Expression of truncated PrP targeted to Purkinje cells of PrP knockout mice causes Purkinje cell death and ataxia

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PrP knockout mice with disruption of only the PrP-encoding region (Zürich I-type) remain healthy, whereas mice with deletions extending upstream of the PrP-encoding exon (Nagasaki-type) suffer Purkinje cell loss and ataxia, associated with ectopic expression of Doppel in brain, particularly in Purkinje cells. The phenotype is abrogated by co-expression of full-length PrP. Doppel is 25% similar to PrP, has the same globular fold, but lacks the flexible N-terminal tail. We now show that in Zürich I-type PrP-null mice, expression of N-terminally truncated PrP targeted to Purkinje cells also leads to Purkinje cell loss and ataxia, which are reversed by PrP. Doppel and truncated PrP probably cause Purkinje cell degeneration by the same mechanism.

Keywords: cerebellar syndrome/Doppel protein/L7 promoter/prion protein/Purkinje cell loss

Introduction

The prion protein (PrP) plays a central role in the pathogenesis of transmissible spongiform encephalopathies (TSEs) such as Creutzfeldt–Jakob disease (CJD) or bovine spongiform encephalopathy (BSE) (Prusiner, 1996; Weissmann *et al.*, 1996; Prusiner *et al.*, 1998; Weissmann, 1999). Mice devoid of PrP are resistant to scrapie and fail to propagate prions (Büeler *et al.*, 1993; Manson *et al.*, 1994b; Sailer *et al.*, 1994; Sakaguchi *et al.*, 1995);

introduction of PrP-encoding transgenes restores susceptibility to the disease (Fischer *et al.*, 1996). The cellular form of the prion protein, PrP^C, is encoded by a single-copy gene (Basler *et al.*, 1986). It is attached by a glycosylphosphatidyl inositol (GPI) anchor to the outer cell surface and is found mainly in the brain, at neuronal synapses (Sales *et al.*, 1998). PrP^C comprises a globular domain consisting of three α -helices, one short antiparallel β -sheet and a single disulfide bond (Riek *et al.*, 1996). The N-terminal half of the molecule is highly flexible (Donne *et al.*, 1997; Riek *et al.*, 1997).

Several PrP knockout mouse lines have been generated, differing in the design of the gene disruption (reviewed in Weissmann and Aguzzi, 1999). Two lines in which only the PrP-coding sequence was disrupted (Büeler *et al.*, 1992; Manson *et al.*, 1994a), to be referred to as ‘Zürich I-type’ mice, developed normally and remained healthy, showing only minor electrophysiological deficits (Collinge *et al.*, 1994; Collinge *et al.*, 1996) and altered circadian rhythm (Tobler *et al.*, 1996). However, three other lines with more extensive gene deletions, ‘Nagasaki-type’ PrP knockout mice, developed Purkinje cell loss followed by ataxia (Sakaguchi *et al.*, 1996; Moore *et al.*, 1999; Rossi *et al.*, 2001). Because the ataxic phenotype could be rescued by a PrP-encoding transgene, it originally was concluded that the phenotype was due to the absence of PrP (Sakaguchi *et al.*, 1996; Nishida *et al.*, 1999), although the conclusion was incompatible with the earlier reports (Weissmann, 1996).

It was found later that the ataxic phenotype of all three Nagasaki-type lines is associated with ectopic expression of the PrP-like protein Doppel (Dpl) in brain (Moore *et al.*, 1999; Li *et al.*, 2000a; Rossi *et al.*, 2001). Dpl is an N-glycosylated, GPI-anchored protein (Lu *et al.*, 2000; Silverman *et al.*, 2000), normally expressed in a variety of tissues but not in postnatal brain (Moore *et al.*, 1999; Li *et al.*, 2000b). Its physiological function is still unknown, but its absence causes male sterility in mice (Behrens *et al.*, 2002). Dpl and PrP show ~25% sequence similarity (Moore *et al.*, 1999), and both comprise three α -helices and one antiparallel β -sheet in a similar fold, with only minor differences (Lu *et al.*, 2000; Silverman *et al.*, 2000; Mo *et al.*, 2001). Dpl is encoded by *Prnd*, located 16 kb downstream of the *Prnp* gene (Moore *et al.*, 1999). The loss of the splice acceptor site of the PrP-encoding *Prnp* exon 3, a consequence of the PrP gene deletion strategy in Nagasaki-type mice, causes formation of chimeric transcripts containing the first two non-coding exons of the *Prnp* locus linked to the Dpl-encoding *Prnd* exon. This places Dpl expression under the control of the *Prnp* promoter (Moore *et al.*, 1999; Li *et al.*, 2000a). The time to onset of disease is inversely correlated with the expression level of Dpl in the brain and is rescued by co-expression of PrP^C (Rossi *et al.*, 2001). Thus, ectopic expression of Dpl

Table I. Zürich I PrP knockout mouse lines expressing truncated PrP (PrP Δ 32–134) under the control of the Purkinje cell-specific L7 promoter

Name of the line ^a	Genotype	Transgene copy number ^b	Age at onset of ataxia (weeks) ^c	Mice analysed
TgF32	<i>Prnp</i> ^{0/0} , Tg L7- Δ PrP/–	130	9	14
TgF37	<i>Prnp</i> ^{0/0} , Tg L7- Δ PrP/–	120	9	21
TgF13	<i>Prnp</i> ^{0/0} , Tg L7- Δ PrP/–	60	11	10
TgF13/ <i>Prnp</i> ^{+/+}	<i>Prnp</i> ^{0/+} , Tg L7- Δ PrP/–	n.d.	>49	8
TgF13/ <i>Tga20</i>	<i>Prnp</i> ^{0/0} , Tg L7- Δ PrP/–, <i>Tga20</i> /–	n.d.	>70	11

^aThe full designations of the lines are *Prnp*^{tm1}-Tg(PrP Δ 134)332 Zbz for TgF32, *Prnp*^{tm1}-Tg(PrP Δ 134)337 Zbz for TgF37, and B6D2F1-Tg(PrP Δ 134)13 for TgF13.

^bTransgenic copy numbers were estimated relative to standards by quantitative PCR using primers SIG and Mut217 (Shmerling *et al.*, 1998). As standards, mouse DNA containing 30 and 40 copies of the transgene cluster PrP Δ 32–80 obtained from D11 and D12 mice were used, respectively (Fischer *et al.*, 1996). Because TgF13 mice showed the highest expression level of Purkinje cell-specific PrP and the most uniform labelling, all complementation and time course experiments were carried out with this line.

^cAverage age of mice when the phenotype was present in 50% of the mice analysed. Earliest onset and 100% morbidity were different for each line, as shown in Figure 1.

n.d., not done.

Fig. 1. Age at onset of ataxic syndrome in mice expressing the truncated PrP (PrP Δ 32–134) under the control of the Purkinje cell-specific L7 promoter. Hemizygous mice (L7- Δ PrP/–) of the lines TgF13 ($n = 10$), TgF32 ($n = 14$) and Tg37 ($n = 21$) were assessed at 2-week intervals for behavioural abnormalities, such as reduced exploratory activities, trembling gait and ataxia. Mice displaying these abnormalities were scored as affected. This syndrome was present in 50% of mice analysed by ~9 and 11 weeks of age, depending on the line (Table I). Introduction of a single wild-type allele (*Prnp*⁺) or a transgenic cluster (*Tga20*) into the TgF13 line abrogated the ataxic phenotype in F₁ offspring (TgF13/*Prnp*^{+/+}; $n = 8$) and (TgF13/*Tga20*/–, *Prnp*^{0/0}; $n = 11$), respectively.

in the absence of PrP, rather than the absence of PrP^C in itself, causes Purkinje cell loss in Nagasaki-type PrP knockout mice. Moore *et al.* (2001) reported that transgenic *Prnp*^{0/0} mice expressing Dpl ubiquitously in brain developed severe ataxia associated with loss of both granule and Purkinje cells. The age of onset was inversely proportional to the level of Dpl expression, and 1-month-old mice were already affected (Moore *et al.*, 2001). Introduction of a hamster PrP-encoding transgene resulted in complete abrogation of the cerebellar syndrome in mice expressing moderate levels, and partial abrogation in animals expressing high levels of Dpl. Both unaltered incubation times and pathology in brain tissue expressing PrP and Dpl upon challenge with different prion strains sustain the conclusion (Behrens *et al.*, 2001) that Dpl is unlikely to be involved in prion pathogenesis (Moore *et al.*, 2001; Tuzi *et al.*, 2002).

We reported earlier that expression in PrP knockout mice of PrP lacking the flexible N-terminal tail

(PrP Δ 32–121 or PrP Δ 32–134) gives rise to a dose-dependent phenotype comprising ataxia and loss of cerebellar granule cells, which is abrogated by co-expression of wild-type PrP (Shmerling *et al.*, 1998). Shmerling *et al.* proposed that the binding of PrP to a putative ligand elicits a signal necessary for cell survival and that a functional homologue of PrP supplies this function in its absence. Expression of truncated PrP was postulated to interfere with signalling by displacing the functional homologue from the ligand without eliciting the survival signal of PrP. PrP, in turn, because of its higher affinity for the ligand, would displace its truncated counterpart and restore the signal (Shmerling *et al.*, 1998).

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 ataxic syndromes (Moore *et al.*, 1999; Weissmann and Aguzzi, 1999). Why, however, should truncated PrP cause degeneration of granule cells while Dpl entails Purkinje cell loss if the mechanism is the same? Perhaps it is because the 'half-genomic' PrP vector used to express the truncated PrP is inactive in Purkinje cells, but very active in granule cells (Fischer *et al.*, 1996; Chiesa *et al.*, 1998), whilst Dpl, under the control of the intact PrP promoter, is strongly upregulated in Purkinje cells and much less so in granule cells (Li *et al.*, 2000a).

To test this hypothesis, we targeted truncated PrP specifically to Purkinje cells of Zürich I-type PrP knockout mice to determine whether the resulting phenotype would be the same as that of Nagasaki-type PrP knockout mice with ectopic expression of Dpl, namely age-dependent Purkinje cell loss followed by ataxia, suppressible by a single wild-type PrP allele.

Results

Purkinje cell loss and ataxia in mice expressing the truncated PrP under the control of the L7 promoter

We expressed PrP devoid of residues 32–134 (PrP Δ 32–134) under the control of the Purkinje cell-specific L7 promoter (Oberdick *et al.*, 1990) in Zürich I *Prnp*^{0/0} mice (Büeler *et al.*, 1992). Three transgenic lines, TgF32, TgF37 and TgF13, were generated and analysed

Fig. 2. Time course of Purkinje cell degeneration in Zürich I *Prnp*^{0/0} mice expressing truncated PrP (PrP^{Δ32–134}) under the control of the Purkinje cell-specific L7 promoter. Immunostaining for calbindin (top row), PrP (middle row) and GFAP (lower row) of sections through cerebella of L7-ΔPrP mice (*TgF13*) are shown. Sections were immunostained for PrP using R340 and TSA amplification as described in Materials and methods. GFAP and calbindin were visualized as described in Materials and methods. Three-week-old mice show normal cerebellar structure with weak gliosis. Truncated PrP is highly expressed in Purkinje cell bodies, axons and dendrites, but not in granule cells. After onset of ataxia, 14-week-old mice present with Purkinje cell loss and moderate gliosis in the molecular layer. At 25 weeks, mice show an extended Purkinje cell loss associated with moderate gliosis. In 49-week-old mice, there is an almost complete Purkinje cell loss, shrinkage of the molecular layer, decrease of granular cells and intense gliosis; a single, remaining Purkinje cell is PrP positive. Age-matched controls (*Prnp*^{0/0}, *Prnp*^{+/+}) show an intact Purkinje cell layer and no gliosis.

(Table I; Figure 1). Truncated PrP was detectable only in Purkinje cells, as judged by immunohistochemistry for PrP (Figure 2). Within the Purkinje cells, truncated PrP is highly expressed in cell bodies, axons and dendrites, as shown in 3-week-old *TgF13* mice (Figures 2 and 3). Mice hemizygous for the transgene cluster on a *Prnp*^{0/0} background showed the first signs of behavioural abnormalities, such as reduced exploratory activities and mild trembling gait, at ~2 months. Mice displaying these abnormalities were scored as affected (ataxic syndrome) (Figure 1). The ataxic syndrome was present in 50% of analysed mice by ~9–11 weeks, depending on the line (Table I). Ataxia and trembling gait increased over time, and footprint analysis revealed a similar pattern to that described for the Nagasaki-type Zürich II PrP knockout mice (Rossi *et al.*, 2001). After ~1 year, the general poor condition of the mice precluded further maintenance.

The *TgF13* line was selected for further analysis, because it was the earliest to reach 100% morbidity (Figure 1). Moreover, *TgF13* mice had the highest level of Purkinje cell-specific expression of the truncated PrP, as judged by immunohistochemistry for PrP (data not shown), and virtually all Purkinje cells were uniformly labelled by immunostaining for PrP (Figures 2 and 3). We analysed cerebellar sections of *TgF13* mice for histopathological changes before and after onset of symptoms

(Figure 2). At 3 weeks of age, there was marginal Purkinje cell loss associated with slight gliosis in the molecular layer, while the granular cell layer was normal. At 14 weeks, when behavioural abnormalities were present in ~80% of the mice (Figure 1), there was severe focal and moderate overall Purkinje cell loss accompanied by astrogliosis in the molecular layer (Figure 2). Purkinje cell bodies varied focally in shape and size, and some were negative for calbindin, a specific Purkinje cell marker. Degeneration progressed over the following months with severe Purkinje cell loss and gliosis in the molecular layer followed by shrinkage of the molecular layer and granule cell loss. At 25 weeks, 3 months after onset of symptoms, the number of Purkinje cell bodies and their dendrites in the molecular layer was dramatically reduced (Figures 2 and 4A) and astrogliosis was evident in both molecular and granule cell layers. At 49 weeks, Purkinje cells had virtually disappeared; the very few remaining Purkinje cells still expressed the (truncated) PrP both in soma and dendrites (Figure 2). Shrinkage of the molecular layer was striking and gliosis was prominent. There was neither Purkinje cell loss nor glial fibrillary acidic protein (GFAP) immunoreactivity in age-matched *Prnp*^{0/0} and *Prnp*^{+/+} mice (Figure 2), indicating that the age-dependent Purkinje cell loss in the transgenic mice was due to L7-specific expression of truncated PrP, rather than to the

Fig. 3. Immunostaining for PrP in Purkinje cells of wild-type, PrP knockout and L7-ΔPrP transgenic mice. Cerebellar sections were immunostained for PrP using R340 and TSA amplification as described in Materials and methods. The truncated PrP is strongly overexpressed in Purkinje cell bodies, axons and dendrites of 3-week-old TgF13 mice (TgF13,Prnp^{0/0}) in comparison with a faint staining in wild-type controls (Prnp^{+/+}). No background staining was found in the same area of PrP knockout mice (Prnp^{0/0}) processed in parallel with all sections shown. Introduction of a single wild-type Prnp⁺ allele in transgenic mice completely abrogated the ataxic phenotype. L7-ΔPrP transgene expression is not decreased in 3-week-old mice (TgF13/–,Prnp^{0/+}) or older mice (data not shown). It is striking that the pathogenic effect of the truncated PrP at levels several times higher than PrP levels in wild-type mice is completely abolished by a single Prnp⁺ allele, suggesting a highly specific effect of wild-type PrP in counteracting the L7-ΔPrP-induced cell degeneration.

absence of PrP. The phenotype of the L7-ΔPrP mice was thus similar to that of the three Nagasaki-type PrP knockout lines exhibiting upregulation of Dpl (Sakaguchi *et al.*, 1996; Moore *et al.*, 1999; Rossi *et al.*, 2001). In Zürich II PrP knockout mice, which are of the Nagasaki-type, there was an average Purkinje cell loss of 40% at 30 weeks, and at 40 weeks 50% of the mice were ataxic (Rossi *et al.*, 2001). As in the case of L7-ΔPrP mice, Dpl-induced Purkinje cell loss progressed over several months and exceeded 80% after 63 weeks (Rossi *et al.*, 2001). Purkinje cell loss was also accompanied by cerebellar astrogliosis (Atarashi *et al.*, 2001; Rossi *et al.*, 2001).

Abrogation of the pathogenic effect of truncated PrP by a Prnp wild-type allele

We next examined whether co-expression of PrP could rescue the phenotype as described for the Nagasaki-type mice (Nishida *et al.*, 1999; Rossi *et al.*, 2001). TgF13 mice (expressing L7-ΔPrP) were crossed with C57BL/6 mice, and the resulting transgenic TgF13/–,Prnp^{0/+} mice were assessed by their home cage behaviour for 1 year. Introduction of the wild-type PrP allele did not downregulate the expression of the truncated PrP in the Purkinje cells (Figure 3). As shown in Figure 1, all eight transgenic TgF13/–,Prnp^{0/+} mice analysed remained healthy for >11 months. Cerebella of transgenic TgF13/–,Prnp^{0/+} mice were analysed for Purkinje cell loss at 25 weeks, 35 weeks and 1 year of age. No obvious reduction in the number of Purkinje cells (Figure 4A) and no astrogliosis

(data not shown) were observed at 25 weeks of age, when severe Purkinje cell loss and reduction of the molecular layer were evident in the absence of a wild-type PrP allele. At 35 weeks, there was a slight to moderate loss of Purkinje cells with a few reactive Bergman glia cells, but no astrogliosis in the molecular layer (data not shown). Cerebella of 1-year-old healthy TgF13/–,Prnp^{0/+} mice showed moderate Purkinje cell loss that was not found in aged-matched Prnp^{0/+} mice (data not shown). In contrast, Purkinje cells were completely absent and shrinkage of the molecular layer was evident in 1-year-old ataxic transgenic TgF13/–,Prnp^{0/0} mice. There was a moderate loss of granule cells, possibly due to disappearance of their Purkinje cell targets, which was not found in Prnp^{0/+} controls (data not shown). Thus, a wild-type PrP allele fully abrogated the clinical manifestations elicited by L7-ΔPrP transgene expression, although it did not completely suppress Purkinje cell loss. Similarly, mice carrying one wild-type PrP and one Zürich II PrP allele showed no clinical symptoms after as long as 100 weeks (Rossi *et al.*, 2001).

Abrogation of the pathogenic effect of truncated PrP by a Tga20 allele cluster expressing PrP in brain with the exception of Purkinje cells

It was suggested earlier that PrP did not have to be expressed in Purkinje cells in order to rescue the Dpl-induced phenotype in Zürich II mice (Rossi *et al.*, 2001) because PrP expressed under the direction of the ‘half-genomic’ PrP promoter (the Tga20 gene cluster) abrogated

Fig. 4. Purkinje cell degeneration in L7-ΔPrP transgenic mice is mitigated by co-expression of PrP. Cerebellar sections were immunostained for calbindin, a marker for Purkinje cells. *Prnp*^{0/0} and wild-type (*Prnp*^{+/+}) controls show normal Purkinje cell layers at 40 and 43 weeks, respectively. (A) L7-ΔPrP mice (*TgF13/-, Prnp*^{0/0}) at 25 weeks presented with severe loss of Purkinje cell dendrites and cell bodies, associated with a reduction of the molecular layer. In the presence of a wild-type allele (*TgF13/-, Prnp*^{+/+}), Purkinje cell loss was completely abrogated at 25 weeks and strongly mitigated at 35 weeks. (B) Introduction of the *Tga20* transgene cluster into L7-ΔPrP mice (*TgF13/-, Tga20/-, Prnp*^{0/0}) resulted in PrP expression in brain, except in the Purkinje cells; in these mice, Purkinje cell loss was practically abrogated at 20 weeks and mitigated at 85 weeks of age. There was also a slight Purkinje cell loss in the age-matched controls (*Tga20/-, Prnp*^{0/0}).

the clinical phenotype, although this promoter is active in brain in general but not in Purkinje cells (Fischer *et al.*, 1996; Rossi *et al.*, 2001). We bred the *Tga20* allele cluster into transgenic *TgF13/-, Prnp*^{0/0} mice. Of 11 double transgenic mice (*TgF13/-, Tga20/-, Prnp*^{0/0}) analysed, none developed behavioural abnormalities within 1 year (Figure 1). At 20 weeks, there was complete suppression of Purkinje cell degeneration in the cerebella of those mice (Figure 4B). However, we found moderate Purkinje cell loss in an 85-week-old double-transgenic mouse, as compared with slight Purkinje cell loss, without astrogliosis, in a 78-week-old control mouse hemizygous for the *Tga20* allele cluster. We cannot exclude the possibility that levels of PrP undetectable by our immunohistochemical analysis sufficed to ensure Purkinje cell survival. Alternatively, perhaps PrP expressed at the synapses of neighbouring cells can supply the protective effect or can be physically transferred, as shown for PrP *in vitro* (Liu *et al.*, 2002) and for other GPI-linked proteins *in vitro* (Anderson *et al.*, 1996) and *in vivo* (Kooyman *et al.*, 1995). In Purkinje cells of wild-type mice, PrP is found at pre- and postsynaptic spines (Haeberle *et al.*, 2000). In the

cerebellum, PrP is also found on other neuronal cells, such as Golgi type II, stellate and basket cells, and to a lesser extent on granule cells (Laine *et al.*, 2001; Liu *et al.*, 2001; Ford *et al.*, 2002).

Discussion

This project aimed to determine whether expression of N-terminally truncated PrP (PrP^{Δ32–134}) targeted specifically to Purkinje cells of Zürich I PrP knockout mice resulted in the same phenotype observed in three lines of Nagasaki-type PrP knockout mice with upregulation of Dpl in brain, namely ataxia and Purkinje cell degeneration, which was abrogated by PrP.

Indeed, transgenic mice overexpressing truncated PrP in Purkinje cells under the control of the L7 promoter developed ataxia and Purkinje cell degeneration similar to that of Nagasaki-type mice, but 8 months earlier than the latter (Rossi *et al.*, 2001). Because onset of ataxia in Nagasaki-type PrP knockout mice is inversely correlated with the expression levels of Dpl in the brain (Rossi *et al.*, 2001), we assume that the earlier onset of the phenotype in

our L7-ΔPrP transgenic lines is due to the high expression level of the truncated PrP. Introduction of a wild-type PrP allele into L7-ΔPrP transgenic *Prnp*^{0/0} mice rescued the phenotype completely: there was no Purkinje cell loss in L7-ΔPrP transgenic *Prnp*^{0/+} at 6 months of age and only a slight reduction of Purkinje cells in mice older than 8 months. Similarly, Purkinje cell degeneration caused by ectopic expression of Dpl in a Nagasaki-type line Zürich II was suppressed in the presence of a wild-type *Prnp* or *Tga20* allele for >20 months, and there was also a mild Purkinje cell loss in older mice expressing the *Tga20* allele (Rossi *et al.*, 2001). Moreover, Dpl-induced Purkinje cell loss in Nagasaki-type lines is accompanied by astrogliosis (Sakaguchi *et al.*, 1996; Rossi *et al.*, 2001). Astrogliosis was present in the cerebellum, but also in the white matter of the forebrain. Because there was no evidence of neuronal damage other than Purkinje cell degeneration, the reason for activation of glia and microglia in the forebrain of Nagasaki PrP-null mice remains unclear (Atarashi *et al.*, 2001). In the cerebellum, astrogliosis occurred several months before the onset of Purkinje cell degeneration (Atarashi *et al.*, 2001). Likewise, astrogliosis is already apparent in 3-week-old L7-ΔPrP transgenic *Prnp*^{0/0} mice, when the Purkinje cell layer appears still normal. Astrogliosis is a prominent histopathological feature in Dpl- and L7-ΔPrP-expressing PrP null mice, which is completely absent in the presence of wild-type PrP. Taken together, the phenotype caused by the L7-controlled overexpression of the truncated PrP (PrPΔ32–134) and its rescue by wild-type PrP strongly suggests that L7-ΔPrP transgenic *Prnp*^{0/0} mice closely mimic Nagasaki-type PrP knockout mice with ectopic expression of Dpl.

The ataxic syndrome is due specifically to overexpression of truncated PrP and not just overexpression of PrP in general, because several fold overexpression of the truncated PrP in Purkinje cells in L7-ΔPrP transgenic mice is completely abrogated by a *Prnp*^{0/+} allele contributing an additional amount of PrP. Our findings support the proposal that the truncated PrP and Dpl cause cell death by the same mechanism, perhaps by interfering with a signalling pathway essential for maintenance of cell viability, which is normally activated by PrP, or, in its absence, by a conjectural functional homologue (Shmerling *et al.*, 1998). Alternatively, truncated PrP and Dpl might activate an apoptotic pathway that normally is kept quiescent by PrP (or a functional homologue). It is of interest in this context that stimulation of differentiated neuronal cells by a monoclonal antibody to PrP leads to activation of the tyrosine kinase Fyn, which has been interpreted as pointing to a signalling function for PrP (Mouillet-Richard *et al.*, 2000). The findings that neuronal cultures of Nagasaki-type PrP knockout mice, which presumably express Dpl, undergo apoptosis more readily than wild-type cultures (Kuwahara *et al.*, 1999) and that PrP protects against Bax-mediated apoptosis (Bounhar *et al.*, 2001) suggest an anti-apoptotic role for PrP. However, other or additional functions have been proposed for PrP, such as cell–cell interactions (Rieger *et al.*, 1997), copper transport (Pauly and Harris, 1998; Brown, 1999) and resistance to oxidative stress (Brown *et al.*, 1999; Wong *et al.*, 2000). Wong *et al.* (2001) proposed that Dpl expression exacerbates oxidative damage that is antagonistic to the protective function of wild-type PrP. A

deeper understanding of the normal function of PrP and Dpl is required to elucidate the processes leading to cell death by ectopic expression of Dpl or of truncated PrP lacking the flexible tail of PrP.

Materials and methods

Generation of transgenic mice

The expression vector L7-PrPΔ32–134 encoding PrPΔ32–134 under control of the L7 promoter was generated by blunt end ligation of the *KpnI*–*NarI* fragment of plasmid PRP123.F (Shmerling *et al.*, 1998) into the blunt *Bam*HI site of plasmid L7-pGEM3 (Oberdick *et al.*, 1990). The vector was digested with *Hind*III and *Eco*RI, purified and injected into fertilized oocytes from *Prnp*^{0/0} mice as described (Shmerling *et al.*, 1998). Line F13 resulted from injection into fertilized oocytes from *Prnp*^{+/+} (F₁ of C57BL/6 × DBA/2) mice. Founders were identified by Southern blot analysis of *Bam*HI-digested tail DNA using the PrP open reading frame probe and mated to *Prnp*^{0/0} Zürich I mice (Büeler *et al.*, 1992) to establish transgenic lines with a *Prnp*^{0/0} background.

Histopathology and immunohistochemistry

Animals were culled, and their brains were removed and fixed for at least 24 h in 4% paraformaldehyde in phosphate-buffered saline (PBS). Fixed samples were embedded in paraffin, sectioned at 1–2 μm and processed for haematoxylin/eosin (H&E) staining, GFAP, calbindin or PrP immunostaining, as described (Rossi *et al.*, 2001). Briefly, GFAP immunostaining was performed by using anti-GFAP rabbit antiserum (1:300; Dako Glostrup, Denmark). For staining of Purkinje cells, sections were stained with anti-calbindin monoclonal antibody (1:5000; Sigma Chemical Company, St Louis, MO), and visualized by a biotinylated rabbit anti-mouse antibody (1:200; Dako) according to the manufacturers' instructions. PrP was detected on microwave-treated sections using polyclonal anti-rabbit antibody R340 (1:200), and visualization was performed with the Tyramide Signal Amplification (TSA) kit (NENTM; Life Science, Boston, MA) according to the manufacturers' instructions.

Behavioural assessment

Mice were analysed at 2-week intervals for behavioural abnormalities, such as reduced exploratory activities, trembling gait and ataxia. Footprints of mice were performed as described (Rossi *et al.*, 2001).

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