

PrP knock-out and PrP transgenic mice in prion research

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Spongiform encephalopathies such as scrapie in sheep, bovine spongiform encephalopathy (BSE) in cattle or Creutzfeldt-Jacob disease (CJD) and Gerstmann-Sträussler-Scheinker syndrome (GSS) in humans is caused by a transmissible agent designated prion. The 'protein only' hypothesis proposes that the prion consists partly or entirely of a conformational isoform of the normal host protein PrP^C, designated PrP^{*1} and that the abnormal conformer, when introduced into the organism, causes the conversion of PrP^C into a likeness of itself. PrP^{*} may be congruent with PrP^{Sc}, a protease-resistant, aggregated conformer of PrP that accumulates mainly in brain of almost all prion-infected organisms.

PrP^C consists of a flexible N-terminal half, comprising Cu²⁺-binding octapeptide repeats, and a globular domain consisting of three α -helices, one short antiparallel β -sheet and a single disulphide bond². It is anchored at the outer cell-surface by a glycosyl phosphatidylinositol (GPI) tail and is present in almost all tissues, however, mainly in brain³.

Compelling linkage between the prion and PrP was established by biochemical and genetic data⁴⁻⁸ and led to the prediction that animals devoid of PrP should be resistant to experimental scrapie and fail to propagate infectivity. This prediction was indeed borne out, adding substantial support to the 'protein only' hypothesis. In addition, the availability of PrP knock-out mice provided an approach to carry out reverse genetics⁹ on PrP, both in regard to prion disease and to its physiological role.

Generation and properties of mice devoid of PrP

PrP is encoded by a single-copy gene¹⁰ that comprises three exons, with the entire reading frame contained in the third exon (Fig. 1b). Several lines of mice devoid of PrP^C have been generated by homologous recombination in embryonic stem cells, using either of two strategies. The 'conservative strategy' involves disruptive modifications restricted to the open reading frame (ORF)^{11,12}. Mice homozygous for the inactivated gene (such as *Prnp*^{0/0} [Zürich I] or *Prnp*^{-/-} [Edinburgh]) develop normally, show no striking pathology and are resistant to prion infection.

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N.B. Colour figures are referred to as 'Plates' and appear in a 'Colour plate section' at the front of this volume

PrP knock-out by the ‘radical strategy’ involves deletion of not only the reading frame, but also of its flanking regions, in particular the splice acceptor site of the third exon (Fig. 1a)¹³. This type of PrP knock-out mouse also develops normally, but exhibits severe ataxia and Purkinje cell loss in later life^{13–16}.

The phenotype of PrP knock-out mice

Prnp^{o/o} (Zürich I) or *Prnp*^{-/-} (Edinburgh) mice develop and reproduce normally^{11,12}; ageing mice show demyelination in the peripheral nervous system, albeit without clinical symptoms¹⁷. Behavioural studies revealed no

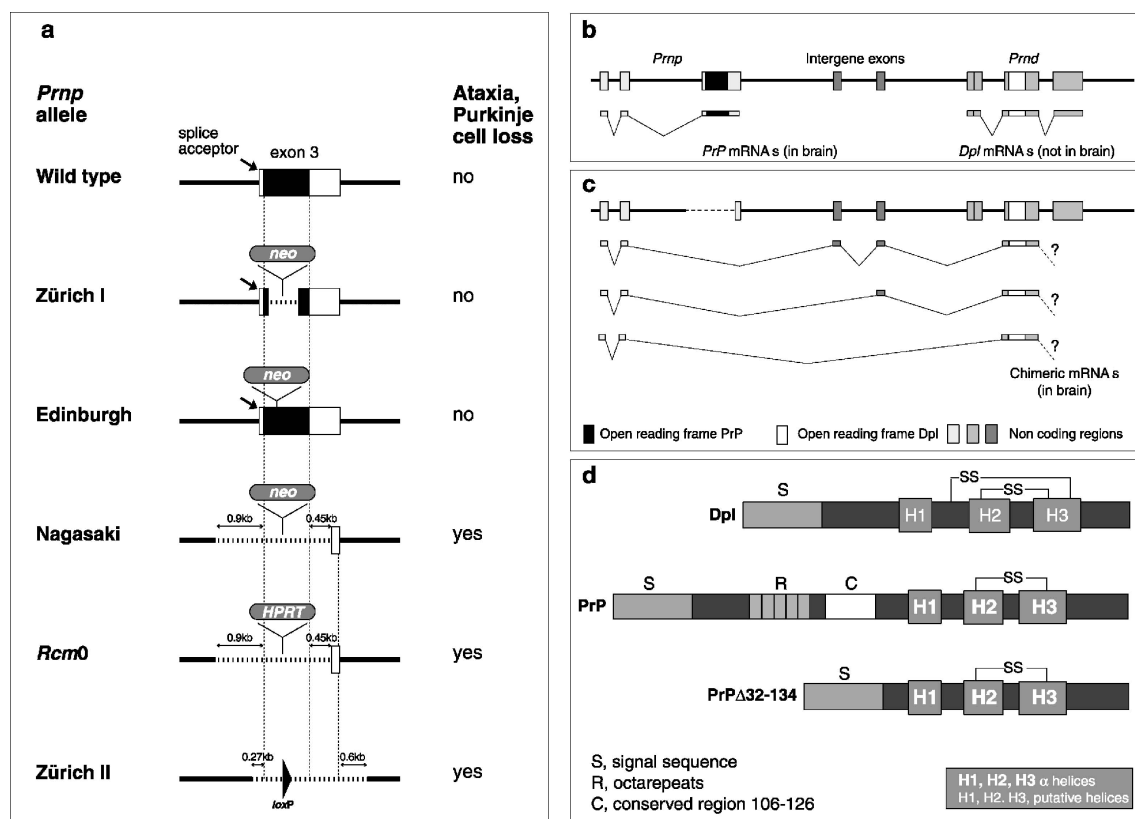


Fig. 1 Knock-out strategies and their consequences. (a) Various strategies used to target *Prnp* by homologous recombination. The black boxes represent PrP ORFs; white boxes, non-coding *Prnp* regions; grey boxes, inserted sequences; dotted line, deleted regions; *neo*, neomycin phosphotransferase; HPRT, hypoxanthine phosphoribosyltransferase; *loxP* (black arrowhead), a 34-bp recombination site from phage P1. (Reprinted, with permission, from Rossi et al, 2001 (copyright *EMBO Journal*)) (b) Coding and non-coding exons of *Prnp*, *Prnd* and intergenic exons of unknown function (c) Exon skipping leads to expression of Doppel under the direction of the *Prnp* promoter. Deletion of the splice receptor site upstream of the third *Prnp* exon entails the formation of several chimeric mRNAs comprising the first two exons of *Prnp*, which are spliced directly or indirectly to the *Dpl*-encoding exon¹⁴. (d) Comparison of domains of *Dpl* with full-length PrP and with PrP lacking residues 32 to 134. (b–d) Reprinted, with permission, from Weissmann and Aguzzi⁵⁶ (copyright *Science*).

significant differences to wild-type mice^{11,18} except for alterations in circadian activity and sleep rhythms^{19,20}. Electrophysiological studies showed that GABA-A receptor-mediated fast inhibition was weakened, long-term potentiation was impaired²¹⁻²⁴, and Ca²⁺-activated K⁺ currents were disrupted in some cells^{25,26}. Biochemical changes reported for Zürich-I-type knock-out mice suggest impairment of enzymatic activity required for anti-oxidant defence²⁷⁻²⁹.

In contrast, mice with extensive deletions in the *Prnp* gene (*Prnp*^{-/-} [Nagasaki], *Rcm0*, *Prnp*^{-/-} [Zürich II]) exhibit severe ataxia and Purkinje cell loss in later life¹³⁻¹⁶. Because this phenotype was abolished by introduction of a PrP transgene, it was attributed¹⁷ to the absence of PrP^C. However, because cerebellar symptoms were not observed in the Zürich I and Edinburgh knock-out lines, they were likely caused not by the absence of PrP but rather by the deletion of sequences flanking the PrP ORF³⁰. The discovery of *Prnd* and the ectopic expression of its product, Doppel (Dpl), in the brains of all three ataxic PrP knockout lines, Nagasaki, Zürich II and *Rcm0*, led to the resolution of this conundrum¹⁴.

Dpl is an N-glycosylated, GPI-anchored protein^{15,31} normally expressed in many tissues but not in post-natal brain¹⁴. Dpl and PrP show about 25% sequence similarity¹⁴ and share similar globular domains^{15,31,32}. However, Dpl lacks a counterpart to the flexible amino-terminal segment of PrP (Fig. 1d). Its physiological function is still unknown, but its absence causes male sterility in mice³³.

Dpl is encoded by *Prnd*, located 16 kb downstream of *Prnp*¹⁴. In brain of wild-type mice, with *Prnd* transcription under control of its own promoter, Dpl is not detected. As consequence of the radical PrP gene deletion strategy, the acceptor splice site of the third *Prnp* exon is lost, causing exon skipping and formation of chimeric transcripts that place *Prnd* transcription under control of the *Prnp* promoter (Fig. 1c)^{14,34}. Time to appearance of disease is inversely correlated with the expression level of Dpl in brain¹⁶, and the phenotype is rescued by co-expression of PrP^C^{16,17}. Thus, ectopic expression of Dpl in the absence of PrP^C, rather than absence of PrP^C itself causes Purkinje cell loss in Nagasaki-type PrP knock-out mice.

How does Dpl cause damage in the brain? It has been proposed that overexpression of Dpl entrains an increase of haem oxygenase 1 and both neuronal and inducible NO synthase, causing oxidative stress deleterious to sensitive neurons; this effect would be counteracted by the antioxidant properties of PrP^C³⁵. Another explanation is discussed below, in connection with the pathogenic properties of an amino-proximally truncated PrP that resembles Dpl in some respects^{14,15}.

PrP knock-out mice are resistant to scrapie

Prnp^{+/+} mice inoculated intracerebrally with mouse-adapted scrapie prions develop clinical symptoms at about 160 days and die about 10

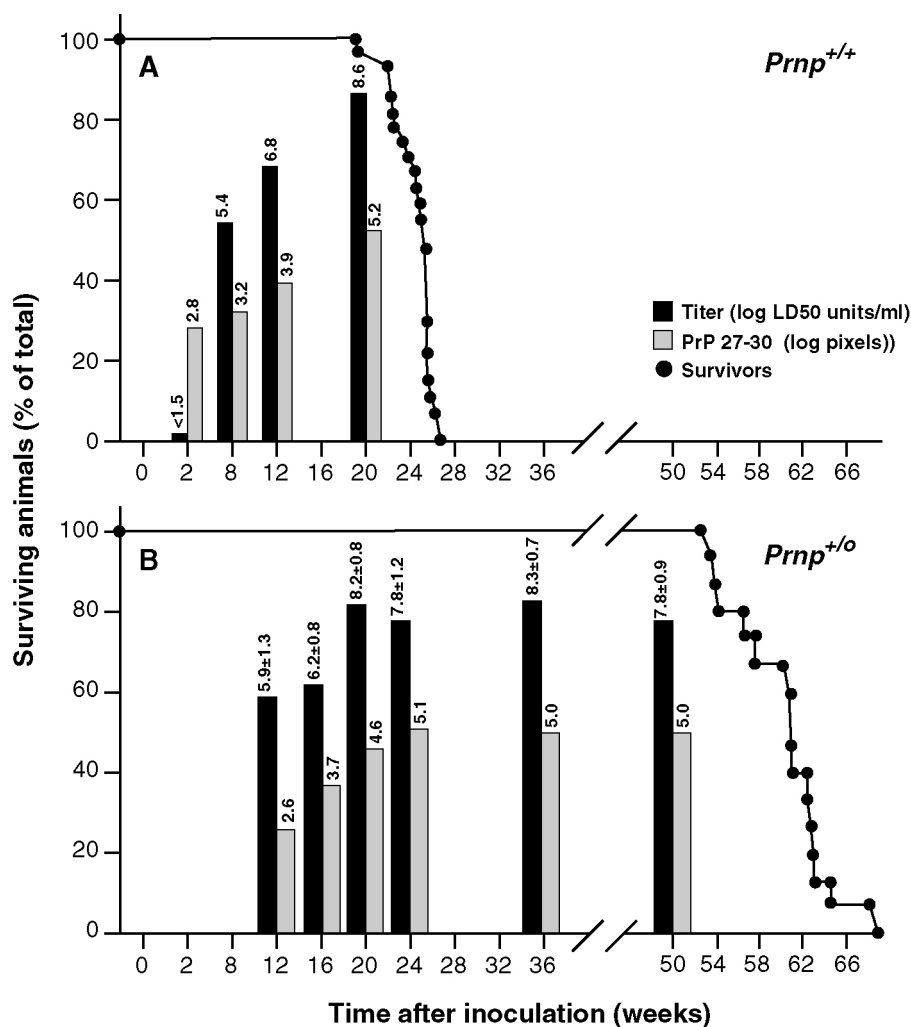


Fig. 2 Survival, prion titres and PrP^{Sc} in brains of scrapie-inoculated mice. (A) Wild-type and (B) *Prnp*^{0/0} mice at various times after inoculation with mouse prions. Although both types of mice have similar levels of prions and PrP^{Sc} at 20 weeks after infection, the wild-type mice succumb within the following 8 weeks, while *Prnp*^{0/0} mice survive for another 30 weeks or longer without clinical symptoms. (Reprinted, with permission, from Büeler *et al*³⁹ (copyright *Molecular Medicine*).

days later (Fig. 2A). In contrast, *Prnp*^{0/0} mice remained free of clinical symptoms for at least 2 years and showed no scrapie-specific pathology as late as 57 weeks after inoculation³⁶. Moreover, *Prnp*^{0/0} [Zürich I] mice challenged with scrapie prions failed to propagate prions in brain and spleen, whereas prion levels in brain and spleen of *Prnp*^{+/+} mice increased

to about 8.6 and 6.9 log LD₅₀ units/ml, respectively, by 20 weeks' post-infection³⁶. Rarely, low-level infectivity was detected in the brains of PrP knock-out mice after intracerebral inoculation, perhaps due to residual inoculum or, less likely, to contamination^{37,38}.

Properties of mice hemizygous for the Prnp^o allele

Mice carrying a single *Prnp* allele (*Prnp*^{o/+} mice) had prolonged incubation times of about 290 days to appearance of disease, as compared to 170 days in the case of *Prnp*^{+/+} mice. The *Prnp*^{o/+} mice harboured high levels of infectivity and PrP^{Sc} by 140 days after inoculation, as did wild-type controls, but survived thereafter for at least another 140 days without showing severe clinical symptoms³⁹. PrP gene dosage affects the timing of disease, but not the final pathology. It has been suggested that the pathological processes must extend to a so-called 'clinical target area' before disease and death ensue⁴⁰. In several instances, prion disease leads to death without substantial accumulation or even detectable levels of PrP^{Sc} in the brain⁴¹⁻⁴⁵, and discrete changes in the postulated target area, which might be in the brain stem or upper spinal cord⁴⁴, have not been routinely searched for. It is of practical interest that under certain conditions clinically healthy mice can harbour high levels of infectivity for long periods of time⁴⁶⁻⁵⁰, because it suggests that apparently healthy cattle or humans could contain infectious agent, a point to consider when animal or human tissues are used for preparing pharmaceuticals or grafts, or blood is transfused.

Transgenesis and gene replacement

Modifications of the genome may be achieved by transgenesis (*i.e.* insertion of cloned genes) or by *in situ* gene replacement using homologous recombination in embryonic stem cells. Gene insertion is usually based on nuclear injection into a one-cell embryo of a DNA segment containing the ORF, which may be mutated in the coding region or placed under the control of a foreign promoter in order to target expression to specific tissues. The DNA segment usually concatenates prior to integration, resulting in random insertion of multiple gene copies into one or few sites of the genome; therefore, each resulting transgenic mouse line is unique. Depending on the site of insertion, expression of the transgene can be silenced or modulated by neighbouring regulatory elements^{51,52}.

In situ gene replacement is based on homologous recombination and allows modification of a resident gene in a variety of fashions. The

normal gene copy number is preserved and the modified gene remains in its physiological environment and is not influenced by alien enhancers or position-dependent silencing. A double replacement gene targeting strategy was used to introduce point mutations into the *Prnp* locus of a murine embryonic stem (ES) cell line lacking hypoxanthine phosphoribosyl transferase (HPRT). In the first step, the entire PrP ORF was replaced with an HPRT minigene by homologous recombination, and successfully targeted clones were isolated by selection in medium lacking HAT (hypoxanthine, adenine, thymidine) and containing gancyclovir, which is lethal for cells lacking HPRT. In the second step the HPRT minigene was replaced with a mutated PrP coding sequence, again by homologous recombination. Cells devoid of HPRT, selected by virtue of their resistance to 6-thioguanine, contained the mutated *Prnp* allele. Selected ES cell clones were injected into blastocysts and mice carrying the modified gene were obtained by appropriate breeding^{45,53-55}.

Transgene vectors for PrP expression

When a certain phenotype is generated by gene ablation – in this case resistance to scrapie – it is important to show that this is indeed the consequence of the targeted modification and not to some unintended event, such as obliteration of an enhancer governing another gene or disruption of an unidentified reading frame. The most effective, albeit not infallible (see below and Weissmann & Aguzzi⁵⁶) strategy to link a phenotype to the ablation of a specific protein is to abrogate the effect of the deletion by introducing into the knock-out animal a cDNA encoding the protein in question.

It is desirable to reproduce the expression pattern of the wild-type gene as closely as possible but, as is the case for most mammalian genes, the location of all elements required for regulation of tissue-specific expression is not known. The 40-kb mouse cosmid I/InJ, derived from the murine *Prnp*^b allele contains 6 kb of upstream sequence, the three exons and two introns, and approximately 18 kb of 3' downstream sequence that include *Prnd*^{14,57}. Because such large cosmid vectors are laborious to work with, the 'half-genomic PrP' expression vector⁵⁸ was constructed by deleting the large intron 2 and all but 2.2 kb of 3' flanking sequence. Thus, the half genomic PrP vector contains about 6 kb *Prnp*^b promoter sequence controlling expression of PrP encoded by the *Prnp*^a locus⁵⁸ and lacks *Prnd*. The expression pattern elicited by the half-genomic construct is similar to that in cosmid-transgenic mice, except that cerebellar Purkinje cells express neither PrP nor PrP mRNA at detectable levels^{16,58}. Perhaps the half-genomic construct lacks a Purkinje-cell-specific enhancer, which could be located in the large

intron or in the distal part of the 3' non-coding region, both of which are absent in the half-genomic construct.

Restoration of susceptibility of Prnp^{0/0} mice to prions by PrP transgenes

Two lines of mice transgenic for the half-genomic *Prnp* construct, expressing PrP at about 3–4 and 6–7 times the level of wild-type mice, were challenged with mouse prions. As shown in Figure 2A, they succumbed to disease even more rapidly than wild-type CD-1 mice⁵⁸, confirming that the incubation times are inversely related to PrP expression levels⁵⁹.

These experiments support the conclusion that the scrapie-resistant phenotype of the Zürich I PrP knock-out mice is indeed due to ablation of PrP. However, it is worth emphasising the pitfalls that may beset the interpretation of knock-out experiments. In the case of the Nagasaki-type PrP knock-out mice described above, extensive deletions of the PrP-encoding exon gave rise to an ataxic phenotype that was reversed by introduction of an intact PrP-expressing transgene, the classical experiment correlating a phenotype with ablation of a gene. Nonetheless, in that case the conclusion was erroneous, because the ataxic phenotype did not result from the deletion of the PrP ORF but from the incidental up-regulation of a deleterious gene product whose pathogenicity was offset by wild-type PrP^C⁵⁶.

Effect of PrP mutations on health and prion disease in the mouse

The ability to reconstitute susceptibility to scrapie in mice devoid of PrP or to replace the wild-type gene by modified counterparts paved the way to the analysis of various consequences of modified PrP expression.

Delineation of PrP regions dispensable for susceptibility to prion disease

Treatment of scrapie prion preparations with protease cleaves off about 60 amino-terminal residues of PrP^{Sc} but does not abrogate infectivity. To ascertain whether amino-terminally truncated PrP^C could serve as substrate for the conversion to PrP^{Sc} and sustain susceptibility to scrapie in the mouse, transgenic *Prnp^{0/0}* [Zürich I] mice expressing PrP that retained the signal sequence but had an amino-proximal deletion were challenged with scrapie prions. PrP knock-out mice overexpressing PrP with deletions from position 32 to 80 ($\Delta 32-80$)⁵⁸ or to 93 ($\Delta 32-93$)⁶⁰ yielded normal mice that, after intracerebral inoculation with scrapie prions, developed disease, propagated prions and exhibited protease-resistant,

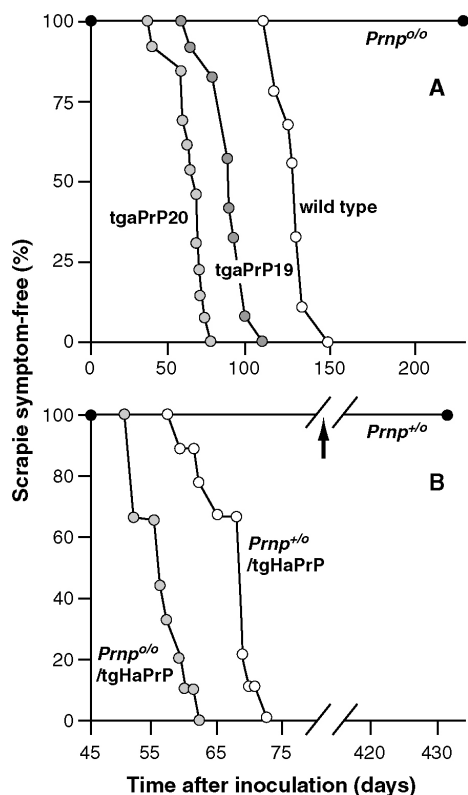
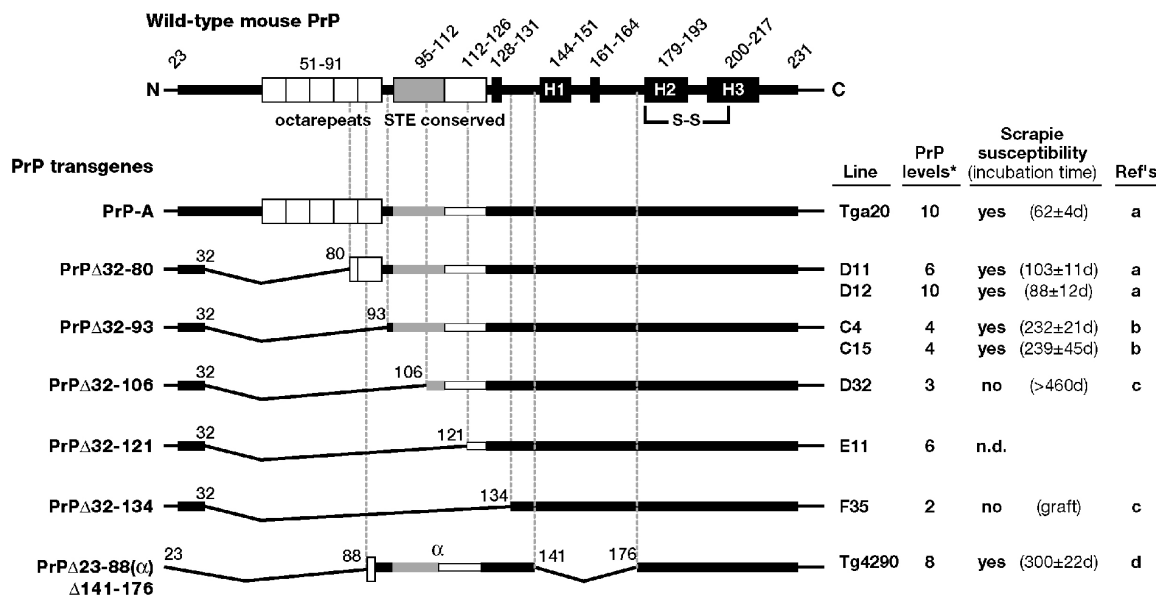


Fig. 3 Susceptibility to scrapie of PrP knock-out mice carrying various transgenes encoding full-length or truncated PrP. (A) *Prnp^{0/0}* mice were rendered transgenic for *Prnp* genes. *tga19/+* mice had 3–4 times the normal PrP^C level; *tga20/+* mice had 6–7 times the normal level. Data from Fischer *et al*⁵⁸. (B) *Prnp^{0/0}* and *Prnp^{0/+}* mice with hamster PrP transgenes were inoculated with the Sc237 isolate of hamster prions. Arrow: one animal died spontaneously without scrapie symptoms and one was killed because of a tumour. Mice with a mouse PrP allele are completely resistant to hamster prions while the presence of hamster PrP in a PrP knock-out mouse renders them very susceptible. The additional presence of a mouse PrP allele reduces susceptibility to hamster prions, as evidenced by increased incubation times. Modified from Büeler *et al*³⁶. (C) Susceptibility to scrapie prions of *Prnp^{0/0}* mice expressing various *Prnp* transgenes. α , 3F4-tag; *relative to wild-type. References: (a) Fischer *et al*⁵⁸; (b) Flechsig *et al*⁴⁴; (c) E Flechsig, I Hegyi, A Aguzzi, C Weissmann, unpublished results; (d) Supattapone *et al*⁶³.



truncated PrP^{Sc}, albeit, in the case of the deletion to residue 93, with longer incubation times and with lower levels of infectivity and PrP^{Sc44}. PrP with a deletion extending to position 106 was unable to restore susceptibility to prions (E Flechsig, I Hegyi, A Aguzzi & C Weissmann, unpublished data).

Thus, at least 60 residues of the amino-proximal region of mature PrP^C are expendable; they include not only the segment that is cleaved off PrP^{Sc} by proteinase K, but also the entire octarepeat region. This is remarkable because amplification of the octarepeat number is associated with familial CJD and GSS^{61,62}. In addition to a partial deletion of the flexible tail (Δ 23–88), the first α -helix (Δ 141–176) could also be removed without abrogating its capacity to restore prion susceptibility to *Prnp*^{0/0} mice. The resulting ‘PrP106’ contains only 106 amino acids, compared to the 208 residues in full-length PrP (Figure 3C)⁶³.

PrP mutations affecting the ‘species barrier’

In many cases, prions originating in one species fail to elicit disease in a different species or do so only inefficiently and/or after a long incubation time. This phenomenon is attributed to a ‘species barrier’⁶⁴; however, at least in the case of mice inoculated intracerebrally with hamster prions, PrP^{Sc} and infectivity may accumulate in the brain despite the absence of clinical symptoms^{46,47}, so that the barrier is not absolute. In many cases, introduction into mice of PrP transgenes containing all or part of the PrP sequence of the prion donor overcomes the species barrier. Thus, *Prnp*^{+/+} mice transgenic for hamster PrP transgenes are susceptible to both mouse and hamster prions^{59,65} while *Prnp*^{0/0} mice containing Syrian hamster PrP transgenes are susceptible to hamster but not mouse-derived prions; interestingly, the presence of a mouse PrP allele diminished the susceptibility to hamster prions (Fig. 2B)³⁶. Similarly, *Prnp*^{0/0} mice expressing human transgenes were susceptible to human sCJD prions, while *Prnp*^{+/+} mice with the same transgene cluster were as resistant to human prions as wild-type mice^{66,67}. Surprisingly, transgenic mice overexpressing human PrP are less susceptible to human variant CJD (vCJD) than wild-type mice⁶⁸. *Prnp*^{0/0} mice transgenic for bovine PrP genes are susceptible to bovine^{69,70} as well as to vCJD and sheep scrapie prions, while mice containing ovine *Prnp*^{VRQ} or *Prnp*^{ARQ} transgenes showed vastly decreased incubation times for sheep scrapie prions as compared to wild-type mice^{71,72}.

Inasmuch as a decrease in incubation time is viewed as a lowering of the ‘species barrier’, it is noteworthy that this can be achieved by mutations in murine PrP that do not increase the similarity to the PrP of the prion donor⁷³. Polymorphisms in the PrP gene have been shown to alter incubation time and susceptibility to prion disease in mice⁵⁴,

sheep⁷⁴, and man^{75,76}, but the mechanism by which this is achieved is not known. Certain polymorphisms of the ovine PrP gene confer resistance to scrapie, raising the possibility of preventing sheep scrapie by breeding resistance alleles into susceptible sheep flocks⁷⁷.

Pathological mouse phenotypes elicited by PrP mutations

Unexpectedly, various PrP mutations have given rise to pathological phenotypes when overexpressed in mice. For example, *Prnp*^{0/0} mice expressing PrP with deletions of the flexible tail extending to amino acid 121 or 134 developed severe ataxia and apoptosis of the cerebellar granule cell layer as early as 1–3 months of age [Plate VII(A)]⁶⁰. Neurons in the cortex and elsewhere expressed truncated PrP at similar levels as granule cells but did not undergo cell death, arguing against an unspecific toxic effect. Strikingly, the pathological phenotype was completely abolished by the introduction of a single wild-type *Prnp* allele, although the level of truncated PrP remained unchanged, exceeding that of the wild-type counterpart. When the truncated PrP was specifically targeted to Purkinje cells of *Prnp*^{0/0} [Zürich I] mice using the L7 promoter, ataxia and Purkinje cell degeneration developed, while the cerebellar granule layer remained unaffected [E Flechsig *et al*, *EMBO J* 2003 (In press); Plate VII(B)]. The phenotype resembled that observed in the three lines of Nagasaki-type PrP knock-out mice with up-regulation of Dpl in brain, which was also abrogated by PrP^{13,16,17}. Because the overall structure of Dpl is remarkably similar to that of the globular domain of PrP lacking the flexible N-terminus, the mechanism of pathogenesis might be the same in both cases^{14,56}. The results have been explained by a model in which truncated PrP^C acts as dominant negative inhibitor of a functional homologue of PrP^C, with both competing for the same putative PrP^C ligand⁶⁰.

Mice expressing PrP with the octarepeats (PrP Δ 23–88) deleted or even, in addition, lacking α -helix-1 and β -sheet-2 (Δ 141–176, ‘PrP106’) remained healthy⁷⁸. However, deletion of either α -helix-2 (Δ 177–202) or α -helix-3 (Δ 201–217) strongly affected correct PrP folding and caused a lethal illness resembling neuronal storage disease⁷⁸.

In cultured cells, mature PrP^C is subject to retrograde transport to the cytosol and degradation by proteasomes. Accumulation of even small amounts of cytosolic PrP was strongly neurotoxic to cultured cells⁷⁹. Wild-type mice transgenic for murine PrP23–230, which promotes accumulation of cytosolic PrP, developed severe ataxia, with cerebellar degeneration and gliosis⁸⁰.

In an attempt to generate spontaneous prion disease in transgenic mice, Scott *et al* substituted Ala by Val at positions 113, 115 and 118 of PrP (‘AV3’) to promote *de novo* β -sheet formation in the flexible tail⁸¹.

Founders indeed developed a fatal neurological disorder⁸²; however, this was not transmissible and no protease-resistant PrP was detected⁸¹.

Transgenic mouse models of inherited prion diseases

More than 20 mutations of the PrP gene have been identified in families suffering from inherited human prion diseases. It has been speculated that mutations in the PrP gene give rise to an unstable PrP^C protein that can spontaneously convert into the abnormal conformer, PrP^{Sc}, whereas sporadic forms of the disease are attributed to rare spontaneous transitions of PrP^C into PrP^{Sc} or to somatic mutations in the PrP. Studies on thermodynamic stability of the C-terminal domain of PrP with those mutations do not generally support the concept of mutation-induced PrP^C destabilisation^{83,84}. Mice transgenic for mutant PrPs associated with familial human prion diseases have failed to develop transmissible disease, with the possible exception of PrP^{P101L}.

Mice overexpressing murine PrP^{P101L} (the counterpart of human PrP^{P102L} linked to familial GSS) 8-fold, on a *Prnp*^{0/0} background, spontaneously developed neurodegeneration at 140 days of age, but little, if any, PrP^{Sc} could be detected⁸⁵. The disease could not be transmitted to wild-type mice, but only to transgenic mice that expressed the same mutation at low levels (2-fold) and developed neurodegeneration only late in life, if at all⁸⁵⁻⁸⁷. It was suggested that the amino acid difference constituted a barrier to transmission to wild-type mice. However, human P102L-linked GSS can be transmitted to both monkeys and wild-type mice^{88,89}. It has been argued that the P101L mutation may be an important susceptibility factor rather than a direct cause of GSS⁹⁰.

Mice expressing PrP with the counterparts of the inherited CJD-linked mutations at T183A or E199K on a PrP null background did not develop any pathological signs^{85,91}. Expression of a mouse PrP version of a nine-octapeptide insertion associated with 'prion dementia' in humans⁹² produced a slowly progressive cerebellar disorder and progressive myopathy⁹³. Because the disease was not transmissible it qualifies as a 'proteinopathy' rather than a 'prion disease'.

Thus, the human familial prion diseases have not been modelled successfully in the mouse.

Transmissible versus non-transmissible PrP-linked disease

Many mutant forms of murine PrP are pathogenic, mostly or perhaps only when overexpressed. It is not surprising that neurons accumulating abnormal forms of protein may suffer damage. With the possible exception⁸⁵ of the neurodegeneration associated with highly overexpressed PrP^{P101L}, none of the PrP-linked diseases in mice were

claimed to be transmissible. It is, therefore, appropriate to distinguish between 'prion diseases', which are transmissible, and 'non-transmissible conformational diseases', or 'non-transmissible proteinopathies' which are not⁸⁸.

In the case of human 'prion diseases', transmission to experimental animals has been achieved for some, but not all, familial cases⁸⁸. Doubtless, in any particular instance, lack of transmission may be due to inadequate recipients, but in view of the results with mice, it is possible that at least some of the familial human spongiform encephalopathies may be truly non-transmissible conformational diseases and, therefore, in view of the definition of prions as transmissible agents, not 'prion diseases'.

Ectopic expression of PrP transgenes

Several questions regarding the cell specificity of prion propagation have been addressed by generating mice that express PrP in only one type of tissue or organ, rather than almost ubiquitously, as in wild-type mice. This was achieved by introducing the PrP ORF linked to tissue- or organ-specific promoters into PrP knock-out mice.

Expression of PrP controlled by the astrocyte-specific glial fibrillary acid protein (GFAP) promoter in PrP knock-out mice rendered the animals susceptible to prions and led to clinical disease and prion propagation. It would thus seem that astrocytes are competent for prion replication. Interestingly, the neuropathology exhibited by these transgenic mice is quite similar to that found in scrapie-infected wild-type mice⁵⁰.

In most mouse scrapie models, infectivity appears in the spleen within days after intracerebral inoculation and rises slowly throughout the lifetime of the mouse, whereas infectivity in the brain starts rising a few weeks after inoculation and reaches titres two orders of magnitude higher than in the spleen^{36,94,95}. In spleen, PrP^{Sc} is found mainly in follicular dendritic cells (FDCs)^{96,97}. Fractionation of scrapie-infected spleen showed that infectivity was associated with B- and T-lymphocytes and with the FDC-containing stromal fraction, but not with neutrophils. Surprisingly, no infectivity was detected in circulating lymphocytes⁹⁸. This raised the question as to whether B- and T-cells were able to propagate prions or whether they acquired them from another source. Transgenic mice overexpressing PrP under the control of the T-cell-specific Ick promoter⁹⁹ showed high levels of PrP in T-cells, both in thymus and spleen but not in brain. Intraperitoneal inoculation led to no symptoms and no infectivity was detected in spleen, thymus or brain up to one year after inoculation¹⁰⁰. Transgenic mice overexpressing PrP under control of the B-cell-specific CD19 promoter had 10–20-fold

higher PrP levels on B-cells compared to wild-type mice¹⁰¹, yet no pathology or prion propagation was observed after intraperitoneal inoculation with scrapie prions. Thus, the presence of PrP does not suffice to support prion replication; maybe location within a particular plasma membrane region is required¹⁰² and/or other components are necessary. Moreover, it follows that prions associated with splenic T- and B-lymphocytes must stem from another source, almost certainly FDCs^{103,104}.

To determine whether spread of prions from the periphery to the CNS is dependent on hematogenous or lymphoreticular tissue (LRS), PrP knock-out mice transgenic for hamster PrP under control of the neuron-specific enolase (NSE) promoter, which express PrP in brain and nerves but not in spleen, lymph nodes or bone marrow, were inoculated with hamster prions orally or intraperitoneally. In both cases, scrapie disease ensued with the same incubation time as in mice expressing hamster PrP under the control of the PrP promoter. Thus, at least after peripheral exposure to high doses of 263K hamster prions, PrP^C-expressing LRS was not required for prion amplification or transport¹⁰⁵.

Concluding remarks

Research on transmissible spongiform encephalopathies went through three phases. The first was dominated by clinical and biological research, including the recognition of transmissibility and the unusual nature of the transmissible agent. In the second phase, biochemistry and recombinant DNA technology led to the isolation of PrP^{Sc} from scrapie-infected brain, to the recognition that it is a conformational isomer of the naturally occurring, host-encoded PrP^C and to the reformulation of the 'protein only' hypothesis. The deepest insights into the central role of PrP in the spongiform encephalopathies, or prion diseases as they came to be called, were made possible by the transgenic and knock-out technologies described above.

Acknowledgements

The Zürich/London prion groups are indebted to M Aguet, A Behrens, T Blättler, H Blüthmann (Hoffmann LaRoche, Basel), HR Büeler, S Brandner, A Cozzio, M Fischer, M Glatzel, I Hegyi, MA Klein, Y Kobayashi, H-P Lipp (Anatomisches Institut, Universität Zürich), F Montrasio, D Rossi, T Rüllicke (Biologisches Zentrallabor, Universitätsspital Zürich), M Prinz, A Raeber, A Sailer, S Sakaguchi, D Shmerling, and P Schwarz for their valuable contributions.

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