expert reviews
in molecular medicine

Transgenic models of the transmissible spongiform encephalopathies

Jean C. Manson and Nadia L. Tuzi

The transmissible spongiform encephalopathies (TSEs) are a group of fatal neurodegenerative diseases that are also transmissible. The PrP protein is central to the disease process and has been hypothesised to be the infectious agent. Polymorphisms in the PrP gene have been linked to the incubation time of TSE disease, and mutations in the human PrP gene have been suggested to result in genetic TSE disease. Several PrP transgenic models have been developed. These models express PrP from different species, with and without PrP mutations, and at different levels of gene expression. This article discusses the contribution of these transgenic models to our present understanding of the TSE diseases.

The transmissible spongiform encephalopathies (TSEs) or prion diseases are a group of fatal neurodegenerative diseases that are also infectious. These diseases include scrapie of sheep and goats, which was first described over 200 years ago, bovine spongiform encephalopathy (BSE) in cattle, and a number of human forms of the disease such as Creutzfelt–Jakob disease (CJD), Gerstmann–Straussler–Scheinker syndrome (GSS), Kuru and fatal familial insomnia.

Although TSE diseases can be efficiently transmitted within a species, the transmission from one species to another usually involves a species barrier and results in long incubation times and low susceptibility (Ref. 1). However, despite this, BSE, which was first described in 1986 and reached epidemic proportions in the UK by 1988, has now been shown to have been transmitted to humans in the form of variant CJD (vCJD) (Refs 2, 3).

In order to address both the normal function of the PrP protein and its precise role in the TSEs, a number of transgenic models have been produced and studied over the past 12 years. These models are described in this review and the contribution of each model to our current understanding of the TSEs is presented and discussed. The review begins with a brief discussion of our current understanding of the structure of PrP and the pathogenesis of TSEs.

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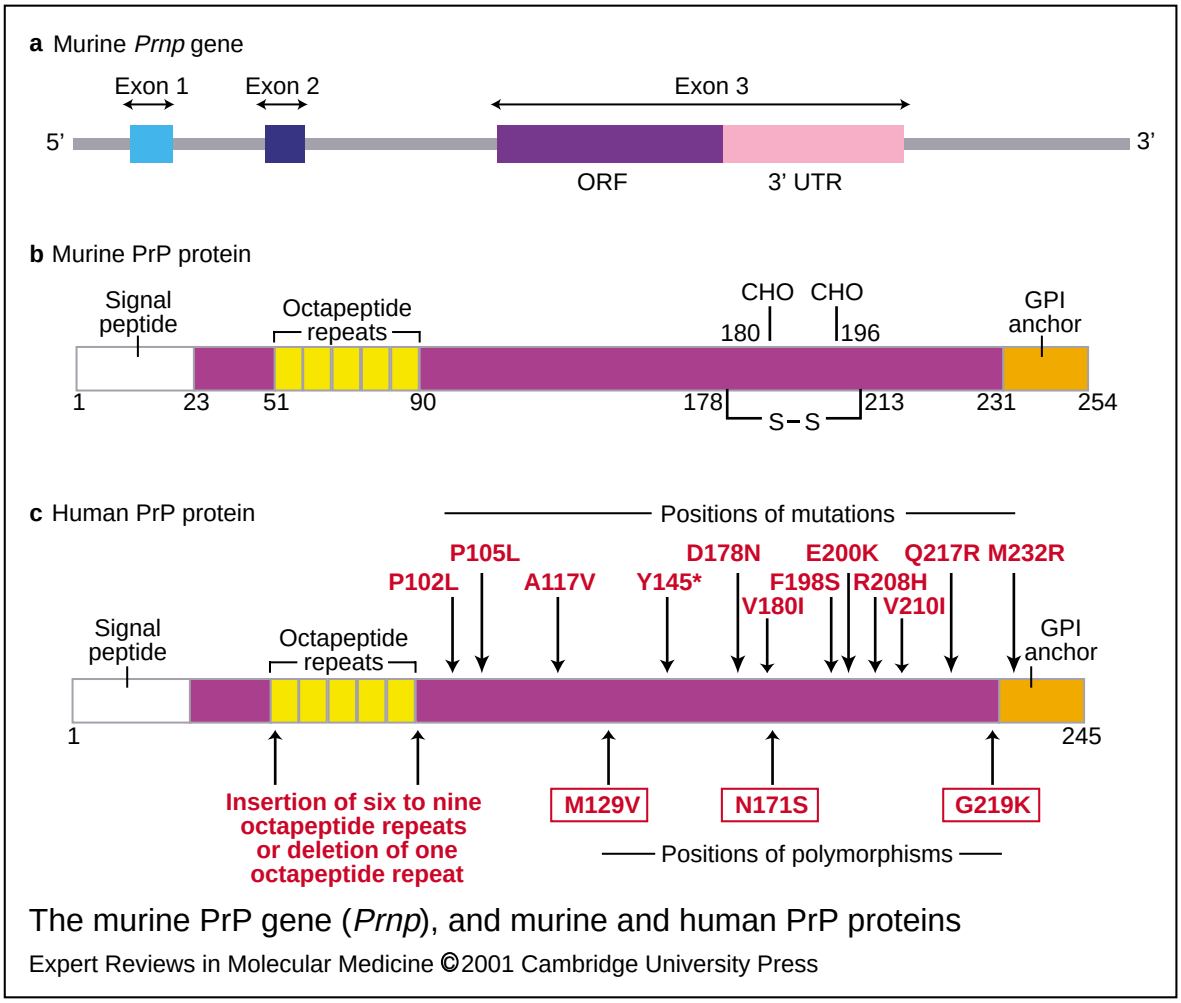
Accession information: (01)00295-2a.pdf (short code: txt001jme); 11 May 2001
ISSN 1462-3994

Structure and function of PrP

The murine PrP gene consists of three exons with the open reading frame residing in the third exon (Fig. 1a), and the messenger RNA (mRNA) (consisting of exons 1, 2 and 3) encodes a protein of approximately 250 amino acid residues. The mRNA from the human PrP gene, *PRNP*, has not been shown to include an exon 2 sequence, although a sequence homologous to murine exon 2 is present within the human gene (Ref. 4). PrP possesses a signal peptide, two potential sites for N-linked glycosylation, a single disulphide bond, and a glycosylphosphatidylinositol (GPI) anchor at its C-terminus (Fig. 1b). The structure of PrP is highly conserved among mammalian species, suggesting an important role for PrP in cell



The murine PrP gene (*Prnp*), and murine and human PrP proteins

Figure 1. The murine PrP gene (*Prnp*), and murine and human PrP proteins. (a) The murine PrP gene (*Prnp*) possesses three exons; exon 3 contains an open reading frame (ORF) and a 3' untranslated region (3' UTR). The human PrP gene (*PRNP*, not shown) possesses two known and one putative exon; the equivalent of murine exon 2 has not been found in human RNA transcripts to date. (b) Murine PrP protein consists of 254 amino acid residues. Features include a 22 amino acid signal peptide, five octapeptide repeats, one disulphide bond (S–S) between cysteine residues 178 and 213, and two potential sites for N-linked glycosylation (CHO) at residues 180 and 196. A glycosylphosphatidylinositol (GPI) anchor is attached to the C-terminus of PrP from residues 231 to 254. (c) Human PrP protein is 245 amino acids in length and has the same structural features, described in (b), as its murine homologue. This diagram indicates in red the mutations, polymorphisms, insertions and deletions that have been found to date in the human PrP gene, and the positions of the mutations (listed above the protein) and polymorphisms (listed in boxes below the protein) are indicated. Amino acids are given in single-letter code. The asterisk indicates a stop codon and therefore this mutation results in a truncated protein **(fig001jme)**.

Accession information: (01)00295-2a.pdf (short code: txt001jme); 11 May 2001
ISSN 1462-3994 ©2001 Cambridge University Press

metabolism. PrP is present in high levels in the brain and at lower levels in other tissues. In the brain, it is found in both neuronal and non-neuronal cells (Refs 5, 6). The location of PrP on the cell membrane, attached via a GPI anchor, has led to suggestions that it may act as a cell receptor or as an adhesion molecule maintaining the architecture of the nervous system. However, although the involvement of PrP in the pathology of the TSEs is well documented, the normal function of PrP is not known.

Polymorphisms encoding Met or Val at amino acid position 129 and Gly or Lys at position 219 (Fig. 1c) of human PrP are associated with the incubation time of, and susceptibility to, human TSEs (Refs 7, 8). Amino acid differences in PrP have also been linked to different incubation times of disease in mice (Ref. 9) and sheep (Ref. 10) following exposure to TSE infectivity. In addition, a number of point mutations and insertions in the human PrP gene (Fig. 1c) appear to lead spontaneously to TSE disease without the addition of an infectious agent (Refs 11, 12). The mechanism by which these polymorphisms alter disease susceptibility or incubation time has not been defined but it has been suggested that the human mutations may destabilise the three-dimensional structure of PrP, making it more likely to convert to the disease-associated PrP^{Sc} isoform. Alternative hypotheses predict that the mutations in PrP make the individual more susceptible to a TSE infectious agent (Ref. 13).

Pathogenesis of TSEs

The pathological features of the TSE diseases include vacuolation in the central nervous system (CNS), loss of neurons and astrogliosis (increase in size and number of astrocytes). However, the underlying mechanism leading to this pathology is not yet understood. Another major pathological feature of these diseases is the abnormal deposition of the host-encoded PrP in the CNS and other tissues of the infected individual. In unaffected individuals, PrP is a protease-sensitive cell-surface glycoprotein (PrP^{C}) anchored in the membrane by a GPI anchor (Ref. 14). During TSE disease, PrP accumulates in and around cells in protease-resistant aggregates of PrP^{Sc}. The amount and distribution of PrP^{Sc} in the brain of an infected individual is dependent on both the strain of TSE agent and on host genetic factors (Ref. 15).

It was originally suggested in 1967 that the infectious agent in the TSEs could be a protein (Ref. 16). This was later expanded upon and gave rise to the prion (protein-only) hypothesis (Ref. 17). This hypothesis now suggests that the TSEs are attributable to a conformational change in PrP that results in a change from a predominantly α-helical form (PrP^{C}) to a β-sheet form (PrP^{Sc}) (Ref. 18). PrP^{Sc} itself is proposed to be the infectious agent and the prion hypothesis predicts that PrP^{Sc} can propagate its own conversion by acting as a template or a seed (Ref. 19), allowing further conversion of PrP^{C} to PrP^{Sc} to occur. If PrP^{Sc} is the infectious agent, it is proposed that the strain characteristics of each of the TSE agents would be maintained through structural differences (Ref. 20) or post-translational modifications, such as glycosylation, in PrP. Alternatively, the conversion of PrP^{C} to PrP^{Sc} may be secondary to the infectious process that is initiated by an additional molecule such as a nucleic acid component that specifies the disease phenotype and strain characteristics associated with TSE disease (Refs 21, 22). While strains of TSE agent are more readily explained by a nucleic acid component, no such molecule has yet been identified.

Results from transgenic models of TSEs

Transgenic approaches

Several transgenic models for the TSEs have been generated by microinjection of a DNA construct containing the PrP gene of interest into the male pronucleus of a fertilised mouse egg (Fig. 2a). The injected eggs are transferred into the oviduct of pseudopregnant mice, and pups resulting from this are screened for the presence of the PrP transgene, usually by using the polymerase chain reaction (PCR). This approach generates transgenic mice in which the transgene is integrated randomly into the murine genome. Although the expression level and distribution of PrP cannot be controlled with this transgenic approach, its use has yielded many interesting and informative TSE models.

In an alternative approach, transgenic mice carrying modifications of the endogenous murine PrP gene have been produced by gene targeting (Fig. 2b). This method can be used to introduce mutations into the PrP gene or to delete or replace parts of the gene. The major advantage of this method is that the altered gene is in the correct genomic location and under the control of the elements that normally regulate the expression

Accession information: (01)00295-2a.pdf (short code: txt001jme); 11 May 2001
ISSN 1462-3994

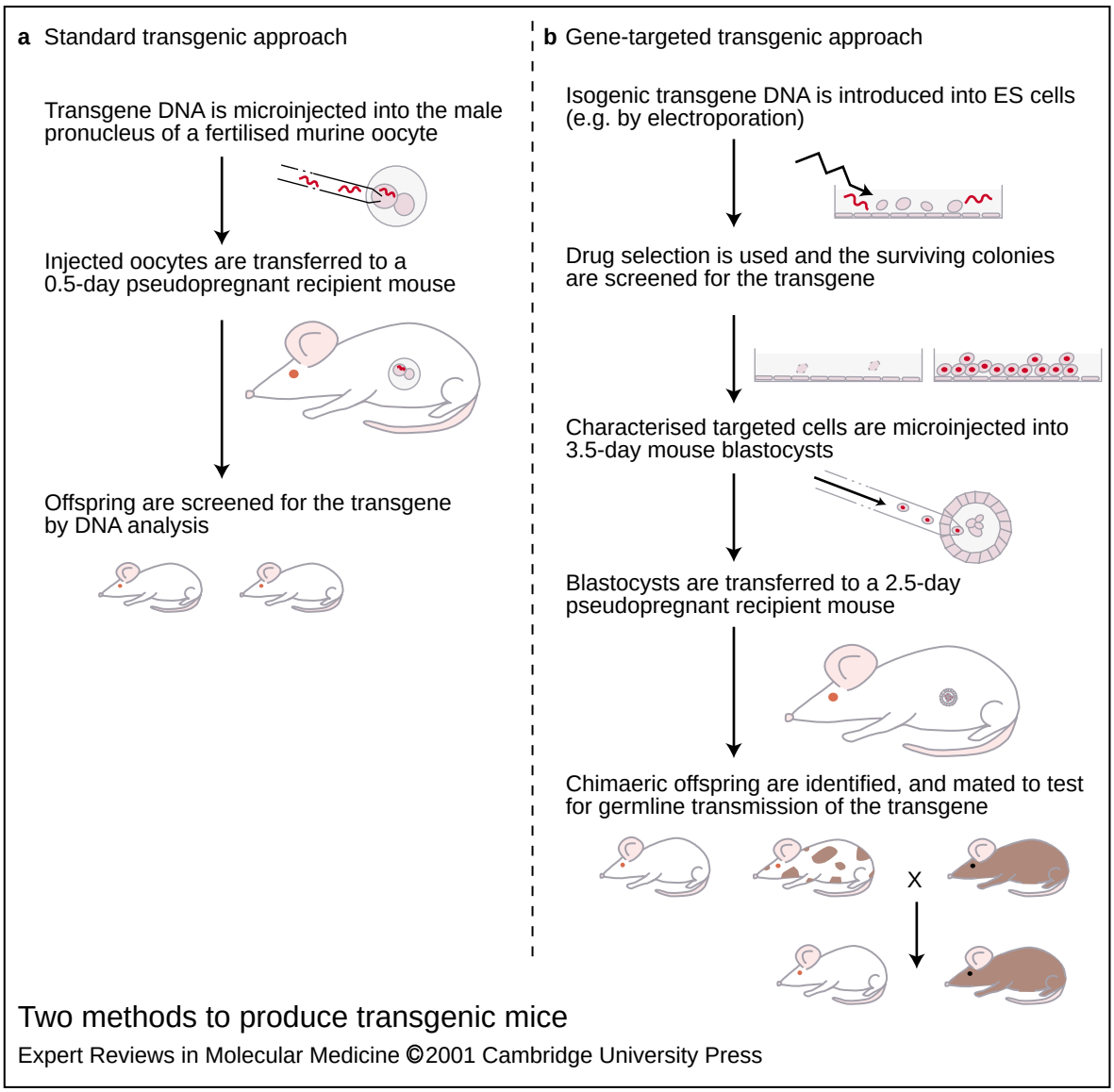


Figure 2. Two methods to produce transgenic mice. (a) Standard transgenic approach. DNA containing the transgene is microinjected into the male pronucleus of a murine oocyte, which is then transferred to a 0.5-day pseudopregnant recipient mouse. Offspring are screened for the presence of the transgene. (b) Gene-targeted transgenic approach. Isogenic DNA [to the embryonic stem (ES) cells being targeted] possessing the transgene is introduced into the ES cells by, for example, electroporation. Drug selection is used and surviving colonies are screened for the presence of the transgene. Characterised targeted ES cells are microinjected into 3.5-day mouse blastocysts and transferred to 2.5-day pseudopregnant recipient mice. The incorporation of targeted ES cells into the offspring is determined by coat colour – i.e. chimaeric mice are produced that possess coat colour of the mouse that donated blastocysts and that of the mouse from which the ES cells were derived. Chimaeric mice are mated to determine whether the targeted ES cells have contributed to the germline. Germline offspring are screened for the presence of the transgene and mated to establish the transgenic line (not shown) **(fig002jme)**.

of the PrP gene. Any alteration in the TSE disease in these animals can therefore be attributed directly to the introduced PrP mutation. Because the only difference in the transgenic mice compared with wild-type mice is the targeted mutation, it is possible to perform experiments with the appropriate controls. Furthermore, this technology allows the comparison of different transgenic mice generated using the same approach.

Accession information: (01)00295-2a.pdf (short code: txt001jme); 11 May 2001
ISSN 1462-3994 ©2001 Cambridge University Press

PrP^C is essential for TSE disease

In order to investigate both the normal function of PrP and its role in the TSEs, a number of different lines of mice have been produced by gene targeting in which the production of PrP has been ablated (Refs 23, 24, 25, 26). Each line of PrP null mice has been produced using a different targeting vector, resulting in deletions of different amounts of the PrP gene (Fig. 3). Mice with no PrP have been shown to be resistant to TSE disease when inoculated with a number of strains of agent that are known to cause TSE disease in mice possessing a functional PrP gene (Refs 27, 28). In the absence of PrP, there is no evidence to suggest that the infectious agent can replicate (Ref. 27), although retention of infectivity for prolonged periods has been observed in the PrP null mice (Ref. 29). These findings clearly implicate PrP as central to the disease process but do not provide proof that PrP is the infectious agent.

Determining the normal function of PrP

The first two lines of PrP null mice to be produced (NPU $Prnp^{-/-}$ and Zurich $Prnp^{-/-}$; Fig. 3) did not show any overt phenotypic abnormalities (Refs 23, 24). By contrast, the third and fourth lines of PrP null mice (Japan $Prnp^{-/-}$ and ICM $Prnp^{-/-}$; Fig. 3) showed an ataxic phenotype that developed at 63–70 weeks (Refs 26, 30). Subsequent analysis of these lines of mice has suggested that this phenotype may not be due to the absence of PrP but rather to the overproduction of a protein known as Doppel (Dpl). This protein is the product of a neighbouring gene, *Prnd*, and shows approximately 25% identity to all known PrPs (Ref. 30). The more extensive deletions in the PrP gene in the third and fourth lines have apparently resulted in the overexpression of the gene that encodes Dpl. The possible involvement of Dpl in TSEs is currently under investigation; however, there is no evidence for disease association between polymorphisms in the human *PRND* gene and human TSEs (Ref. 31).

PrP null mice have been used extensively to examine possible functions of PrP and, despite the lack of overt phenotypic abnormalities in these mice, a number of more subtle differences have been identified. These studies have suggested possible roles for PrP in synaptic transmission (Ref. 32), circadian rhythm, sleep (Ref. 33), copper metabolism and cellular resistance to oxidative stress (Ref. 34). However, it should be noted that since PrP is not present throughout the life of these null mice, other genes might compensate for the loss of PrP function. The abnormalities detected in these mice may therefore result from these compensatory mechanisms, rather than from the absence of PrP. Models in which the PrP gene can be deleted at specific time points in specific tissues might be necessary to reveal the function of PrP. The development of these models is discussed later.

Replication and transport of infectivity

PrP is expressed in both neuronal and non-neuronal cells of the CNS. The propagation of infectivity in the TSEs is thought to occur in neuronal cells. Indeed, transgenic mice have been produced that express hamster PrP only in neuronal cells and these mice are highly susceptible to hamster scrapie agent (Ref. 35). This demonstrates that, in the case of intracerebral inoculation within a species, non-neuronal cells are not required to support the disease process. However, it has also been shown that transgenic mice devoid of murine PrP but expressing hamster PrP transgenes under an astrocyte-specific promoter can accumulate infectivity and develop disease after inoculation with hamster scrapie (Ref. 36). This suggests a role for astrocytes in TSE pathogenesis, perhaps by an indirect toxic effect on neurons.

Natural routes of infection of the TSEs usually occur through the peripheral tissues of the animal rather than directly through the CNS. To investigate this in more detail, PrP null mice were grafted with brain material from wild-type mice or transgenic mice overexpressing murine PrP^C. After inoculation with a TSE agent via CNS and peripheral routes, the transfer of infectivity from the periphery to the CNS was found to be dependent on cells expressing PrP in the periphery (Ref. 37). The precise mechanisms of transport from the periphery to the CNS might differ for different strains of TSEs (Refs 38, 39, 40). Since replication and transfer of infectivity occurs, often over many years, before the neurodegeneration of the CNS, the peripheral route might be an important area for therapeutic intervention in these diseases. Transgenic models that allow for temporal and tissue specificity of PrP expression might enable these mechanisms to be established and the potential for therapeutic intervention to be investigated.

Accession information: (01)00295-2a.pdf (short code: txt001jme); 11 May 2001
ISSN 1462-3994

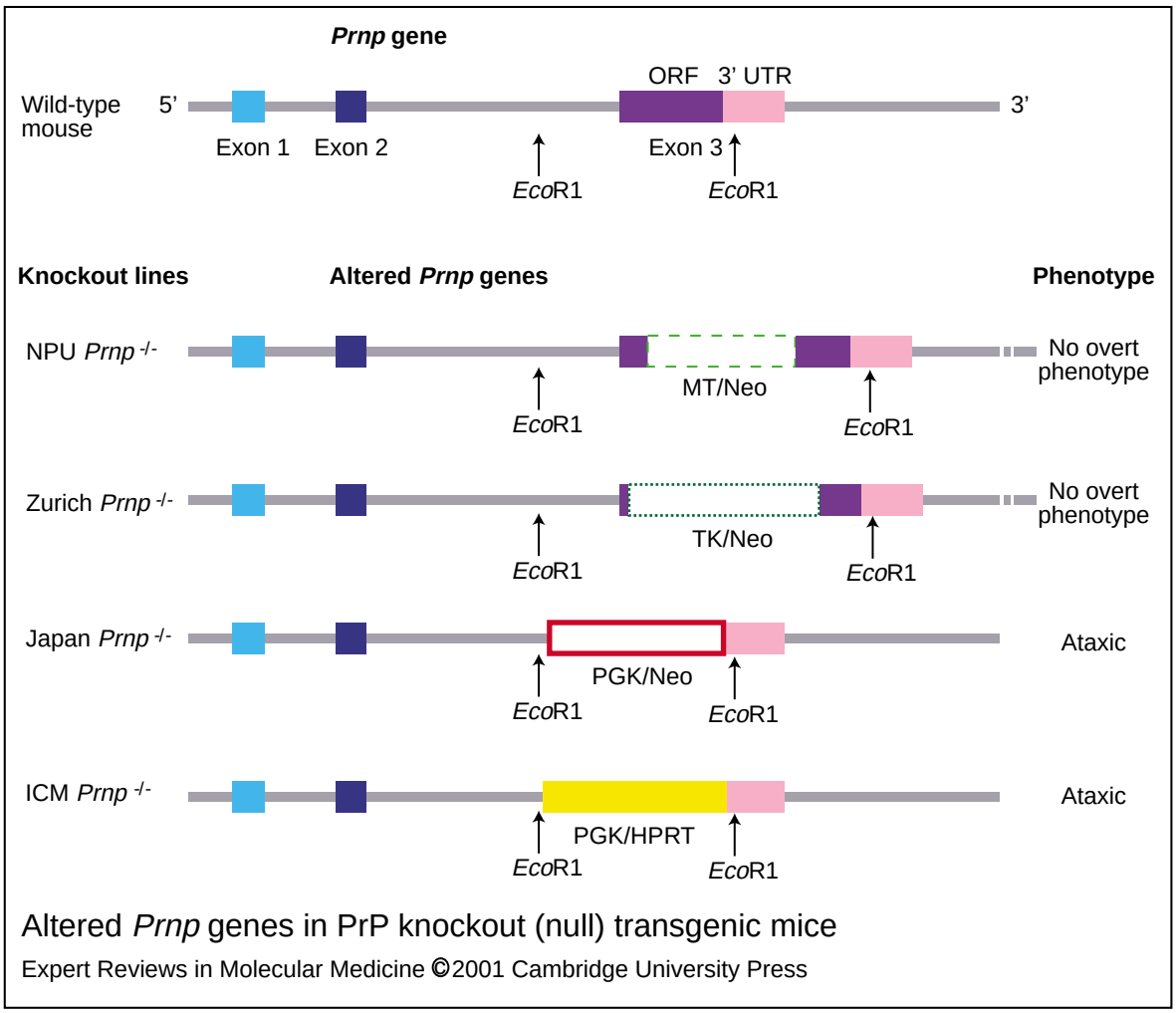


Figure 3. Altered *Prnp* genes in PrP knockout (null) transgenic mice. The mouse PrP gene (*Prnp*) possesses 3 exons, with the open reading frame (ORF) in exon 3. The position of two *Eco*RI restriction enzyme sites are indicated. In the first three knockout models shown (NPU *Prnp*$^{-/-}$, Zurich *Prnp*$^{-/-}$ and Japan *Prnp*$^{-/-}$) a neomycin resistance (Neo) cassette (white, outlined box) was used as the selection gene, under the control of three different promoters: the models NPU *Prnp*$^{-/-}$ (Ref. 24), Zurich *Prnp*$^{-/-}$ (Ref. 27) and Japan *Prnp*$^{-/-}$ (Ref. 26) contain the Neo casette directed by the promoters for mouse metallothionein (MT), human HSV-1 thymidine kinase (TK) and mouse phosphoglycerate kinase (PGK), respectively. The Neo cassettes in the NPU and Zurich *Prnp*$^{-/-}$ mice were contained within exon 3 but in the Japan *Prnp*$^{-/-}$ line a region 5' to exon 3 was removed during insertion. In the fourth knockout model, ICM *Prnp*$^{-/-}$, the ORF and a region 5' to exon 3 was replaced with the hypoxanthine phosphoribosyltransferase (HPRT) drug selection cassette (yellow box) driven by the PGK promoter (Ref. 25). The Japan *Prnp*$^{-/-}$ and ICM *Prnp*$^{-/-}$ mice develop ataxia (defined by an abnormal gait) at approximately 70 and 63 weeks, respectively, whereas the NPU *Prnp*$^{-/-}$ and Zurich *Prnp*$^{-/-}$ mice appeared to be normal throughout their normal lifespan. These null lines of transgenic mice were generated by first introducing the DNA targeting construct containing the altered *Prnp* gene into embryonic stem (ES) cells. Cells possessing the desired alteration were isolated using the appropriate drug selection. The targeted cells were then injected into mouse blastocysts as illustrated in Figure 2 to allow the generation of transgenic mice **(fig003jme)**.

Expression levels of PrP alter the disease incubation time

Transgenic models have been produced in which PrP genes are over- or underexpressed, and these models have demonstrated a relationship between TSE incubation time and the expression level of the PrP gene. Overexpression of a hamster PrP gene in transgenic mice has shown

Accession information: (01)00295-2a.pdf (short code: txt001jme); 11 May 2001
ISSN 1462-3994 ©2001 Cambridge University Press

that high levels of PrP lead to shorter incubation times than those of wild-type mice following inoculation with a hamster strain of scrapie (Table 1; Ref. 41). Overexpression of a murine PrP gene also demonstrated shortening of incubation time, following inoculation with a murine scrapie strain (Table 1; Ref. 42).

A similar relationship between PrP expression level and incubation time has been demonstrated in mice that have decreased levels of PrP. During the production of PrP null mice, heterozygous animals were produced with only one functional copy of the murine PrP gene *Prnp*. The level of PrP in these mice is lower than that in wild-type mice. When inoculated with a number of different strains of TSE, these animals consistently have incubation times that are longer than those of their wild-type littermates (Table 1; Refs 27, 28).

These models clearly demonstrate that there is a relationship between PrP expression level and the incubation time of TSE diseases. However, altered susceptibility or incubation time resulting from different expression levels of PrP has not been demonstrated in the naturally occurring TSE diseases. Nevertheless, the possibility is currently under investigation through studies examining the sequences that control PrP gene expression (e.g. promoter and 3' untranslated regions) and the polymorphic variants of these sequences that might alter the expression of PrP.

Overexpression of PrP and spontaneous neurological disease

Overexpression of a number of PrP transgenes has been shown to lead to development of disease in the CNS. Transgenic mice expressing high

Table 1. PrP gene dosage influences transmissible spongiform encephalopathy (TSE) incubation time (tab001jme)

Species/transgene	Transgene/gene copy no.	TSE agent	Incubation period (days)	Ref.
WT *Prnp*[b] mice	2[a]	RML[b]	140	42
Mouse *Prnp*[b] Tg	2[c]	RML[b]	107	42
Mouse *Prnp*[b] Tg	36[c]	RML[b]	86	42
WT hamster	2[a]	Sc237[d]	89	41
Hamster PrP Tg	30–50[e]	Sc237[d]	75	41
WT mice	2[a]	Sc237[d]	>148	41
WT mice	2[a]	ME7[b]	160	28
WT x PrP $^{-/-}$ mice	1[a]	ME7[b]	284	28
PrP $^{-/-}$ mice	0	ME7[b]	>500	28
WT mice	2[a]	301V[b]	227	28
WT x PrP $^{-/-}$ mice	1[a]	301V[b]	320	28
PrP $^{-/-}$ mice	0	301V[b]	>500	28

[a] Endogenous PrP gene only
[b] Mouse-passaged TSE (brain from a mouse challenged with a TSE and inoculated into another mouse and the process repeated)
[c] Haploid transgene copy number
[d] Hamster-passaged TSE (brain from a hamster challenged with a TSE and inoculated into another hamster and the process repeated)
[e] Diploid transgene copy number
Abbreviations: *Prnp*, murine PrP gene; PrP $^{-/-}$, PrP knockout (null) mice; Tg, transgene; WT, wild type.

Accession information: (01)00295-2a.pdf (short code: txt001jme); 11 May 2001
ISSN 1462-3994

levels of the murine PrP gene with a proline to leucine mutation at amino acid 101 develop neurodegeneration, spongiform changes in the brain and astrogliosis (Ref. 43). Overexpression of a wild-type murine PrP gene in transgenic mice has also been shown to lead to the development of a lethal neurological disease, involving spongiform changes in the brain and muscle degeneration (Ref. 44). In addition, several of these spontaneous neurological diseases are also reported to be transmissible, since a neurological disease developed when mice were inoculated with brain material from the transgenic mice that had the spontaneous neurological disease (Refs 43, 44).

Whether the overexpression of PrP in transgenic mice reflects features of the natural TSEs in other species or whether it results in clinical artifacts that do not accurately mimic the disease process has yet to be established. Some of the PrP transgenes that have been used to express PrP in transgenic mice also include the recently described *Prnd* gene encoding Dpl (Refs 43, 44). Since Dpl overexpression has been postulated to lead to ataxia in mice (Ref. 30), it might be partly responsible for some of the neurological disease developing spontaneously in these animals. However, this requires further investigation.

Polymorphisms in PrP alter the disease incubation time

Allelic forms of murine PrP differing at residues 108 and 189 have been shown to be closely associated with the gene *Sinc*/*Prni*, which controls survival time of mice exposed to scrapie (Ref. 45). *Sinc* comprises two alleles, *s7* and *p7*, which programme short and prolonged incubation times, respectively (Ref. 46). Gene targeting was used to establish whether *Sinc, Prni* and *Prnp* are the same gene. The PrP gene associated with the *Sinc s7* allele (Leu108; Thr189) was altered by gene targeting in embryonic stem (ES) cells to that associated with a *Sinc p7* mouse (Pro108; Val189) (Ref. 9). The gene-targeted mice inoculated with a mouse-passaged BSE strain (i.e. brain material sub-passaged in mice from a mouse previously inoculated with cattle brain infected with BSE) showed a dramatic alteration in incubation time (113 days) when compared with their wild-type littermates (244 days). The transgenic mice produced in these experiments were identical to the wild-type mice, apart from the amino acid 108 and 189 alterations introduced into the PrP gene. Therefore, the difference in incubation time could be attributed entirely to these polymorphic differences. It was thus established that *Sinc*, *Prni* and *Prnp* were indeed the same gene.

Allelic forms of PrP are also associated with altered incubation time or susceptibility in other species. Polymorphisms at positions encoding amino acids 129 and 219 are associated with human TSEs (Refs 7, 8), and polymorphisms at positions encoding amino acids 136, 154 and 171 are associated with TSE susceptibility in sheep (Ref. 10). Transgenic models overexpressing PrP genes encoding the two polymorphisms at position 129 (Met129 and Val129) have been produced to study human TSE disease. In one study, transgenic mice with a PrP transgene composed entirely of human *PRNP* and with the Val129 polymorphism were no more susceptible to human TSEs than were non-transgenic mice when the endogenous murine PrP was also present. However, if the endogenous murine PrP gene was removed by crossing the transgenic mice onto a PrP null background, these mice became susceptible to several human TSEs (Ref. 47). By contrast, a second study using the same line of transgenic mice showed increased susceptibility of the transgenic mice to CJD in the presence of the endogenous murine PrP gene (Ref. 48). These disparities might be explained on the basis of differences in human TSE strains used in the studies, although this remains to be established. PrP transgenes consisting of a chimaeric gene composed of mouse and human PrP DNA (with murine sequence coding for the C-terminus of PrP) were also produced. These transgenic mice were susceptible to human TSEs regardless of whether the endogenous murine PrP was present or not. These experiments have led to the suggestion that an additional component, referred to as protein X, binds at the C-terminus of PrP and is required for infectivity of TSEs. The researchers speculate that this macromolecule might act as a molecular chaperone for PrP^{Sc} (Ref. 47).

Transgenic mice expressing a human transgene with Met129 appear to be more susceptible to TSE infectivity derived from a Met129 individual (Ref. 47). However, mice expressing a human transgene encoding Val129 appear equally susceptible to TSE infectivity from a Val129 or Met129 individual (Ref. 3). Further study is consequently required before the mechanism

Accession information: (01)00295-2a.pdf (short code: txt001jme); 11 May 2001
ISSN 1462-3994

underlying PrP polymorphisms and disease susceptibility can be understood.

The species of PrP influences susceptibility

In vitro assay systems (Ref. 49) and mouse models (Ref. 50) have suggested that homology between host PrP^{C} and the PrP^{Sc} associated with infectivity facilitates the infectious process in the host. Thus, by introducing an appropriate PrP gene into transgenic mice, the species barrier can be overcome. This was demonstrated using transgenic mice expressing a hamster PrP gene. These were found to be susceptible to a hamster strain of scrapie, in contrast to wild-type mice, which did not succumb to disease with this agent (Ref. 41). However, apparently incompatible PrP sequences can also result in disease transmission. BSE has been passed through many species with different PrP sequences (e.g. mice and sheep) and yet has retained its strain identity (Ref. 51). This phenomenon has also recently been demonstrated in transgenic animals overexpressing a bovine PrP transgene. These mice are not only susceptible to BSE but are also susceptible to vCJD and sheep scrapie (Ref. 52). Thus, although these results have demonstrated that PrP is at least partly responsible for the species barrier, it is also clear that factors other than the primary sequence of PrP are also involved in transmission between species.

PrP mutations and human TSEs

While polymorphisms in PrP have been demonstrated to alter the incubation time of TSEs following exposure to an infectious agent, several point mutations, deletions and insertions in the human PrP gene appear to lead directly to spontaneous genetic disease. Many human TSEs linked to mutations in the PrP gene have also been shown to be transmissible to rodents and primates (Ref. 53). It has been hypothesised that these mutations alter the structure or processing of the protein, resulting in a destabilisation of the PrP molecule (Refs 54, 55, 56). This in turn might make it more likely for PrP^{C} to convert to PrP^{Sc} without any requirement for an exogenous infectious agent.

Transgenic models have been produced to investigate several of these human TSEs that are linked to PrP mutations. The Leu102 mutation in human PrP has been shown to be closely linked to GSS in family studies by lod score analysis (Ref. 57). The equivalent mutation (Leu101) was introduced into a murine PrP transgene and mice were generated with 64 copies of the transgene (Ref. 43). These mice produced eightfold more PrP protein than wild-type mice produced and developed a spontaneous neurological disease. It was later reported that some mice expressing a level of the transgene equivalent to the endogenous murine PrP also developed a neurological disease (Ref. 58). The neurological disease that developed in the overexpressing Leu101 mice included spongiform change, astrogliosis and amyloid plaques that were shown to contain PrP, although further analysis demonstrated that the PrP was not protease-resistant PrP (i.e. not PrP^{Sc}). Moreover, this spontaneous disease could be transmitted to transgenic mice expressing the Leu101 transgene and to hamsters (Ref. 43). This model thus suggests that the Leu102 mutation might indeed result in genetic disease that is initiated by an unstable PrP.

In contrast to these results, a gene-targeted model of the Leu101 mutation was recently developed and mice homozygous or heterozygous for this mutation showed no sign of spontaneous neurological disease up to 899 days of age (Ref. 13). Furthermore, no disease could be transmitted from these mice to either transgenic or non-transgenic mice. However, it was demonstrated that the introduction of a single amino acid alteration into the endogenous murine PrP gene altered the susceptibility of the transgenic mice (homozygous for Leu at amino acid 101; 101LL) to TSE infectivity (Table 2). This model therefore provides no evidence for a spontaneous neurological disease associated with the Leu101 mutation, but does provide evidence that this mutation alters susceptibility of the mice to TSE infectivity (Ref. 13). In addition, this model provides no evidence that the Leu101 mutation leads to an inherently unstable molecule, since Leu101 transgenic mice, identical in every other respect to the wild-type mice, can have longer incubation times than the wild-type mice when infected with some strains of TSE (Ref. 13). It has therefore not yet been definitively established whether the human TSEs associated with mutations in the PrP gene represent genetic disease or altered genetic susceptibility to TSE infectivity.

Other transgenic models have also been produced that overexpress PrP transgenes with

Accession information: (01)00295-2a.pdf (short code: txt001jme); 11 May 2001
ISSN 1462-3994

Table 2. Gene-targeted transgenic mice expressing PrP with the Pro101Leu mutation[a] (tab002jme)

Mouse line	No. with clinical disease/total	TSE inoculum	Incubation period (days)
101LL[b]	15/15	GSS[c]	288
101PP[d]	1/7	GSS[c]	450
101LL[b]	18/18	GSS-LL[e]	148
101PP[d]	16/16	GSS-LL[e]	226
101LL[b]	18/18	ME7[f]	338
101PP[d]	10/10	ME7[f]	161

[a] Data taken from Ref. 13
[b] Gene-targeted transgenic mice expressing PrP protein with leucine (L) at position 101
[c] Brain material from patient with GSS
[d] Non-transgenic mice expressing PrP protein with proline (P) at position 101
[e] Brain material from GSS-challenged 101LL tansgenic mice
[f] Mouse-passaged TSE agent
Abbreviations: GSS, Gerstmann–Straussler–Scheinker disease; TSE, transmissible spongiform encephalopathy.

mutations associated with human TSEs (Refs 59, 60). One of these mutations, Ala117Val, has been demonstrated to be associated with a transmembrane form of PrP. This has been detected in both transgenic mice and in a patient with the Ala117Val mutation (Ref. 61), neither of which have been shown to transmit TSE. This has led to the hypothesis that the transmissibility of TSE diseases might be due to PrP^{Sc}, but that the neurodegeneration might be caused by a transmembrane PrP that cannot itself transmit disease (Ref. 62).

Future transgenic models for TSE research

The transgenic models of the TSEs produced to date have provided a wealth of information on these diseases. In particular, these studies have shown that PrP is central to the disease process and that the amount of PrP controls incubation time. Furthermore, polymorphisms in PrP have been demonstrated to control incubation time in mice, and models have been developed for examining polymorphisms in other species. It has also been established that the species barrier is, at least in part, controlled by PrP, and it has been shown that a single amino acid alteration in PrP can dramatically alter incubation time.

Despite these advances, many fundamental questions still remain to be answered. Is PrP the infectious agent? How are strains of agent generated and maintained? How does infectivity reach the CNS from the peripheral tissues and can interference in this process block the onset of disease? These questions are being addressed with the transgenic models currently available, but also with the generation of new models in which gene targeting is being used to introduce specific mutations into the endogenous murine PrP gene to examine susceptibility, incubation times and strains of agent. The species of PrP can also be altered through gene targeting to develop models with no barrier to TSE agents from other species. In addition, models are being developed that will allow the expression of PrP at different time points in specific tissues (Fig. 4). This might be achievable using systems that allow regions of DNA, flanked by recognition tags, to be excised by transgenes that express enzymes capable of such a function. Therefore, by controlling the expression of the excision enzyme, using tissue-specific promoters to drive enzyme expression and/or drug-induced activation of the enzyme, expression of the gene of interest (e.g. PrP) can be controlled, in a temporal and/or spatial manner. Such models should allow the pathogenic events leading to neurodengeneration in the CNS to be

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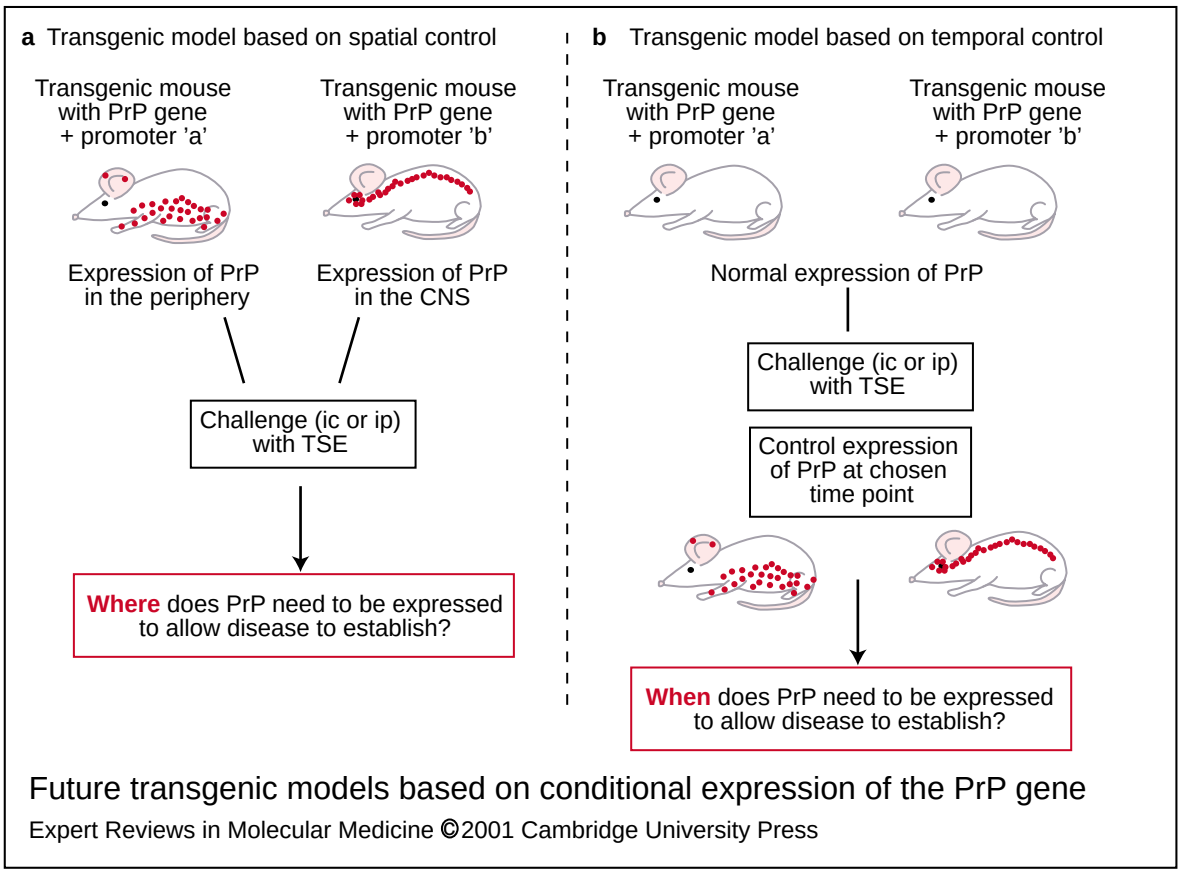

Figure 4. Future transgenic models based on conditional expression of the PrP gene. (a) Spatial control. By using specific promoters, the PrP gene could be expressed in the periphery (left) or in the central nervous system (right) as indicated in red. When these animals are challenged with TSE (transmissible spongiform encephalopathy) agent, either intracerebrally (ic) or intraperitoneally (ip), it will be possible to determine where PrP must be expressed to allow disease to establish. (b) Temporal control. Following normal expression of PrP and TSE-challenge, at a chosen time point PrP expression could be controlled to determine whether disease progression is altered (red indicates where PrP expression is prevented). The normal function of PrP could also be investigated by temporal control of PrP expression in the adult animal, therefore avoiding the possibility of gene compensation occurring for the lack of PrP expression. Both of these models will provide information important for the development of potential therapeutic intervention **(fig004jme)**.

identified and the potential for therapeutic intervention in these diseases to be investigated.

Conclusions

The work described in this review has shown that transgenic mouse models have already been invaluable in helping us achieve a better understanding of the TSE diseases. They have allowed us to determine that PrP is essential for the TSE diseases to occur and that differences in PrP sequence can affect incubation periods. However, it has also highlighted that many questions still remain unanswered. Without doubt, the present and future transgenic models will continue to generate information that will lead to much better knowledge of this fascinating group of diseases. This in turn will enable us to begin to develop potential therapeutic interventions with the aim of treating and/or preventing TSE infection.

Acknowledgements and funding

We thank Patricia McBride (Neuropathogenesis Unit, Institute for Animal Health, Edinburgh, UK) and Robert Somerville (Neuropathogenesis Unit, Institute for Animal Health, Edinburgh, UK) for reviewing this article. The Institute for Animal Health, the Medical Research Council, and the Biotechnology and Biological Sciences Research Council provided funding.

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Further reading, resources and contacts

Biotechnology and Biological Sciences Research Council home page.

http://www.iah.bbsrc.ac.uk/

The Edinburgh TSE Initiative website, planned for May 2001. It will contain public pages describing the research of members of the group and include links to their own websites and publication lists. There will also be a members-only area.

http://www.tse.ac.uk/

Information on BSE provided by the UK's Ministry of Agriculture, Fisheries and Food.

http://www.maff.gov.uk/animalh/bse/index.html

The report of the Phillips Inquiry into BSE and vCJD in the UK, published October 2000.

http://www.bse.org.uk/

Review of BSE controls to protect public health and the food chain are being reviewed by the UK's Food Standards Agency.

http://www.foodstandards.gov.uk/bsereview.htm

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ISSN 1462-3994

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Figures

Citation details for this article

Jean C. Manson and Nadia L. Tuzi (2001) Transgenic models of the transmissible spongiform encephalopathies. Exp. Rev. Mol. Med. 11 May, http://www-ermm.cbcu.cam.ac.uk/01002952h.htm

Accession information: (01)00295-2a.pdf (short code: txt001jme); 11 May 2001
ISSN 1462-3994