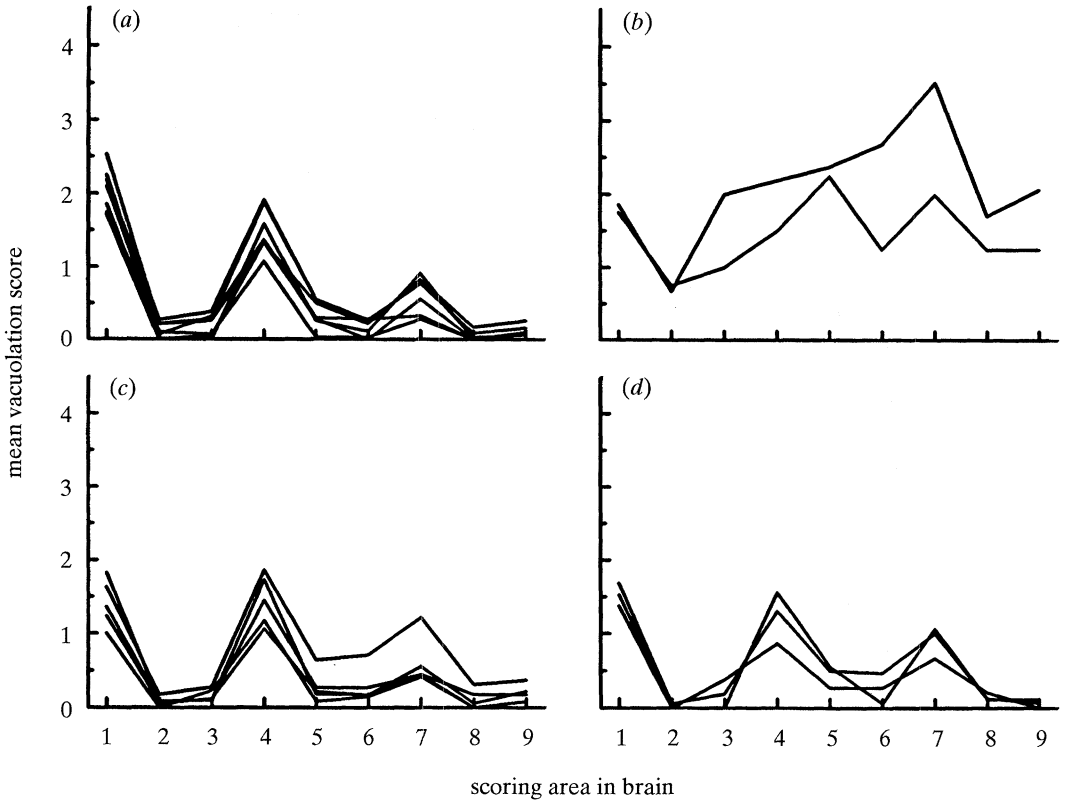

Figure 1. Lesion profiles in RIII mice for (*a*) the seven BSE transmissions from cattle, (*b*) the two positive transmissions from natural sheep scrapie, (*c*) the transmissions from three cats, the kudu and the nyala and (*d*) the transmissions from the experimentally BSE-infected sheep, goat and pig.

between mice of different *Sinc* genotypes, but also, surprisingly, between mouse strains of the same *Sinc* genotype; this was particularly marked in the approximately 100 day difference in incubation periods between RIII and C57BL mice. The incubation period in the F_1 cross between C57BL and VM was over 700 days, well beyond the incubation period range in the two parental mouse strains. This apparent overdominance of one *Sinc* allele has been observed previously for some mouse-passaged scrapie strains (Bruce *et al.* 1991). The pathology produced in mice was also very similar for each BSE source; for example, figure 1*a* shows the uniformity of lesion profiles in RIII mice for the seven transmissions.

(*b*) *Transmissions from natural sheep scrapie*

Transmissions were also attempted from the brains of three sheep with natural scrapie, collected since 1985 and representing three different breeds (Greyface, Dorset Horn and Cheviot). Only one source, the Greyface (sheep 1), produced disease in all mouse genotypes (table 1). The Dorset Horn source (sheep 2) produced neither clinical disease nor pathology in any of the mice within their lifespan. With the Cheviot source (sheep 3), clinical disease has been seen only in the RIII and C57BL groups and in one IM and one C57BL × VM mouse, after long incubation periods (this experiment is still in progress 800 days after injection). Neither of the positive transmissions showed any resemblance to BSE. There were no large differences in incubation periods between C57BL and RIII mice and, for the Greyface source, the incubation period in the F_1 cross lay between those of the two parental strains. For the two positive scrapie transmissions, the lesion profiles in RIII mice (figure 1*b*) differed from each other and from the lesion profiles in the BSE transmissions.

The BSE transmissions have also been compared with a series of transmissions from natural sheep and goat scrapie, performed by Alan Dickinson in the early 1970s (Dickinson 1976). The results of these previous transmissions were extremely variable, in both the incidence of disease and the incubation periods in recipient mice, but none resembled BSE. These results suggest that the strains of agent causing scrapie and BSE are different, but this could still be consistent with the proposition that BSE was derived originally from sheep scrapie. Previous studies have shown that the characteristics of scrapie strains may sometimes be changed permanently by passage through another species and this has been interpreted as the selection of variant strains which replicate more readily in the new host species (Kimberlin *et al.* 1989). It is possible that the high temperatures involved in rendering and the subsequent accidental passage of the infection through cattle have selected variant strains from sheep scrapie (Taylor 1993).

(*c*) *Transmissions from spongiform encephalopathies in other species*

Similar transmissions were set up from three cats, one greater kudu and one nyala with spongiform

Table 2. *Mean incubation periods (days + s.e.m.) in C57BL, RIII and VM subpassage lines from one BSE and the Greyface natural sheep scrapie source, at second mouse-to-mouse passage*

		mouse strain or cross			
source	passage line (agent strain)	RIII	C57BL	VM	C57BL × VM
BSE	RIII (301R)	167 ± 1	190 ± 1	334 ± 8	523 ± 5
	C57BL (301C)	188 ± 3	207 ± 3	363 ± 8	565 ± 9
	VM (301V)	223 ± 5	267 ± 5	116 ± 3	244 ± 2
scrapie	RIII (201R)	169 ± 2	171 ± 2	322 ± 4	255 ± 4
	C57BL (201C)	172 ± 1	164 ± 4	331 ± 6	248 ± 5
	VM (201V)	216 ± 10	203 ± 10	308 ± 15	266 ± 3

encephalopathy. As no fresh-frozen brain was available from one of the cats, the kudu and the nyala, formol-fixed brain, leached with saline, was used in these transmissions. The transmission results from all of these sources showed striking similarities to the BSE transmissions, although there was more variation between sources than was seen with BSE (table 1). Part of this variation may reflect a loss of titre due to formol fixation of three of the source brains. Nevertheless, all transmissions showed the same ranking of incubation periods as BSE in the five mouse strains, with large differences between RIII and C57BL mice and with very long incubation periods in the C57BL × VM cross. The lesion profiles in RIII mice, were closely similar to those in the BSE transmissions (figure 1*c*). These results confirm the suspicion that the cats, greater kudu and nyala were infected with the BSE agent.

Further transmissions to mice were achieved from a Cheviot sheep, a goat and a pig, all of which had developed clinical disease after experimental infection with BSE (Foster *et al.* 1993; Dawson *et al.* 1990); the sheep came from the 'negative' line of a flock at NPU which has been selectively bred on the basis of response to experimental challenge with scrapie (Foster & Dickinson 1988). Each of these sources gave patterns of incubation periods and lesion profiles which were similar to those seen in direct BSE transmissions from cattle (table 1, figure 1*d*).

These results show that the BSE agent has retained its identity on passage through six species and that differences in donor species have had little influence on the disease characteristics in mice. Transmission to mice therefore provides a method for testing any suspicion that BSE has spread to another species. However, a difference in transmission characteristics would not necessarily mean that the source was unrelated to BSE, as passage of scrapie through different species has sometimes led to a change in the properties of the isolate (Kimberlin *et al.* 1989).

3. ISOLATION OF MOUSE-PASSAGED STRAINS OF BSE AND SCRAPIE

Further mouse-to-mouse passages have been set up from one BSE source and the Greyface scrapie source, by i.c. injection of 1% brain inocula. Separate passage lines have been established in the RIII, C57BL and VM mouse strains. As in previous studies with other isolates, the incubation period in the mouse strain used for passage shortened dramatically at the first mouse-to-mouse passage; in all three BSE passage lines the large RIII/C57BL difference was reduced at this stage. Although the mouse-passaged isolates may not yet be fully stable, the incubation period characteristics after two passages in mice indicate that different strains of agent have been isolated from the BSE and scrapie sources (table 2). The results also show that, for each source, different agent strains have been isolated in the two *Sinc* genotypes of mouse, but the same strain has been isolated in C57BL and RIII mice.

The two strains from BSE (301V and 301C) differ from all strains previously isolated from sheep and goat scrapie (Bruce *et al.* 1992). On the other hand, the strain isolated in $Sinc^{s7}$ mice from the scrapie source (201C) closely resembles the ME7 strain, which, in previous studies, has been derived from the majority of natural sheep scrapie sources (Dickinson 1976). The $Sinc^{p7}$-passaged isolate from this source (201V) does not resemble any previously characterized strains.

4. COMPONENTS OF THE SPECIES BARRIER

Previous studies in which well characterized scrapie strains were passaged between hamsters, rats and mice have shown the species barrier to have a number of contributing factors (Kimberlin *et al.* 1987, 1989); these include a reduced efficiency of infection on interspecies transmission, differences in pathogenesis between first and subsequent passages in a new species and the selection of strains of agent which replicate more quickly in the new host species. The studies described above show clearly that there is a species barrier effect on transmission of BSE from cattle to RIII, C57BL or VM mice, but that the extent of this effect differs according to the mouse strain. Further investigations with BSE have confirmed that the species barrier is a complex phenomenon, involving a number of separate components.

Firstly, the level of infectivity in a BSE source brain has been estimated by end-point titration in the two $Sinc^{s7}$ mouse strains; serial tenfold dilutions were injected i.c. into groups of RIII and C57BL mice and the titre was estimated using the Kärber method, as described previously (Fraser *et al.* 1992). The titres measured by assay in RIII and C57BL mice were

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Table 3. *Incidence of disease and incubation periods in mice injected with 10% BSE brain homogenate by different routes and with different volumes of inoculum*

route of infection and volume of inoculum	number positive/ number injected	mean incubation period (days ± s.e.m.)
20 µl i.c.	8/8	360 ± 13
20 µl i.c. + 20 µl i.p.	10/10	340 ± 11
20 µl i.c. + 100 µl i.p.	8/8	312 ± 4
20 µl i.c. + 500 µl i.p.	10/10	317 ± 5
20 µl i.p.	5/7	330 ± 9
100 µl i.p.	9/9	314 ± 6
500 µl i.p.	8/8	315 ± 7

$10^{5.16}$ and $10^{5.18}$ i.c. ID_{50} units g^{-1} respectively. This is more than 1000-fold lower than maximum brain titres, measured in the same way, in most mouse scrapie models. Therefore the relatively long incubation periods seen on primary transmission of BSE are partly due to a relatively low effective dose of infection. Although it is possible that cow brain contains fewer potentially infectious particles than mouse brain in absolute terms, a more likely explanation is that the efficiency of the assay is lower when there is a change of species. This could be due to a less efficient interaction between agent and host receptors, or a more effective clearance and inactivation of infectivity in the inoculum by the mouse when this infectivity is associated with 'foreign' tissue, as has been suggested previously for hamster-passaged scrapie transmitted to mice (Kimberlin *et al.* 1975).

In a second experiment comparing routes of infection, the incubation period in RIII mice following i.c. injection with a high dose of BSE was slightly longer than the incubation period in mice infected i.p. with the same inoculum (table 3). When the two routes were combined the incubation period was similar to that produced by an i.p. injection alone. However, the fact that there were survivors in the group injected i.p. with 20 µl suggests that, despite its shorter incubation period, the i.p. route is less efficient than the i.c. route. The i.c. and i.p. routes have also been found to produce similar incubation periods in some transmissions of natural scrapie to mice (Kimberlin 1993). These results are of interest because studies with mouse-passaged scrapie strains have demonstrated consistently that the incubation period in mice following i.p. infection is longer than that following direct i.c. infection, usually by about 50% (Bruce *et al.* 1991).

When there is no change in passaging species, an i.c. injection establishes infection directly in the brain; following an i.p. injection, on the other hand, infection spreads from peripheral organs such as spleen, via peripheral nerves, to the spinal cord and finally ascends to the brain (Kimberlin & Walker 1988). The fact that the i.c. and i.p. incubation periods in some interspecies transmissions are not greatly different implies that the i.c. injection has failed to establish infection directly in the brain and is therefore the equivalent of infection by a peripheral route. It is possible that the agent is not recognized by receptors in the brain when it is associated with 'foreign' tissue components, but must be processed in some way in peripheral organs before it can infect nervous tissue.

5. PrP AND THE SPECIES BARRIER

It is clear that host PrP plays a central role in controlling the pathogenesis of scrapie and related diseases. In mice PrP is almost certainly encoded by the *Sinc* gene; the biological effects of *Sinc* are likely to depend on the differences in PrP amino acid sequence, consistently observed between $Sinc^{s7}$ and $Sinc^{p7}$ mice (Westaway *et al.* 1987; Hunter *et al.* 1992). Recent studies in mice lacking PrP strongly suggest that this protein is necessary for agent replication (Büeler *et al.* 1993). Moreover, it has been proposed that PrP is also an essential component of the infectious agent, either alone (Prusiner 1982) or in close association with an agent-specific molecule (Dickinson & Outram 1988).

The fact that the BSE agent has maintained its identity on passage through six different species adds to previous evidence that scrapie-like agents have an informational molecule which determines disease characteristics (Kimberlin *et al.* 1989; Bruce 1993); the nature of this informational molecule is still a matter for speculation. In 'protein-only' models, it is suggested that the agent is composed solely of PrP which carries information in the form of self-perpetuating post-translational modifications (Prusiner 1992). On the other hand, the 'virino hypothesis' proposes that the agent consists of an agent-specific informational molecule, closely associated with a host component (PrP) (Dickinson & Outram 1988; Bruce *et al.* 1992); in this model the informational molecule is most likely to be an unidentified small nucleic acid.

The phenotypic properties of scrapie strains depend on precise interactions between the infection-specific informational molecule and the *Sinc* or PrP genotype of the host (Bruce *et al.* 1991). It has been suggested that these properties may be influenced by the degree of compatibility between donor and recipient PrP proteins (Carlson *et al.* 1989). This does not appear to be an important factor when scrapie is transmitted between the two *Sinc* genotypes of mouse (Bruce 1993). However, mismatching of PrPs could make a significant contribution to the comparative difficulty in transmitting scrapie-like diseases between species (Scott *et al.* 1989; Prusiner *et al.* 1990). In the studies described here the species barrier effect on transmission to mice was similar for each of the six BSE-infected

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species, suggesting a simple recognition by the recipient mice that the inocula were 'foreign' rather than a precise interaction between donor and recipient PrPs.

A surprising feature of the primary BSE transmissions is the very large incubation period difference between the two *Sinc*^{s7} mouse strains, RIII and C57BL, a difference which is substantially reduced on subsequent serial passage in either mouse strain. This RIII/C57BL difference clearly does not reflect the selection of different BSE strains (see table 2), although it remains possible that the agent strain isolated in both mouse strains differs from the major strain present in the cattle sources. Nor does it reflect differences in the effective dose of infection (Fraser *et al.* 1992). Investigations into the details of pathogenesis in the two mouse strains are still in progress. It is known that, like all other *Sinc*^{s7} mouse strains so far tested, RIII and C57BL mice have leucine at codon 108 of the PrP gene and threonine at codon 189 (A.D. Bennett, personal communication). This leaves a number of possible explanations for the RIII/C57BL difference, the simplest being a previously unrecognized polymorphism in the mouse PrP gene. However, if the PrP sequence is identical in the two mouse strains, the difference in incubation period could be related to the regulation of expression of the PrP gene or to the action of other genes unrelated to PrP.

A complete analysis of the role of PrP in the species barrier will depend on studies in transgenic animals carrying foreign or altered PrP genes. Such studies have so far been difficult to interpret because of complicating effects of transgene copy number, endogenous gene expression and the use of uncloned scrapie isolates. The most promising approach for future work is the insertion of foreign PrP genes into 'null' mice, lacking their own PrP (Büeler *et al.* 1993). In designing experiments of this type, it will be important to keep in mind the results of studies in conventional animals, showing that the species barrier is a complex phenomenon, involving effects on efficiency of infection, details of pathogenesis and selection of strains of agent.

The authors thank Michael Dawson, Gerald Wells and Martin Jeffrey for their help in supplying tissues for transmission. These studies were partially funded by MAFF (grant numbers SE1403 and CSA1853).

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