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SYMPOSIUM: Transgenic Models of Neurodegeneration

Transgenic and Knockout Mice in Research on Prion Diseases

Alex J. Raeber[1], Sebastian Brandner[1], Michael A. Klein[1], Yves Benninger[1], Christine Musahl[1], Rico Frigg[1], Christiane Roeckl[1], Michael B. Fischer[1], Charles Weissmann[2], Adriano Aguzzi[1]

[1] Institute of Neuropathology, Department of Pathology, Schmelzbergstr. 12, University Hospital, 8091 Zürich, Switzerland,
[2] Institute of Molecular Biology, Abteilung 1, University of Zürich, Winterthurerstr. 190, 8057 Zürich, Switzerland.

Since the discovery of the prion protein (PrP) gene more than a decade ago, transgenetic investigations on the PrP gene have shaped the field of prion biology in an unprecedented way. Many questions regarding the role of PrP in susceptibility of an organism exposed to prions have been elucidated. For example mice with a targeted disruption of the PrP gene have allowed the demonstration that an organism that lacks PrP^c is resistant to infection by prions. Reconstitution of these mice with mutant PrP genes allowed investigations on the structure-activity relationship of the PrP gene with regard to scrapie susceptibility. Unexpectedly, transgenic mice expressing PrP with specific amino-proximal truncations spontaneously develop a neurologic syndrome presenting with ataxia and cerebellar lesions. A distinct spontaneous neurologic phenotype was observed in mice with internal deletions in PrP. Using ectopic expression of PrP in PrP knockout mice has turned out to be a valuable approach towards the identification of host cells that are capable of replicating prions. Transgenic mice have also contributed to our understanding of the molecular basis of the species barrier for prions. Finally, the availability of PrP knockout mice and transgenic mice overexpressing PrP allows selective reconstitution experiments aimed at expressing PrP in neurografts or in specific populations of hemato- and lymphopoietic cells. Such studies have shed new light onto the mechanisms of prion spread and disease pathogenesis.

Corresponding author:
Alex J. Raeber, Institute of Neuropathology, Schmelzbergstr. 12, University Hospital, CH-8091 Zürich; Tel.: (01) 255 2151; Fax: (01) 255 4402; E-mail: raeber@molbio1.unizh.ch

Introduction

Prion diseases or transmissible spongiform encephalopathies (TSE) are neurologic disorders caused by transmissible pathogens termed prions. While the prototype of all prion diseases, scrapie in sheep and goats, has been known for more than two centuries, a new form of animal prion disease designated bovine spongiform encephalopathy (BSE) has since its first recognition in 1986 developed into an epizootic (7, 144). The emergence of a new variant form of Creutzfeldt-Jakob disease (vCJD) in young people in the UK has raised the possibility that BSE has spread to humans by dietary exposure (30, 145). This fearful scenario has recently been supported by experimental evidence claiming that the agent causing BSE is indistinguishable from the vCJD agent (1, 4, 21, 56).

Progress in understanding prion diseases has been achieved by developing better assay systems to detect prions using mice and hamsters. This has led to the purification of the infectious agent and the discovery of the prion protein (PrP) (13, 101) followed by the molecular cloning of the PrP cDNA (31, 96) and gene (8). Mutations in the host-encoded PrP gene were shown to be genetically linked to human prion diseases (61). In addition, the PrP gene controls many features of prion diseases such as incubation time, species barrier and strain specificity (for reviews, see (5, 105, 112, 138).

Since the discovery of the PrP gene, transgenetic investigations of prion diseases have become a fruitful area of research on these once enigmatic disorders. In this overview, we summarize the transgenic mouse models developed in the past years and how they contributed to the current understanding of the key role of PrP in the development of transmissible spongiform encephalopathies.

PrP and the molecular biology of prions

One of the hallmarks of prion diseases is the accumulation in the central nervous system (CNS) of an abnormal isoform (PrP^{Sc} or PrP-res) of the host-encoded prion protein (PrP^{C} or PrP-sen) (89, 96, 107). Although the exact physical nature of the transmissible agent is still controversial, a growing body of experimental data now supports the 'protein only' hypothesis which postulates that the agent is devoid of nucleic acid and consists solely of an abnormal conformer of the cellular prion protein, PrP^{C} (53, 101). It has been proposed that the partially protease-resistant and detergent-insoluble PrP^{Sc} is congruent with the infectious agent (101). Two distinct models have been postulated to explain the mechanism by which a misfolded form of PrP could catalyze the refolding of native PrP molecules into the abnormal conformation: (i) the template assistance model, and (ii) the nucleation-polymerization model. In the first model, a PrP^{Sc} monomer promotes the conformational conversion of PrP^{C}, or of a partially destabilized intermediate, to the PrP^{Sc} conformation. In this model PrP^{Sc} is inherently more stable than PrP^{C}, but kinetically inaccessible (103). In the second model, the formation of PrP^{Sc} is initiated by an aggregate of PrP^{Sc} acting as a seed in a nucleation-dependent polymerization process. In contrast to the template assistance model, the PrP^{Sc} monomer is less stable than PrP^{C} but is stabilized upon binding to the PrP^{Sc} aggregate (50, 66). Consistent with the latter model, cell-free conversion studies indicate that PrP^{Sc} aggregates are able to convert PrP^{C} into a protease-resistant PrP isoform (29).

By virtue of its location at the outer surface of cells, anchored by phosphatidylinositol glycolipid (131), PrP is a candidate for a signaling, cell adhesion or (less likely) transport function. PrP is expressed on many cell types, including neurons (80), astrocytes (93), lymphocytes (28) and appears to be developmentally regulated during mouse embryogenesis (84). Although PrP is predominantly found in brain tissue, high levels are also present in heart, skeletal muscle and kidney whereas it is barely detectable in the liver (10). Recently, several candidate proteins that bind PrP^{C} have been reported. Among them are the amyloid precursor-like protein 1 (Aplp1) (147), the human laminin receptor precursor (114) and an uncharacterized 66-kDa membrane protein (88). Evidence that any of these interactions are physiologically significant is, however, still missing.

Human prion diseases and genetic linkage to the prion protein

The human prion diseases are characterized by extended incubation periods ranging from several months to decades followed by a progressive clinical phase presenting with severe dementia and ataxia. Clinical disease is invariably leading to death within as little as a few weeks but up to >1 year. The neuropathological features include profound astrocytic gliosis, spongiosis and neuronal cell death. A typical, albeit not invariable, finding is the presence of amyloid plaques consisting, at least in part, of PrP^{Sc}.

The human prion diseases comprise the three etiologies of TSE's in general, namely sporadic, genetic and infectious forms of the disease. Most cases of Creutzfeldt-Jakob disease (CJD) occur sporadically with a frequency of $1{:}10^{6}$ – $1{:}10^{7}$ per year, worldwide. A compilation of Swiss epidemiological data of the last three years has yielded an incidence in excess of $2{:}10^{6}$ per year (I.Hegyi and A. Aguzzi, unpublished). In these patients, mutations of the PrP coding sequence are not found. The genetic forms of prion diseases comprise most cases of Gerstmann-Straeussler-Scheinker disease (GSS), the familial forms of CJD and fatal familial insomnia (3, 104). The first mutation in the PrP gene found to be genetically linked to hereditary forms of GSS was the codon 102 Pro>Leu mutation (61) that has since been found in many GSS families throughout the world (52, 79). By now, all genetic forms of prion disease have been linked to mutations in the human PrP gene.

The prototype of the infectious form of TSEs in humans is kuru, a disease that assumed epidemic proportions in Fore people in Papua New Guinea by spreading through ritualistic cannibalism. Iatrogenic transmission of CJD to young individuals occurred by the administration of growth hormone or gonadotropin of cadaveric origin, which was presumably contaminated with CJD prions. A new variant form of CJD affecting predominantly young people in the UK is thought to be caused by dietary exposure to BSE prions (82). A hallmark of all human prion diseases is that they can be transmitted to experimental animals including mice. How can these different features of prion diseases be explained by the protein-only hypothesis? 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}. Sporadic forms of the disease would have to be explained by a very rarely occurring spontaneous transition of PrP^{C} into PrP^{Sc} or by somatic mutations in the PrP gene. Once conversion has started it would be followed by autocatalytic propagation.

The Prusiner group introduced the leucin substitution

corresponding to the human GSS mutation at codon 102 into the murine *Prnp* gene. One transgenic line harboring this modified transgene developed a spontaneous neurological disease with spongiform degeneration of the brain (62). In addition they could show that infectious prions were synthesized *de novo* by transmitting brain extracts of these mice to transgenic animals harboring the same mutant PrP transgene but expressing it at lower levels which did not lead to spontaneous illness (132). An inherent problem with these studies was the fact that the mutant protein was overexpressed several fold compared to wild-type mice. Considering that overexpression of wild-type PrP by itself from a cosmid PrP transgene causes a spontaneous disease phenotype in transgenic mice (140), it was desirable to study the mutation in the context of the endogenous PrP gene. Using a two-step gene targeting strategy in embryonic stem cells, the 101 Pro>Leu mutation (equivalent to codon 102 in the human PrP gene) was introduced into the endogenous murine PrP gene (92). Mice either homozygous or heterozygous for the codon 101 mutation were generated from gene targeted embryonic stem cells. Surprisingly, these mice remained healthy for more than 650 days without showing any signs of spontaneous CNS disease and no abnormal pathology (91). This shows that the presence of the mutated PrP at physiological levels is not sufficient to cause spontaneous neurodegeneration in mice.

Phenotypes of PrP knockout mice

According to the 'protein only' hypothesis, PrP^{C} is a substrate for the PrP^{Sc}-mediated conversion of PrP^{C} into new PrP^{Sc} molecules. An important corollary to this hypothesis is that an organism lacking PrP^{C} should be resistant to scrapie and unable to propagate the infectious agent. Büeler and colleagues (24) generated mice with a targeted disruption of the *Prnp* gene using homologous recombination in embryonic stem cells. In the disrupted *Prnp* allele 184 codons of the *Prnp* coding region (254 codons) were replaced by a drug-resistance gene as selectable marker. A second line of PrP knockout mice was generated by Manson and coworkers (85) employing the strategy of inserting a selectable marker into a unique KpnI site of the PrP open reading frame, thereby disrupting but not deleting the *Prnp* coding region. Sakaguchi and colleagues generated a third line of PrP knockout mice by replacing the whole PrP ORF and in addition about 250 bp of the 5' intron and 452 bp of 3' untranslated sequences with a drug-resistance gene (120). Both the Büeler and Sakaguchi mice were on a mixed genetic (129/Sv x C57BL) background whereas the Manson mice were bred on a pure 129/Ola background.

Although it was proposed that PrP, as a ubiquitously expressed neuronal protein, may have housekeeping function (8), homozygous PrP knockout mice generated by Büeler (24) and Manson (85) were viable with no behavioral impairment and showed no overt phenotypic abnormalities, suggesting that PrP^{C} does not play a crucial role in development or function of the nervous system (24, 85). However, electrophysiological defects such as weakened GABA-A receptor-mediated fast inhibition and impaired long-term potentiation in the hippocampus were reported for these two lines of PrP knockout mice when compared to their corresponding wild-type counterparts, indicating that PrP might play a role in synaptic plasticity (35, 87). Interestingly, this electrophysiological phenotype could be rescued by a transgene encoding human PrP (143). In contrast, no electrophysiological abnormalities were found by others in the hippocampus (83) or in the cerebellum (55) using the line of PrP null mice generated by Büeler et al. (24). Tobler *et al*. (136) reported altered sleep patterns and rhythms of circadian activity in the Büeler and Manson mice. The $Prnp^{-/-}$ mice by Sakaguchi *et al*. (120) presented with the most severe phenotype consisting of progressive ataxia from 70 weeks of age. Analysis of the brains of affected animals revealed an extensive loss of cerebellar Purkinje cells (119). Because no such phenotype was observed in the other two lines of PrP knockout mice, it is likely that this phenotype is not due to the lack of PrP but rather to the deletion of flanking sequences. Interestingly, a Purkinje cell-specific enhancer was proposed to be contained within the second intron of *Prnp* (44).

Prnp$^{0/0}$ mice are resistant to scrapie

All three lines of PrP null mice were identical in regard to their resistance to scrapie. $Prnp^{0/0}$ mice generated by Büeler *et al*. were inoculated with the RML isolate of mouse-adapted prions and remained healthy for their whole life-span whereas all wild-type (129/Sv x C57BL/6) mice developed clinical scrapie symptoms at 158 ± 11 days and died of scrapie at 171 ± 11 days after inoculation (23). Similar results were obtained with the other two lines of PrP null mice challenged with the ME7 isolate (86) and the mouse-adapted Fukuoka-1 strain of CJD prions (120). All three lines of PrP null mice showed a complete lack of scrapie typical neuropathology following inoculation with prions.

An additional consequence of the corollary mentioned above is that prion propagation should not occur

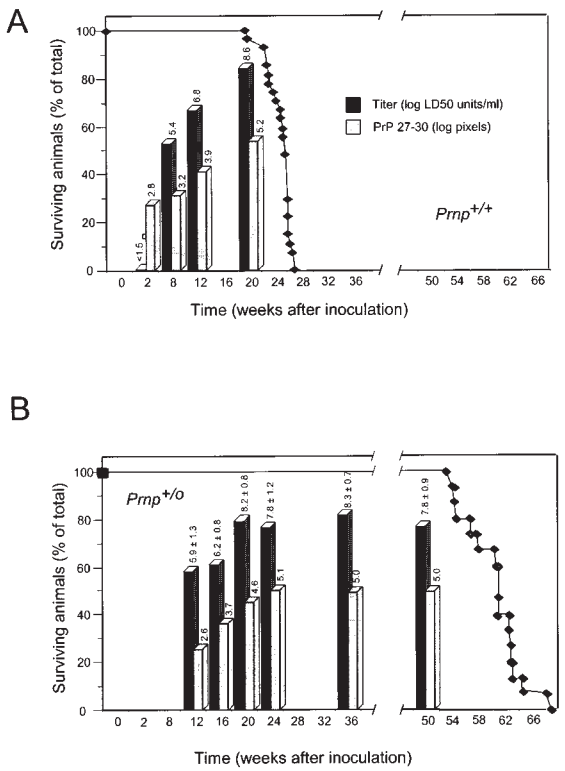

Figure 1. Prion titers and PrP^{Sc} in brains of mice at various times after inoculation with mouse prions.
Prion titers (in the 10% brain homogenate) as determined by titration in indicator mice and PrP^{Sc} (protease-resistant PrP) quantified on immunoblots from brains of (**A**) *Prnp*$^{+/+}$ and (**B**) *Prnp*$^{0/+}$ mice at various times after inoculation. From Büeler et al. (22).

in PrP knockout mice. Indeed, animals lacking both *Prnp* alleles were unable 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 LD_{50} units/ml respectively, by 20 weeks post infection (23, 118). Surprisingly, mice heterozygous for the disrupted *Prnp* gene (*Prnp*$^{0/+}$) showed also partial resistance to scrapie infection as manifested by prolonged incubation times of about 290 days as compared to about 160 days in the case of *Prnp*$^{+/+}$ mice (Figure 1). Although times to onset of symptoms and rate of disease progression seem to correlate with the steady-state levels of PrP^{C} in the host, final severity of the disease as assessed by the extent of scrapie pathology and levels of prion infectivity were not dependent on the PrP^{C} level (22, 86). This suggests that the amount of PrP^{C} protein in the brain is a rate limiting step in the development of the disease and paves the way to therapeutic efforts aimed at reducing the production of the normal PrP^{C} isoform. Interestingly, *Prnp*$^{0/+}$ mice harbored high levels of infectivity and PrP^{Sc} by 140 days after inoculation but survived thereafter for at least another 140 days without showing severe clinical symptoms (22) (Figure 1). These findings suggest that despite high levels of prion infectivity and PrP^{Sc} in the brain, clinical disease may not manifest in an organism. Whether this important finding is also true for humans and cattle remains to be seen.

Restoration of scrapie susceptibility of *Prnp*$^{0/0}$ mice

Mice devoid of PrP are viable and resistant to scrapie. To unequivocally show that this phenotype is due to the disruption of the PrP gene, it was necessary to demonstrate that reintroduction of PrP into *Prnp*$^{0/0}$ mice is able to restore scrapie susceptibility. In a first experiment, this was achieved by crossing *Prnp*$^{0/0}$ mice with transgenic mice expressing a Syrian hamster PrP gene. These mice were very susceptible to hamster prions with average incubation times of about 56 days but much less susceptible to mouse prions (300 days incubation time) (23). In a different approach, murine *Prnp* genes were introduced by transgenesis into *Prnp*$^{0/0}$ mice and were shown to restore scrapie susceptibility of *Prnp*$^{0/0}$ mice in a dose-dependent fashion. Two lines of transgenic mice (*tga19* and *tga20*), which contained about 30 copies of a *Prnp* transgene lacking the 10 kb intron 2 ('half-genomic' *Prnp*: phgPrP) (Figure 2) and overexpressed PrP in the brain 3-4 (*tga19*) and 6-7 (*tga20*) fold as compared to wild-type brain, showed enhanced susceptibility to scrapie with incubation times of 87 ± 13 (*tga19*) and 64 ± 9 (*tga20*) days, respectively (44) (Figure 3A). This confirmed an inverse relationship between the steady-state level of PrP^{C} in the brain and the incubation time for scrapie as reported earlier (108).

While overexpression of PrP from a cosmid *Prnp* transgene caused a lethal neurologic disease associated with scrapie-like spongiform degeneration in the brain, demyelination of the sciatic nerve and muscle degeneration in old transgenic mice (140), no such phenotype was observed in *Prnp*$^{0/0}$ mice overexpressing PrP from the half-genomic *Prnp* transgene (44). Since PrP expression levels were similar in these transgenic mice, it seems unlikely that overexpression of PrP^{C} is sufficient to account for this novel phenotype. Alternatively, different expression patterns due to the constructs used are most likely to be responsible for the observed neurologic syndrome. Incidentally, it was found that transgenic mice generated with the half-genomic *Prnp* transgene showed no detectable PrP RNA in Purkinje cells. This suggests that one or more control elements responsible for Purkinje cell- specific expression are absent from the

half-genomic construct (44).

Structure-function studies on the PrP gene

The fact that *Prnp*$^{0/0}$ mice are resistant to scrapie and that susceptibility to prions can be restored by the introduction of PrP transgenes opened the possibility to study the structure-function relationship of PrP with regard to scrapie susceptibility.

Limited proteolysis truncates the N-terminus of PrP^{C} to form PrP27-30 without loss of infectivity arguing that at least 60 amino terminal residues of PrP^{Sc} are not required for infectivity (59, 89). It had been shown that PrP^{C} lacking residues 23-88 can be converted into protease-resistant PrP in scrapie-infected neuroblastoma cells (117). The question then arose as to whether N-terminally truncated PrP molecules can support prion replication in mice. For that purpose, PrP transgenes harboring N-terminal deletions were introduced into PrP deficient mice by transgenesis. Construction of the N-terminal truncated PrP genes was such that the signal peptide and nine additional amino acids were retained to ensure correct processing and localization in the cell (Figure 2). It was demonstrated that mutant PrP with amino-proximal deletions of residues 32-80 (lines *tgd11* and *tgd12*) (44) and 32-93 (lines C4 and C15) (128) corresponding to truncations of 49 and 63 residues, respectively, are capable of restoring scrapie susceptibility (Figure 3B), prion replication and formation of truncated PrP^{Sc}. These experiments demonstrate that the octapeptide region encompassing residues 51-90 of murine PrP seems to be dispensable for scrapie pathogenesis. This is remarkable in view of the fact that additional octapeptide repeats instead of the normal 5 segregate with affected individuals in families with inherited CJD (51).

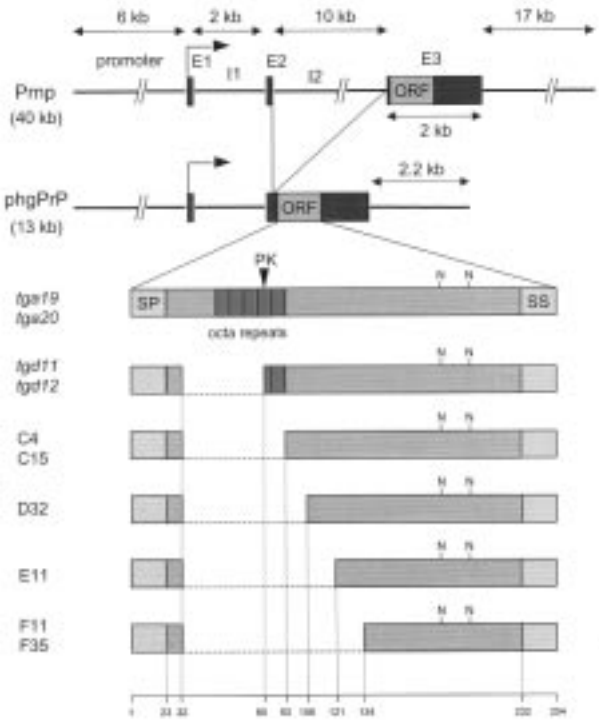

Figure 2. Wild-type and mutant PrP genes.
Prnp: a cosmid-derived clone (cos6.I/LnJ-4 (142)) encoding PrP comprising three exons (E1, E2 and E3), two introns (I1 and I2), the promoter region and 17 kb of 3'-flanking sequence. phgPrP: the half-genomic PrP clone derived from the cosmid by deletion of the intron 2 and only 2.2 kb of 3'-flanking sequence. Transgenic mouse lines containing wild-type (tga19, tga20) PrP transgenes and mutant PrP transgenes with N-terminal deletions of residues 32-80 (tgd11, tgd12), 32-93 (C4, C15), 32-106 (D32), 32-121 (E11) and 32-134 (F11, F35) (Modified from Fischer et al. (44) and Shmerling et al. (129)). Arrowhead denotes the cleavage site to proteinase K (PK) in PrPSc ; N, asparagine-linked glycosylation at residues 181 and 197; SP, signal peptide (residues 1-22); SS, signal sequence for glycolipid anchoring (residues 232-254). Relevant amino acid positions of the deletions are shown at the bottom.

Spontaneous phenotype in mice expressing truncated PrP

NMR structure determinations of full-length mature PrP have revealed a highly flexible amino-terminal tail that lacks ordered secondary structures extending from residue 23 to 121 (41, 116). The carboxy-terminal part of PrP consists of a stably folded globular domain (65, 115). The flexible tail, part of which is protease-sensitive in PrP^{Sc}, comprises the most conserved region of PrP across all species examined (121). It was proposed that the flexible tail may play a role in the conformational transition of PrP^{C} to PrP^{Sc} by initiating the structural rearrangements form α-helices to β-sheets (100, 116). To further analyse the importance of the flexible tail in regard to scrapie susceptibility, amino-proximal deletions of residues 32-121 and 32-134 were generated and the transgenes introduced into PrP-deficient mice (Figure 2). Unexpectedly, mice overexpressing these truncated PrP transgenes (lines E11, F11 and F35) developed severe ataxia and neuronal death limited to the granular layer of the cerebellum, as early as 1-3 months of age (Figure 4). No pathological phenotype was observed in transgenic mice with shorter deletions encompassing residues 32-80, 32-93 and 32-106, respectively. The selective degeneration of granule cells of the cerebellum argues against an unspecific toxic effect elicited by the truncated PrP. This is further supported by the fact that neurons in the cortex and else-

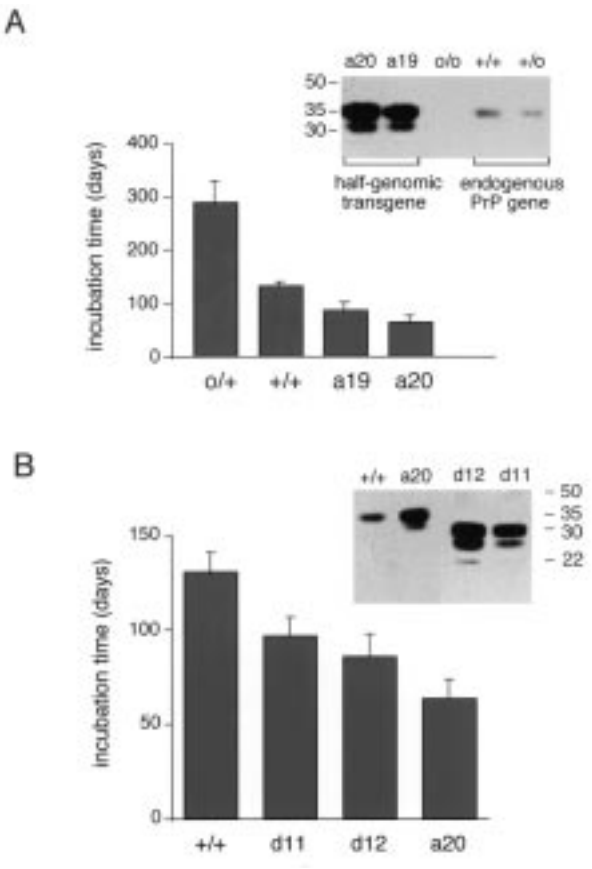

Figure 3. Scrapie incubation time of mice expressing wild-type and mutant PrP genes after inoculation with mouse prions.
(**A**) *Prnp*$^{0/+}$ mice (o/+) express about half the wild-type PrP level and develop scrapie after 290 ± 33 days. *Prnp*$^{+/+}$ mice (+/+) show scrapie symptoms after 133 ± 9 days. Mice hemizygous for the half-genomic wild-type PrP transgene expressing 3-4 times (*tga19*) and 6-7 times (*tga20*) wild-type PrP level had scrapie symptoms after 87 ± 13 days and 64 ± 9 days, respectively. Insert shows immunoblot of total mouse brain homogenates probed with the anti-PrP polyclonal antibody R073. Modified from Fischer et al. (44).
(**B**) *Prnp*$^{+/+}$ mice, tga20 mice, and mice hemizygous for the half-genomic mutant PrP transgene lacking residues 32-80 and expressing 6 times (tgd11) and 10-12 times (tgd12) the wild-type PrP level had incubation times of 97 ± 9 and 86 ± 11 days after inoculation, respectively. Insert shows immunoblot of total mouse brain homogenates probed with the anti-PrP polyclonal antibody 1B3. Modified from Fischer et al. (44).

where express truncated PrP at similar levels but do not undergo cell death by apoptosis. Strikingly, the pathological phenotype was completely abolished by the introduction of one copy of a wild-type *Prnp* allele. These results are consistent with a model in which truncated PrP acts as dominant negative inhibitor of a functional homologue of PrP, with both competing for the same putative PrP ligand (129).

Although the functional role of PrP remains elusive, there is recent evidence *in vitro* and *in vivo* that the octarepeats in the flexible tail of PrP^{C} exhibit binding sites for copper (17, 60). Interestingly, the cerebellar defects are only apparent in transgenic mice with PrP deletions encompassing the whole flexible tail. Therefore, it is conceivable that the flexible tail is involved in a signal transduction pathway which may be modulated by copper.

A different spontaneous neurologic phenotype was reported in mice carrying PrP transgenes with internal deletions corresponding to either of the two carboxy-proximal α-helices. Two transgenic mouse lines generated on the *Prnp*$^{0/0}$ background expressing mutant PrP with deletions of residues 23-88 and either residues 177-200 or 201-217 developed CNS dysfunction and neuropathological changes characteristic of a neuronal storage disease (94). Since deletion of residues 23-88 alone did not lead to a spontaneous phenotype it was concluded that ablation of either of the two carboxy-terminal alpha-helices is sufficient to cause this novel CNS illness. Ultrastructural studies indicated extensive proliferation of the endoplasmic reticulum and revealed accumulation of mutant PrP within cytoplasmic inclusions in enlarged neurons. Since both Asn-linked glycosylation sites are located within residues 177-200 it is conceivable that aberrant glycosylation affects processing of the mutant PrP. However, it is unlikely that altered glycosylation of PrP is sufficient to account for neuronal storage disease because transgenic mice expressing hamster PrP with point mutations that block Asn-linked glycosylation did not show this spontaneous disease phenotype (36).

The species barrier

Transmission of prions from one species to another is usually accompanied by a prolongation of the incubation period in the first passage and incomplete penetrance of the disease. Subsequent passage in the same species occurs with high frequency and shortened incubation times (98). This so-called species barrier can be overcome by introducing into the recipient host PrP transgenes derived from the prion donor. Thus, transgenic mice harboring Syrian hamster (Sha) PrP transgenes developed hamster scrapie with a latency of 75 days following inoculation with hamster prions while wild-type littermates failed to show symptoms after more than 500 days. Brains of hamster prion infected Tg(SHaPrP) mice contained high levels of hamster prions, hamster PrP^{Sc}, and a distribution of the pathological lesions characteristic for hamster scrapie. This finding

demonstrated that the PrP gene profoundly influences the species specificity of prions and consequently modulates scrapie susceptibility, incubation times and neuropathology (123). In a next experiment, several lines of Tg(SHaPrP) mice with various transgene copy numbers and PrP expression levels were analysed for their susceptibility to hamster scrapie. These studies showed that the length of the incubation time after inoculation with hamster prions is inversely correlated with the steady-state level of $HaPrP^C$ in the brains of these mice. Moreover inoculation of Tg(SHaPrP) mice with hamster prions led to the production of about 10^9 LD_{50} units of hamster prions and <10 LD_{50} units of mouse prions. Similarly, inoculation with mouse prions resulted in the accumulation of high levels of mouse prions in the brains of scrapie sick animals and no detectable hamster infectivity. These results suggest that the prion inoculum dictates which prions are synthesized in a mouse containing both murine and hamster PrP genes. To explain these data within the framework of the 'protein only' hypothesis, Prusiner proposed that efficient interaction of PrP^{Sc} with host-derived PrP^C requires homology between the two (108).

A further series of experiments by the Prusiner group were aimed at defining the regions of the PrP molecule involved in determining species specificity. Chimeric PrP genes composed of portions from the Syrian hamster and mouse PrP genes were introduced into transgenic mice (126). These transgenic mice allowed for a detailed mapping of the molecular domains of the PrP gene responsible for homotypic interactions between PrP^{Sc} in the inoculum and host PrP^C. The brains of sick transgenic mice contained prions with an artificial host range favoring propagation in mice that express the corresponding chimeric PrP and were also transmissible, albeit at reduced efficiency, to non-transgenic mice and hamsters (124). These findings add considerable strength to the notion that homotypic interactions between cellular and pathological isoforms of PrP are a fundamental pathogenetic principle in spongiform encephalopathies.

Crossing the species barrier for transmission of pri-

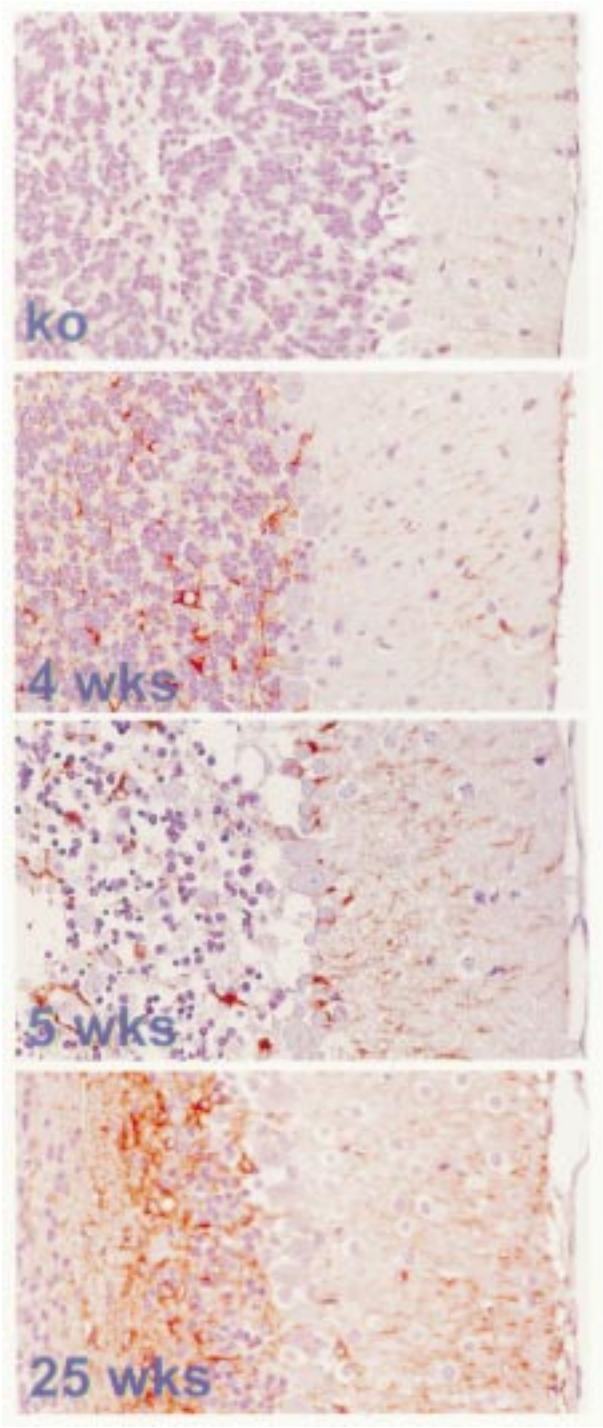

Figure 4. Cerebellar sections from mice expressing D32-134 truncated PrP at different ages, showing progressive degeneration of the granule cell layer.
Development of the cerebellum proceeds normally until early postnatal life, and leads to formation of molecular layer, Purkinje cell layer and granule cell layer (right to left in the figure) of normal thickness. However, at 4 weeks some degree of pathological astrogliosis can already be discerned. At 5 weeks, massive degeneration of granule cells by apoptosis is ongoing. Note strong gliosis affecting also the molecular layer. At the end stage of disease, mice suffer from a profound cerebellar syndrome and the thickness of the granule cell layer is considerably reduced (129). In some areas of the cerebellar cortex, granule cells disappear completely (not shown).

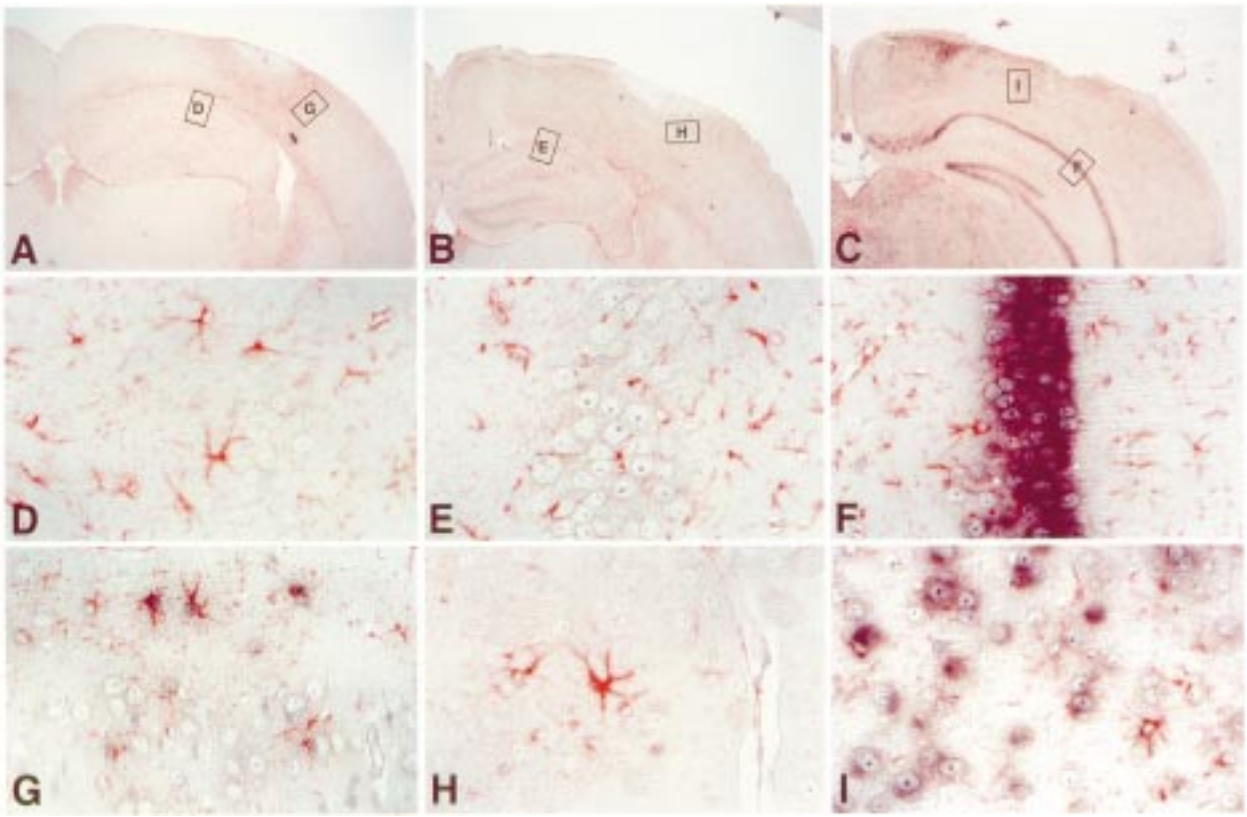

Figure 5. Astrocyte-specific PrP expression in *Prnp*$^{0/0}$ mice transgenic for hamster PrP (HaPrP) under control of the glial fibrillary acidic protein (GFAP) gene promoter.
In situ hybridization for HaPrP mRNA (black) and immunohistochemistry for GFAP (red) on brain sections of *Prnp*$^{0/0}$ mice harboring a PrP transgene driven by the GFAP promoter (left column), *Prnp*$^{0/0}$ mice (middle column) and *Prnp*$^{+/+}$ mice (right column). To enhance transcription from the GFAP promoter, mice were subjected to 'cryolesioning' of the right frontoparietal cortex for 60 sec. At low power magnification (**A**,**B** and **C**), the necrotic zone and the penumbra of the cryolesion appear demarcated by reactive astrocytes. Labeled rectangles indicate the detailed view of the respective area, hippocampus or penumbra zone of the cortex. In transgenic mice, coexpression of HaPrP mRNA (black) and GFAP (red) is visible after strong activation of the GFAP promoter in the penumbra zone of the cryolesion (**G**), while HaPrP mRNA is barely detectable in resting astrocytes (**D**). *Prnp*$^{0/0}$ mice show activated astrocytes around cryolesioned areas (H) and resting astrocytes in the hippocampus (**E**) but no PrP mRNA. *Prnp*$^{+/+}$ mice show PrP mRNA in hippocampal (**F**) and cortical neurons (**I**) but not in activated (**I**) or resting (**F**) astrocytes.

ons from humans to mice turned out to be much more difficult than between hamsters and mice. This might be explained by the sequence differences of the corresponding PrP genes. While hamster PrP shows only 16 amino acid differences compared to mouse PrP, human PrP differs from mouse PrP at 28 of 254 amino acids. Human prions were found to infect wild-type mice with an efficiency of only about 10% and with incubation times exceding 500 days (134). Introduction of human PrP transgenes into wild-type mice was not sufficient to abrogate resistance to human prions (134, 135). Introduction of the transgene array into a PrP null background by crossing Tg(HuPrP) mice with *Prnp*$^{0/0}$ mice abrogated the species barrier to human prions, resulting in incubation times of 250-270 days. Moreover, mice expressing chimeric human-mouse PrP (MHu2M) transgenes on a *Prnp*$^{+/+}$ background were susceptible to human prions with incubation times of 210-240 days. Surprisingly, crossing the transgene array into the MoPrP gene ablated background rendered the mice only slighly more susceptible to human prions with incubation times of 180-220 days. It was concluded that endogenous mouse PrP^{C} interferes with the conversion of human PrP^{C} to human PrP^{Sc} and this was proposed to be due to a species specific factor X interacting with the COOH-terminal domain of PrP (135).

Similar transgenetic studies were applied to the investigation of transmission characteristics of BSE prions to mice. Unexpectedly, mice expressing a bovine PrP transgene on the *Prnp*$^{0/0}$ background were suscepti-

ble to BSE prions with incubation times ranging from 230 to 360 days but Tg(MBo2M) mice harboring a chimeric bovine-mouse PrP transgene were resistant to BSE prions. This puzzling result was explained by amino acid differences in the C-terminus between mouse, bovine, and human PrP that constitute an epitope which was proposed to modulate interspecies transmission of prions (127).

In summary, a wealth of transgenetic studies support an important role for PrP in the transmission of prions between different species. In addition, recent data suggest that the PrP gene might be required for the long-term persistence of prions in a host which is normally resistant to foreign prions. In that particular study, it was demonstrated that very low levels of hamster prions persist for more than 700 days in the brain and spleen of wild-type mice inoculated intracerebrally with hamster prions but the mice failed to develop clinical disease. Surprisingly, hamster prions were undetectable in brain and spleen of *Prnp*$^{0/0}$ mice arguing that PrP^{C} is required for the long-term persistence of prions (109). This might be explained if wild-type but not *Prnp*$^{0/0}$ mice were immune tolerant to PrP^{Sc} from a different species. In fact, *Prnp*$^{0/0}$ mice are able to mount an immune response to PrP (16, 78, 106) and therefore might be able to clear hamster prions efficiently (6). This observation raises the possibility that BSE prions might also persist in various 'resistant' species, including humans, after exposure to BSE-contaminated cattle-derived products (33).

Prion strains

Distinct isolates or 'strains' of prions were first recognized in goats with scrapie where two different clinical manifestations, described originally as 'scratching' and 'drowsy' were identified (99). Prion strains were originally characterized by their incubation time and the distribution of vacuolar lesions in the brain. Studies of scrapie in inbred mice led to the identification of a single autosomal gene, *Sinc* or *Prni*, controlling incubation time of mice infected with mouse prion strains (27, 38, 64). Short and long incubation times for the ME7 strain of murine prions segregated with two different alleles of the *Prnp* gene, *Prnp*^{a} and *Prnp*^{b}, encoding prion proteins which differ at two amino acid residues (141). *Prnp*^{a} is characterized by residue 108 Leu and 189 Thr and *Prnp*^{b} encodes 108 Phe and 189 Val. In contrast to the ME7 strain, the mouse-adapted BSE strain 301V shows long incubation times for the *Prnp*^{a} allele and short incubation times for the *Prnp*^{b} allele (19). Classical genetic studies and study of transgenic mice failed to resolve the question as to whether *Sinc/Prni* and *Prnp* were closely linked or congruent (26, 63, 142).

Using a two-step double replacement gene targeting strategy in embryonic stem cells (92), the *Prnp*^{a} allele was changed to encode the *Prnp*^{b}-specific residues 108 Phe and 189 Val within the context of the *Prnp*^{a} allele. Following challenge with 301V prions, mice homozygous for the targeted *Prnpa*$^{[108F189V]}$ allele had an average incubation time of 133 days, while wild-type 129/Ola mice (*Prnp*^{a}) developed disease after about 244 days. The profound shortening of the incubation time in gene-targeted mice with only two amino acid differences at codon 108 and 189 demonstrates unequivocally that *Sinc/Prni* and the PrP gene are congruent (90).

Although these studies clearly establish an important role for the endogenous PrP gene in the host response to a particular prion strain they cannot explain why different prion strains can be propagated, without changing their properties, in the same mouse strain homozygous for the PrP gene. It was argued that such a phenomenon could best be accounted for by the existence of an additional component within the infectious agent (20, 39, 68, 137). To explain prion strains by the protein-only hypothesis, it was suggested that different chemical or conformational modifications of PrP^{Sc} might be responsible for the strain properties. Whereas the amino acid sequence of PrP^{Sc} is likely to be the same for strains derived from the same host, strain-specific differences might be encoded by the asparagine-linked oligosaccharides of PrP^{Sc}. Studies with transgenic mice expressing PrP mutated at one or both of the glycosylation consensus sequences indicate that prion strains are not encoded by the sugars (36). Alternatively, it was proposed that PrP^{Sc} derived from the same precursor might assume different conformations which can be stably propagated by a non-genetic mechanism.

Evidence that PrP^{Sc} acquires strain-specific properties, which manifest themselves in differential susceptibility to protease digestion has been reported for two different strains of transmissible mink encephalopathy in hamsters (11) and for various CJD isolates (97). Protease-treated PrP^{Sc} displays three distinct bands corresponding to different glycoforms of the same protein. Based on the fragment size and the relative abundance of the individual bands, three distinct patterns (PrP^{Sc} types 1-3) were defined for sporadic and iatrogenic CJD cases. In contrast, all cases of vCJD exhibited a novel pattern, designated type-4 pattern. Moreover, extracts from the brains of BSE-infected cattle, cats or kudu that were thought to have acquired BSE, and macaques that were infected experimentally with BSE, all showed type-4 pattern (34). Even more intriguingly, transmis-

sion of BSE or vCJD to mice produced mouse PrP^{Sc} with a type-4 pattern indistinguishable from the original inoculum (56). These findings strongly support the view that vCJD is the human counterpart of BSE (21). Furthermore transmission of CJD and FFI isolates to transgenic mice expressing chimeric human-mouse PrP genes have shown that the PrP^{Sc} strain phenotype is preserved upon passage to the new host providing further support for the hypothesis that strains are encoded by the tertiary structure of PrP^{Sc} (133). It has been shown that molecular strain typing by Western blot analysis can be used in the differential diagnosis of vCJD (57). However, some concern towards the general applicability of glycoform ratio analysis for strain typing has been expressed (130). More powerful methods for resolution of various glycoforms perhaps in conjunction with a PrP^{Sc}-specific reagent such as the recently described antibody 15B3 (78) may eventually serve as diagnostic tool for prion strains.

But how does a strain emerge on its passage from the original host through a variety of intermediate hosts? More than 20 different strains of mouse prions are known (37) and it has been suggested that new strains arise by mutation and selection of a putative nucleic acid within the infectious particle (20, 74). New evidence regarding this issue has been provided by using transgenic mice expressing a chimeric mouse-hamster (MH2M) PrP transgene. Using serial transmission to Tg(MH2M) mice as an intermediate host, it was shown that two prion strains, Me7 and Sc237, derived from completely different primary sources converged to yield identical strains with respect to incubation time and pathology. These striking results suggest that prion strain characteristics change depending on the sequence of PrP encoded by the host during multiple serial transmissions. These studies further imply that prion strain diversity is limited to a finite and highly restricted number of conformations of PrP^{Sc} that can be adopted by the sequence of PrP encoded by the host (125).

Ectopic expression of PrP in PrP knockout mice

The finding that PrP null mice are unable to replicate prions shows that PrP is a necessary host factor for prion replication. However, is PrP also sufficient for prion replication or are additional, perhaps cell- or tissue-specific factors required for prion propagation? This question was addressed by generating transgenic mice that express PrP ectopically in distinct cell types.

Because both neurons and astrocytes express PrP, it is not clear which cell type in the CNS is capable of generating infectivity. It was reported that transgenic mice expressing HaPrP under the control of the neuron-specific enolase promoter are highly susceptible to hamster prions. In these mice, HaPrP expression was found exclusively in neurons and not in glial cells or cells within the spleen or lymph nodes. Thus, neuron-specific PrP expression is sufficient to sustain scrapie infection, and PrP expression in non-neuronal cells, in particular astrocytes and cells of the lymphoreticular system, is not required, at least in the case of intracerebral inoculation (110).

The possibility that astrocytes might also contribute to the natural disease process is suggested not only by the findings that astrocytes express PrP, but also that in at least one model they are the earliest site of PrP^{Sc} accumulation in the brain (40). In addition, astrocytic activation occurs very early in the disease process, leading to physiological effects such as impairment of the blood-brain barrier (32, 146). To study the role of astrocytes in prion-elicited pathogenesis, $Prnp^{0/0}$ mice expressing HaPrP under the control of the glial fibrillary acidic protein (GFAP) promoter were generated. These mice expressed HaPrP only in astrocytes and not in neurons (Figure 5). After inoculation with hamster prions, these mice developed neurologic disease and accumulated in their brains infectivity and $HaPrP^{Sc}$ to high levels (113). These findings demonstrate that not only neurons but also astrocytes are capable of prion replication. Interestingly, scrapie neuropathology in transgenic animals was strikingly similar as in wild-type mice. How astrocytes are involved in the pathogenesis of prion diseases and whether indirect effects perhaps mediated by cytokines (25) play a role in the disease process remains to be elucidated. Growing evidence also incriminates microglial cells in prion-elicited pathogenesis. Recently, it was demonstrated that in a tissue culture model the neuronal damage elicited by a fragment of PrP is dependent on PrP^{C} and on microglial cells (18). It will be interesting to learn whether transgenic mice expressing PrP exclusively in microglial cells are susceptible to scrapie.

Prion replication in cells of the central nervous system seems to be the cardinal event in scrapie pathogenesis. Although accumulation of the infectious agent in lymphoid organs always precedes invasion of the brain (43) (48), the lymphoreticular system (LRS) is not essential for the development of disease after intracerebral inoculation. However, peripheral uptake of prions is epidemiologically more relevant than intracerebral administration of prions. In particular BSE and vCJD for which a common agent has been demonstrated (21, 56), but also scrapie, are due to oral transmission of pri-

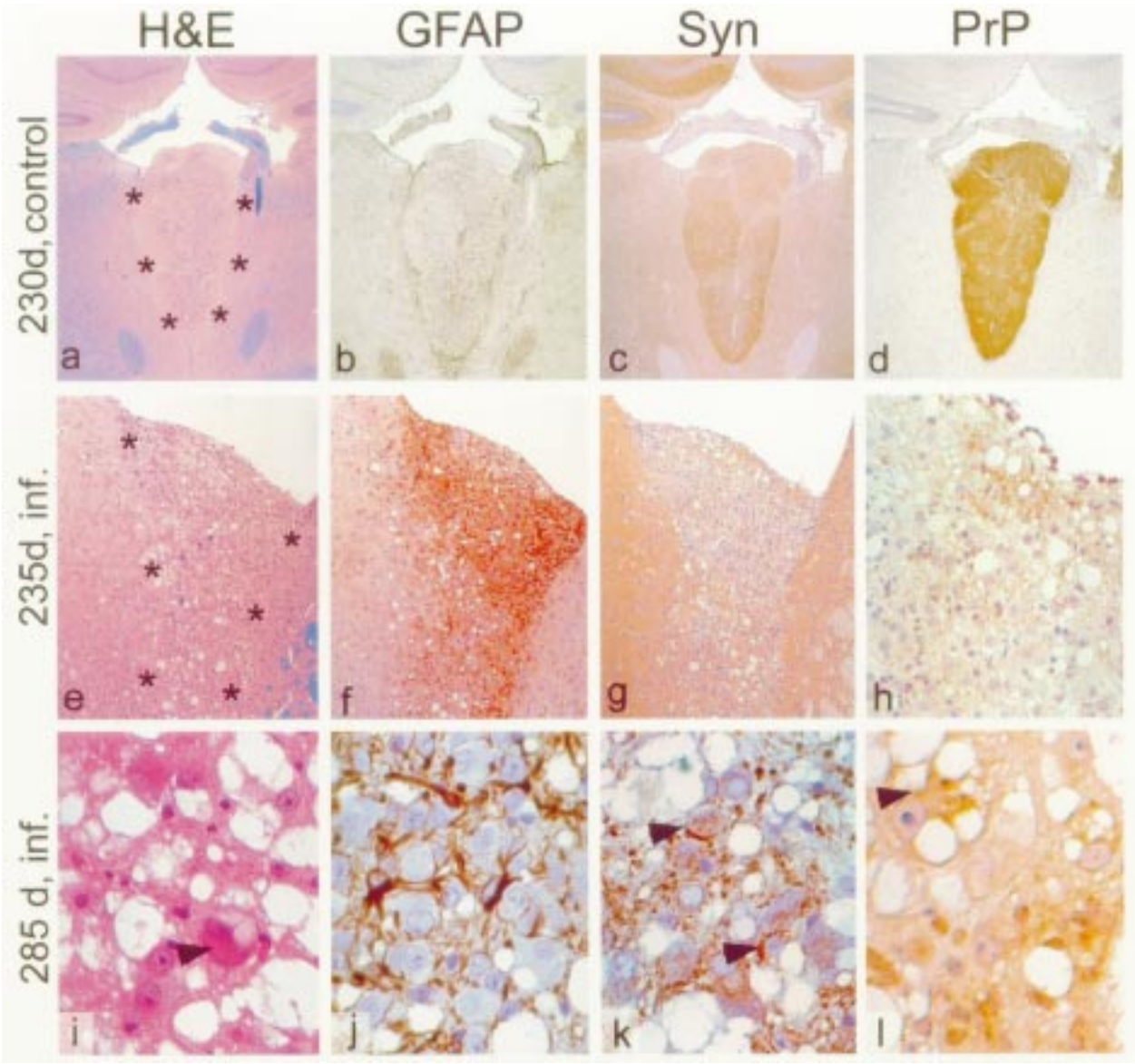

Figure 6. Typical appearance of non-infected and scrapie-infected neural grafts in the brain of a *Prnp*$^{0/0}$ mouse.
Upper row (**a**, **b**, **c**, **d**): healthy control graft 230 days after mock inoculation. The graft is located in third ventricle of the recipient mouse (**a**, see asterisks, hematoxylin-eosin), and shows no spongiform change, little gliosis (**b**, immunostain for GFAP) , and strong expression of synaptophysin (**c**) and of PrPC (**d**). Middle row (**e**, **f**, **g**, **h**): scrapie-infected graft 235 days after inoculation with increased cellularity (**e**), brisk gliosis (**f**) and a significant loss of synaptophysin (**g**) and PrP (**h**) staining intensity is shown. Bottom row: high magnification of a similar graft shows characteristic pathological changes in a chronically infected graft. (**i**) Appearance of large vacuoles and ballooned neurons (arrow). In the GFAP immunostain (**j**), astrocytes appear wrapped around densely packed neurons. Granular deposits and intracytoplasmic accumulation of synaptophysin (**k**) and PrP immunoreactivity (**l**) in the cytoplasm of neurons.

ons. Which cell types in the lymphoreticular system are targets for the scrapie agent is not known. Involvement of follicular dendritic cells has been postulated based on immunohistochemical detection of PrPSc in these cells (76).

Transgenic *Prnp*$^{0/0}$ mice harbouring a PrP transgene driven by the heterologous IRF1-promoter/Eμ-enhancer expressed high levels of PrP on B and T lymphocytes and only low levels in brain. Following intraperitoneal inoculation with mouse scrapie, these mice propagated prions early on in spleen and thymus at a level similar as in wild-type mice, but infectivity was below detectabili-

ty in the brain at six months post inoculation. Transgenic *Prnp*$^{0/0}$ mice containing a PrP transgene controlled by the *lck* promoter overexpressed PrP on T cells about 100-fold compared to wild-type T cells but lacked PrP expression on B cells. Surprisingly, these mice were resistant to scrapie following intraperitoneal inoculation with mouse prions and were unable to propagate prions in thymus, spleen and brain (111). These findings show that PrP expression is not sufficient for prion replication in T cells and that perhaps cell-specific factors such as a prion receptor, a chaperone or a protein X are required (135).

Neurografts in prion research

Because *Prnp*$^{0/0}$ mice show normal development and behavior (24, 85), it has been argued that scrapie pathology may come about because PrP^{Sc} deposition is neurotoxic (45), rather than by depletion of cellular PrP^{C} (35). In the latter case, lack of PrP^{C} might result in embryonic or perinatal lethality, especially since PrP^{C} is encoded by a unique gene for which no related family members have been found. However, acute depletion of PrP^{C} may be much more deleterious than its lack throughout development since the organism may then not have the time to enable compensatory mechanisms.

To address the question of neurotoxicity, we exposed brain tissue of *Prnp*$^{0/0}$ mice to a continuous source of PrP^{Sc}. To this end, we grafted neural tissue overexpressing PrP into the brain of PrP-deficient mice. After intracerebral inoculation with scrapie prions, the grafts accumulated high levels of PrP^{Sc} and infectivity and developed severe histopathological changes characteristic for scrapie. Substantial amounts of graft-derived PrP^{Sc} migrated into the host brain and even in areas distant from the grafts, substantial amounts of infectivity were detected (15, 44). Nonetheless, even 16 months after transplantation and infection with prions, no pathological changes were detected in the PrP-deficient tissue, not even in the immediate vicinity of the grafts or the PrP deposits. The above results suggest that PrP^{Sc} is inherently non-toxic and PrP^{Sc} plaques found in spongiform encephalopathies may be an epiphenomenon rather than a cause of neuronal damage. It is conceivable that PrP^{Sc} is only toxic when it is formed and accumulated within the cell but not when presented from outside.

Because the host mice harboring a chronically scrapie-infected neural graft did not develop any sign of disease, they enabled us not only to study the effects of prions on the surrounding tissue but were also an ideal medium to assess all changes occurring during the progression of scrapie disease in neuroectodermal tissue. With increasing length of the incubation time, grafts underwent progressive astrogliosis and spongiosis which was accompanied by loss of neuronal processes within the grafts and subsequent destruction of the neuropil (Figure 6). The terminal stage of the disease (435 days after inoculation) was characterized by an increase of cellular density in the graft probably due to astroglial proliferation and a complete loss of neurons. Intriguingly, in vivo imaging with magnetic resonance imaging using gadolinium as contrast enhancing medium, a progressive disruption of the blood-brain barrier in scrapie infected grafts was detected during the course of the disease (14). These findings confirmed several predictions about the pathogenesis of spongiform encephalopathies, mainly that scrapie leads to selective neuronal loss while astrocytes and perhaps other neuroectodermal cells, while being affected by the disease, can survive and maintain their phenotypic characteristics for very long periods of time.

Disruption of blood-brain barrier function is a finding that has been reported for experimental hamster scrapie (32) but was not found in human spongiform encephalopathies. The localized blood-brain barrier disruption in chronically infected grafts might contribute to the spread of prions from grafts to the surrounding brain, as described previously (15). And may account for the accumulation pattern of protease-resistant PrP within the white matter and in brain areas surrounding the grafts. Another explanation might be that vasogenic diffusion from the affected graft towards the host brain is a mechanism contributing to prion spread within the central nervous system if the blood-brain barrier is impaired due to astrocytic activation and/or damage during prion disease.

Spread of prions in the central nervous system

Intracerebral inoculation of scrapie-infected brain homogenate into suitable recipients is the most effective method for transmission of spongiform encephalopathies and may even facilitate circumvention of the species barrier. However, prion diseases can also be initiated by feeding (7, 75, 139) by intravenous and intraperitoneal injection (69) as well as from the eye by conjunctival instillation (122), corneal grafts (42) and intraocular injection (47). The latter method has proved particularly useful to study neural spread of the agent, since the retina is a part of the central nervous system (CNS) and intraocular injection does not produce direct physical trauma to the brain, which may disrupt the blood-brain barrier and impair other aspects of brain

physiology. The assumption that spread of prions occurs axonally rests mainly on the demonstration of diachronic spongiform changes along the retinal pathway following intraocular infection (47).

It has been repeatedly shown that expression of PrP^C is required for prion replication (23, 118) and also for neurodegenerative changes to occur (15). To investigate whether spread of prions within the CNS is dependent on PrP^C expression in the visual pathway, PrP-producing neural grafts were used as sensitive indicators of the presence of prion infectivity in the brain of an otherwise PrP-less host.

Following inoculation with prions into the eye of grafted *Prnp*$^{0/0}$ mice, none of the grafts showed signs of spongiosis, gliosis, synaptic loss, or PrP^{Sc}. In one instance, the graft of an intraocularly inoculated mouse was assayed and found to be devoid of infectivity. Therefore, it was concluded that infectivity administered to the eye of PrP-deficient hosts cannot induce scrapie in a PrP-expressing brain graft (16).

Engraftment of *Prnp*$^{0/0}$ mice with PrP^C-producing tissue might lead to an immune response to PrP (106) and possibly to neutralization of infectivity. Indeed, analysis of sera from grafted mice revealed significant anti-PrP antibody titers (16) and it was shown that PrP^C presented by the intracerebral graft (rather than the inoculum or graft-borne PrP^{Sc}) was the offending antigen. In order to definitively rule out the possibility that prion transport was disabled by a neutralizing immune response, the experiments were repeated in mice tolerant to PrP namely the *Prnp*$^{0/0}$ mice transgenic for the PrP coding sequence under the control of the *lck*-promoter described above. These mice overexpress PrP on T-lymphocytes, but were resistant to scrapie and did not replicate prions in brain, spleen and thymus after intraperitoneal inoculation with scrapie prions (111). Engraftment of these mice with PrP-overexpressing neuroectoderm did not lead to the development of antibodies to PrP after intracerebral or intraocular inoculation, presumably due to clonal deletion of PrP-immunoreactive lymphocytes. As before, intraocular inoculation with prions did not provoke scrapie in the graft, supporting the conclusion that lack of PrP^C, rather than immune response to PrP, prevented prion spread (16). Therefore, PrP^C appears to be necessary for the spread of prions along the retinal projections and within the intact CNS.

These results indicate that intracerebral spread of prions is based on a PrP^C -paved chain of cells, perhaps because they are capable of supporting prion replication. When such a chain is interrupted by interposed cells that lack PrP^C, as in the case described here, no propagation of prions to the target tissue can occur. Perhaps prions require PrP^C for propagation across synapses: PrP^C is present in the synaptic region (46) and certain synaptic properties are altered in *Prnp*$^{o/o}$ mice (35, 143). Perhaps transport of prions within (or on the surface of) neuronal processes is PrP^C-dependent. Within the framework of the protein-only hypothesis (53, 102), these findings may be accommodated by a "domino-stone" model in which spreading of scrapie prions in the CNS occurs *per continuitatem* through conversion of PrP^C by adjacent PrP^{Sc} (2).

Spread of prions from extracerebral sites to the CNS

Epidemiologically more relevant than the intracerebral transmission is the oral uptake of prions which is thought to be responsible for the BSE epidemic and for transmission of BSE to a variety of species including humans (21, 56). Prions can find their way through the body to the brain of their host, yet histopathological changes have not been identified in organs other than the CNS. But the prions may multiply silently in 'reservoirs' during the incubation phase of the disease. In the case of the mouse, one such reservoir may be the immune system and many studies point to the importance of prion replication in lymphoid organs which always precedes prion replication in the CNS, even if infectivity is administered intracerebrally (43). Infectivity can accumulate in all components of the lymphoreticular system (LRS), including lymph nodes and intestinal Peyer's patches, where prions replicate almost immediately after oral administration of prions to mice (72). Recently, it was shown that in human vCJD and in sheep scrapie, PrP^{Sc} accumulates in the lymphoid tissue of tonsils in such large amounts that it can easily be detected with antibodies on histological sections (58).

Although a wealth of early studies points to the existence of prion replication in lymphoid organs, little is known about which cells support prion propagation in the lymphoreticular system. Whole-body ionizing radiation studies in mice (49) after intraperitoneal infection have suggested that the critical cells are long-lived. The follicular dendritic cell (FDC) would be a prime candidate, and indeed PrP^{Sc} accumulates in such cells of wild-type and nude mice (which have a selective T-cell defect) (76). Moreover, intraperitoneal infection does not lead to replication of prions in the spleen nor to cerebral scrapie in mice with severe combined immunodeficiency (SCID) whose FDC are thought to be functionally impaired (95). Reconstitution of SCID mice with wild-type spleen cells restores susceptibility to scrapie

after peripheral infection (81). These findings suggest that components of the immune system are required for efficient transfer of prions from the site of peripheral infection to the CNS.

Using a panel of immune-deficient mice inoculated intraperitoneally with prions, Klein et al. (77) found that defects affecting T cells had no apparent effect, but that all mutations that disrupted the differentiation of B cells prevented the development of clinical scrapie. These results argue for a crucial role of B cells in the development of scrapie after peripheral infection. But do B cells suffice to transport prions all the way from the periphery to the CNS? This is unlikely, since lymphocytes do not normally cross the blood-brain barrier unless they have a specific reason to do so (e.g. during an inflammatory reaction). How then prions might spread in the body? Perhaps, prions administered to peripheral sites are first brought to lymphatic organs by mobile immune cells such as B cells. One the other hand, perhaps the role of B cells may consist in triggering maturation of some other cell types such as FDC's. Once infection has been established in the LRS, prions invade peripheral nerve endings (54, 73) and reach the CNS where further spread occurs transsynaptically and along fiber tracts (70, 71). Is it possible to interfere with this chain of events without resorting to ablation of a functional immune system such as in the case of SCID mice? Again, PrP^C may offer an intriguing handle. PrP^C is crucial for prion spread within the CNS (16) and it is conceivable that PrP^C is also required for spread of prions from peripheral sites to the CNS. Indeed, PrP-expressing neurografts in *Prnp*$^{0/0}$ mice did not develop scrapie histopathology after intraperitoneal or intravenous inoculation with prions and no infectivity was detectable in the spleen. Following reconstitution of the host lymphohaemopoietic system with PrP-expressing cells, prion titers in the spleen were restored to wild-type levels but, surprisingly, PrP-expressing grafts failed to develop scrapie upon intraperitoneal or intravenous infection with prions (12). These findings suggest that transfer of infectivity from the spleen to the CNS is crucially dependent on the expression of PrP in a tissue compartment interposed between the LRS and the CNS that cannot be reconstituted by bone marrow transfer. Indirect evidence suggests that this compartment may comprise part of the peripheral nervous system (9, 67).

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