Transmissible spongiform encephalopathies (TSEs) or prion diseases are a group of neurodegenerative diseases including Creutzfeldt-Jakob disease (CJD) in humans, bovine spongiform encephalopathy (BSE) in cattle and scrapie in sheep. These diseases are characterized by the accumulation of an abnormal isoform of the cellular prion protein (PrPC), termed PrPSc, in the brain of the affected individuals. PrP Sc is thought to be the principal component of the infectious agent (23).

Experimental transmission to laboratory rodents has been achieved with TSE agents from many naturally affected species. In these studies, the concept of the species barrier was encountered following experimental inoculation with TSE agents derived from a different species (21). The adaptation of diseases and TME seems plausible (11, 12, 24, 25). TME was experimentally transmitted to hamsters, but has never been successfully transmitted to mice (2, 18, 30). The species barrier between TME and mice could therefore be termed as an ‘absolute’ one.

The coding region of the mink PrP gene was sequenced and the deduced amino acid sequence showed a similarity to mouse PrPc of around 90% in the mature protein (17) (Fig. 1A). There is strong evidence that transmission of TSEs is tightly controlled by the PrP-encoding gene (PRNP for humans, Pmnp for mice and PrP gene for all other species). Studies with transgenic animals have shown that the species barrier encountered during transmission from nonmurine TSE strains to mice is overcome by introducing the respective nonmurine PrP transgene into mice (5, 8, 26, 28, 29, 34). Subsequent investigations, however, have revealed that the expression of a donor-derived PrP transgene may not always be sufficient to erase the species barrier to TSE transmissions and host and strain-specific factors may play a role as well (6, 14, 31, 32).

In the report provided here, we have expanded these transgenic studies to TME and introduced the mink PrP gene into transgenic mice.

Generation of transgenic mice. The construct cosMink was derived from the vector cosSHa.Tet (27) and contained the coding region of the mink PrP gene under the control of the hamster PrP gene promoter (Fig. 1B). The vector does not contain the gene coding for Doppel (20). The large NotI fragment of cosMink was injected into fertilized oocytes of either FVB or C57BL/6 mice. The offspring were screened for the

Transmissible mink encephalopathy (TME) is a rare disease of the North American mink, which has never been successfully transmitted to laboratory mice. We generated transgenic mice expressing the mink prion protein (PrP) and inoculated them with TME or the mouse-adapted scrapie strain 79A. TME infected mink PrP-transgenic mice on a murine PrP knockout background. The absolute species barrier between the infectious agent of TME and mice was therefore broken. Following TME and 79A infection of mice carrying both mink and murine PrPc, only proteinase-resistant PrP homologous to the incoming agent was detectable. The presence of the murine PrPc prolonged the incubation time of TME substantially.

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Transmissible mink encephalopathy (TME) of the North American mink (Mustela vison) was first recognized in 1947 and subsequently described as a TSE in 1965 by Hartsough and Burger (13, 19). Only very few outbreaks of this disease in farmed mink have been described and the cause for these outbreaks is unknown. As scrapie and BSE were experimentally transmitted to mink and, vice versa, TME was transmitted to sheep and cattle, a causal link between the ruminant diseases and TME seems plausible (11, 12, 24, 25). TME was experimentally transmitted to hamsters, but has never been successfully transmitted to mice (2, 18, 30). The species barrier between TME and mice could therefore be termed as an ‘absolute’ one.

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the coding region of the mink PrP gene is highlighted in exonic sequences of the hamster PrP gene are represented by white boxes and the coding region of the mink PrP gene is highlighted in gray.

presence of the transgene by using PCR and four founder animals were identified (MK7, MK27, MK39, and MK45). The copy number of the transgene was determined by a densitometric analysis of a Southern blot hybridization of EcoRI-digested genomic DNA (Table 1). Expression of the transgene in brain tissue was detected by Western blot analysis using the antibody L42 (35). The expression levels of the transgenic protein in MK7, MK27, and MK45 were compared with the expression of PrP in the brain of an American mink and were 200%, 50%, and 100% respectively, while no synthesis of the transgenic protein was visible in MK39 (Table 1; Fig. 2).

Infection of lines MK7 and MK45 with TME on a murine PrP-deficient background. Lines MK7 and MK45 were selected for transmission experiments and were crossed with Prnp<sup>0/0</sup> mice (4) over two generations to generate mice hemizygous for the transgene and homozygous for Prnp<sup>0/0</sup>. A TME agent did not infect C57BL/6 or FVB inbred mice after more than 800 days (Table 2). In contrast, transgenic mice expressing the mink PrP gene on a Prnp<sup>0/0</sup> background were readily infected with the TME agent and succumbed to disease with a mean incubation time of 164 days (MK7/Prnp<sup>0/0</sup>) or 312 days (MK45/Prnp<sup>0/0</sup>). The absolute species barrier of mice towards the TME agent was therefore broken by the introduction of the mink PrP gene and the incubation time was inversely proportional to the level of mink PrP gene expression.

TABLE 1. Transgenic mouse lines carrying the mink PrP gene

<table>
<thead>
<tr>
<th>Line no.</th>
<th>Background</th>
<th>Copy no./haploid genome</th>
<th>Expression</th>
</tr>
</thead>
<tbody>
<tr>
<td>MK7</td>
<td>FVB</td>
<td>4–5</td>
<td>200%</td>
</tr>
<tr>
<td>MK27</td>
<td>C57BL/6</td>
<td>1–2</td>
<td>50%</td>
</tr>
<tr>
<td>MK39</td>
<td>C57BL/6</td>
<td>2–3</td>
<td>50%</td>
</tr>
<tr>
<td>MK45</td>
<td>FVB</td>
<td>4–5</td>
<td>100%</td>
</tr>
</tbody>
</table>
PrPSc deposited in the brain of terminally ill animals was further examined by Western blot analysis. These analyses benefited from two antibodies, L42, which detected mink PrP but not mouse PrP, and Ra3153 (37), which detected mouse PrP but not mink PrP (Fig. 4A). Following proteinase K (PK) digestion, the typical banding pattern of three PrPSc isoforms between about 20 and 30 kDa was visible in brain homogenates of all terminally ill animals, but not in normal mice or mice that proved to be resistant to TME infection (Fig. 4A and 4B). The distribution of the three PrPSc-specific bands, which most likely represent un-, mono-, and diglycosylated PrPSc, was clearly different in animals infected with TME from those infected with 79A. TME-infected animals presented with an overrepresentation of diglycosylated PrPSc, while in 79A-infected mice, the unglycosylated and monoglycosylated forms of PK-resistant PrPSc were prominent.

It is noteworthy that the use of a gene chimeric for the mink and mouse PrP genes similar to the human-mouse chimera used by Telling and colleagues was not necessary to generate mice susceptible to TME (15, 31, 32). The mink sequence in the C-terminal end of the protein seems fully sufficient to interact with the postulated host-specific protein X to support the conversion process between the incoming PrPSc and the residual PrPC. The amino acids 167, 171, 214, and 218 (numbered according to the murine PrP) postulated to form the discontinuous, protein X-binding epitope are all identical between the murine and the mink PrP (Fig. 1A), which is in line with this hypothesis.

Infection of lines MK7 and MK45 with TME on a murine PrP background. In a separate line of breeding, the transgenic animals were crossed with nontransgenic inbred mice (FVB) instead of Prnp0/0 mice. These mice produced the mink PrP along with the murine PrP and were inoculated with the TME agent and scrapie strain 79A. The TME agent caused disease in MK7/FVB mice after a mean incubation time of 459 days, which was dramatically prolonged compared with the incubation time of 164 days in MK7/Prnp0/0 mice (Table 2). TME did not cause disease in the lower-expressing MK45/FVB within 800 days. The presence of the mink PrP gene had therefore a strong inhibitory effect on the disease progression of TME in mice expressing mink PrP genes.

In sharp contrast to the TME agent, the mouse-adapted scrapie strain 79A infected both transgenic lines on normal background with a mean incubation time of 149 (MK7/FVB) and 167 days (MK45/FVB). These incubation times are comparable to or even slightly shorter than the 173 days following infection of normal mice with 79A. The presence of the mink PrP gene substantially reduced the incubation time to 312 days in MK7/FVB and 121 in MK45/FVB. These findings are in line with the hypothesis that the mink PrP sequence in the C-terminal end of the protein is sufficient to mediate the interaction with the postulated host-specific protein X to support the conversion process between the incoming PrPSc and the residual PrPC.
digestion, only murine PrPSc was visible in 79A-infected mice (Fig. 4B) for MK7/FVB mice infected with TME, while after PK digestion of MK7/FVB mice and MK45/FVB mice infected with 79A and TME, only mink PrPSc could be detected in TME infected animals (Fig. 4B). Similar to an experiment expressing the murine PrP gene in transgenic mice and infecting them with either hamster- or mouse-adapted scrapie (22), the infecting agent determined the type of PrP that accumulated in the brain of the diseased transgenic mouse. It is particularly noteworthy that also the banding pattern of the PK-resistant PrP corresponded the pattern of the inoculum. This banding pattern is considered as a strain signature and it has been shown in experimentally infected normal and transgenic mice to be faithfully transmitted (7, 16, 33).

One possible hypothesis that would explain these findings in mice producing both mink and mouse PrPSc is that mouse PrPSc does not interact with mink PrPSc and, therefore, the interaction of the incoming mouse PrPSc (79A) with the homologous mouse PrPSc can progress with the same efficiency as in the absence of mink PrP, i.e., in the normal mouse. The incoming mink PrPSc (TME), however, can interact with mouse PrPSc, but the conversion of this heterologous complex either cannot progress efficiently or converted mouse PrPSc cannot bind mouse or mink PrPSc or, if bound, cannot convert mink or mouse PrPSc. The homologous mink PrP conversion takes place, but is much less efficient in the presence of endogenous mouse PrPSc than without mouse PrPSc, i.e., in the MK7-mice on a Prnp0/0 background. It is important to note that the amino acid residues 183, 202, and 204 (numbering according to the murine PrP), which define part of the PrPSc/PrPSc interface according to the publication by Scott and colleagues (28), all differ between the murreine and the mink PrP. The nature of the interaction between the mink PrPSc and the mouse PrPSc or the mouse PrPSc and the mink PrPSc or lack thereof could be tested in in vitro conversion assays.

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