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Genetics of prion diseases and prion diversity in mice

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SUMMARY

Linkage of the prion protein (PrP) and scrapie incubation time genes in mice provided strong evidence for the central role of PrP in determining susceptibility to prion disorders. Considerable evidence now argues that the prion protein and incubation time genes are identical. The mouse prion protein gene (*Prn-p*) may act both quantitatively and qualitatively in modulating prion incubation time. Differences at positions 108 and 189 between PrP-A and PrP-B allotypes can place constraints on interaction between the normal cellular and the scrapie-specific isoforms of PrP (PrP^{C} and PrP^{Sc}), although the supply of PrP^{C} available for post-translational conversion to PrP^{Sc} can also influence incubation time. Results using transgenic (Tg) mice in studies on scrapie 'strains' or isolates suggest that incubation time characteristics of scrapie isolates can be explained by these two properties of PrP. The final section of this report discusses the novel finding that uninoculated Tg mice overexpressing wild-type (wt) PrP transgenes spontaneously develop a late-onset degenerative neuromyopathy, broadening the spectrum of prion diseases and providing new information on PrP function in both normal and pathological states.

1. INTRODUCTION

Inbred strains and genetic variants of the laboratory mouse have been used to provide much of our understanding of experimental scrapie. The initial observation of an extraordinarily long scrapie incubation time in the VM stock of mice compared with other mouse strains (Dickinson & MacKay 1964), and the subsequent demonstration that the effect was due to a single gene, designated *Sinc* (Dickinson *et al.* 1968), profoundly influenced the direction of scrapie research for nearly 30 years. Based on the microbiological paradigm, multiple 'strains' of scrapie agent were defined on the basis of incubation times and other isolate properties in mice of different *Sinc* genotypes (Dickinson & Meikle 1971). Among the many scrapie isolates were some that were true-breeding and exhibited distinct properties in the same inbred strain of mice, justifying the premise of a host-independent informational macromolecule, thought to be a polynucleotide, specified by the infectious agent.

Biochemical fractionation for scrapie infectivity in Syrian hamsters, a species of severely limited use in genetic studies but ideal for rapid scrapie bioassay (Prusiner *et al.* 1982), led to the identification and isolation of prion protein (PrP) (Bolton *et al.* 1982). A cDNA encoding PrP was recovered from scrapie-infected hamster brain and used to demonstrate that the PrP gene was chromosomal and not present in the infectious agent (Oesch *et al.* 1985; Basler *et al.* 1986); this PrP clone also provided a probe for genetic studies in mice. A survey of common inbred strains of mice suggested an exceptionally long prion incubation time in I/LnJ mice (Kingsbury *et al.* 1983), and formal backcross analysis demonstrated linkage between a locus (*Prn-i*) controlling incubation times for the Chandler scrapie isolate and prion protein gene (*Prn-p*) restriction fragment length polymorphisms (Carlson *et al.* 1986). *Prn-i* and *Sinc* are synonymous, but most, if not all, effects attributed to their two alleles reflect differences at codons 108 and 189 between the *a* and *b* alleles of *Prn-p* (Westaway *et al.* 1987).

The ability to manipulate the genome through transgenesis freed mouse geneticists from reliance on naturally occurring mutations in studying experimental scrapie. For example, a number of mutations in the human PRNP gene segregate with familial prion disease loci (Prusiner 1991); spontaneous neurodegeneration in transgenic (Tg) mice expressing a codon 101 mutant *Prn-p* transgene (equivalent to human codon 102) indicates this single amino acid substitution as a direct cause of Gerstmann-Sträussler-Scheinker (GSS) disease (Hsiao *et al.* 1990). Similarly, results from Tg mice expressing Syrian hamster PrP genes demonstrated that preferential interaction between homologous PrP molecules provided an explanation for the species barrier to scrapie transmission (Scott *et al.* 1989). Novel genes can also be introduced into the mouse germline; mice expressing chimeric mouse–hamster PrP genes produce prions with novel properties and emphasize the importance

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of PrP primary structure in constraining the behaviour of distinct scrapie isolates (Scott *et al.* 1993).

Mice deleted for PrP coding sequences through homologous recombination reinforce the concept that PrP is essential for susceptibility to inoculated prions and for replication of infectivity (Büeler *et al.* 1993). PrP^{C}, the normal cellular isoform, is converted to PrP^{Sc}, its disease-specific post-translational variant. PrP^{Sc} is the major, and possibly sole, component of infectious prion particles (Prusiner 1991). The supply of PrP^{C} that is available for conversion to PrP^{Sc} dramatically influences prion incubation time; mice with only a single functional copy of *Prn-p* have extraordinarily long scrapie incubation times (Büeler *et al.* 1993), whereas extra copies of PrP as transgenes shorten incubation time in inverse proportion to the amount of PrP^{C} expressed (Prusiner *et al.* 1990).

In this report, we will discuss the use of formal and molecular mouse genetics as tools for understanding the biochemical processes involved in prion diseases. First, we will outline the evidence that the prion protein and incubation time genes are identical. The second part will emphasize the use of Tg mice for studies on scrapie 'strains' or isolates. We will argue that our results, and those of others, suggest that properties of scrapie isolates can be explained by constraints on interaction between PrP^{C} and PrP^{Sc}, and by PrP^{C} concentration. The final section presents the novel finding that uninoculated mice overexpressing wild-type (wt) PrP transgenes spontaneously develop a late-onset degenerative neuromyopathy (Westaway *et al.* 1994*a*), broadening the spectrum of prion diseases and providing new information on PrP function in both normal and pathological states.

2. THE PRION PROTEIN AND PRION INCUBATION TIME GENES ARE EQUIVALENT

Genetic linkage studies in mice provided the first evidence that the prion protein gene might modulate susceptibility to prion diseases (Carlson *et al.* 1986). Backcross and restriction fragment length polymorphism (RFLP) analyses using I/LnJ (prolonged incubation time for the Chandler scrapie isolate) and NZW/LacJ (short incubation time) mice demonstrated that incubation period was controlled by a single dominant gene linked to *Prn-p*. The sequence of genomic clones encompassing the entire open reading frames of the NZW/LacJ ($Prn\text{-}p^{a}$) and I/LnJ ($Prn\text{-}p^{b}$) alleles provided compelling evidence that *Prn-p* itself is responsible for control of scrapie incubation time (Westaway *et al.* 1987). The two alleles encode distinct proteins, PrP-A and PrP-B, which differ at residues 108 (Leu/Phe) and 189 (Thr/Val).

However, the conclusion that *Prn-p*, rather than a distinct linked locus, controlled prion incubation time in mice was tempered by the reproducible appearance of mice with discordant incubation time phenotype and *Prn-p* genotype among offspring of test crosses. Presumptive recombination frequencies between *Prn-p* and *Prn-i* ranged from 0.015 ± 0.015 to 0.059 ± 0.04 in four independent crosses in two laboratories (Carlson *et al.* 1986; Carlson *et al.* 1988; Race *et al.* 1990). Unfortunately, the lethal nature of the scrapie bioassay precluded progeny testing to determine whether the discordant mice were truly *Prn-p*–*Prn-i* recombinants. Results from transgenic mice were also compatible with the hypothesis of an incubation time gene distinct from *Prn-p* (Westaway *et al.* 1991). Expression of $Prn\text{-}p^{b}$ transgenes not only failed to reproduce the long incubation time phenotype of normal $Prn\text{-}p^{b}$ mice, but caused shorter incubation times than non-Tg animals.

Taking advantage of easily scored mutations flanking *Prn-p*, experiments were designed to obtain a population of mice that theoretically would be enriched for recombinants between *Prn-p* and *Prn-i* (Carlson *et al.* 1993). *Prn-p* is located between the wellhaarig (*we*) and undulated (*un*) mutations on chromosome 2; *we*/*we* mice have a wavy first coat whereas *un* homozygotes have a shortened, kinked tail. A backcross with *we* and *un* in coupling allowed visual screening for mice with only one of the two mutant phenotypes suggesting recombination in the vicinity of *Prn-p*. DNA typing for loci within the *we*–*un* interval localized the crossover position in each recombinant. Offspring of recombinant mice were inoculated with scrapie prions to determine whether crossover between *Prn-p* and a putative prion incubation time gene had occurred. Each mouse had the scrapie incubation time predicted by its *Prn-p* genotype. As shown in figure 1, these results excluded all but ≈ 0.67 centiMorgans (cM) proximal and ≈ 0.22 cM distal from *Prn-p* as a possible location for *Prn-i*. The two strains of mice used in these 'recombinant capture' studies were very closely related, in contrast to earlier crosses between more distantly related strains that generated the putative *Prn-p*–*Prn-i* recombinants. We conclude that the apparent genetic dissociation of incubation time and *Prn-p* genotype reflects simultaneous inheritance of unlinked loci with minor influences on prion diseases. The effects of such non-*Prn-p* loci are evident in mice as dramatic differences in incubation times between inbred strains that carry the same *Prn-p* alleles (Carlson *et al.* 1988). Similarly, incomplete penetrance of pathogenic mutations in PRNP in humans may reflect the effects of other genes on prion disease susceptibility.

The finding that over-expression of $Prn\text{-}p^{b}$ transgenes from long incubation time mice shortens rather than delays disease onset for the Chandler scrapie isolate (Westaway *et al.* 1991) cannot be taken as evidence for a distinct incubation time locus, because the amount of PrP^{C} that is available dramatically influences scrapie incubation time. In mice transgenic for a Syrian hamster (SHa) PrP gene, the incubation time for hamster prions is inversely correlated with the amount of $SHaPrP^{C}$ expressed as determined with monoclonal antibodies that do not cross-react with mouse PrP (Prusiner *et al.* 1990). Although lacking antibodies that discriminate between the PrP-A and PrP-B allotypes, a similar trend is seen in Tg ($Prn\text{-}p^{b}$) mice, with high transgene copy number lines having shorter incubation times than lines with few copies of the transgene (Westaway *et al.* 1991). Prolongation of

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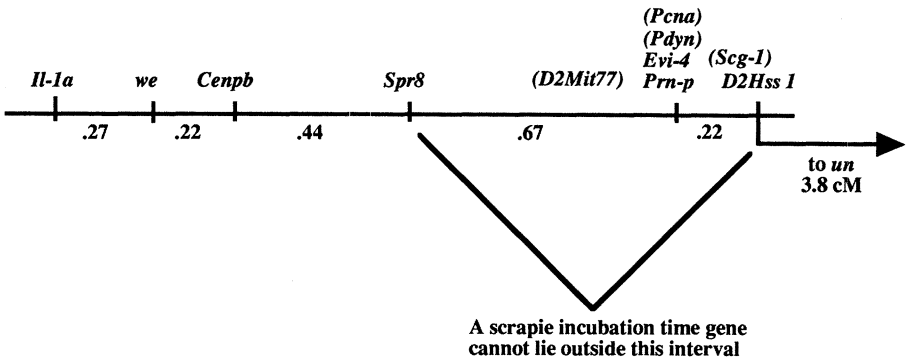

Figure 1. Limits on the location of a putative prion incubation time gene. Genetic map of the *Prn-p* region of mouse chromosome 2 based on results from 451 offspring of a B10.UW/Sn × (B6.I/Co × B10.UW)F1 backcross (Carlson *et al.* 1993); map distances are in centiMorgans. Scrapie incubation time in offspring of *Spr8–Prn-p* and *Prn-p–D2Hss1* recombinant mice were concordant with *Prn-p* genotype, indicating that a chromosome 2 incubation time locus cannot lie outside the *Spr8–D2Hss1* interval. Loci mapped in other crosses (Siracusa *et al.* 1990; Zuberi & Roopenian 1993) are enclosed in parentheses and their approximate position indicated. Recombination between *Prn-p* and *Pcna* (proliferating cell nuclear antigen), *Pdyn* (prodynorphin) or *Evi-4* (ecotropic integration site 4) have not been observed; *Evi-4* and *Prn-p* have been physically linked in a single yeast artificial chromosome (YAC) clone (Westaway *et al.* 1994*b*).

scrapie incubation period is a consequence of reduction in PrP^{C} expression. Mice with only a single functional *Prn-p* allele with the other ablated through homologous recombination have greatly prolonged incubation times.

Prn-p may act both quantitatively and qualitatively in modulating prion incubation time. The differences in PrP-A and PrP-B primary structure places constraints on interaction with PrP^{Sc} (Carlson *et al.* 1989), and the supply of PrP^{C} available for post-translational conversion to PrP^{Sc} can also influence incubation time (Westaway *et al.* 1991; Büeler *et al.* 1993). Although there is no obvious difference in *Prn-p*^{a} and *Prn-p*^{b} mRNA levels, polymorphisms near the 5′ untranslated exons and in the large intron raise the possibility of differential regulation (Westaway *et al.* 1994*b*).

3. ALLOTYPIC PREFERENCE IN PrP^{C}–PrP^{Sc} INTERACTION CAN ACCOUNT FOR INCUBATION TIME PROPERTIES OF DISTINCT SCRAPIE ISOLATES

The existence of true-breeding prior isolates or strains with distinct properties demonstrates that the prion carries information independent of the host (Dickinson & Meikle 1971; Bruce & Dickinson 1987; Bruce *et al.* 1991). One of the most striking properties used in classifying scrapie isolates is incubation time profile in mice with *a*/*a*, *b*/*b*, and *a*/*b* *Prn-p* genotypes. Scrapie isolate behaviour in Tg mice is providing new clues to the nature of prion-specified information. Two examples, both using Tg(*Prn-p*^{b})15 mice that harbour three copies of *Prn-p*^{b} in the transgene array, will illustrate. The Chandler and related isolates are the most widely used mouse scrapie strains; the *b* allele of *Prn-p* behaves in a dominant fashion and prolongs scrapie incubation time (table 1). Paradoxically, Tg mice expressing *Prn-p*^{b} from long incubation time mice had shorter incubation times than non-Tg mice (Westaway *et al.* 1991). As shown in table 1, the abbreviating effect of transgene expression on scrapie incubation time is greatly reduced when the three-copy transgene array is expressed by *Prn-p*^{a}/*Prn-p*^{b} heterozygous mice compared with *Prn-p*^{a} homozygotes (Carlson *et al.* 1993). This could be interpreted as evidence for a distinct prion incubation time locus. A more economical explanation postulates that PrP^{C}-A is converted to PrP^{Sc} much more efficiently by the Chandler isolate-specific PrP^{Sc} isoform than is PrP^{C}-B, and invokes the sensitivity of incubation time to PrP^{C} concentration (figure 2). Rather than attributing the long incubation time of *Prn-p* heterozygous mice to an active effect of the *b* allele, we propose that the reduced amount of PrP^{C}-A is responsible. Transgene expression in *Prn-p*^{a} homozygous mice has no effect on the supply of PrP^{C}-A, and additional PrP^{C}, even the less efficiently converted PrP^{C}-B, can only shorten incubation time. Under this scenario, the effect of expression of an 'authentic' *Prn-p*^{b} allele in prolonging the incubation time of Tg (*Prn-p*^{b}) 15 mice simply reflects reduction in

Table 1. *Effect of transgene expression on incubation times for the Chandler scrapie isolate*

mice	Prn-p genotype	Prn-p transgenotype	incubation time/d
C57BL/6J (B6)	a/a	−/−	143 ± 4
non-Tg 15	a/a	−/−	130 ± 3
B6.I	b/b	−/−	360 ± 16
B6.I × non-Tg 15	a/b	−/−	255 ± 7
Tg 15 (+/−)	a/a	bbb/−	112 ± 3
Tg 15 (+/+)	a/a	bbb/bbb	107 ± 5
B6.I × Tg 15 (+/−)	a/b	bbb/−	166 ± 2

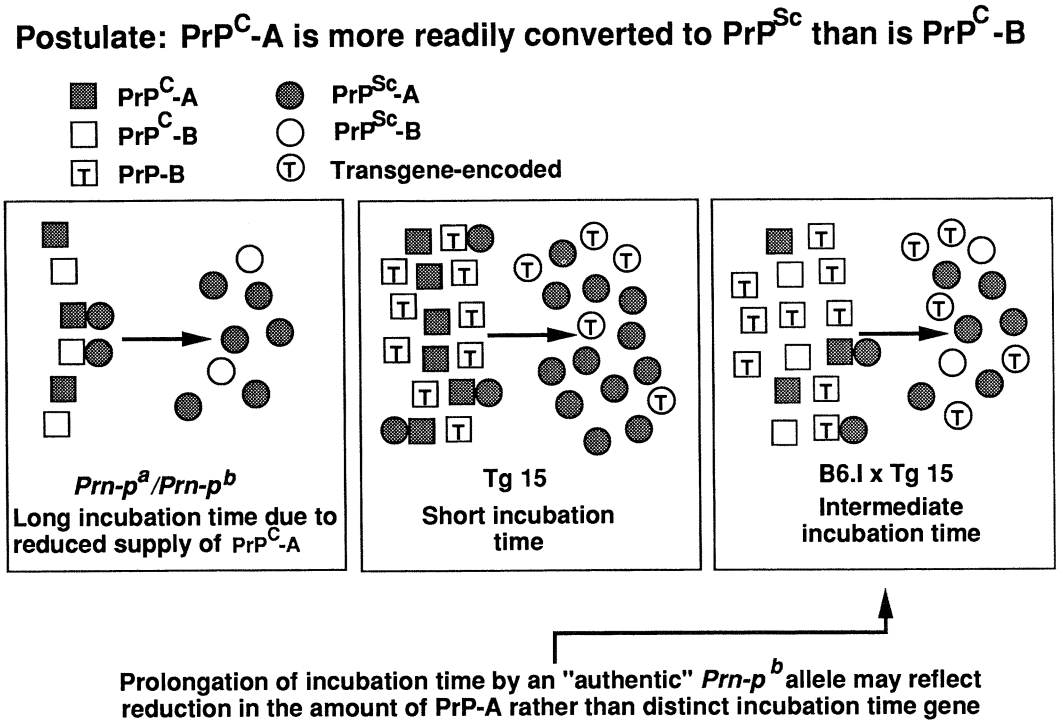

Figure 2. Models for incubation time behaviour of the Chandler scrapie isolate. The postulate that Chandler-specific PrP^{Sc} converts PrP^{C}-A to PrP^{Sc} more efficiently than PrP^{C}-B can explain the incubation time results presented in table 1. See text for details.

PrP^{C}-A concentration, rather than something lacking in the transgene.

The incubation time for the 22A scrapie isolate is shorter in *Prn-p*^{b} mice than in *Prn-p*^{a} animals (Dickinson & Meikle 1971). In further contrast with more commonly used isolates, *Prn-p* heterozygous mice have longer incubation times than either parent. This overdominance led Dickinson & Outram (1979) to conclude that *Sinc* encoded the replication site for the scrapie agent and that this replication site was a dimer. Overdominance does suggest that the target for PrP^{Sc} may be a PrP^{C} dimer or multimer. Using Tg mice to increase the ratio of PrP-B to PrP-A over that seen in *Prn-p*^{a}/*Prn-p*^{b} heterozygous mice shortens 22A incubation time (table 2). The assumptions for the cartoon in figure 3 are that PrP^{C}-B is more readily converted to PrP^{Sc} than is PrP^{C}-A, and that PrP^{C}-A:PrP^{C}-B dimers are very resistant to conversion to PrP^{Sc}. It is also possible that overdominance simply reflects reduction in supply of PrP^{C}-B. Regardless, it is clear that increasing the supply of PrP^{C}-B through transgenetics shortens 22A incubation time.

Although constraints placed by PrP primary structure on formation of host PrP^{Sc} by distinct scrapie isolates can explain incubation time properties, we must emphasize that the nature of PrP^{C}-PrP^{Sc} interaction remains a mystery. In addition to incubation time properties, scrapie isolates differ in the location and type of pathological lesions within the brain (Bruce *et al.* 1976; Hecker *et al.* 1992; see also articles by Bruce and by DeArmond, this symposium). Whether this reflects covalent modifications or alternative conformations specific to distinct cell types is unknown, but the biochemical nature of prion-specified information is one of the most intriguing problems in biology today.

4. SPONTANEOUS NEUROMUSCULAR DISEASE IN MICE OVEREXPRESSING WILD-TYPE PRION PROTEIN TRANSGENES

As discussed above, Tg mice have provided exciting new information on the nature of prion diseases. During the course of some of these experiments we observed that Tg mice with high copy numbers of wild-type (wt) PrP genes succumbed to spontaneous neurologic disease (Westaway *et al.* 1993). Disease was observed in mice expressing SHaPrP, Mouse (Mo) PrP-B, and Sheep (She) PrP transgenes. Within each Tg line, there were a variety of clinical signs whose spectrum overlapped those seen in other lines (table 3). For example, Tg(SHaPrP+/+)7 mice (+/+

Table 2. *Influence of PrP-B transgene expression on incubation times of 22A scrapie isolate*

mice	Prn-p genotype	Prn-p transgenotype	incubation time/d
B6	a/a	−/−	405 ± 2
B6.I	b/b	−/−	194 ± 10
B6 × B6.I	a/b	−/−	508 ± 14
Tg 15 (+/−)	a/a	bbb/−	395 ± 12
Tg 15 (+/+)	a/a	bbb/bbb	286 ± 15

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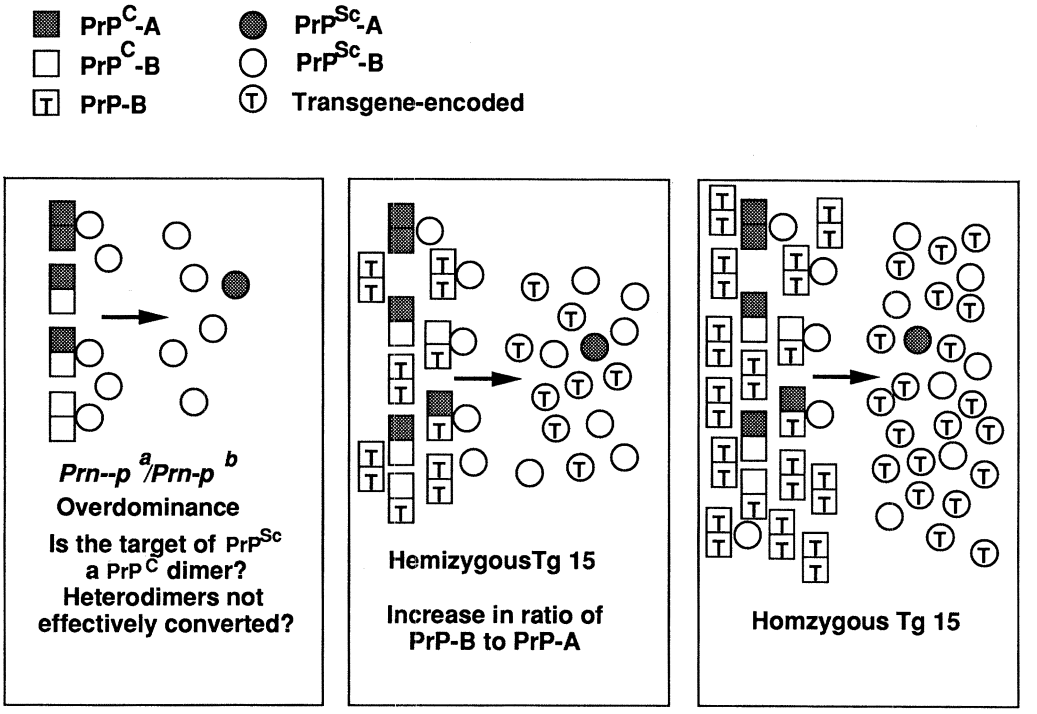

Figure 3. Models for incubation time behaviour of the 22A scrapie isolate. See text for details.

indicates homozygosity for the transgene array) developed progressive disease presenting with tremors, ataxia, head-bobbing and an abnormal hunched posture, whereas the majority of Tg(Prn-p^{b}+/−)94 mice (+/− indicates hemizygosity for the transgene array) presented with hind-limb paresis progressing to paralysis. Age at onset of disease is related to the amount of PrP^{C} expressed. The comparison between homozygous Tg(SHaPrP+/+)7 and hemizygous Tg(SHaPrP+/−)7 mice is particularly revealing. Homozygous Tg7 mice have ≈120 copies of SHaPrP and develop disease at 468 ± 8 (s.e.m.) days and die at 546 ± 5 days (figure 4). In contrast, no hemizygous Tg7 animals with ≈60 copies of the transgene array showed any sign of neurologic illness before 650 days, as illustrated in figure 5. Homozygous Tg7 mice express 367 ± 34 μg of SHaPrP per gram of brain protein, whereas hemizygous Tg7 animals had 195 ± 12 μg per gram. No clinical disease was evident at similar ages in Tg(SHaPrP)71 and Tg(SHaPrP)81 mice that expressed 128 ± 12 μg and 56 ± 7 μg per gram of the hamster transgene, nor in non-Tg animals or in Tg mice expressing β-amyloid precursor protein. A similar relation is seen in mice expressing mouse PrP-B, with disease occurring only in high copy number lines.

Results from pathologic examination are available for Tg(SHaPrP+/+)7 and Tg(*Prn-p*^{b}+/−)94 lines. These Tg mice with signs of neurologic dysfunction exhibited similar changes in the CNS with focal spongiform degeneration of the grey matter, localized to the stratum lacunosum moleculare of the hippocampus, the superior colliculus and midbrain tegmentum. Mild astrogliosis accompanied the spongiform change. Although similar to experimental scrapie, these changes were more focal and much less intense than those found in mice inoculated with rodent prion isolates, and seemed insufficient to account for the profound clinical signs. The hind-limb paresis in Tg94 and gait abnormalities in Tg7 led to examination of the spinal cord, sciatic nerve and muscle. Although no obvious pathology in spinal cord was noted, dramatic pathological changes were seen in both skeletal muscle and peripheral nerve. We stress that none of these changes were seen in age-matched controls.

Quadriceps muscle from clinically ill mice had scattered degenerating fibres, increased numbers of fibres with central nuclei, active phagocytosis of fibres,

Table 3. *Transgenic mice expressing wild-type PrP: spontaneous disease presentation*

transgene	Tg line	copy number	clinical signs	age range of onset/d
SHaPrP	Tg(SHaPrP+/+)7	≈120	tremor, kyphosis, gait abnormalities, head bobbing	220–619
SHaPrP	Tg(SHaPrP+/−)7	≈60	similar to Tg(SHaPrP+/+)7	653–750
SHaPrP	Tg(SHaPrP+/−)81	≈30	none	not applicable
MoPrP-B	Tg(*Prn-p*^{b}+/−)94	31	hind-limb paresis and paralysis, kyphosis	524–733
MoPrP-B	Tg(*Prn-p*^{b}+/−)15	3	none	not applicable
ShePrP	Tg(ShePrP)[a]	'high'	truncal ataxia, tremor	100–146
APP$_{695}$[b]	Tg(HuMtAPP$_{695}$)	3–4	none	not applicable

[a] Two individual founders; one was sterile, the other did not transmit a high copy number array.
[b] Human β-amyloid precursor protein under the control of the human metallothein II promoter (Beer *et al.* 1991).

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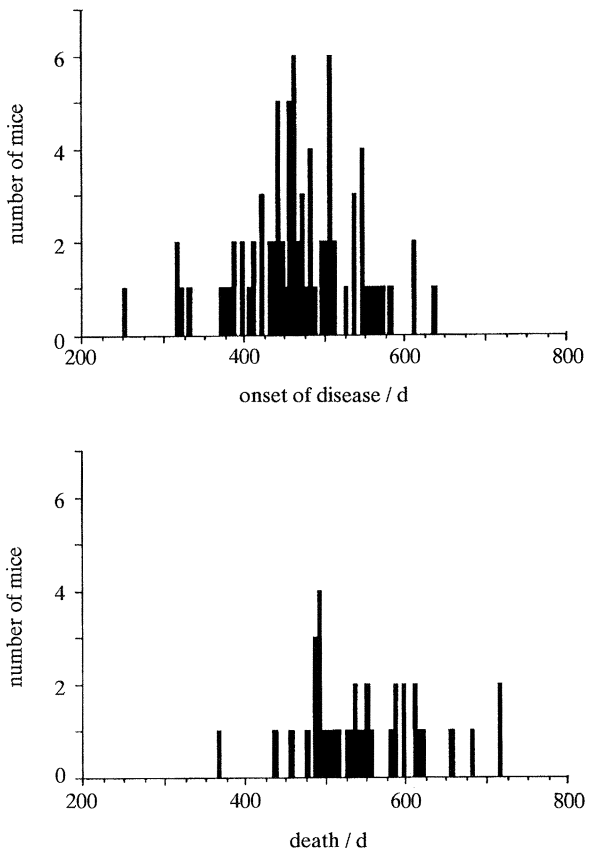

Figure 4. Spontaneous neuromyopathy in Tg(SHaPrP+/+)7 mice Transgene homozygous Tg7 mice (94) were examined twice weekly for signs of neurologic dysfunction, and 81 of these developed illness. The age of onset of disease is shown in the top histogram with each bar representing a 5-day interval; 12 mice died without showing obvious clinical signs; these mice are included in the lower panel showing age at death. Of the 18 mice with illness, 38 were observed until death; the remainder were killed for biochemical and pathological evaluation.

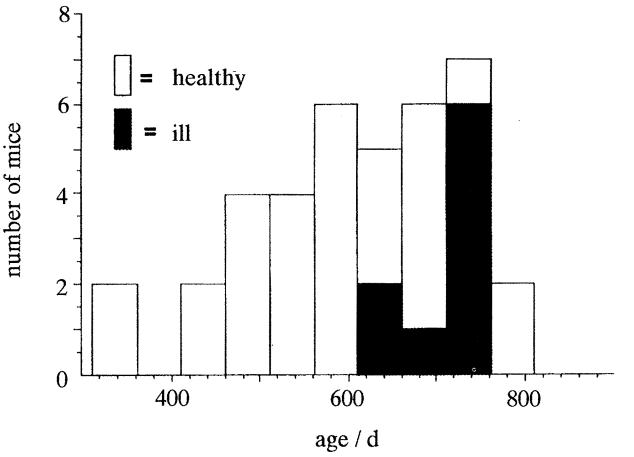

Figure 5. Very late onset spontaneous disease in Tg(SHaPrP+/−)7 mice. Transgene hemizygous Tg7 mice (38) are under observation. Only 9 mice, all over 650 days of age, have developed clinical signs of neurologic dysfunction similar to that seen in homozygous Tg7 mice (see figure 4).

and large variation in fibre size. Similar changes were found in all skeletal muscle groups including the diaphragm and intercostals; cardiac and smooth muscle appeared to be unaffected. Mitochondrial hyperplasia was also evident. The PrP transgenes are expressed in skeletal muscle with relative levels of transgene-encoded mRNA related to transgene copy number. Immunoblots revealed $SHaPrP^C$ in muscle of Tg(SHaPrP) mice; although antibodies capable of discriminating between PrP-A and PrP-B are not available, polyclonal antibody revealed a stronger signal in Tg (*Prn-p*^{b}) mice than in non-Tg animals. It is likely that muscle degeneration resulted from overexpression of wtPrP. In addition to these primary myopathic changes, fibre type grouping indicated neurogenic rearrangement.

As suggested by the grouping of Type I fibres that are normally evenly distributed among Type II fibres, there are significant abnormalities in the sciatic nerves of Tg7 and Tg94 mice. There were large numbers of thinly myelinated axons, which suggest demyelination followed by remyelination. There was also a mild loss of large myelinated axons. Again, none of these changes were seen in non-Tg mice or in mice with low copy numbers of wtPrP transgenes.

In contrast to experimental scrapie in rodents and familial prion diseases in humans, accumulation of protease-resistant PrP^{Sc} was not associated with spontaneous neuromuscular disease in mice overexpressing wtPrP. The PrP present in the brain and muscle of clinically ill mice was susceptible to proteinase K digestion. Although some faint immunoreactivity has been seen in some proteinase-treated samples, it had the same mobility as undigested samples, unlike retention of a strong signal and a shift to a lower apparent molecular mass seen with PrP^{Sc}. This suggests that the neuromyopathy was not caused by infection with exogenous prions.

Although probably due to excessive amounts of wtPrP, the mechanism of disease is unknown. PrP^C levels in brain and muscle are not obviously different between clinically ill and young Tg mice, suggesting that the late onset of disease does not reflect a requirement for accumulation of PrP, and suggesting accumulation of damage resulting from excess PrP^C. Toxicity in tissue culture has been demonstrated for a PrP synthetic peptide corresponding to residues 106–126 of human PrP (Forloni *et al.* 1993). Some known PRNP mutations that cause familial prion disease might possibly exert their effects by decreasing the turnover of PrP^C. Similarly, prion disease pathology might reflect local elevation in concentration due to accumulation of PrP^{Sc} rather than acquisition of novel toxic properties arising from the conversion of PrP^C to PrP^{Sc}. Skeletal muscle from scrapie-infected mice had no pathological changes.

Although PrP^{Sc} is not apparent in Tg(SHaPrP+/+)7 mice, brain extracts from clinically ill mice have transmitted neurologic disease to hamsters (D. Groth & S. Prusiner, unpublished results). Although additional work is needed to confirm and extend these findings, transmissibility of disease by $wtPrP^C$ has important implications for naturally occurring prion diseases of humans and animals. It is also possible that

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overexpression of PrP^{C} might lead to the formation of PrP^{Sc}-like molecules capable of transmitting disease.

These novel neurologic syndromes in Tg mice expressing high levels of $wtPrP^{C}$ extend the spectrum of prion diseases and re-emphasize transgenic mice as essential tools in discovery.

REFERENCES

Basler, K., Oesch, B., Scott, M. *et al.* 1986 Scrapie and cellular PrP isoforms are encoded by the same chromosomal gene. *Cell* **46**, 417–428.

Beer, J., Salbaum, J.M., Schlichtmann, E. *et al.* 1991 Transgenic mice in Alzheimer's disease. In *Alzheimer's disease: basic mechanisms, diagnosis and therapeutic strategies* (ed. K. Iqbal, D. R. C. McLachlan, B. Winblad & H. M. Wisniewski), pp. 473–478. New York: J. Wiley & Sons.

Bolton, D.C., McKinley, M.P. & Prusiner, S.B. 1982 Identification of a protein that purifies with the scrapie prion. *Science, Wash.* **218**, 1309–1311.

Bruce, M.E., Dickinson, A.G. & Fraser, H. 1976 Cerebral amyloidosis in scrapie in the mouse: Effect of agent strain and mouse genotype. *Neuropathol. appl. Neurobiol.* **2**, 471–478.

Bruce, M.E. & Dickinson, A.G. 1987 Biological evidence that the scrapie agent has an independent genome. *J. gen. Virol.* **68**, 79–89.

Bruce, M.E., McConnell, I., Fraser, H. & Dickinson, A.G. 1991 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. *J. gen. Virol.* **72**, 595–603.

Büeler, H., Aguzzi, A., Sailer, A. *et al.* 1993 Mice devoid of PrP are resistant to scrapie. *Cell* **73**, 1339–1347.

Carlson, G.A., Kingsbury, D.T., Goodman, P.A. *et al.* 1986 Linkage of prion protein and scrapie incubation time genes. *Cell* **46**, 503–511.

Carlson, G.A., Goodman, P.A., Lovett, M., *et al.* 1988 Genetics and polymorphism of the mouse prion gene complex: control of scrapie incubation time. *Molec. Cell Biol.* **8**, 5528–5540.

Carlson, G.A., Westway, D., DeArmond, S.J., Peterson-Torchia, M. & Prusiner, S.B. 1989 Primary structure of prion protein may modify scrapie isolate properties. *Proc. natn. Acad. Sci. U.S.A.* **86**, 7475–7479.

Carlson, G.A., Ebeling, C., Torchia, M., Westaway, D. & Prusiner, S.B. 1993 Delimiting the location of the scrapie prion incubation time gene on chromosome 2 of the mouse. *Genetics* **133**, 979–988.

Dickinson, A.G., Meikle, V.M.H. & Fraser, H.G. 1968 Identification of a gene which controls the incubation period of some strains of scrapie agent in mice. *J. comp. Pathol.* **78**, 293–299.

Dickinson, A.G. & Meikle, V.M.H. 1971 Host-genotype and agent effects in scrapie incubation: Change in allelic interaction with different strains of agent. *Molec. gen. Genet.* **112**, 73–79.

Dickinson, A.G. & Outram, G.W. 1979 The scrapie replication-site hypothesis and its implications for pathogenesis. In *Slow transmissible disease of the nervous system*, vol. 2 (ed. S. B. Prusiner & W. J. Hadlow), pp. 13–31. New York: Academic Press.

Dickinson, A.G. & MacKay, J.M.K. 1964 Genetical control of the incubation period in mice of the neurological disease, scrapie. *Heredity* **19**, 279–288.

Forloni, G., Angeretti, N., Chiesa, R. *et al.* 1993 Neurotoxicity of a prion protein fragment. *Nature, Lond.* **362**, 543–546.

Hecker, R., Taraboulos, A., Scott, M. *et al.* 1992 Replication of distinct prion isolates is region specific in brains of transgenic mice and hamsters. *Genes Dev.* **6**, 1213–1228.

Hsiao, K., Scott, M., Foster, D., Groth, D.F., DeArmond, S.J. & Prusiner, S.B. 1990 Spontaneous neurodegeneration in transgenic mice with mutant prion protein. *Science, Wash.* **250**, 1587–1590.

Kingsbury, D.T., Kasper, K.C., Stites, D.P., Watson, J.C., Hogan, R.N. & Prusiner, S.B. 1983 Genetic control of scrapie and Creutzfeldt-Jakob disease in mice. *J. Immun.* **131**, 491–496.

Oesch, B., Westaway, D., Wächli, M. *et al.* 1985 A cellular gene encodes scrapie PrP 27-30 protein. *Cell* **40**, 735–746.

Prusiner, S.B., Cochran, S.P., Groth, D.F., Bowman, K.A. & Martinez, H.M. 1982 Measurement of the scrapie agent using an incubation time interval assay. *Ann. Neurol.* **11**, 353–358.

Prusiner, S.B., Scott, M., Foster, D. *et al.* 1990 Transgenetic studies implicate interactions between homologous PrP isoforms in scrapie prion replication. *Cell* **63**, 673–686.

Prusiner, S.B. 1991 Molecular biology of prion diseases. *Science, Wash.* **252**, 1515–1522.

Race, R.E., Graham, K., Ernst, D., Caughey, B. & Chesebro, B. 1990 Analysis of linkage between scrapie incubation period and the prion protein gene in mice. *J. gen. Virol.* **71**, 493–497.

Scott, M., Foster, D., Mirenda, C. *et al.* 1989 Transgenic mice expressing hamster prion protein produce species-specific scrapie infectivity and amyloid plaques. *Cell* **59**, 847–857.

Scott, M., Groth, D., Foster, D. *et al.* 1993 Propagation of prions with artificial properties in transgenic mice expressing chimeric PrP genes. *Cell* **73**, 979–988.

Siracusa, L.D., Silan, C.M., Justice, M.J. *et al.* 1990 A molecular genetic linkage map of mouse chromosome 2. *Genomics* **6**, 491–504.

Westaway, D., Goodman, P.A., Mirenda, C.A., McKinley, M.P., Carlson, G.A. & Prusiner, S.B. 1987 Distinct prion proteins in short and long scrapie incubation period mice. *Cell* **51**, 651–662.

Westaway, D., Mirenda, C.A., Foster, D. *et al.* 1991 Paradoxical shortening of scrapie incubation times by expression of prion protein transgenes derives from long incubation time mice. *Neuron* **7**, 59–68.

Westaway, D., DeArmond, S.J., Cayetano-Canlas, J. *et al.* 1994*a* Degeneration of skeletal muscle, peripheral nerves and the CNS in mice overexpressing wild-type prion proteins. *Cell* **76**, 117–129.

Westaway, D., Cooper, C., Turner, S., DaCosta, M., Carlson, G.A. & Prusiner, S.B. 1994*b* Structure and polymorphism of the mouse prion protein gene. *Proc. natn. Acad. Sci. U.S.A.* (In the press.)

Zuberi, A.R. & Roopenian, D.C. 1993 High resolution mapping of a minor histocompatibility antigen gene on mouse Chromosome 2. *Mamm. Genome.* **4**, 516–522.

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