Chimeric elk/mouse prion proteins in transgenic mice

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Chronic wasting disease (CWD) of deer and elk is a highly communicable neurodegenerative disorder caused by prions. Investigations of CWD are hampered by slow bioassays in transgenic (Tg) mice. Towards the development of Tg mice that will be more susceptible to CWD prions, we created a series of chimeric elk/mouse transgenes that encode the N terminus of elk PrP (ElkPrP) up to residue Y168 and the C terminus of mouse PrP (MoPrP) beyond residue 169 (mouse numbering), designated Elk3M(SNIVVK). Between codons 169 and 219, six residues distinguish ElkPrP from MoPrP: N169S, T173N, V183I, I202V, I214V and R219K. Using chimeric elk/mouse PrP constructs, we generated 12 Tg mouse lines and determined incubation times after intracerebral inoculation with the mouse-passaged RML scrapie or Elk1P CWD prions. Unexpectedly, one Tg mouse line expressing Elk3M(SNIVVK) exhibited incubation times of 70 days when inoculated with RML prions; a second line had incubation times of 90 days. In contrast, mice expressing full-length ElkPrP had incubation periods of 250 days for RML prions. Tg(Elk3M,SNIVVK) mice were less susceptible to CWD prions than Tg(ElkPrP) mice. Changing three C-terminal mouse residues (202, 214 and 219) to those of elk doubled the incubation time for mouse RML prions and rendered the mice resistant to Elk1P CWD prions. Mutating an additional two residues from mouse to elk at codons 169 and 173 increased the incubation times for mouse prions to 300 days, but made the mice susceptible to CWD prions. Our findings highlight the role of C-terminal residues in PrP that control the susceptibility and replication of prions.

INTRODUCTION

Chronic wasting disease (CWD) is a fatal prion disease of the cervid family, including deer, elk and moose (Sigurdson, 2008; Williams, 2005). CWD is highly communicable among cervids. Over 90% of deer and elk in closed domesticated and game herds have been reported to be infected with CWD prions (Williams, 2005). Why CWD is so contagious among cervids is unknown, but shedding of prions in faeces probably leads to grassland contamination (Tamgüney et al., 2009b). In addition, CWD prions have been identified in tissues and bodily secretions from both ill and asymptomatic animals; these include the central nervous system (CNS), the lymphoreticular system, muscle, blood and saliva (Angers et al., 2006; Fox et al., 2006; Mathiason et al., 2006; Miller et al., 2004; Nichols et al., 2009; Sigurdson et al., 1999; Tamgüney et al., 2012).

Prions are composed solely of PrPSc, an aberrantly folded, infectious isoform of the benign cellular prion protein (denoted PrPC). Conversion of PrPC to PrPSc probably requires direct interaction between the two conformers, in which PrPSc acts as a template or seed for PrPC (Prusiner et al., 1990). The formation of prions is influenced by many factors, including the genetic background of the host, expression levels of PrPC, the primary structures of both host PrPC and infecting PrPSc, and the strain of PrPSc (Barron et al., 2001; Carlson et al., 1988, 1989; Manson et al., 1999; Scott et al., 1989, 2005). Transmission experiments of CWD prion isolates from white-tailed deer, mule deer and elk to transgenic (Tg) mice expressing deer, elk, sheep, cattle or human PrPC suggest that the transmission barrier for CWD prions among different species of the cervid family is low, whereas the transmission barrier for CWD prions among different species of the cervid family is low, whereas the transmission barrier for CWD prions to sheep, cattle and humans is high (Browning et al., 2004; Kong et al., 2005; LaFauci et al., 2006; Tamgüney et al., 2006). In contrast, sheep and sheep-passaged bovine spongiform encephalopathy (BSE) prions transmit readily to Tg mice expressing elk PrP (ElkPrP) (Green et al., 2008; Tamgüney et al., 2009a).

Tg mice expressing chimeric PrP molecules have facilitated studies of the species barrier and helped to identify critical
PrP residues that control prion transmission or de novo prion generation (Giles et al., 2010, 2012; Korth et al., 2003; Scott et al., 1993; Telling et al., 1994, 1995). Residues at the C terminus of chimeric human/mouse PrP transgenes were identified to be critical in facilitating transmission of human prions to Tg mice (Telling et al., 1995). Introduction of elk residues S169N and N173T into mouse PrP (MoPrP; mouse numbering) was found to result in the de novo generation of transmissible prions in Tg mice (Sigurdson et al., 2009).

To interrogate further the role of the C-terminal residues in the conversion of PrP<sup>C</sup> into PrP<sup>Sc</sup>, we created 12 Tg mouse lines on a background where the wild-type (wt) mouse PrP gene had been ablated (Büeler et al., 1992). We constructed a series of chimeric elk/mouse PrP transgenes that encode the N terminus of ElkPrP up to residue Y168 and the C terminus of MoPrP beyond residue 169 (mouse numbering), designated Elk3M(SNIVVK). Between codons 169 and 219, six residues distinguish ElkPrP from MoPrP; namely, N169S, T173N, Y174F, Q180R, V202I, and I214V (Fig. 1). For each Tg line, we determined incubation times after intracerebral inoculation with mouse-passaged scrapie (RML strain) or mouse-passaged CWD (Elk1P) prions. Unexpectedly, one Tg mouse line expressing Elk3M(SNIVVK) exhibited incubation times of <70 days when inoculated with mouse RML prions; a second line had incubation times of <90 days. In contrast, Tg mice expressing full-length ElkPrP had incubations of >250 days. The Tg(Elk3M,SNIVVK) mice were less susceptible to Elk1P prions than the Tg(ElkPrP) mice. We found that changing three C-terminal mouse residues (202, 214 and 219) to those of elk doubled the incubation time for mouse RML prions and rendered the mice resistant to Elk1P prions. Mutating an additional two residues from mouse to elk at codons 169 and 173 increased the incubation times for RML prions to >300 days, but made the mice susceptible to Elk1P prions.

The results of the studies reported here not only highlight the critical role of the C-terminal residues in prion transmission from one species to another, but also argue for a more complex set of rules than was previously appreciated in studies of human and bovine prion transmission to Tg mice (Scott et al., 1993, 2005; Telling et al., 1995).

**RESULTS**

**Tg mice expressing chimeric elk/mouse PrP**

Earlier, we and others established lines of Tg mice expressing PrP<sup>C</sup> from American elk (*Cervus elaphus nelsoni*) that are susceptible to infection with CWD prions (Browning et al., 2004; Kong et al., 2005; LaFauci et al., 2006; Tamgüney et al., 2006, 2009a). To study the influence of the C-terminal residues of ElkPrP<sup>C</sup> on conversion into PrP<sup>Sc</sup>, we generated 12 Tg mouse lines expressing chimeric elk/mouse PrP<sup>C</sup> (Table 1). All chimeric PrP molecules had the same N terminus of ElkPrP up to residue Y168 (mouse numbering) and the C terminus of MoPrP beginning at Q222. Between codons 169 and 221, ElkPrP and MoPrP share the same sequence except at six codons: 169, 173, 183, 202, 214 and 219 (Fig. 1a, b). Chimeric PrP with mouse residues at all six C-terminal positions was designated Elk3M(SNIVVK). We generated five additional chimeric elk/mouse PrP constructs by reverting selected mouse residues at these six codons back to elk (Fig. 1c). In the 12 Tg mouse lines established from each of these chimeric PrP constructs, the mice expressed varying levels of chimeric PrPs (1–12 ×) relative to wt PrP in FVB/N mice (Table 1). Eleven of the 12 lines showed no signs of CNS dysfunction for >600 days (data not shown). One line, Tg(Elk3M,NTIVVK)16033 mice, expressed chimeric PrP at a level 12-fold greater than that found for wt PrP in FVB/N mice; these mice developed spontaneous disease at a median age of 342 days, but did not show any proteinase K (PK)-resistant PrP<sub>Sc</sub> by Western blotting, PrP aggregates by immunohistochemistry, or other distinguishing neuropathological lesions, and were excluded from further study (data not shown). We restricted our observation period to 600 days, because a few Tg mice from some lines develop spontaneous neurological dysfunction after this time (Telling et al., 1996; Colby et al. 2010).

**Transmission of prions to Tg mice expressing chimeric PrP**

We inoculated Tg mice expressing ElkPrP, as well as Tg mice expressing chimeric elk/mouse PrP, with mouse RML prions or Elk1P prions, a CWD field isolate from elk that was passaged twice in Tg(ElkPrP<sup>+/+</sup>) mice (Table 1). Mouse RML prions transmitted disease to two lines of Tg(ElkPrP) mice, in 258 and 398 days (Fig. 2a). Unexpectedly, exchanging the C terminus of ElkPrP for MoPrP altered the susceptibility for RML prions, which caused disease in 67 and 88 days in two lines of Tg(Elk3M,SNIVVK) mice (Fig. 2b). While Elk1P prions transmitted CNS dysfunction to two Tg(ElkPrP) mouse lines in 99 and 123 days (Fig. 2a), their transmission to FVB, Tg(MoPrP-A)4053 and Tg(Elk3M,SNIVVK) mice was prolonged and inefficient (Fig. 2b, c, Table 1). Western blots of brain homogenates prepared from terminal, euthanized mice showed protease-resistant PrP<sub>27–30</sub> after limited digestion with PK (Fig. 3a–c).

Changing three C-terminal residues in Tg(Elk3M,SNIVVK) mice from mouse to elk generated Tg(Elk3M,NTIIR) mice. This change doubled the incubation time for mouse RML prions to 139 and 145 days, and rendered the mice resistant to Elk1P prions (Fig. 2d, Table 1). Western blot analysis showed PK-resistant PrP<sub>Sc</sub> in the brains of Tg(Elk3M,NTIIR) mice inoculated with RML prions (Fig. 3d).

Introduction of two additional ElkPrP residues (total of five) into the C terminus prolonged the incubation times to 312 and 378 days in Tg(Elk3M,NTIIR) mice after inoculation with RML prions (Fig. 2e, Table 1); the chimeric PrP expression levels in these Tg mice were 1 × <i>+</i> similar to those...
found in FVB mice, which had an incubation period of 133 days (Table 1). In the Tg(Elk3M,NTIIIR) mice, five C-terminal mouse residues were converted to elk, and I183 remains mouse-specific (Fig. 1). Notably, the incubation times for RML prions in Tg(Elk3M,NTIIIR) mice were similar to those for Tg(ElkPrP) mice. However, the incubation times for Elk1P prions in the Tg(Elk3M,NTIIIR) mice were quite prolonged (>350 days) with not all mice becoming ill, compared with those in Tg(ElkPrP) mice, with incubation periods of 99 and 123 days (Table 1). Western blotting of the brains of ill Tg(Elk3M,NTIIIR) mice inoculated with RML or Elk1P prions showed protease-resistant PrPSc (Fig. 3e).

Analysis of a single-residue change at the C terminus is provided by the Tg(Elk3M,NNIVVK) mice with a 16 level of transgene expression (Fig. 2f). Tg18401 mice expressed Elk3M(NNIVVK) at lower levels than the Tg(Elk3M,SNIVVK) lines (2–36). Despite this lower expression level, all seven Tg(Elk3M,NNIVVK) mice inoculated with Elk1P prions developed disease after 364 days, compared with only 50% of Tg(Elk3M,SNIVVK) mice developing signs of CNS dysfunction after Elk1P inoculation (Table 1). The brains of the Tg(Elk3M,NNIVVK) mice inoculated with RML or CWD prions harboured protease-resistant PrPSc (Fig. 3d).

Reverting four of six residues in Elk3M(SNIVVK) from mouse to elk produced a large increase in the incubation time of Tg(Elk3M,NTVIVK) mice inoculated with mouse RML prions; these Tg mice became ill at 216 and 231 days (Fig. 2g, Table 1). Elk1P prions caused CNS dysfunction in the Tg(Elk3M,NTVIVK) mice in 389 and 439 days. Western blotting of the brains of Tg(Elk3M,NTVIVK) mice inoculated with RML or Elk1P prions showed protease-resistant PrPSc (Fig. 3f).

When the six C-terminal mouse residues in the Elk3M(SNIVVK) transgene were changed to elk, the resulting Tg(Elk3M,NTVIIR) transgene were changed to elk, the resulting Tg(Elk3M,NTVIIR) mice had incubation times of 330 and 368 days (Fig. 2h, Table 1) after inoculation with mouse RML prions. These incubation times were similar to those observed in Tg(ElkPrP) mice (258 and 367 days) for RML prions (Table 1). Curiously, Elk1P prions resulted in prolonged incubation times in Tg(Elk3M,NTVIIR) mice, of 265 and 246 days (Table 1), compared with passage in Tg(ElkPrP) mice, with incubation periods of 99 and 123 days. Western blotting of brain homogenates from Tg(Elk3M,NTVIIR) mice inoculated with RML or Elk1P prions showed protease-resistant PrPSc (Fig. 3g).
mice showed protease-resistant PrPSc after limited digestion. Interestingly, transmission of Elk1P prions to mice expressing Elk3M(SNIVVK) (Fig. 3b) and MoPrP (Fig. 3c) resulted in predominantly monoglycosylated PrP, whereas primarily diglycosylated PrP resulted following transmission to other lines.

Neuropathology in Tg mice

Histological analysis of the brain sections from symptomatic Tg mice expressing elk/mouse PrP inoculated with Elk1P prions showed deposition of PrPSc and reactive astrocytic gliosis characteristic of prion disease (Fig. 4). For the mice expressing Elk3M(SNIVVK), Elk3M(SNIIR) or Elk3M(NNIVVK) constructs (data not shown), no distinguishing features were observed; however, other constructs led to distinct phenotypes. With inoculation of Elk1P prions, focal vacuolation occurred in a few regions of the brains of Tg(Elk3M,NTVIIR) and Tg(Elk3M,NTIIIR) mice (Fig. 4a, e), whereas vacuolation was distributed to all brain regions in Tg(Elk3M,NTVIVK) mice (Fig. 4i). PrP plaques were found surrounding the lateral ventricles in Tg mice expressing Elk3M(NTVIIR) and Elk3M(NTIIIR) (Fig. 4b, f), but were also found with varying abundance throughout the brain (Fig. 4d, h). The largest number of plaques with the broadest brain distribution occurred in Tg(Elk3M,NTVIIR) mice (Fig. 4d). In comparison, the number and distribution of plaques were reduced by approximately 50 % in Tg(Elk3M,NTIIIR) mice (Fig. 4h). Different patterns of PrP deposition were identified in three Tg(Elk3M,NTVIVK) mice (Fig. 4l): one resembled the pattern in Tg(Elk3M,NTVIIR) mice, one resembled the pattern in Tg(Elk3M,NTIIIR) (Fig. 2).

Table 1. Transmission of RML and Elk1P prions in Tg mice expressing mouse, elk, and chimeric elk/mouse PrP

c.i., Confidence interval.

<table>
<thead>
<tr>
<th>Mouse line*</th>
<th>PrP expression (fold)†</th>
<th>RML</th>
<th>Elk1P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Median incubation time with 95 % c.i. (days)</td>
<td>n/n0‡</td>
</tr>
<tr>
<td>FVB/N</td>
<td>1</td>
<td>133 (119, 150)</td>
<td>5/5</td>
</tr>
<tr>
<td>Tg(MoPrP)4053</td>
<td>8</td>
<td>54 (47, 57)</td>
<td>8/8</td>
</tr>
<tr>
<td>Tg(ElkPrP)12577</td>
<td>2</td>
<td>398 (280, 508)</td>
<td>6/6</td>
</tr>
<tr>
<td>Tg(ElkPrP+/+)12584</td>
<td>6</td>
<td>258 (165, 400)</td>
<td>7/8</td>
</tr>
<tr>
<td>Tg(Elk3M,SNIVVK)12316</td>
<td>3</td>
<td>67 (48, 71)</td>
<td>7/8</td>
</tr>
<tr>
<td>Tg(Elk3M,SNIVVK)12336</td>
<td>2–3</td>
<td>88 (83, 92)</td>
<td>8/8</td>
</tr>
<tr>
<td>Tg(Elk3M,SNIIR)23029</td>
<td>3</td>
<td>145 (134, 158)</td>
<td>8/8</td>
</tr>
<tr>
<td>Tg(Elk3M,SNIIR)23048</td>
<td>2–3</td>
<td>139 (132, 163)</td>
<td>8/8</td>
</tr>
<tr>
<td>Tg(Elk3M,NTVIIR+/+)18108</td>
<td>1</td>
<td>378 (329, 359)</td>
<td>4/5</td>
</tr>
<tr>
<td>Tg(Elk3M,NTVIIR)20909</td>
<td>1</td>
<td>312 (256, 354)</td>
<td>5/5</td>
</tr>
<tr>
<td>Tg(Elk3M,NNIVVK)18401</td>
<td>1</td>
<td>193 (160, 202)</td>
<td>8/8</td>
</tr>
<tr>
<td>Tg(Elk3M,NTVIIR)16048</td>
<td>4–6</td>
<td>216 (196, 225)</td>
<td>8/8</td>
</tr>
<tr>
<td>Tg(Elk3M,NTVIIR)16036</td>
<td>2–3</td>
<td>231 (204, 260)</td>
<td>8/8</td>
</tr>
<tr>
<td>Tg(Elk3M,NTVIIR)20840</td>
<td>4</td>
<td>330 (187, 482)</td>
<td>5/5</td>
</tr>
<tr>
<td>Tg(Elk3M,NTVIIR)20841</td>
<td>2</td>
<td>368 (242, 498)</td>
<td>5/7</td>
</tr>
</tbody>
</table>

*Elk residues within the region of interest are shown in bold and underlined. Mice homozygous for the transgene are denoted by ‘+/+’.
†Compared with PrPC in wild-type FVB/N mouse brain.
‡n, No. of ill animals; n0, no. of inoculated animals without intercurrent disease.
§When fewer than half of the inoculated mice developed disease, individual incubation periods are listed in italics.
||Data from Telling et al. (1996).
¶Data from Tamgüney et al. (2009a).
#Data from Tamgüney et al. (2006).
C-terminal PrP residues control prion transmission

(a) ElkPrP

(b) Elk3M, SNIVVK

(c) MoPrP

(d) Elk3M, SNIIIIR

(e) Elk3M, NTIIIR

(f) Elk3M, NNIVVK

(g) Elk3M, NTVIIR

(h) Elk3M, NTVIIR

Mice without disease (%) vs. Incubation time (days)
Tg(Elk3M,NTIIIR) mice, and the third mouse showed a completely different pattern, which consisted of PrP staining surrounding arteries and arterioles, and no freestanding plaques (Fig. 4l). To determine whether the PrP plaques are amyloid, they were stained with thioflavin S. The PrP plaques in Tg(Elk3M,NTVIVK) mice (Fig. 4g, k) stained strongly with thioflavin S, and those in Tg(Elk3M,NTVIIR) mice stained weakly (Fig. 4c).

**DISCUSSION**

Our findings show that single-residue substitutions in the C-terminal segment of PrP alter the susceptibility of Tg mice to a particular prion strain. The expression of elk residues in the C-terminal PrP segment plays a critical role in the formation of ElkPrPSc after infection with Elk1P prions. Based on earlier studies with chimeric PrP transgenes (Giles et al., 2010, 2012; Korth et al., 2003; Scott et al., 2005; Telling et al., 1995), we hypothesized that Tg(Elk3M,SNIVVK) mice would be more susceptible to cervid prions than Tg(ElkPrP) mice. Our finding that Tg(Elk3M,SNIVVK) mice are more susceptible to mouse RML prions than Elk1P prions was unanticipated. Reverting individual residues in the mouse segment of Elk3M(SNIVVK) to elk did not create a strategy for constructing Tg mice with greater susceptibility to cervid prions. As expected, the incubation times for RML prions lengthened when the mouse residues in the C-terminal segment were mutated to elk. Notably, Tg(Elk3M,NTVIIR) and Tg(ElkPrP) mice showed greater than twofold differences in their incubation periods for Elk1P prions. These mice express PrP constructs that differ at four residues at the extreme C terminus, arguing that residues at the extreme C terminus control strain selectivity in prion replication.

Reverting mouse S169 in Elk3M(SNIVVK) to the elk residue (N) doubled the incubation time for RML prions but facilitated transmission of Elk1P prions, underscoring...
the importance of this residue for the formation of CWD prions (Table 1). Mutating S→N at residue 169 and N→T at 173 (mouse numbering) in MoPrP created a rigid loop that was first recognized when the nuclear magnetic resonance (NMR) solution structure of recombinant ElkPrP was solved (Gossert et al., 2005). The chimeric MoPrP(S169N,N173T) transgene led to spontaneous disease, which was transmissible (Sigurdson et al., 2009). The importance of N169 for CWD prion replication has also been emphasized in a protein misfolding cyclic amplification study, in which brain homogenates from species expressing N169 supported amplification of CWD prions and those expressing S173 did not, with only one exception (Kurt et al., 2009).

CWD prions passedag in Tg mice expressing ElkPrP generally show a glycosylation profile with a predominant band of diglycosylated PrP (Tamgini et al., 2006). Here, transmission of Elk1P prions to Tg mice expressing MoPrP or Elk3M(SNIVVK) resulted in primarily monoglycosylated PrP. This observation may indicate a modification of the Elk1P prion strain following passage in some lines of mice. Strain adaptation may also become more evident should second passage of this inoculum result in shorter incubation times.

Because CWD prions cause widespread PrP amyloid deposition in the brains of cervids (Bahmanyar et al., 1985), we examined the amyloid in the brains of our mice. Tg mice expressing wt ElkPrP exhibited widespread PrP amyloid plaques after inoculation with CWD prions (Browning et al., 2004; Kong et al., 2005; Tamgini et al., 2006; Trifilo et al., 2007). In the Tg lines expressing chimeric elk/mouse PrP and inoculated with Elk1P prions, the highest PrP plaque load was found in Tg(Elk3M,NTVIIR) mice, which express chimeric PrP that differs from ElkPrP at only four residues at the extreme C terminus. In Tg(Elk3M,NTIIIR) and Tg(Elk3M,NTVIVK) mice expressing one or two, respectively, fewer C-terminal elk residues than Tg(Elk3M,NTVIIR) mice, the PrP plaque burden was lower after Elk1P inoculation (Fig. 4). In Tg mice expressing chimeric PrP with even fewer C-terminal elk residues [Tg(Elk3M,NTIIIR), Tg(Elk3M,NNIVVK) and Tg(Elk3M, SNIVVK) mice], PrP amyloid plaques were not found. Interestingly, this phenotype was not related to the incubation period (Table 1).

Because cervids are susceptible to experimental infection with scrapie and BSE prions, it will be important to ascertain whether any of the Tg mouse lines established here...
could be used to differentiate between scrapie, BSE and CWD prions in cervids (Hamir et al., 2004; Martin et al., 2009). In a recent report, the residue expressed at codon 169 in voles and mice was correlated with differential susceptibility to scrapie and BSE prions (Agrimi et al., 2008). Previous work also showed that certain lines of Tg mice expressing chimeric bovine/mouse PrP were differentially susceptible to infection with BSE, variant Creutzfeldt–Jakob disease (CJD) and scrapie prions (Scott et al., 2005). The differential results for Elk1P and RML prions in these lines may help to unravel the complexity of the crucial residues for strain-specific transmission of prions. Defining the regulatory signals carried within the C-terminal region of PrP that influence PrPSc formation may aid in deciphering how prion strains are replicated with high fidelity as well as in developing effective therapeutics for CJD and other prion diseases.

**METHODS**

**Chimeric elk/mouse PrP constructs.** The ORFs encoding MoPrP-A and cervid PrP were cloned in earlier studies (Carlson et al., 1994; Tamgüney et al., 2006). The C-terminal sequence of cervid PrP was changed to that of MoPrP-A using a unique Kpn restriction site common to both PrP sequences, resulting in a chimeric PrP molecule encoding cervid PrP residues 1–95 and MoPrP thereafter (mouse numbering). All further mutations were obtained by site-directed mutagenesis using a QuickChange Multi Site-Directed Mutagenesis kit (Stratagene). Mutations were introduced sequentially at N96S, L108M, M137L and M138V; 5′-GATCCATTTTl 1 % (w/v) brain homogenates (for S169N and N173T); 5′-GAGTGC- GTCAATATCCGGTCAAGCAGGCAGGCAGTGTC-3′ (for 1183V); 5′-C TTCAGAGCAGCTATCCAAATGTGGAGCCCGG-3′ (for V202I); and 5′-CAGATGTCAGACCTCCATCCCCAGAGGAGGAGGTCACGG-3′ (for V214I and K219R). The amino acids expressed in the resulting constructs are denoted following 'Elk3M'.

Complete sequences of all constructs were determined and archived by using Vector NTI Advance software (Invitrogen).

**Source of Tg mice.** All Tg mouse lines were generated using the cosShA-Tet cosmid vector for transgenic expression as described previously (Scott et al., 1992). With the exception of Tg(MoPrP)4053 mice, all Tg mice originating from Zech/PmrP4053 mice that do not express endogenous MoPrP (Büeler et al., 1992), and were maintained by breeding with FVB/PmrP4053 mice. Tg(MoPrP)4053 mice express endogenous PrP at 8 × the level of wt FVB/N mice (Charles River Laboratories; Carlson et al., 1994). Tg(ElkPrP)12577 and Tg(ElkPrP)+/+ )12584 mice have been described previously (Tamgüney et al., 2006, 2009a). The Tg(Elk3M, NTIIIR)12584 line was generated using the Human PrP expression vector hHuM-P (Safar et al., 2002) and developed with the enhanced chemiluminescent detection system (GE Healthcare Biosciences).

**Western blotting.** For Western blotting analysis, 10% (w/v) brain homogenates were prepared in PBS with 2% (w/v) N-lauroylsarcosine sodium salt (Sigma-Aldrich) by three runs of 15 s each in a Precellys 24 homogenizer (MO BIO Laboratories) expressing MoPrP-A, was originally provided by William Hadlow, Rocky Mountain Laboratory, Hamilton, MT, USA. For RML prions, 10% (w/v) brain homogenates in Ca2+/Mg2+-free PBS (pH 7.4) were obtained by applying three repeated strokes of 15 s each using a Kinematica Polytron Generator with a PTA-20 tip (Brinkman Instruments).

**Nomenclature.** Tg(Elk3M, SNIVVK) mice express the chimeric Elk3M (SNIVVK) construct, which has the elk sequence up to residue 168 and mouse sequence beginning at residue 169 (mouse numbering). Tg mice expressing chimeric elk/mouse PrP constructs with mutations are designated by Elk3M, followed by the amino acids expressed at codons 169, 173, 183, 202, 214 and 219, respectively. The mouse PrP residues at these positions are SNIVVK; the elk residues are NTIVIR (Fig. 1). Mice homozygous for the transgene are denoted by +/+.

**Prion isolates and transmission studies.** Elk1P was derived from the elk CWD isolate 03-12609 (Elk1, provided by Michael W. Miller, Wildlife Research Center, Fort Collins, CO, USA), characterized in an earlier study (Tamgüney et al., 2006), and maintained by passaging twice in Tg(ElkPrP+)12584 mice. Brain homogenates (10%, w/v) in PBS from Tg(ElkPrP+)12584 mice infected with the serially passaged Elk1P inoculum were obtained by three 30 s strokes of a PowerGen homogenizer (Fisher Scientific).

The RML prion strain, which was derived from the Chandler isolate (Chandler, 1961) passed in Swiss CD-1 mice (Charles River Laboratories) expressing MoPrP-A, was originally provided by William Hadlow, Rocky Mountain Laboratory, Hamilton, MT, USA. For RML prions, 10% (w/v) brain homogenates in Ca2+/Mg2+-free PBS (pH 7.4) were obtained by applying three repeated strokes of 15 s each using a Kinematica Polytron Generator with a PTA-20 tip (Brinkman Instruments).

Brain homogenates were diluted in 5% (w/v) bovine albumin fraction V and PBS to obtain final 1% (w/v) brain homogenates used for inoculation. Mice were inoculated with 30 μl 1% (w/v) brain homogenate using a 27-gauge, disposable hypodermic syringe inserted into the right parietal lobe. The clinical status of inoculated mice was assessed daily and neurological dysfunction three times weekly. CNS dysfunction was determined based on standard diagnostic criteria; mice were euthanized following evidence of progressive neurological dysfunction (Carlson et al., 1988; Scott et al., 1993). All mouse studies were approved by the UCSF Institutional Animal Care and Use Committee.

The Kaplan–Meier function was used to calculate median incubation periods (Kaplan & Meier, 1958); mice with intercurrent illness were censored at the time of euthanasia, and 95% confidence intervals were determined (Brookmeyer & Crowley, 1982). Calculations were performed with Stata/IC 10.0 (StataCorp).

**Western blotting.** For Western blotting analysis, 10% (w/v) brain homogenates were prepared in PBS with 2% (w/v) N-lauroylsarcosine sodium salt (Sigma-Aldrich) by three runs of 15 s each in a Precellys 24 homogenizer (MO BIO Laboratories). Samples of 5% brain homogenates were incubated with 20 μg PK ml−1 (New England Biolabs) for 1 h at 37 °C. PK was inactivated with 1 mM PMSF (Sigma-Aldrich) and samples were centrifuged at 100 000 g for 1 h at 4 °C. Pellets were resuspended in 100 μl PBS containing 2% (w/v) N-lauroylsarcosine sodium salt, before 100 μl 2 × NuPage LDS sample buffer (Invitrogen) was added and the samples were boiled for 5 min. For electrophoresis, 5–20 μl undigested and PK-digested samples were loaded onto the gels. SDS gel electrophoresis and Western blotting were performed using NuPAGE Novex 4–12% Bis-Tris gels and the iBlot dry blotting system (Invitrogen). PrP was detected with recFab HuM-P bound covalently to HRP (Safar et al., 2002) and developed with the enhanced chemiluminescent detection system (GE Healthcare Biosciences).
Neuropathology. Mouse brains were removed and frozen on dry ice or immersion-fixed in 10% buffered formalin and embedded in paraffin. Vacuolation was evaluated using 8 μm-thick brain sections that were stained with haematoxylin and eosin (H&E). PrPSc was detected in formalin-fixed tissue sections after hydrolytic autoclaving with recFab HuM-P, as described previously (Muramoto et al., 1997). PrP plaque scores were determined by assigning a value from 0 to 4 based on the number of plaques in the tissue section: ‘0’ indicates no plaques, ‘1’ denotes a few plaques, ‘2’ was given for some plaques, ‘3’ was assigned for a moderate number of plaques, and ‘4’ signifies many plaques. Thioflavin S staining of PrP amyloid plaques was performed as described previously (Tamgune et al., 2006).

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