EMBO reports

scientific report

The 37 kDa/67 kDa laminin receptor is required for PrP^{Sc} propagation in scrapie-infected neuronal cells

Christoph Leucht[1], Steve Simoneau[2], Clémence Rey[1], Karen Vana[1], Roman Rieger[1], Corinne Ida Lasmézas[2] & Stefan Weiss[1+]

[1]Laboratorium für Molekulare Biologie-Genzentrum-Institut für Biochemie der LMU München, Munich, Germany, and

[2]CEA Laboratory for Prion Pathogenesis, Service de Neurovirologie DRM/DSV, Fontenay-aux-Roses, France

The accumulation of PrP^{Sc} in scrapie-infected neuronal cells has been prevented by three approaches: (i) transfection of ScMNB cells with an antisense laminin receptor precursor (LRP) RNA-expression plasmid, (ii) transfection of ScN2a cells and ScGT1 cells with small interfering RNAs (siRNAs) specific for the LRP mRNA, and (iii) incubation of ScN2a cells with an anti-LRP/LR antibody. LRP antisense RNA and LRP siRNAs reduced LRP/LR expression and inhibited the accumulation of PrP^{Sc} in these cells. The treatments also reduced PrP^{c} levels. The anti-LRP/LR antibody, W3, abolished PrP^{Sc} accumulation and reduced PrP^{c} levels after seven days of incubation. Cells remained free of PrP^{Sc} after being cultured for 14 additional days without the antibody, whereas the PrP^{c} level was restored. Our results demonstrate the necessity of the laminin receptor (LRP/LR) for PrP^{Sc} propagation in cultured cells and suggest that LRP/LR-specific antibodies could be used as powerful therapeutic tools in the treatment of transmissible spongiform encephalopathies.

EMBO reports 4, 290–295 (2003)
doi:10.1038/sj.embor.embor768

INTRODUCTION

Transmissible spongiform encephalopathies (TSEs) are a group of neurodegenerative disorders that include Creutzfeldt–Jakob disease (CJD) in humans, bovine spongiform encephalopathy (BSE) in cattle and scrapie in sheep (Aguzzi & Weissmann, 1998; Prusiner *et al*., 1998; Weissmann, 1999; Lasmézas & Weiss, 2000). The main pathogenic event in the development of TSEs is the conversion of PrP^{C}, the normal cellular form of the prion protein, to PrP^{Sc}. An important feature of PrP^{Sc} is its partial resistance to proteases, which makes it biochemically distinguishable from PrP^{C} (Caughey & Raymond, 1991). Recently, we identified the laminin receptor (LRP/LR) as the cell-surface receptor for PrP^{C} (Gauczynski *et al*., 2001a). Heparan sulphate proteoglycans (HSPGs) have been shown to function as cofactors or co-receptors for the binding of PrP^{C} to the LRP/LR (Hundt *et al*., 2001). The LRP/LR has been shown to interact directly with PrP^{C} in the yeast two-hybrid system (Rieger *et al*., 1997). This interaction was confirmed by pull-down assays in cotransfected COS-7 cells and co-infected insect cells (Rieger *et al*., 1997). Furthermore, increased levels of the LRP were found in the brain, spleen and pancreas of scrapie-infected mice and hamsters, as well as in scrapie-infected neuroblastoma cells, which are a well characterized *in vitro* model for scrapie infection (Rieger *et al*., 1997). These data suggest a link between the LRP/LR and prion propagation.

The non-integrin LRP/LR is a multifunctional protein that is required for cell differentiation, movement and growth (for review see Gauczynski *et al*., 2001b). The LRP cDNA encodes a 37-kDa precursor protein (LRP), also known as p40, and has been cloned from different species by several groups. This protein has been reported to be ribosome-associated, to bind to the histones H2A, H2B and H4 and to be the precursor of the metastasis-associated 67-kDa mature high-affinity laminin receptor (LR) (for a review, see Gauczynski *et al*., 2001b; Leucht & Weiss, 2002). The 67-kDa LR is consistently upregulated in aggressive carcinomas, suggesting a role in cell homeostasis and cohesion. The amino-acid sequence of the receptor is highly conserved throughout evolution, with at least 98.3% homology between the mouse, human and bovine sequences, and 99% homology between the rat and human sequences (for a review, see Gauczynski *et al*., 2001b; Leucht & Weiss, 2002). Published data suggest the existence of at least six LR genes in the mouse genome; one of these is localized on chromosome nine, and at least two copies are thought to be functional (Douville & Carbonetto, 1992). Using TRIBE–MCL, an algorithm for the detection of protein families (Enright *et al*., 2002), five LR genes were identified when the program was used to search the latest mouse draft genome sequence (Mouse Genome Sequencing Consortium, 2003, available at http://www.ensembl.org). The LRP gene on chromosome nine has seven exons and six introns but, in contrast with earlier results (Douville & Carbonetto, 1992), no LRP/LR gene on chromosome six has been identified. Interestingly, genes that affect susceptibility to prions have been identified on mouse chromosome nine (Stephenson *et al*., 2000).

[1]Laboratorium für Molekulare Biologie-Genzentrum-Institut für Biochemie der LMU München, Feodor-Lynen Str. 25, D- 81377 Munich, Germany
[2]CEA, Laboratory for Prion Pathogenesis, Service de Neurovirologie DRM/DSV, BP 6, F-92265 Fontenay-aux-Roses Cedex, France
[+]Corresponding author. Tel: +49 89 2180 76951; Fax: +49 89 2180 76999;
E-mail: weiss@lmb.uni-muenchen.de

Received 3 June 2002; revised 3 December 2002; accepted 19 December 2002

PrP-specific antibodies have successfully been used in preventing prion propagation *in vitro* and *in vivo* as follows: first, the accumulation of PrP^{Sc} in scrapie-infected neuroblastoma cells was inhibited by PrP-specific antibodies (Peretz *et al*., 2001); second, scrapie infection was abolished by transgenic expression of PrP-specific antibodies in mice (Heppner *et al*., 2001). The epitope recognized by the antibody that has the most potent effect on PrP^{Sc}, D18, consists of amino-acid residues 132–156 of PrP, which

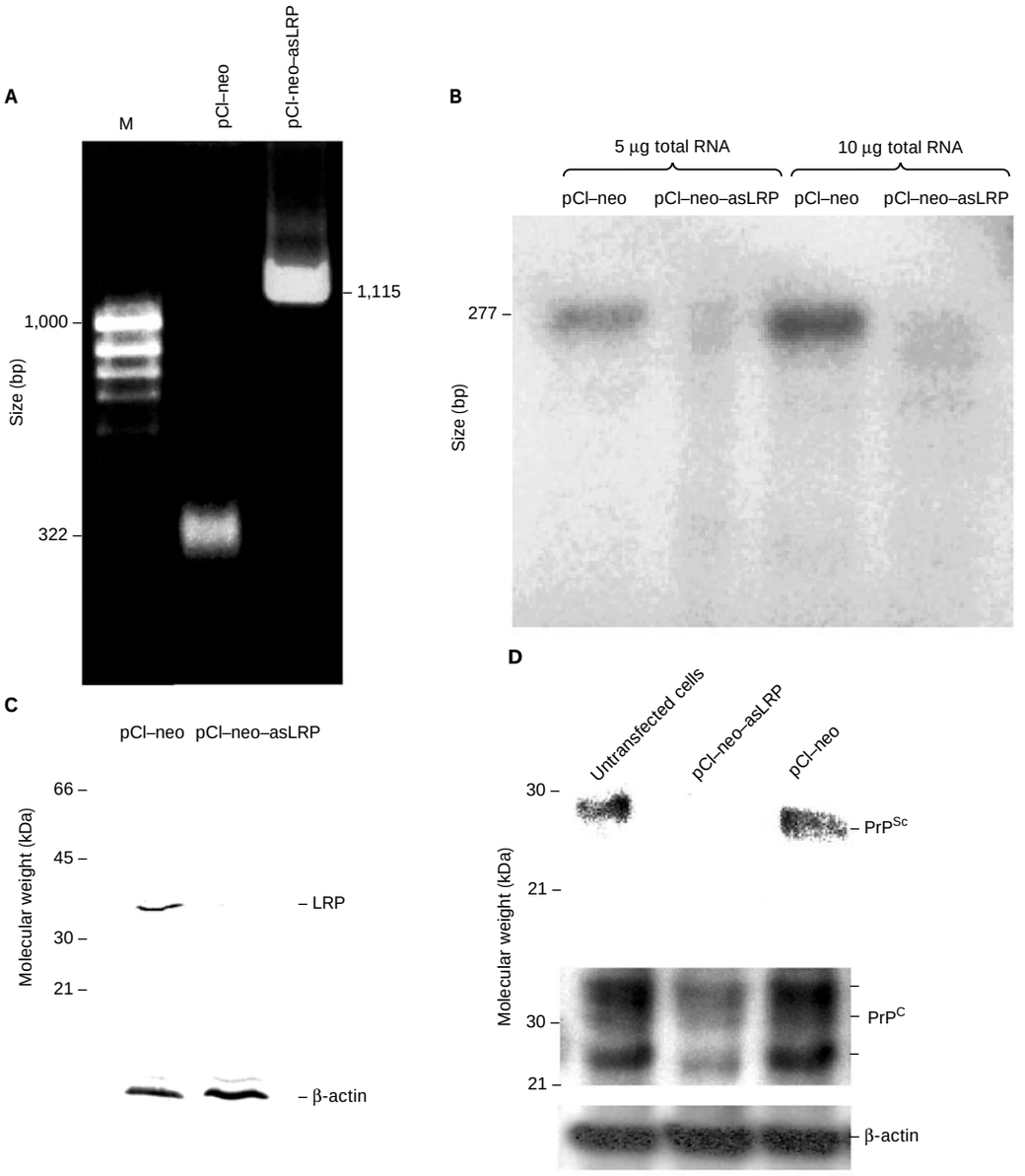

Fig. 1 | Abolition of PrP^{Sc} propagation using laminin receptor precursor (LRP) antisense RNA. (A) Analysis by PCR with reverse transcription of total RNA extracts from transfected ScMNB cells. Oligodesoxythymidine-primed complementary DNA was amplified by PCR using specific primers for the pCI–neo plasmid. This gave a 322-bp cDNA fragment for the pCI–neo transfected cells and a 1,115-bp cDNA fragment for the pCI–neo–asLRP transfected cells. (B) A ribonuclease protection assay was carried out on total RNA from cells transfected with either pCI–neo or pCI–neo–asLRP; the RNA was then separated using a 5% acrylamide/urea gel. 5 µg or 10 µg of total RNA was used, and in both cases the level of LRP messenger RNA was reduced by 80–85% in cells transfected with pCI–neo–asLRP (quantified by posphorimaging). (C) Western blot analysis of cell lysates from pCI–neo- and pCI–neo–asLRP-transfected ScMNB cells assayed 48 hours after transfection. LRP was detected using the polyclonal anti-LRP/LR antibody, W3. β-actin was detected using an anti-β-actin antibody as loading control. (D) ScMNB cells were transfected with pCI–neo and pCI–neo–asLRP. The PrP^{Sc} content of ScMNB cells was analysed 72 h after transfection. The monoclonal anti-PrP antibody SAF70 was used for PrP^{Sc} detection and the SAF32 antibody was used for detection of PrP^{C}.

includes helix A (residues 144–154). Because PrP residues 144–179 have been shown to constitute a binding site for the LRP/LR (Hundt *et al*., 2001) we investigated whether an antibody directed against the LRP/LR, the cellular receptor for PrP^{C} (Gauczynski *et al*., 2001a), can also be used to interfere with the metabolism of PrP^{Sc}. To ablate LRP/LR expression from all putative LRP/LR-encoding genes we used an antisense RNA and a small interfering RNA (siRNA) approach. We investigated whether these strategies had an effect on prion propagation in several scrapie-infected cell systems.

RESULTS AND DISCUSSION

Antisense LRP RNA prevents PrP^{Sc} propagation

To produce LRP antisense messenger RNA, we cloned a region of LRP complementary DNA from nucleotide positions 65–901 into the expression plasmid pCI-neo in the antisense orientation to produce the pCI–neo–asLRP plasmid. After transient transfection of pCI–neo–asLRP into ScMNB cells we confirmed antisense LRP RNA expression in these cells (Fig. 1A). The level of LRP mRNA was greatly reduced 48 h after transfection (Fig. 1B). Using phosphoimaging, this reduction was quantified and LRP mRNA levels were found to be 80–85% of normal LRP mRNA expression levels. A similar reduction in target mRNA levels has been shown in other studies that have used the antisense RNA method to downregulate the expression of myelin basic protein (Katsuki *et al*., 1988; 80% reduction) and *Wnt-1* (Erickson *et al*., 1993; up to 98% reduction). At the level of protein expression, no LRP protein was detected by western blotting 48 h after transfection (Fig. 1C). Analysis of cells 72 h after transfection showed an absence of PrP^{Sc} propagation (Fig. 1D) in cells with reduced LRP levels (Fig. 1C). Levels of PrP^{Sc} were unaffected in cells transfected with pCI–neo as compared with untransfected cells (Fig. 1D). In ScMNB cells we were able to detect only the diglycosylated form of PrP using the SAF70 antibody, whereas in ScN2a and ScGT1 cells we observed the classic three-band pattern. We observed a reduction in PrP^{C} level after antisense LRP RNA transfection (Fig. 1D), which might be caused by an altered PrP^{C} metabolism. Previous studies have indicated that PrP^{C} internalization is highly dependent on the presence of the LRP/LR at the cell surface (Gauczynski *et al*., 2001a), where the LRP/LR binds PrP^{C} through two distinct domains: the octapeptide region and the region encompassing amino acids 144–179 of PrP^{C} (Hundt *et al*., 2001). This is consistent with a recent study, in which it was found that the octapeptide region is essential for internalization of PrP^{C} (Nunziante *et al*., 2003). Hence, the altered PrP^{C} levels seen in this study are likely to be due to perturbed metabolism of the protein.

LRP-specific siRNAs prevent PrP^{Sc} propagation

SiRNAs were used to verify the results obtained using the LRP antisense RNA construct. This method has been used succesfully in other studies to knock down target-gene expression levels (Elbashir *et al*., 2001). We tested four different LRP-specific siRNAs for their ability to repress LRP expression in ScN2a cells. All of them repressed LRP synthesis (Fig. 2A). Figure 2B shows data from a time-course experiment carried out to analyse the effect of siRNA-LRP3 on PrP^{Sc} propagation in ScN2a cells. Seventy-two hours after transfection, PrP^{Sc} propagation was completely abolished by siRNA-LRP3, whereas siRNA-LRP1, siRNA-LRP4 and a control siRNA (lamin A/C, described in Elbashir *et al*., 2001) had a smaller effect

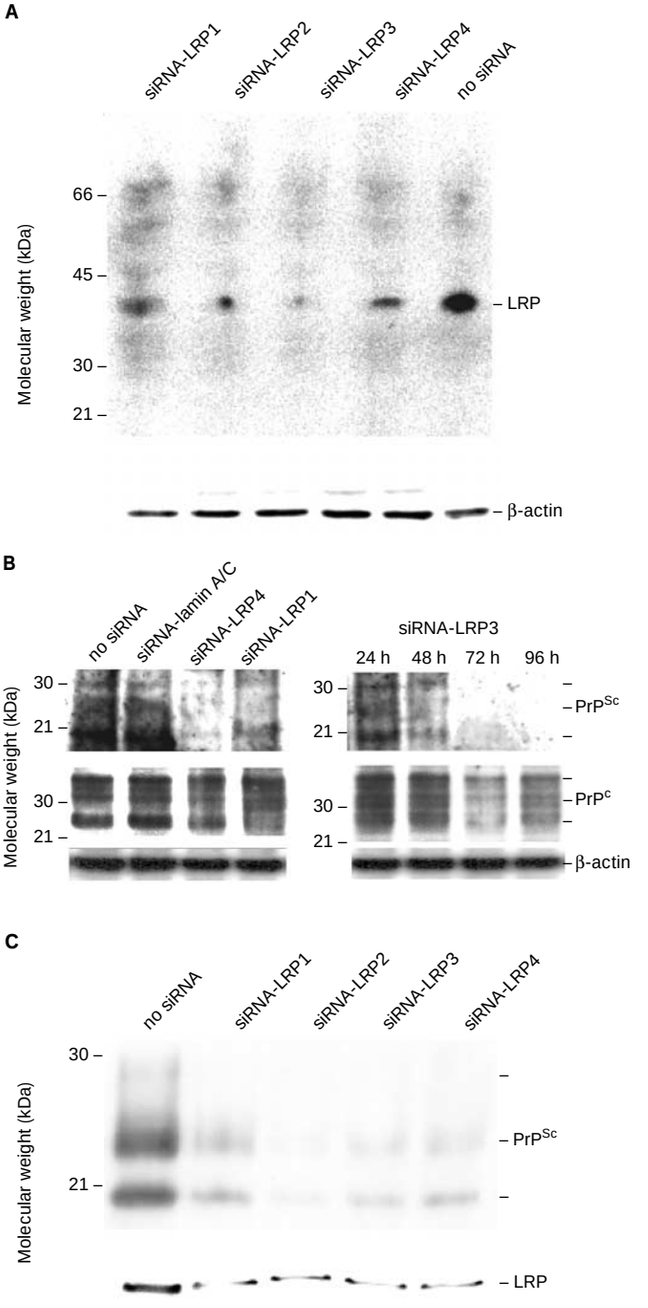

Fig. 2 | Inhibition of PrP^{Sc} propagation using small interfering RNAs. (A) Western blot analysis of ScN2a cells transfected with small interfering RNAs (siRNAs). Cells were analysed 72 h after transfection using the polyclonal anti-laminin receptor (LR/LRP) antibody W3. (B) The effect of siRNAs on PrP^{Sc} propagation was assayed 72 h after transfection (left panel). The time-dependent effect of siRNA-LRP3 on PrP^{Sc} propagation (right panel) was analysed using the SAF70 antibody; PrP^{C} was detected with the SAF32 antibody. β-actin was detected using an anti-β-actin antibody as a loading control (A, B). (C) Western blot analysis of siRNA-transfected ScGT1 cells at 72 h after transfection. The cells were analysed using the monoclonal antibodies LR43512 (lower panel) and SAF84 (upper panel). All samples were normalized to equal protein concentrations.

(siRNA-LRP1+ siRNA-LRP4) or no effect (control) on PrP^{Sc} levels. PrP^{C} levels were reduced in the presence of siRNA-LRP3. The same effects were observed with LRP antisense RNA 72 h after transfection. In contrast to PrP^{Sc}, PrP^{C} levels increased 96 h after transfection, probably due to a decrease in siRNA effectiveness with time.

We also tested the efficiency of the reduction of LRP expression using siRNAs in ScGT1 cells, which show a robust PrP^{Sc} phenotype (that is, these cells propagate PrP^{Sc} over a long period of time). The results were consistent with those obtained using ScN2a cells, with a strong reduction of PrP^{Sc} correlated with LRP downregulation (Fig. 2C).

Anti-LRP/LR antibody W3 prevents PrP^{Sc} accumulation

LRP/LR-specific antibodies have been used successfully to compete with recombinant prion proteins for binding to the LRP/LR in different mammalian cell types (Gauczynski *et al*., 2001a), showing that the LRP/LR has a crucial role in the metabolism of PrP^{C}. Using the LRP/LR-specific antibody, W3 (Rieger *et al*., 1997), in ScN2a cells we observed a reduction of PrP^{Sc} to undetectable levels (Fig. 3A, B). The antibody was used at concentrations of 6–64 μg ml^{-1}. At a concentration of 12 μg ml^{-1} a reduction in PrP^{Sc} levels was observed. At a higher concentration (64 μg ml^{-1}), PrP^{Sc} accumulation was completely abolished after incubation for three days, indicating a dose-dependent effect (Fig. 3A). In a timecourse experiment, we found a complete clearance of PrP^{Sc} after incubation for one week, using an antibody concentration of 32 μg ml^{-1} (Fig. 3B). These results are consistent with a previous study, in which different anti-PrP antibodies were used to reduce PrP^{Sc} levels in cultured cells (Peretz *et al*., 2001; Table 1). In that study, PrP antibody concentrations of 1.2–10.0 μg ml^{-1} were sufficient to clear PrP^{Sc} from ScN2a cells after one week of incubation (Table 1).

We also incubated ScN2a cells in which PrP^{Sc} had been previously cleared by W3 for a further two weeks without any antibody, and showed that no PrP^{Sc} reappeared (Fig. 3B). PrP^{C} levels in W3-treated cells were reduced after seven days of incubation with W3, but were completely restored after a further two-week incubation in the absence of the antibody (Fig. 3B).

Table 1 | Efficacy of anti-PrP and anti-LRP/LR antibodies in clearance of PrP^{Sc} from ScN2a cells

Antibody	Anti-LRP/LR	Anti-PrP[1]			
Incubation time	1 week	1 week			
Antibody	W3	D18	D13	R1	R2
Effective Concentration (μg ml^{-1})	32	1.2	2.5	10	10

[1]Data taken from Peretz *et al.* (2001).

Role of the LRP/LR in PrP^{Sc} propagation in cultured cells

The knock down of the LRP/LR on the cell surface by LRP antisense RNAs or by siRNAs, and the blockage of LRP/LR binding sites by the W3 anti-LRP/LR antibody are most likely to interfere with PrP levels by blocking the PrP internalization process. However, some PrP^{C} can still be synthesized and transported through the secretory pathway to the cell surface (Figs 1D, 2B and 3). Conversion of PrP^{C} into PrP^{Sc} is thought to take place either at the cell membrane or in the endocytic pathway. Thus, it is possible that due to the lack of PrP^{C} within the endocytic pathway no PrP^{Sc} can be formed, resulting in a time-dependent reduction in PrP^{Sc} (Figs 2B and 3B). It is also possible that the LRP/LR has a function in the conversion of PrP^{C} to PrP^{Sc}, and that the absence of the LRP/LR from the cell surface affects PrP^{Sc} formation. PrP^{Sc} propagation cannot be restored after cessation of the incubation with anti-LRP/LR antibody (Fig. 3B) due to the absence of any PrP^{Sc} to re-initiate the conversion process. In contrast, PrP^{C} levels were completely restored after cessation of incubation with the anti-LRP/LR antibody (Fig. 3B). Furthermore, depletion or blockage of the LRP/LR on the cell surface might directly prevent PrP^{Sc} binding and internalization. In summary, our results show that the LRP/LR is not only involved in PrP^{C} metabolism, as demonstrated in previous reports (Gauczynski *et al*., 2001a; Hundt *et al*., 2001), but also has a crucial role in prion propagation. The fact that LRP/LR-specific antibodies are able to clear PrP^{Sc} from neuroblastoma cells provides possibilities for the development of new experimental therapies for TSEs.

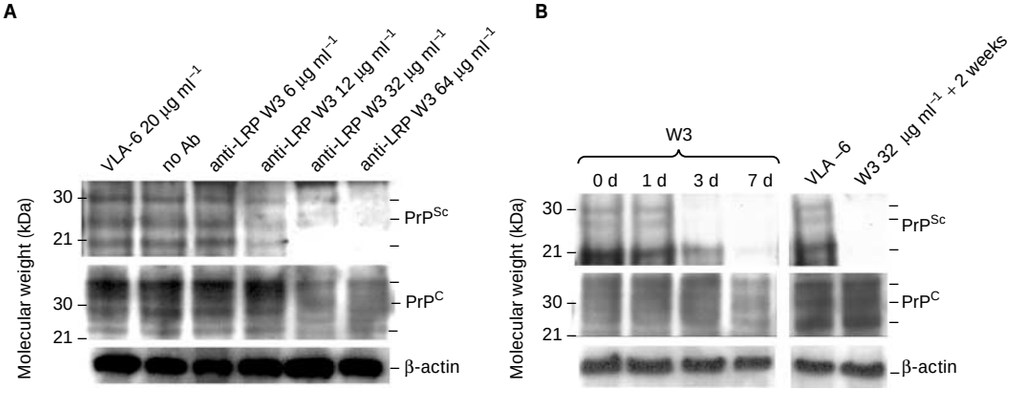

Fig. 3 | The effect of the W3 anti-laminin receptor (LRP/LR) antibody on PrP^{Sc} propagation. (A) ScN2a cells were incubated with W3 at varying concentrations. The PrP^{Sc} content was determined after a 72 h incubation with W3. An anti VLA-6 (integrin-type laminin receptor) antibody was used as a control. PrP^{Sc} was detected using the A7 polyconal antibody; PrP^{C} was detected with the SAF32 antibody. (B) ScN2a cells were incubated with W3 at 32 μg ml^{-1} for varying durations. The last lane shows W3-treated ScN2a cells after an additional 2-week incubation without any antibody. PrP^{Sc} was detected with the SAF 70 antibody, PrP^{c} was detected with the SAF32 antibody. β-actin was detected using an anti-β-actin antibody as a loading control.

METHODS

Construction of pCI–neo–asLRP. Bases 65–901 of the LRP cDNA were amplified by PCR with reverse transcription (RT–PCR) from total RNA isolated from N2a cells, introducing the restriction sites *Nhe*I and *Sma*I. The LRP cassette was cloned in an antisense orientation into the plasmid pCI–neo using the *Nhe*I and *Sma*I sites to produce pCI–neo–asLRP. Cloning was confirmed by sequencing.

Cell culture. ScMNB and ScN2a cells (both lines are neuroblastoma cells chronically infected with scrapie) were grown in DMEM, 10% fetal bovine serum, 2 mM Glutamax (Invitrogen), 100 units ml^{-1} penicillin and 10 μg ml^{-1} streptomycin sulphate, at 37 °C with 5% CO_2. ScN2a cells were produced as described previously (Bosque & Prusiner, 2000). ScGT1–7 cells (GT1 hypothalamic neuronal cells, chronically infected with the Chandler scrapie isolate) were provided by S. Lehmann, and were cultured as described previously (Mange *et al.*, 2000), with the exception that DMEM was replaced with Opti-MEM (Gibco Life Sciences).

Inhibition studies using the W3 antibody. ScN2a cells (1×10^6) were incubated in normal growth medium (DMEM, 10% fetal bovine serum, 2mM Glutamax) supplemented with the purified polyclonal anti-LRP/LR antibody, W3, at varying concentrations. After incubation with the antibody, the cells were harvested, lysed and analysed by western blotting.

Inhibition studies using LRP antisense RNA. ScMNB cells were grown in six-well plates to 60% confluence. Cells were transfected with pCI–neo–asLRP and pCI–neo (control plasmid) using Lipofectamine (Invitrogen) in accordance with the manufacturer's instructions. Transfection efficiencies were determined using a chloramphenicol acetyltransferase construct, and were estimated to be approximately 80% on average (data not shown). Cells were harvested 72 h after transfection, lysed and analysed by western blotting.

Inhibition studies using small interfering RNAs. Four pairs of complementary 21-nucleotide RNAs corresponding to regions of the LRP cDNA were made (Ambion). As a control, the lamin A/C RNA duplex was used (Elbashir *et al.*, 2001). The single-stranded complementary RNAs were annealed in annealing buffer (provided by the manufacturer) for 1 min at 90 °C, followed by incubation for 1 h at 37 °C. The RNA duplexes were transfected into ScN2a cells (cultured in Opti-MEM medium, Invitrogen) using Oligofectamine (Invitrogen) in accordance wih the manufacturer's instructions. ScGT1-7 cells were seeded in 60-mm petri dishes (5×10^5 cells per dish) and transfected the following day with 10 μg of each of the 21-nucleotide RNA pairs using Exgen 500 (Fermentas) in accordance with the manufacturer's instructions.

Ribonuclease protection assays. Total RNA was purified from transfected ScMNB cells and used in a ribonuclease protection assay using the RPA III kit (Ambion). An antisense riboprobe was made by *in vitro* transcription from pCI–neo–asLRP, following linearization of the plasmid with *Eco*RI, in the presence of [α^{32}P]UTP. The antisense riboprobe was combined with the total RNA and the mixture was then precipitated. The precipitates were dissolved in hybridization buffer, denatured and hybridized with the total RNA. This was followed by incubation with RNAse for 30 min at 37 °C, followed by inactivation of the RNAse and ethanol precipitation of the RNA. Protected RNA fragments were separated on a 5% acrylamide/urea gel and visualized using a Storm 860 phosphorimager equipped with ImageQuant software.

Reverse-transcriptase-PCR. Total RNA was purified from transfected ScMNB cells and cDNA synthesis was carried out using an oligo(dT) primer in an RT reaction. The resulting cDNA was then amplified by PCR using a 5′-oligodeoxyribonucleotide corresponding to the 3′-end of the cytomegalovirus promoter and a 3′-oligodeoxyribonucleotide corresponding to a sequence in the 5′-region of the simian virus 40 polyadenylation signal. PCR products were separated on 1% agarose gels and stained with ethidium bromide.

Western blotting. Cytoplasmic lysates were made using a buffer containing 10 mM Tris–HCl, pH 7.5, 100 mM NaCl, 10 mM EDTA, 0.5% Triton X-100 and 0.5% sodium deoxycholate. After centrifugation, the total protein content of the lysates was measured (BCA-Protein Assay, Pierce) and equal amounts of protein from each lysate were analysed. For PrP^{Sc} detection, cell lysates were digested with proteinase K (20 μg ml^{-1}) for 1 h at 37 °C. The reaction was stopped by the addition of Pefabloc (1 mM) and the proteins were denatured using 6 M guanidine hydrochloride. Samples were boiled in SDS sample buffer and analysed on an SDS–polyacrylamide gel containing 12.5% acrylamide. For PrP^{C} or PrP^{Sc} detection (from ScN2a cells), 10% Bis–Tris gels with MES running buffer (NuPAGE, Invitrogen) were used. Proteins were blotted on a polyvinylidene difluoride membrane, blocked and incubated overnight with the monoclonal antibodies SAF70, SAF32 or SAF84 (diluted 1:5,000 in blocking solution) or A7 (diluted 1:2,500 in blocking solution) for PrP detection. The polyclonal anti-LRP/LR antibody, W3 (Rieger *et al.*, 1997) (1:2,000), or the monoclonal antibody 43512 (1 μg ml^{-1}) were used for LRP/LR detection and anti-β actin antibody (Chemicon) (1:5,000) for β-actin detection. After washing with TBS/0.05% Tween 20 the blot was incubated for 1 h with a peroxidase-conjugated secondary antibody (Sigma) (1:2,500). Detection was carried out by enhanced chemiluminescence (Western Lightning, NEN).

ACKNOWLEDGEMENTS

We acknowledge support by grants QLRT-2000-02085 and FAIR-CT-98-7020 from the European Union. S.W. acknowledges support by grants 01-KO-0106, 0313012 (both Bundesministerium für Bildung u. Forschung), LMU 3 and LMU 4 (Bavarian Prion Research Foundation). We thank J. Grassi for providing us with the SAF70, SAF32 and SAF84 antibodies, A. Bürkle for ScMNB cells, H.M. Schätzl for the A7 antibody, and K. Krüger, A. Pahlich, K. Töpolt, S. Janetzky and S. Hengge for excellent technical assistance. C.L. thanks M. Buschbeck for helpful discussions. S.W. thanks R. Grosschedl and E.-L. Winnacker for valuable advice and continuous support.

REFERENCES

Aguzzi, A. & Weissmann, C. (1998) Prion diseases. *Haemophilia*, **4**, 619–627.

Bosque, P.J. & Prusiner, S.B. (2000) Cultured cell sublines highly susceptible to prion infection. *J. Virol.*, **74**, 4377–4386.

Caughey, B. & Raymond, G.J. (1991) The scrapie-associated form of PrP is made from a cell surface precursor that is both protease- and phospholipase-sensitive. *J. Biol. Chem.*, **266**, 18217–18223.

Douville, P.J. & Carbonetto, S. (1992) Genetic linkage analysis in recombinant inbred mice of P40, a putative clone for the high-affinity laminin receptor. *Mamm. Genome*, **3**, 438–446.

Elbashir, S.M., Harborth, J., Lendeckel, W., Yalcin, A., Weber, K. & Tuschl, T. (2001) Duplexes of 21-nucleotide RNAs mediate RNA interference in cultured mammalian cells. *Nature*, **411**, 494–498.

Enright, A.J., Van Dongen, S. & Ouzounis, C.A. (2002) An efficient algorithm for large-scale detection of protein families. *Nucleic Acids Res.*, **30**, 1575–1584.

Erickson, R.P., Lai, L.W. & Grimes, J. (1993) Creating a conditional mutation of *Wnt-1* by antisense transgenesis provides evidence that *Wnt-1* is not essential for spermatogenesis. *Dev. Genet.*, **14**, 274–281.

Gauczynski, S. *et al.* (2001a) The 37kDa/67kDa laminin receptor acts as the cell-surface receptor for the cellular prion protein. *EMBO J.*, **20**, 5863–5875.

Gauczynski, S., Hundt, C., Leucht, C. & Weiss, S. (2001b) Interaction of prion proteins with cell surface receptors, molecular chaperones, and other molecules. *Adv. Protein Chem.*, **57**, 229–272.

Heppner, F.L. *et al.* (2001) Prevention of scrapie pathogenesis by transgenic expression of anti-prion protein antibodies. *Science*, **294**, 178–182.

Hundt, C. *et al.* (2001) Identification of interaction domains of the prion protein with its 37-kDa/67-kDa laminin receptor. *EMBO J.*, **20**, 5876–5886.

Katsuki, M., Sato, M., Kimura, M., Yokoyama, M., Kobayashi, K. & Nomura, T. (1988) Conversion of normal behavior to shiverer by myelin basic protein antisense cDNA in transgenic mice. *Science*, **241**, 593–595.

Lasmézas, C.I. & Weiss, S. (2000) in *Microbial Foodborne Diseases. Mechanisms of Pathogenicity and Toxin Synthesis* (eds Cary, J.W., Linz, J.E. & Bhatnagar, D.), 495–537. Technomic, Lancaster , PA, USA.

Leucht, C. & Weiss, S. (2002) in *BSE-Wahnsinn und Wirklichkeit* (eds Brem, M. & Müller, M.), Vol. 87, 39–54. Nova Acta Leopoldina, Halle, Germany.

Mange, A., Nishida, N., Milhavet, O., McMahon, H.E.M., Casanova, D. & Lehmann, S. (2000) Amphotericin B inhibits the generation of the scrapie isoform of the prion protein in infected cultures. *J. Virol*. **74**, 3135–3140.

Nunziante, M., Gilch, S. & Schätzl, H.M. (2003) Essential role of the prion protein N-terminus in subcellular trafficking and half-life of PrP^{c}. *J. Biol. Chem.*, **278**, 3726–3734.

Peretz, D. *et al.* (2001) Antibodies inhibit prion propagation and clear cell cultures of prion infectivity. *Nature*, **412**, 739–743.

Prusiner, S.B., Scott, M.R., DeArmond, S.J. & Cohen, F.E. (1998) Prion protein biology. *Cell*, **93**, 337–348.

Rieger, R., Edenhofer, F., Lasmezas, C.I. & Weiss, S. (1997) The human 37-kDa laminin receptor precursor interacts with the prion protein in eukaryotic cells. *Nature Med.*, **3**, 1383–1388.

Stephenson, D.A., Chiotti, K., Ebeling, C., Groth, D., DeArmond, S.J., Prusiner, S.B. & Carlson, G.A. (2000) Quantitative trait loci affecting prion incubation time in mice. *Genomics*, **69**, 47–53.

Weissmann, C. (1999) Molecular genetics of transmissible spongiform encephalopathies. *J. Biol. Chem.*, **274**, 3–6.