The Role of PrP in Health and Disease

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Abstract: Transmissible spongiform encephalopathies (TSEs) such as scrapie in sheep, bovine spongiform encephalopathy (BSE) in cattle or Creutzfeldt-Jacob disease (CJD) and Gerstmann-Sträussler-Scheinker syndrome (GSS) in humans, are caused by an infectious agent designated prion. The "protein only" hypothesis states that the prion consists partly or entirely of a conformational isofrom of the normal host protein PrP\( ^{C} \) and that the abnormal conformer, when introduced into the organism, causes the conversion of PrP\( ^{C} \) into a likeness of itself. Since the proposal of the "protein only" hypothesis more than three decades ago, cloning of the PrP gene, studies on PrP knockout mice and on mice transgenic for mutant PrP genes allowed deep insights into prion biology. Reverse genetics on PrP knockout mice containing modified PrP transgenes was used to address a variety of problems: mapping PrP regions required for prion replication, studying PrP mutations affecting the species barrier, modeling familial forms of human prion disease, analysing the cell specificity of prion propagation and investigating the physiological role of PrP by structure-function studies. Many questions regarding the role of PrP in susceptibility to prions have been elucidated, however the physiological role of PrP and the pathological mechanisms of neurodegeneration in prion diseases are still elusive.

1. PRIONS AND THE PRION PROTEIN

Prions and Prion Strains

Prion diseases are neurodegenerative disorders of the central nervous system leading to motor dysfunction, dementia and death. They are accompanied by a broad spectrum of neuropathological changes, such as spongiform degeneration, astrocytic gliosis, neuronal death and frequently the deposition of amyloid plaques in the brain [1, 2]. Among brain diseases associated with the accumulation of aggregated proteins or amyloid plaques involving various host proteins, such as the APP-derived βA4 peptide in Alzheimer’s disease, huntingtin in Huntington’s disease or the prion-protein-derived PrP\(^{Sc} \) in prion diseases, only prion diseases have so far been found to be transmissible. Transmissibility, first shown for sheep scrapie in 1936 [3], was later used as hallmark of prion diseases [4]. The agent’s unusual resistance to UV irradiation led to the idea that it might be devoid of nucleic acid [5]. The "protein only" hypothesis [6] in its updated version proposes that the prion consists partly or entirely of a conformational isofrom of the normal host protein PrP\( ^{C} \), designated PrP* [7], and that the abnormal conformer, when introduced into the organism, causes the conversion of PrP\( ^{C} \) into a likeness of itself [8]. PrP* may be congruent with PrP\(^{Sc} \), a protease-resistant, aggregated conformer of PrP that accumulates mainly in the brain of almost all prion-infected hosts. PrP\(^{Sc} \) co-purifies with prion infectivity [9] and tryptic peptides of PrP\(^{Sc} \) purified from scrapie-infected hamster enabled the cloning of Prnp, the gene encoding PrP [10, 11]. Surprisingly at the time, Prnp was found to be transcribed to the same extent in healthy and infected organisms [11, 12].

The resistance of PrP\(^{Sc} \) to protease is attributed to its β-sheet-rich conformation, which distinguishes it from the α-helix-rich, protease-sensitive PrP\(^{C} \) [13]. Originally, Huang et al. (1995) proposed an anti-parallel β-sheet structure for PrP\(^{Sc} \) [14], but an electron microscopy study suggests a parallel β-helix [15]. This fold is considered unusually stable and found in other amyloid-forming proteins [16]. Various strains of prions may be recovered from the same host species [17, 18] and can be stably propagated in one inbred mouse line, causing disease with distinct incubation times and specific patterns of spongiform changes in the brain [19-21]. It is believed that strains differ in that they are associated with different conformations of PrP\(^{Sc} \). This hypothesis is supported by the finding that the protease-resistant moieties of PrP\(^{Sc} \) from at least some different strains are of different size [20, 22-26]. Eight different strains have been distinguished by virtue of their relative affinity for a monoclonal antibody against a particular epitope that is fully accessible in PrP\(^{C} \) but partially occluded in PrP\(^{Sc} \) [27]. Moreover, the ratio of diglycosylated to monoglycosylated forms of PrP\(^{Sc} \) provides a molecular fingerprint for some strains [28].

The Cellular Prion Protein

PrP\(^{C} \) is encoded by a single-copy gene located on chromosome 2 in mouse (Prnp) and on
Figure 1. PrP knockout strategies and their consequences.

(A) Two strategies have been used to target the open reading frame (ORF) of PrP in the third Prnp exon by homologous recombination. Gene disruptions restricted to the ORF that retain the indicated splice site do not cause a pathological phenotype (conservative strategy). Gene deletions that extend beyond the ORF and remove the indicated splice site elicit up-regulation of Dpl and cause a pathological phenotype (radical strategy). Knockouts were performed by insertion of HPRT (hypoxanthine phosphoribosyl transferase) into the ORF or replacement of ORF-containing gene segments by neo (neomycin phosphotransferase) or loxP, a 34-bp recombination site from phage P1 (black arrowhead). Deleted sequences are shown by dotted lines. Modified from [64]. (B) Coding and non-coding exons of the PrP gene (Prnp), the Dpl gene (Prnd) and intergenic exons of unknown function. In the brain of wild-type mice, the promoter of Prnp is active, but the promoter of Prnd is silent. A PrP-like gene (PRNT) was found downstream of Dpl gene (PRND) in humans [211]. (C) Exon skipping leads to expression of Dpl under the control of the Prnp promoter in the brain. Deletion of the splice site caused formation of chimeric mRNA transcripts comprising the first two exons of Prnp, non-coding exons and the Dpl-encoding exon. (D) Comparison of protein domains of Dpl with full-length PrP and with PrP lacking its flexible region, PrPΔ32-134 (B-C modified from [81]).

chromosome 20 (PRNP) in humans [29]. A PrP gene has been found in all vertebrates examined and is highly conserved [30]. Maturation of the primary translation product results in removal of the N-terminal signal sequence of 22 amino acids, replacement of 23 residues at the C-terminus by a glycosylphosphatidylinositol (GPI) anchor and glycosylation at two asparagines residues. PrP<sup>C</sup> comprises a globular domain consisting of three α-helices, one short antiparallel β-sheet and a single disulfide bond [31]. The N-terminal half of PrP<sup>C</sup> is highly flexible [32, 33] and contains five octapeptide repeats (Figure 4A). NMR studies have shown PrP to be monomeric in solution, but X-ray crystallographic analysis of a crystallized form of PrP revealed a dimeric structure [34]. The dimer comes about through domain swapping of the third α-helix and a rearrangement of the disulfide bond to link the two polypeptide chains.

PrP<sup>C</sup> is attached by the GPI anchor to the outer surface of the cells, in cholesterol-rich microdomains (rafts) or caveolae [35-37] and undergoes endocytosis and recycling [38-40]. Interestingly, the N-terminal half of PrP contains a targeting signal for rafts [41, 42]. In cell culture, endocytosis of PrP<sup>C</sup> is stimulated by copper ions [43] and is abrogated if the copper-binding octarepeats of PrP<sup>C</sup> are deleted [44, 45]. Cu<sup>2+</sup> binds both to the octapeptide region and the histidines 96 and 111 of the flexible tail [46, 47], but the functional significance of this binding is
still unknown (see section 2). PrP C is found predominantly in the brain, particularly in some, albeit not all, neurons [48, 49] and at a lower level in glia [50] as well as in Schwann cells [51]. In neurons, PrP C is localized at synapses [52-54], but also in the soma, dendrites [55] and in elongating axons [54]. PrP C is also present in heart and skeletal muscle [56], in follicular dendritic cells (FDCs) [57], non-follicular dendritic cells [49], and in some lymphocytes [58, 59], but it is barely detectable in liver [56].

2. GENERATION AND PROPERTIES OF MICE DEVOID OF PRP

PrP Knockout Mouse Lines

Mouse PrP is encoded by a single-copy gene that comprises three exons. The entire coding sequence is contained in the third exon. Several lines of mice devoid of PrP C have been generated by homologous recombination in embryonic stem cells, using either of two strategies (Figure 1A). The 'conservative strategy' involves disruptive modifications restricted to the open reading frame (ORF) [60, 61]. Mice homozygous for the disrupted gene, such as Prnp o/o[Zürich I] or Prnp o/o[Edinburgh], develop normally, show no striking pathology and are resistant to prion infection [62]. PrP knockouts by the 'radical strategy' involve deletion of not only the ORF, but also of its flanking regions, in particular the splice acceptor site of the third exon (Figure 1A). This type of PrP knockout mouse (Prnp o/o[Nagasaki], Rcm o/o and Prnp o/o[Zürich III]) also develops normally but exhibits severe ataxia and Purkinje cell loss in later life [63-65].

The Phenotype of PrP Knockout Mice

Prnp o/o[Zürich I] and Prnp o/o[Edinburgh] mice develop and reproduce normally [60, 61]; ageing mice show demyelination in the peripheral nervous system, albeit without clinical symptoms [66]. Behavioural studies revealed no significant differences to wild-type mice [60, 67], except for alterations in circadian activity and sleep rhythms [68, 69]. Electrophysiological studies showed that GABA-A receptor-mediated fast inhibition was weakened, long-term potentiation (LTP) was impaired [23, 70, 71], afterhyperpolarization potentials (AHPs) in hippocampal neurons were reduced [23, 72] and Ca2+ - activated K+ currents were disrupted in some cells [73, 74]. Moreover, reorganization of mossy fibers in hippocampus has been described [75]. Biochemical changes reported for Zürich-I-type knockout mice suggest impairment of enzymatic activity required for anti-oxidant defense [76-78].

Two lines of "conditional PrP knockout" mice have been generated on the basis of the 'conservative strategy'. A PrP-expressing transgene under the control of a doxycycline-repressible promoter was introduced into Prnp o/o[Zürich I] mice, however repression was incomplete and resulted in basal PrP C levels about 5-15 % of wild type. No histopathological changes appeared after repression and no clinical disease after prion inoculation was noted, but prions accumulated nonetheless, albeit at a low level [79]. Mallucci et al. generated Prnp o/o mice that were transgenic for both a foxP-flanked cassette containing the PrP ORF under control of a Prnp promoter element and Cre recombinase under the control of the neurofilament heavy chain (NFH) promoter [72]. Expression of the recombinase resulted in the ablation of PrP in neurons at 9 weeks of age without entailing histopathological changes, but expression of PrP was still detectable in non-neuronal cells, albeit at very low levels. No clinical disease was observed for up to 400 days after prion inoculation.

In contrast, PrP knockout lines with extensive deletions in the Prnp gene exhibit severe ataxia and Purkinje cell loss in later life [63-65]. This phenotype was attributed to the absence of PrP C because it was rescued by introduction of a PrP transgene [66]. Because the phenotype was not observed in two PrP knockout lines generated by the 'conservative strategy', it was suggested that the ataxic phenotype was likely caused by the deletion of sequences flanking the PrP gene and not by the absence of PrP [80]. As illustrated in Figure 1, this puzzle was solved three years later with the discovery of a novel gene, designated Prnd [81]. It was found that the phenotype is associated with ectopic expression of the Prnd product, Doppel (Dpl), in brains of all three ataxic PrP knockout lines [64, 65, 82]. Prnd is located 16 kb downstream of Prnp and is not transcribed in brain of wild-type mice (Figure 1B). As consequence of the radical PrP knockout strategy, the splice acceptor site of the PrP-encoding exon 3 is lost and chimeric transcripts containing the first two non-coding exons of the Prnp locus linked to the Dpl-encoding Prnd exon are formed. This places Dpl expression under the control of the Prnp promoter and Dpl transcripts have been preferentially found in Purkinje cells [83], explaining the Purkinje cell degeneration. Time to appearance of Purkinje cell loss and ataxia was inversely correlated with the expression level of Dpl in brain of PrP knockout mice and the phenotype was rescued by co-expression of PrP C [64, 66]. Thus, ectopic expression of Dpl in the absence of PrP C rather than absence of PrP C itself causes ataxia and Purkinje cell loss in all three ataxic PrP knockout lines.

It is worth emphasizing the pitfalls that may beset the interpretation of knockout experiments. In the case of the ataxic PrP knockout lines, extensive deletions of the PrP-encoding exon gave rise to an ataxic phenotype that was reversed by introduction of a 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 coding sequence,
Figure 2. Model to explain the cerebellar syndrome caused by both PrP devoid of its flexible region and Dpl, and abrogation of the syndrome by wild-type PrP.

The model proposes that the globular part of PrP, comprising the three $\alpha$-helices, binds to the conjectured ligand $L_{PrP}$ and the flexible N-terminal tail of PrP elicits a survival signal (star). In the absence of PrP the same signal is elicited by binding of $\pi$ - a proposed functional homology of PrP- to the ligand, explaining why PrP knockout mice generated by the conservative strategy show no phenotype. PrP devoid of its flexible tail as well as Dpl can bind to the ligand with higher affinity than $\pi$ however fail to elicit a signal, thus exerting a dominant negative effect on $\pi$ and giving rise to a pathological phenotype. If wild-type PrP is co-expressed with the truncated PrP or Dpl, it displaces these inactive ligands and restores the survival signal, thereby abrogating the pathological phenotype. The two-step mechanism of binding and activation is discussed in section 7. Modified from [207]. With permission from Cambridge press.

How is brain damage caused by Dpl expression and why does PrP prevent it? Dpl is an N-glycosylated, GPI-anchored protein normally expressed in many tissues except in post-natal brain [84]. Its physiological function is still unknown, but its absence causes male sterility in mice [85]. Dpl is not involved in prion pathogenesis [86-88]. While Dpl and PrP show about 25 % sequence similarity and share similar globular domains [84, 89], Dpl lacks a counterpart to the flexible N-terminal half of PrP (Figure 1D). It has been proposed that ectopic expression of Dpl elicits an increase of heme 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$^C$ [90, 91]. However, further studies did not found evidence that Dpl elicits enhanced oxidative damage to brain proteins [92] or reduced activities of enzymes involves oxidative stress, such superoxide dismutase (SOD) or glutathione reductase activity [93]. Another explanation is based on the structural similarities between Dpl and the truncated PrP lacking the flexible tail [81]. Expression of this truncated PrP targeted to Purkinje cells of Prnp$^{0/0}$ [Zürich I] mice also caused Purkinje cell loss and ataxia (as in ataxic PrP knockout lines), that are also suppressed by wild-type PrP [94]. Thus, Dpl and the truncated PrP might cause Purkinje cell degeneration by the same mechanism proposed in Figure 2 and further discussed in section 7.

Possible Physiological Functions of PrP$^C$

Several physiological roles for PrP$^C$ have been proposed, in particular cell adhesion, signalling, neuroprotection and metabolic functions related to
Figure 3. Incubation time and prion titers in brains of scrapie-inoculated mice.

Prion titers in the brain of wild-type (Prnp\(^{+/+}\)) and heterozygous (Prnp\(^{0/+}\)) mice throughout the incubation time after intracerebral inoculation. Although both types of mice have similar levels of prion infectivity at 20 weeks after infection, the wild-type mice succumb within the following 8 weeks, while Prnp\(^{0/+}\) mice survive for another 30 weeks or longer without clinical symptoms. Data from [62, 122].

Its copper-binding properties. Because PrP binds Cu\(^{2+}\) tightly [46, 47] and Cu\(^{2+}\) stimulates endocytosis of PrPC, a role in copper ion transport has been proposed [43, 52]. Another suggestion is that PrPC protects cells against Cu\(^{2+}\) by scavenging it [52, 95] or against oxidative stress by enhancing Cu,Zn superoxide dismutase (SOD) [96] and/or glutathione reductase activity [97]. Recombinant PrP possesses Cu\(^{2+}\)-dependent SOD activity, albeit at low levels [98]. However, the claim that the level of SOD activity in brain tissue significantly varied with expression level of PrPC is a subject of controversy [76, 93, 99]. Despite the missing link between the physiological function of PrPC and its copper-binding properties, there is some evidence that oxidative stress homeostasis is altered in the absence of PrPC [76, 100]. It has also been suggested that Cu\(^{2+}\) may serve to impart a defined tertiary structure to PrPC [101] and/or to target it to caveolae-like domains [43, 45] and thereby support signalling, as shown for other GPI-anchored proteins [102]. Studies on differentiated neuronal cells, in which antibody-mediated cross-linking of PrPC led to caveolin-dependent activation of the tyrosine kinase Fyn [103] led to the proposal that PrPC is a signal transduction protein. PrP interacts with the 37-kDa / 67-kDa laminin receptor precursor [104-106] and heparan sulfate [107], supporting a possible role in cell adhesion and/or signalling. An anti-apoptotic role for PrPC has been suggested because neuronal cultures derived from Dpl-expressing Nagasaki mice undergo apoptosis more readily than wild-type controls [108]. In addition, PrPC protects cells against Bax-mediated apoptosis [109] and rescued retinal neurons from apoptosis [110]. In the latter case, the stress-inducible protein 1 (STI1) was proposed to be the 67-kDa cell surface ligand for PrP [111] that triggers this neuroprotection [112].

Almost a dozen other proteins have been reported to interact with PrP, using various screening strategies. However, the physiological significance, if any, of interactions with proteins, such as APLP1 or a tyrosine phosphatase (non-receptor type) [113], N-
Figure 4. Delineation of PrP regions expendable for prion propagation in PrP knockout mice.

(A) Scheme of the murine PrP sequence. The globular part of PrP comprises three α-helices (H1-H3) and a short antiparallel β-sheet (β1, β2). The five octarepeats, the stop-transfer effector site (STE) and the `optional´ transmembrane (TM) region containing a highly conserved sequence motif are located in the flexible amino terminal region. The single disulfide bond (S-S) and the glycolipid (GPI) attachment site are shown. (B) Susceptibility of PrP knockout mice expressing various PrP transgenes after intracerebral inoculation with mouse prion (RML). Tag, 3F4 epitope (**); References. (a) [140]; (b) [133] (c) E.F, I Hegyi, A Aguzzi and CW, unpublished results; (d) [148]; (e) [149].

CAM [114], Bcl-2 [115, 116], synapsin Ib or Grb2 [117], dystroglycan [118] and the laminin gamma-1 chain [119] needs to be established. The profusion of interactions and functions attributed to PrP underlines the current state of ignorance.

PrP Knockout Mice are Resistant to Scrapie

The finding that mice devoid of PrP (Prnp<sup>o/o</sup>) are resistant to scrapie provides one of the main supports for the "protein only" hypothesis. Prnp<sup>o/o</sup> mice remained free of clinical symptoms for at least 2 years and showed no scrapie-specific pathology as late as one year after inoculation [62]. In contrast, wild-type C57BL/6 mice (Prnp<sup>+/+</sup>) inoculated intracerebrally with mouse-adapted scrapie prions developed clinical symptoms at about 160 days and died about 10 days later (Figure 3). Moreover, Prnp<sup>o/o</sup>[Zürich I] mice challenged with scrapie prions failed to propagate prions in brain and spleen, whereas prion levels in brain and spleen of wild-type mice increased to about 8.6 and 6.9 log LD<sub>50</sub> units/ml of 10% homogenate respectively, by 140 days post infection. Rarely, low-level infectivity was detected in the brains of PrP knockout mice after intracerebral inoculation, perhaps due to residual inoculum or, less likely, to contamination [120, 121].

Properties of Mice Hemizygous for the Prnp<sup>o</sup> Allele

Mice carrying a single Prnp allele (Prnp<sup>o/+</sup> mice) had prolonged incubation times of about 290 days to appearance of disease, as compared to 160 days in the case of Prnp<sup>+/+</sup> mice [122]. The Prnp<sup>o/+</sup> mice harbored high levels of infectivity and PrP<sub>Sc</sub> by 140 days after inoculation, as did wild-type controls, but survived thereafter for at least another 140 days without showing severe clinical symptoms (Figure 3). PrP gene dosage affects the timing of disease but not the final pathology [122]. In summary, whereas in wild-type mice inoculated with prions the increase in prion titer and PrP<sub>Sc</sub> levels is followed within weeks by the onset of scrapie symptoms and death, Prnp<sup>o/+</sup> mice remained free of symptoms for many months despite similar levels of scrapie infectivity and PrP<sub>Sc</sub>
levels. This finding argues against the idea that accumulation of PrPSc is sufficient to cause clinical disease.

3. BRAIN PATHOLOGY AND PRION DISEASE

Accumulation of PrPSc and neuropathological changes (Figure 5) such as spongiosis, astrocytic gliosis and neuronal death are typical hallmarks of prion diseases, however there is a broad spectrum of presentation in the brain of affected individuals [1, 2]. Onset of clinical symptoms is not necessarily correlated with the overall accumulation of PrPSc and pathology. In several instances, prion disease leads to death without substantial accumulation or even detectable levels of PrPSc in the brain [123-126]. Conversely, clinically healthy mice can harbor high levels of PrPSc and infectivity for long periods of time or during their lifetime [122, 127-131]. It has been suggested that the pathological processes must extend to a so-called “clinical target area” before clinical disease and death ensue [132]. Thus, discrete changes in the postulated target area, which might be in the brain stem or upper spinal cord [133] and which have not been routinely searched for, might control the onset of disease. Many questions related to the pathological mechanism of prions are still unsolved [134, 135]. The findings that neuropathology can develop independent of the level of PrPSc argues that PrPSc itself might not be highly neurotoxic and that other forms of PrP could be responsible for prion-induced neurodegeneration [7]. Lingappa and co-workers suggested that an unusual transmembrane form of PrP, designated CtmPrP, might be induced by the conversion of PrPC into PrPSc and be toxic to neurons. They found that levels of CtmPrP correlate well with the neuropathological changes in brains of certain transgenic mice and patients with the GSS [136, 137]; however, it is unclear whether this mechanism is a general one rather than being specific for mutations within or near the ‘optional’ transmembrane region of PrP (Figure 6A). Recently, Lindquist and colleagues showed that accumulation of cytosolic PrP (see section 5 and Figure 7B) was highly toxic and proposed that this may contribute to the neuronal degeneration in prion diseases [138].

4. TRANSGENIC STUDIES ON PRP

When a certain phenotype is generated by gene ablation –such as ataxia in some PrP knockout lines or 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 elimination of enhancers regulating another gene, disruption of an unidentified gene or gain of function as described for Dpl [81]. The most effective, albeit (as shown above) not infallible strategy to link a phenotype to the knockout of a specific protein is to abrogate the effect of the deletion by introducing into the knockout animal a transgenic vector encoding the protein in question. Such gene insertion is usually performed by nuclear injection into a one-cell embryo of a DNA segment containing the coding sequence under the control of a promoter aimed at reproducing the expression pattern of the wild-type gene as closely as possible.

![Image of brain and spinal cord sections showing PrP expression](image)

**Figure 5.** Neuropathological features of prion disease in mice. Brain and spinal cord sections from RML-prion-infected, scrapie-sick wild-type (Prnp+/+) mice and Prnpo/o mice expressing PrP devoid of the five octarepeats (PrPA32-93) were stained with hematoxylin-eosin (HE), or with antibodies against either glial fibrillary acidic protein (GFAP) or microtubule-associated protein 2 (MAP-2). In the brain of wild-type mice, HE and GFAP staining, respectively, show spongiosis and proliferation of reactive astrocytes in the thalamus and brain stem. Surprisingly, spongiosis and astrogliosis were absent in both these brain regions of the transgenic mice. However, in the spinal cord of transgenic mice, gliosis and motor neuron loss were as in wild-type mice, as shown in cervical sections stained with GFAP and MAP-2, respectively. Data published in [133].
Transgene Vectors for PrP Expression

Three vector types have been generated and most commonly used to express the wild-type PrP gene: the PrP cosmid, the PrP minigene, designated "half-genomic PrP vector" and the "cosTet" vector. The 40-kb mouse cosmid, derived from the murine Prnp<sup>a</sup> allele contains 6 kb of the promoter sequence, the three exons and two introns and approximately 18 kb of 3’ downstream sequence that includes Prnd [64, 65, 139]. Because such large cosmid vectors are laborious to work with, the "half-genomic PrP" expression vector 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 the same promoter sequence as the cosmid, but the coding sequence is derived from the Prnp<sup>a</sup> locus and Prnd is deleted [64, 140]. The expression pattern elicited by the half-genomic construct is similar to wild type, except that cerebellar Purkinje cells express neither PrP nor PrP mRNA at detectable levels [140]. Perhaps the half-genomic PrP vector 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. The lack of Purkinje-cell-specific PrP expression has also been found in mice expressing mutated PrP [138, 141] under control of MoPrP vector.XhoI [142], a construct similar to the half-genomic vector. The third vector, called "cosTet", encompasses the two exons and one intron of the hamster Prnp gene and approximately 24 and 6 kb of the 5’ and 3’ flanking region, respectively, and lacks the Dpl ORF [143].

Restoration of Susceptibility of Prnp<sup>0/0</sup> Mice to Prions by PrP Transgenes

The "protein only" hypothesis requires not only the demonstration that resistance to scrapie is due to the absence of PrP, but it is also necessary to show that reintroduction of PrP in PrP knockout mice restores scrapie susceptibility. Thus, murine Prnp genes were introduced by transgenesis into Prnp<sup>0/0</sup> mice. Two transgenic lines of mice expressing PrP at about 3-4 and 6-7 times the level of wild-type mice were challenged with mouse prions. They developed disease after 3 and 2 months, respectively, compared to about 5 months in the case of wild-type (C57BL/6) mice [140]. This confirmed that the incubation times are inversely related to PrP expression levels, as reported earlier in a different context [144] and sustained the conclusion that the scrapie-resistant phenotype of the Zürich I PrP knockout mice is indeed due to ablation of PrP.

5. EFFECT OF PRP MUTATIONS ON SUSCEPTIBILITY OF MICE TO PRION DISEASE

The ability to restore susceptibility to scrapie in PrP knockout mice by introduction of PrP encoding transgenes opened the way for reverse genetics on PrP. Reverse genetics was first established as an approach to the elucidation of structure-function relationships of the genome of the phage Qβ, a classical model in the early days of the molecular biology [145]. To determine the function of a known gene, a mutation is introduced in the gene of interest and the resulting effect or phenotype is analyzed. In contrast, classical genetics involves screening for a particular phenotype followed by the identification of the responsible gene. Here we focus on four questions that were addressed by reverse genetics: Which parts of PrP are essential for sustaining prion propagation? Is the species barrier affected by PrP mutations? Is expression of PrP with mutations causing familial prion diseases in humans sufficient to elicit transmissible disease in mice? Is expression of PrP sufficient to enable a cell to propagate prions?

Mapping of PrP Regions Required for Prion Propagation

A number of transgenic mouse lines expressing PrP with deletions have been generated in order to delineate the minimal sequence of PrP required to sustain prion replication (Figure 4). Treatment of prion preparations with protease cleaves off about 60 amino terminal residues of PrP<sup>Sc</sup> [146] without abrogating infectivity [147]. This raised the question as to whether amino terminally truncated PrP<sup>D</sup> could serve as substrate for the conversion to PrP<sup>Sc</sup> and sustain susceptibility to scrapie in mice. Prnp<sup>0/0</sup> mice expressing PrP with deletions from position 32 to 80 (Δ32-80) [140] or to 93 (Δ32-93) [133] were normal and after intracerebral inoculation with mouse prions (RML) developed disease, propagated prions and accumulated protease-resistant, truncated PrP<sup>Sc</sup>. Removal of the five octarepeats (PrP<sup>Δ</sup>32-93) resulted in a longer incubation time and a lower level of both infectivity and PrP<sup>Sc</sup> [133]. The longer incubation time might due to the reduced conversion efficiency of the truncated PrP [133, 148]. Surprisingly, brains of terminally sick mice expressing PrP<sup>Δ</sup>32-93 failed to show histopathology typical of mouse scrapie; however, in the spinal cord, gliosis and motor neuron loss were as in terminally sick wild-type controls (Figure 5). PrP with deletions extending to position 106 or 134 was unable to restore susceptibility to prions (E.F., I.Hegyi, A.Aguzzi and C.W., unpublished data) (Figure 4B). Thus, at least 60 residues of the amino proximal region of mature PrP<sup>C</sup>, which contain the entire octarepeat region, are dispensable. This is remarkable because amplification of the octarepeat region is associated with familial CJD and GSS (Figure 6A). Amazingly, PrP that in addition to a partial deletion of the flexible tail (Δ23-88), lacks the first α-helix and one β-sheet (Δ141-176) still confers prion susceptibility to Prnp<sup>0/0</sup> mice [149]. The resulting "PrP106" contains only 106 amino acids, compared to the 208 residues in full-length PrP (Figure 4B). However, PrP106<sup>Sc</sup> miniprions are only transmissible to mice expressing...
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Figure 6. Modeling inherited human prion disease in mice.

(A) Scheme of the human PrP sequence. Mutations causing inherited human prion diseases are shown above the line. Polymorphisms that influence the onset as well as the phenotype of the disease are indicated below the line. Structural elements and indicated regions as described in Fig. 4A. Data from [2]. (B) Modeling inherited prion disease in mice expressing PrP transgenes with mutations associated with inherited human prion diseases. Single amino acid mutations (P102L, T183A, E200K) and one nine-octapeptide insertion (PG14) were analyzed for phenotypic abnormalities in transgenic mice. In addition, a triple mutation (AV3) designed to promote spontaneous transitions, in which three alanines (113, 115, 118) were substituted to valines, was studied. In some cases a pathological phenotype resulted but was not transmissible to wild-type mice. These diseases therefore qualify as proteinopathies and not as prion diseases, whose hallmark is transmissibility (see section 5). References. (a) [165]; (b) [164]; (c) [169]; (d) [141]; (e) [171]; (f) [136].

Disease is not transmissible to wild-type mice, but only to mice expressing the same mutation at lower level.

PrP Mutations Affecting the “Species Barrier”

In many cases, prions originating in one species fail to elicit disease in another species or do so only inefficiently, after a long incubation time. This phenomenon is attributed to a “species barrier” [150], however the barrier is not absolute. For example, in mice inoculated intracerebrally with hamster prions, PrPSc and infectivity may accumulate in the brain despite the absence of clinical symptoms [130, 131]. Frequently, introduction into mice of PrP transgenes containing all or part of the PrP sequence of the prion donor overcomes the species barrier. Thus, transgenic mice expressing hamster PrP on a wild-type background are susceptible to both mouse and hamster prions [144, 151], while mice with the same transgene on a Prnp\(^{o/o}\) background are susceptible to hamster, but not mouse prions [62]. Interestingly, the presence of a mouse PrP allele diminished the susceptibility to hamster prions [62]. Similarly, Prnp\(^{o/o}\) mice expressing human transgenes were susceptible to human sporadic CJD prions, while Prnp\(^{+/+}\) mice with the same transgene cluster were as resistant to human prions as wild-type mice [152, 153]. Surprisingly, transgenic mice overexpressing human PrP are less susceptible to human variant CJD (vCJD) than wild-type mice [19]. Prnp\(^{o/o}\) mice transgenic for bovine PrP\(^{\text{ARQ}}\) genes are susceptible to bovine [154, 155] as well as to vCJD and sheep scrapie prions, while mice containing ovine transgenes (Prnp\(^{\text{VRQ}}\) or Prnp\(^{\text{ARQ}}\)) showed vastly shortened incubation times for sheep scrapie as
compared to wild-type mice [156, 157]. Although the shortening of the incubation time is viewed as a lowering of the “species barrier”, it is noteworthy that this effect is also observed in transgenic mice expressing murine PrP with amino acid substitutions that do not increase the sequence similarity between donor and host PrP<sup>C</sup> [158]. Polymorphisms in the PrP gene have been shown to alter incubation time and susceptibility to prion disease in mice, sheep and man, but the mechanism by which this is comes about is not known [2, 159]. 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 flocks [160]. Surprisingly, sheep carrying resistance alleles against sheep scrapie are only partly protected against intracerebrally inoculated BSE prions [161].

**Modeling Familial Forms of Human Prion Disease in Mice**

More than 20 different mutations of the PrP gene have been identified in families suffering from familial human prion diseases, such as GSS and familial CJD (Figure 6A). It has been speculated that mutated PrP genes give rise to an unstable PrP<sup>C</sup> proteins that can spontaneously convert into abnormal PrP<sup>Sc</sup> conformers whereas sporadic forms of the disease are attributed to rare spontaneous transitions of PrP<sup>C</sup> into PrP<sup>Sc</sup> or to somatic mutations in the PrP gene. However, studies on thermodynamic stability of the C proximal globular domain of PrP with those mutations do not generally support the concept of mutation-induced PrP<sup>C</sup> destabilization [162, 163]. Several attempts have been made to elicit transmissible prion disease in mice by expressing mutated PrPs associated with inherited prion diseases. So far, all models have failed to develop transmissible disease, the hallmark of prion diseases, with the possible exception of PrP<sup>P<sub>101L</sub></sup> (Figure 6B). Mice overexpressing murine PrP<sup>P<sub>101L</sub></sup> (the counterpart of human PrP<sup>P102L</sup> linked to familial GSS) 8-fold, on a Pr<sup>0/0</sup> background, spontaneously developed neurodegeneration at 140 days of age, but little if any PrP<sup>Sc</sup> could be detected [164]. 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 spontaneously developed neurodegeneration only late in life [164-166]. It was suggested that the amino acid difference constituted an artificial transmission barrier to wild-type mice. However, human P102L-linked GSS can be transmitted to both monkeys and wild-type mice [4, 167]. Thus, it has been argued that the P101L mutation may be an important susceptibility factor rather than a direct cause of GSS [158, 168].

In addition to the P102L-linked GSS mutation, two different mutations associated with inherited CJD in human were analysed. Pr<sup>0/0</sup> mice expressing PrP with the counterparts of the human CJD-linked mutations at T183A or E200K did not develop any pathological signs [164, 169]. Furthermore, expression of a mouse PrP version of a nine-octapeptide insertion associated with CJD in humans produced a slowly progressive cerebellar disorder and progressive myopathy [141, 170]. Because the disease was not transmissible, it qualifies as a “proteinopathy” rather than a “prion disease”. In a further attempt to generate spontaneous prion disease in transgenic mice, Ala was replaced by Val at positions 113, 115 and 118 of PrP (“AV3”) to promote de novo β-sheet formation in the flexible tail [171]. Although founders developed a fatal neurological disorder [136], this was not transmissible and no protease-resistant PrP<sup>C</sup> was detected [171]. Thus, with one possible exception, the human familial prion diseases have not been modeled successfully in the mouse.

**Transmissible Versus Non-Transmissible PrP-Linked Disease**

Many PrP mutations are pathogenic in mice, mostly or perhaps only when overexpressed. It is not surprising that neurons accumulating abnormal forms of protein may suffer damage. With the possible exception of the disease caused by overexpression of PrP<sup>P101L</sup> [164, 165], none of the PrP-linked diseases in mice were shown to be transmissible (Figure 6B). Thus, it is appropriate to distinguish between “prion diseases”, which are transmissible, and “non-transmissible conformational diseases”, or “non-transmissible proteinopathies” which are not [4]. In the case of human “prion diseases”, experimental transmission to animals has been achieved for some but not all familial cases [4]. 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”. Beyond the nine-octapeptide insertion and the AV3 mutations that cause non-transmissible neurodegeneration in the mouse, further examples of this type of mutation have been described.

**Internal Deletions of α-Helices Causing a Storage Disease**

Mice expressing PrP with the four octarepeats (PrP<sup>Δ23-88</sup>) deleted or even, in addition, lacking the first α-helix and one β-sheet (Δ141-176, “PrP106”) remained healthy [172]. However, deletion of either the second α-helix (Δ177-202) or third α-helix (Δ201-217) strongly affected correct PrP folding and caused a lethal illness resembling neuronal storage disease [172]. As summarized in Figure 7, deletions of all three α-helices have been analysed. Pr<sup>0/0</sup> mice expressing low levels of a 61-amino acid PrP protein (PrP61) that contains only the amino acid
sequence 88-141 and the GPI-attachment site (residues 221-231) developed a neurological disorder at few months of age and died few days later. PrP61-mediated neurotoxicity was not prevented by co-expression of wild-type PrP and the disease was not transmissible to mice expressing PrP106 [173].

Expression of Cytosolic PrP Causing a Cerebellar Disorder in Wild-type Mice

In cultured cells, mature PrPC 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 cells [174]. Wild-type mice transgenic for murine PrP23-230, which lacks the signal sequence, accumulated cytosolic PrP and developed severe ataxia with cerebellar degeneration and gliosis [138]. Although the mechanism for the formation of cytosolic PrP species (retro-translocation or inefficient translocation of PrP due to over-expression) is debated, it appears that PrPC located partly or entirely in the cytosol is extremely toxic and may contribute to neuronal degeneration in prion disease [175].

6. INVESTIGATING THE MECHANISM OF PRION PROPAGATION AND SPREAD BY ECTOPEIC EXPRESSION OF PRP

Several questions regarding the cell specificity of prion propagation and the spread of prions through the body have been addressed by generating mice that express PrP in only one type of tissue or organ, rather than almost ubiquitously, as wild-type mice do (Table 1). Because PrP is expressed in both neurons and astrocytes, the question arose whether PrP expression in each cell type alone is sufficient for prion propagation. Expression of hamster PrP under control of the neuron-specific enolase (NSE) promoter, which is considered to be neuron specific, rendered transgenic Prnp/o mice susceptible to hamster prions [176]. Thus, PrP expression in neurons is sufficient for prion propagation. It was surprising to find that expression of PrP targeted to...
astrocytes of Prnp<sup>o/o</sup> mice by the glial fibrillary acid protein (GFAP) promoter also rendered mice susceptible to intracerebrally administered prions and led to clinical disease and prion propagation [127]. Thus, not only do astrocytes appear to be competent for prion replication, but also the brain pathology exhibited by the terminally scrapie-sick transgenic and wild-type mice is quite similar.

### Table 1. Ectopic expression of PrP in PrP null mice and cell specificity of prion replication*

<table>
<thead>
<tr>
<th>Ectopic expression of PrP</th>
<th>Promoter</th>
<th>Prion propagation</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neurons</td>
<td>NSE</td>
<td>Yes</td>
<td>[176]</td>
</tr>
<tr>
<td>Astrocytes</td>
<td>GFAP</td>
<td>Yes</td>
<td>[127]</td>
</tr>
<tr>
<td>T lymphocytes</td>
<td>lck</td>
<td>No</td>
<td>[192]</td>
</tr>
<tr>
<td>B lymphocytes</td>
<td>CD19</td>
<td>No</td>
<td>[193]</td>
</tr>
<tr>
<td>Hepatocytes</td>
<td>transthyretin</td>
<td>Yes</td>
<td>[203]</td>
</tr>
<tr>
<td>Muscle cells</td>
<td>α-actin</td>
<td>Yes</td>
<td>[203]</td>
</tr>
</tbody>
</table>

*Ectopic expression of PrP in the indicated cell types of PrP knockout mice was accomplished by using cell-specific promoter elements, such as neuron-specific enolase (NSE) for neurons, glial fibrillary acid protein (GFAP) for astrocytes, lck for T cells and CD19 for B cells.

“Natural” transmission of prions, as it occurs in BSE, vCJD, kuru, scrapie and chronic wasting disease (CWD), usually comes about by ingestion of the transmissible agent. The relative resistance of prions to protease digestion probably allows a significant proportion of the infectious agent to survive passage through the digestive tract [177-179]. It is not clear how prions pass through the intestinal mucosa. M cells, which are portals for antigens and pathogens [180-182], may be involved in the transport of prions [183]. Thus, after oral uptake, the infectious agent may penetrate the mucosa through M cells and reach Peyer's patches, where they are found early on [177] as well as the enteric nervous system [184]. The numbers of Peyer’s patches [185] and the presence of functional follicular dendritic cells (FDCs) [186] correlates with the efficiency of oral infection. Depending on the host, other tissues of the lymphoreticular system (LRS), in particular the spleen, but also lymph nodes [187], are sites in which prions replicate and accumulate. Recent reports suggest that myeloid dendritic cells mediate the transport to the lymph nodes and the spleen [188, 189], but also other cells can be involved [190]. In spleen, PrP<sup>Sc</sup> is found mainly in FDCs. Further studies found mouse prions also associated with spleen-derived B and T cells and with the FDC-containing stromal fraction, but not with neutrophils. No infectivity was detected in circulating lymphocytes in this model [191]. To address the question whether B and T cells were able to propagate prions or whether they acquired them from another source, PrP was specifically targeted to B or T cells: Prnp<sup>o/o</sup> mice expressing PrP under the control of the T-cell-specific lck promoter showed high levels of PrP in T cells, both in thymus and spleen, but not in brain [192]. No clinical symptoms became apparent and no infectivity was detected in spleen, thymus or brain of these mice up to one year after intraperitoneal inoculation. Prnp<sup>o/o</sup> mice expressing PrP under control of the B-cell-specific CD19 promoter had 10-20 fold higher PrP levels on B cells compared to wild-type mice, yet no pathology or prion propagation was observed after intraperitoneal inoculation with scrapie prions [193]. Thus, the presence of PrP on T or B cells does not suffice to support prion replication and prions associated with splenic T and B lymphocytes must stem from another source, most likely FDCs [194, 195].

From the spleen and likely from other sites, prions proceed along the peripheral nervous system to finally reach the brain, either directly via the vagus nerve [196, 197] or via the spinal cord, under involvement of the sympathetic nervous system [132, 178]. The finding that Prnp<sup>o/o</sup> mice with a PrP-expressing brain graft developed scrapie pathology in the graft after intracerebral [198], but not after intraperitoneal inoculation [199] shows that PrP is required for the spread of prions from the periphery to the brain. After reconstitution of the hematopoietic system of those graft-bearing Prnp<sup>o/o</sup> mice with PrP-expressing cells, prion accumulation in the spleen was as in wild-type animals, nonetheless, intraperitoneal inoculation failed to produce scrapie pathology in the neurografts [200]. Thus, transfer of infectivity from spleen to the CNS is crucially dependent on the expression of PrP in a tissue compartment that cannot be reconstituted by bone marrow transfer, likely the peripheral nervous system [132, 178]. This was shown indeed to be the case using Prnp<sup>o/o</sup> mice transgenic for hamster PrP under control of the NSE promoter [176], in which PrP was expressed exclusively in neurons of both brain and peripheral nerves. After inoculation with hamster prions, either orally or intraperitoneally, scrapie disease ensued with the same incubation time as in mice expressing hamster PrP under the control of the PrP promoter [201]. Thus, at least after peripheral exposure to high doses of hamster prions, PrP<sup>Sc</sup>-expressing LRS was not required for prion replication or transport. Although PrP expression on neurons is sufficient for the spread of prions from the periphery into the brain, the underlying mechanism is still unknown, because the velocity of the propagation (approximately 1 mm per day) corresponds neither to fast nor to slow axonal transport [132, 202]. Possibly, Schwann cells are involved, which expressed PrP in mice and are able to propagate prions in culture [51]. Further transgenic studies have shown that expressing PrP under the control of the α-actin promoter in Prnp<sup>o/o</sup> mice allows prion replication in muscle [203]. Inoculation of mice expressing PrP under the direction of the liver-specific transthyretin promoter/enhancer resulted in low levels of prion infectivity in the liver, but also in the brain [203].
From the experiments with B and T lymphocytes, it can be concluded that the presence of PrP on the cell surface does not suffice to support prion replication; maybe location within a particular membrane region is required [204] and or other components are necessary, such as the postulated factor X [152] or a receptor.

7. EXPLORING THE PHYSIOLOGICAL ROLE OF PRP BY TRANSGENIC STUDIES

Despite the nearly normal phenotypes of Zürich-I Prnp\(^{+/o}\) mice, it cannot be excluded that PrP might have important functions, since the absence of PrP might be compensated during embryogenesis or subsequently by some functionally homologous protein(s). For instance, knockout mice lacking either APP or the APP-like protein APLP-2 remain healthy, whereas the knockout of both genes is lethal, suggesting functional redundancy of both genes [205]. If indeed a functional homologue of PrP exists, and if this homologue and PrP have a common ligand, it might be possible to interfere with their function by a mutated version of PrP, which binds and blocks the ligand thereby impeding function. A candidate for such a dominant negative PrP mutant was discovered when screening a series of amino terminal deletions [206] (Figure 7B).

Deletions of the Flexible Tail Causing a Cerebellar Syndrome in PrP Knockout Mice that is Suppressed by Wild-type PrP

Transgenic Prnp\(^{+/o}\) mice expressing PrP with amino proximal deletions were generated by using the “half-genomic vector” and analyzed for phenotypic abnormalities. Removal of amino acids 32 to 93 (octarepeats) or 32 to 106 did not show an abnormal phenotype [206]. However, Prnp\(^{+/o}\) 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 [206]. 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. The truncated PrP was not expressed in Purkinje cells, for reasons explained in section 4, and these were spared from the disease. 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 [206]. To map the PrP region that suppresses the defect, mice expressing PrP with the largest, pathogenic deletion (PrP\(^{Δ32-134}\)) were crossed with mice expressing PrP with shorter deletions. PrP lacking the octarepeats (PrP\(^{Δ32-93}\)) still completely suppressed the syndrome, whereas PrP\(^{Δ32-106}\) could not abolish the defect anymore. However, the duration of the disorder to the terminal state was prolonged by 2 months [126]. When the truncated PrP was specifically targeted to Purkinje cells of Prnp\(^{o/o}\) [Zürich I] mice using the L7 promoter, ataxia and Purkinje cell degeneration developed, while the cerebellar granule layer remained unaffected [94]. The phenotype resembled that observed in the ataxic PrP knockout lines with up-regulation of Dpl in brain, which was also abrogated by PrP.

Prnp\(^{o/o}\) mice expressing Dpl ubiquitously in brain developed ataxia associated with loss of both granule and Purkinje cells. Introduction of a hamster PrP transgene resulted in complete abrogation of the phenotype in mice expressing moderate levels, and partial abrogation in mice expressing high levels of Dpl [87]. 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 [64, 81, 94].

Although overexpression of a variety of PrP mutations gives rise to non–transmissible clinical disease, usually associated with accumulation of insoluble and/or toxic forms of PrP, in no case was the phenotype abrogated by the co-expression of wild-type PrP other than in that of the “Shmerling syndrome” described above (Figure 7). The findings have been explained by a model in which truncated PrP\(^{C}\) or Dpl acts as dominant negative inhibitor of a functional homologue of PrP\(^{C}\), with both competing for the same putative PrP\(^{C}\) ligand [206, 207]. As shown in (Figure 2), we proposed a two-step mechanism according to the address-message concept [208], in which binding of the globular part of PrP to its ligand (address) is the first step and activation of responses with the N-terminal tail of PrP (message) is the second step. Dpl and truncated PrP would bind to the conjectured ligand and prevent its activation, but because wild-type PrP would have a higher binding affinity, it overcomes the inhibition. Examples of such bipartite function of molecules include several hormones [208] or bacterial flagellin when acting as an elicitor in plants [209]. Recently, it has been shown that the Dpl-induced ataxic phenotype in Prnp\(^{−/−}\) [Nagasaki] is suppressed in the presence of hamster PrP or a mouse/hamster chimeric PrP (MHM2), but not by PrPMM2 devoid of amino acids 23-88 [93]. Alternative models to explain the pathological effect of Dpl and the truncated PrP are discussed elsewhere [210].

8. CONCLUDING REMARKS

The first major insight into the nature of TSEs came about with the discovery of transmissibility and was dominated by experiments at the biological level, leading to the recognition of the peculiar properties of the transmissible agent. The next phase was initiated with the isolation and biochemical characterization of PrP\(^{Sc}\) and PrP\(^{C}\), the cloning of the PrP gene and the establishment of the genetic linkage between PrP and TSEs. In a third phase, experimentation on transgenic and
knockout mice proved the essential role of PrP in susceptibility to TSEs and the propagation of the infectious agent, and yielded insights into molecular events underlying the disease. However, despite intense research the physiological role of PrPc is still not understood. Above all, the structure of the infectious entity is not yet known and while there is strong evidence that some conformer of PrP is an essential constituent, it is neither clear that it is the only one nor that the conformer is the protease-resistant, aggregated molecule originally designated as PrPSc. There may still be many surprises awaiting us in the field.

REFERENCES


