Molecular cloning of a mink prion protein gene

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Transmissible mink encephalopathy (TME) is a rare disease which is presumably transmitted to ranch-raised mink from scrapie-infected sheep offal or bovine spongiform encephalopathy-infected cattle products. Although the infectious agent of TME has not been isolated, there is circumstantial evidence that TME is caused by prions. The experimental host range of TME includes sheep, cattle, monkeys and hamsters. However, TME has never been transmitted to mice. Since experiments in transgenic animals have shown that the prion protein (PrP) gene modulates the susceptibility, incubation time and neuropathology of prion-induced disease, we have started to analyse the mink PrP gene. PrP, as deduced from a genomic DNA sequence, consists of 257 amino acids and overall shows similarity of 84 to 90% with the sequences of the PrPs of other mammalian species. It remains to be determined whether these differences in the primary structure of PrP will explain the peculiar host range of TME.

Transmissible mink encephalopathy (TME) is a rare disease of ranch-raised mink which was first reported in Wisconsin in 1947. Since that time, only 22 additional outbreaks have been recorded worldwide (Marsh, 1991). The clinical, pathological and biochemical features of TME suggest that it is a prion disease very similar to scrapie in sheep and goats, bovine spongiform encephalopathy (BSE) in cattle, chronic wasting disease in mule deer and elk, and Creutzfeldt-Jakob disease, kuru and the Gerstmann-Sträussler-Scheinker syndrome in humans (Marsh & Hanson, 1969; Kimberlin & Marsh, 1975; Marsh & Kimberlin, 1975).

Epidemiological studies have associated the occurrence of TME with the feeding of contaminated food items, presumably scrapie-contaminated sheep offal. Recent epidemiological findings (Marsh et al., 1991) suggest that the disease may be transmitted to mink from BSE-infected fallen cattle. Experimentally, TME is readily transmissible to ferrets, squirrel monkeys, cattle and hamsters. In contrast to BSE, TME has not been shown to be transmissible to mice, and scrapie is not transmissible to mice after mink passage (Marsh et al., 1991).

Recent experiments have shown that the prion protein (PrP), which, in its scrapie isoform (PrPSc), co-purifies with the infectious agent of prion diseases, the prion, has a normal cellular isoform (PrPc) which plays a crucial role in the determination of incubation time, the specificity of pathological changes and species barriers (Westaway et al., 1987; Scott et al., 1989). In addition, mutations and insertions in the PrP gene have been linked to inheritable human prion diseases (Hsiao et al., 1989; Owen et al., 1989), and transgenic animals expressing one of these mutations (Leu for Pro at codon 101 in the mouse) spontaneously develop neurodegeneration and consequently die of a disease very similar to scrapie (Hsiao et al., 1990). To elucidate the role mink PrP might play in the pathogenesis and transmission of TME, we cloned and analysed the mink PrP gene.

RNA from mink brain tissue was prepared by a CsCl gradient procedure (Chirgwin et al., 1979). Total RNA (10 μg) was separated on a formaldehyde–agarose gel and transferred to a Hybond-N membrane (Amersham). RNA on the filters was hybridized to an 864 bp fragment of the human PrP gene containing the entire open reading frame (ORF) (Hsiao et al., 1989), and labelled by random priming with [α-32P]dATP (Feinberg & Vogelstein, 1983). Hybridization was performed in 1% BSA, 7% SDS, 0.5 mM-Na2HPO4 and 1 mM-EDTA. The membranes were washed at 65°C in 3 x SSC, 10 mM-NaH2PO4/Na2HPO4, 10 x Denhardt’s solution and 5% SDS, followed by a 10 min wash in 1 x SSC, 1% SDS. Mink DNA was prepared from liver tissue by a modified proteinase K procedure (Gross-Bellard et al., 1973). For Southern blot analysis, 10 μg DNA was digested with BamHI, BglII or EcoRI, run through a 0.7% agarose gel and transferred to a Hybond-plus membrane (Amersham). Hybridization and washing conditions were identical to Northern blot conditions.

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Mink liver DNA was partially digested with Sau3A and size-fractionated on a sucrose gradient as described by Maniatis et al. (1982). A genomic DNA library was prepared using BamHI-digested λ phage EMBL3. Phage plaques were screened with the 32P-labelled 864 bp fragment of the human PrP gene (see above); hybridization was for 12 h at 65 °C. Hybridization and washing conditions were as described for Northern and Southern blotting. Approximately 400,000 phage plaques were screened, one of which gave a positive hybridization signal. The insert was cloned into a plasmid (pMa) and subsequently sequenced following a variation of the dideoxynucleotide chain termination method (Chen & Seeburg, 1985) using a T7 sequencing kit (Pharmacia) and specially synthesized oligonucleotides.

Southern blot analysis was used to detect PrP genes in a number of mammalian species (Westaway & Prusiner, 1986). Cloning and sequencing has shown that in all species analysed, the PrP gene is a single copy gene with an uninterrupted protein-coding ORF in one exon. Southern blot analysis using a human PrP DNA probe containing the entire PrP ORF revealed single bands after digestion of mink genomic DNA with BamHI, BglII or EcoRI (Fig. 1). These findings are compatible with a single compact PrP gene in mink.

PrP mRNAs from different species show considerable variation in size, ranging from 2 kb to 4.6 kb (Westaway et al., 1987; Goldmann et al., 1990). Using a human PrP DNA probe containing the entire ORF in Northern blot analysis of total brain RNA, we estimated the size of mink PrP mRNA to be 2-3 kb (Fig. 2), within the size range of rodent and human PrP mRNAs.

A genomic mink DNA library was prepared from mink liver DNA, plaques were screened for PrP-related sequences by hybridization to a human PrP gene probe and a positive clone was isolated. Large parts of the mink PrP gene, including the coding region and 3' untranslated region, were sequenced.

The sequenced ORF consists of 771 nucleotides, followed by a putative 3' untranslated sequence of approximately 1650 nucleotides. The nucleotide sequence surrounding the AUG initiation codon (ATCATGG) is consistent with the consensus sequence (ANNATGG) for eukaryotic initiation sites (Kozak, 1983). At position 772, after codon 257 of this ORF, there is a stop codon (TGA), which is followed by a long trailer with the possible polyadenylation signals TATAAA at positions 2305 and 2316 and ATTAAA at position 2342. Both are variants of the consensus sequence (AATATAA) preceding the polyadenylation site of most eukaryotic mRNAs (Proudfoot & Brownlee, 1976), which are also found in the human 3' untranslated region.

The deduced mink PrP sequence consists of 257 amino acids and has an Mr of 28,941 prior to post-translational modifications. A region of 24 amino acids at the N terminus is typical of a signal peptide (von Heijne, 1985), with a hydrophobic core (LLVLFVA) and a small uncharged residue (C) at the putative signal sequence cleavage site. We predict that the mature protein commences at the lysine residue at codon 25, and, before post-translational modification, has an Mr of 25,816. There are two possible asparagine-linked glycosylation sites at positions 185 and 201, and there is also an extremely hydrophobic C-terminal sequence.

Southern blot analysis revealed a single copy PrP gene in a number of mammalian species. The protein-coding region has been found to be located in one uninterrupted ORF in one exon in all species investigated. Southern blot and sequence analysis of genomic mink DNA shows that the organization of the mink PrP gene is identical to that of other mammalian species.

We have deduced that the mink PrP consists of 257 amino acids, but as only one genomic clone was sequenced, no information is available about possible
mink PrP polymorphisms. The deduced protein shows 84 to 95% overall similarity with the PrPs from other species. Genomic DNA sequence similarity ranges from 81% when compared to mouse, to 87%, when compared to cattle. At the amino acid level, the greatest similarity is also observed between the mink and sheep PrP sequences (Table 1). The structure of the 3’ untranslated region is most closely related to that of the human trailer.
Table 1. Similarity between the mink PrP sequence and other known PrP sequences

<table>
<thead>
<tr>
<th>PrP</th>
<th>Nucleotide identity (%)</th>
<th>Amino acid identity (%)</th>
<th>Amino acid identity (excluding signal peptide) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse</td>
<td>81.19</td>
<td>84.43</td>
<td>87.98</td>
</tr>
<tr>
<td>Hamster</td>
<td>82.36</td>
<td>84.82</td>
<td>88.41</td>
</tr>
<tr>
<td>Cow</td>
<td>86.64</td>
<td>93.00</td>
<td>93.99</td>
</tr>
<tr>
<td>Sheep</td>
<td>87.03</td>
<td>93.77</td>
<td>94.85</td>
</tr>
<tr>
<td>Human</td>
<td>84.69</td>
<td>87.55</td>
<td>89.70</td>
</tr>
</tbody>
</table>

Both are approximately 1650 bp in total length and the similarity is 75%.

The putative signal sequence of 24 amino acids is most closely related to the signal sequence in sheep and cattle PrPs, with a stretch of five identical amino acids [(M/V)KSHI] found only in these three species. Two glycine codons are inserted between codons 30 and 31 and codons 89 and 90; these are also present in the sheep and bovine sequences. Other amino acid residues identical only in sheep, cattle and mink PrPs are leucine at codon 138, valine at codon 184, alanine at codon 230 and isoleucine at codon 233. Three amino acid residues which differ in mink and mouse PrPs are identical in mink and hamster PrPs: methionine at codon 113 (codon 108 in the mouse), isoleucine at codon 209 and alanine at codon 236. Codon 108 in mice with short or intermediate scrapie incubation times encodes leucine; in mice with long incubation times it encodes phenylalanine. In mink and hamster PrPs as well as those of sheep and humans it encodes methionine.

Recently, Goldgaber (1991) found that the DNA strand opposite to the PrP transcriptional unit contains a large ORF, which for simplicity was named anti-PrP. There are no stop codons in this large ORF in any human or animal genome. However, the strand complementary to the mink PrP gene shows numerous stop codons in two reading frames. The third reading frame has a stop signal at the position complementary to codon 249. This codon, which is TCT in human, cattle and sheep PrP genes and TCC in hamster, rat and mouse PrP genes, is TCA in that of mink. There are a number of possible initiation sites following this stop codon on the complementary strand, although the longest possible ORF follows codon 181. Thus, our sequencing data may be an argument against the anti-PrP hypothesis. Should there be a twofold evolutionary pressure on the ORF of the PrP gene, this would apply only to the first 181 codons.

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References


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