UPSTREAM AUGs MODULATE PRION PROTEIN TRANSLATION *IN VITRO*

B. SCHRÖDER[1*], R. NICKODEMUS[1], T. JÜRGENS[1], W. BODEMER[2]

[1]Department of Virology, Paul Ehrlich Institute, Langen, Germany; [2]German Primate Center, Kellnerweg, Göttingen, Germany

Received May 17, 2002; accepted September 27, 2002

Summary. – The level of expression of the host PrP gene (*PRNP*) has been shown to affect the progression to a disease, transmissible spongiform encephalopathy (TSE). In order to define sequences that are responsible for translation of *PRNP* mRNA we have investigated a region comprising its 5'-leader sequence. Most remarkable, it consists of an almost identical Kozak mRNA sequence and two AUG initiation codons which seem to modulate translation of the prion protein mRNA *in vitro*. Although transcriptional regulation of the prion protein *PRNP* gene had been expected to dominate the translational modulation, our observations point to a translational regulation of the mouse prion protein synthesis controlled by ribosomal entry and usage of AUG codons.

Key words: prion; PrP^{C}; PrP^{SC}; transmissible spongiform encephalopathy; translation; regulation; expression; promoter; reinitiation

Introduction

Prion diseases are neurodegenerative disorders characterized by accumulation of a post-translationally modified pathological form of a host-encoded glycoprotein PRP (PRP^{C}), designated PrP^{SC} (prion protein). The expression level of the gene encoding PrP^{SC} (*PRNP*) has been shown to alter the incubation period of scrapie in mice. Transgenic mice containing multiple copies of the *PRNP* gene and elevated levels of PrP^{c} had reduced incubation periods (Scott *et al.*, 1989).

PrP is a neuronal membrane protein and its pathogenic isoform, PRP^{SC} appears to play a key role in the development of TSE. Only 10% of human TSE are associated with mutations within the *PRNP* sequence encoding PrP. Sporadic forms, about 90% of all cases, have not yet been correlated to a specific genetic lesion except to a methionine-valine polymorphism at codon 129, as considered to be a predisposing factor for the Creutzfeldt-Jakob disease (Collinge *et al.*, 1991; Alperovitch *et al.*, 1999). However, the expression level of PRP^{SC} has been shown to alter the incubation period of scrapie in mice. Transgenic mice containing multiple copies of the *PRNP* gene and elevated levels of PrP^{c} have reduced incubation periods (Scott *et al.*, 1989). Mice carrying only one copy of *PRNP* gene and a reduced level of cellular PrP have extended incubation times compared to mice over expressing *PRNP*. Westaway *et al.* (1994) have described functional domains and regulatory regions of the murine *PRNP* gene. Baybutt and Manson (1997) have assessed functional regions of the murine *PRNP* gene promoter by experimental approaches. They have concluded that a full promoter activity requires 80 nucleotides (nt) upstream of the transcription initiation site. It appears to be typical for housekeeping genes that they have no AT-rich box but rather two Sp1 transcription factor-binding sites. These sites, 5' to the transcription initiation site, are all that is required for expression of *PRNP* gene in neuronal N_2A cells. The intron 1 contains nt elements that appear to suppress its expression. Baybutt *et al.* (1997) have also described polymorphism of the sequence located

*E-mail: schroebj@ing.boehringer-ingelheim.com; fax: +496132-772947. Present address: Boehringer Ingelheim Vetmedica GmbH, Biologicals Research and Development, 55216 Ingelheim/Rhein, Germany.

Abbreviations: Cm = chloramphenicol; CNS = central nervous system; RT = room temperature; TSE = transmissible spongiform encephalopathy; uORF = upstream ORF

immediately downstream of the Ap1 binding site with a consensus sequence of $C_{5\text{-}8}AC_{5\text{-}8}AC_7$. Operating together, the enhanced synthesis of cellular PrP may be associated with degeneration of skeletal muscles, peripheral nerves and CNS. These findings enabled us to analyze the mechanisms controlling PrP mRNA translation. Recently, Lemaire-Vieille *et al.* (2000) have shown that an unexpected splicing operates to remove the sequences of the non-translated 5'-leader of bovine PRP mRNA.

Materials and Methods

Isolation, amplification, cloning and sequencing of a 648 bp fragment from genomic DNA. Genomic DNA was isolated from mice (strain C57Bl6) using standard procedures. A 648 bp fragment clone 3124 (Fig. 1) was amplified using the primers MuPrP24 (5'GCACTCGAGGCTGCGTCGCATCGGTGGCAG GTAA-3') and MuPrP31 (5'-GCACTCGAGCAGGAGATTGAG GGAGAA GCATTGCTCT-3'). All other DNA fragments were generated by PCR using the clone 3124 as template. The fragments were cloned into the pGEM-3Z vector (Promega) and then subcloned into the pBLCAT 3, pBLCAT2 and pBLCAT2-XhoI vectors (data not shown). The sequencing and sequence analysis were performed using the Perkin Elmer Sequencer System 377 according to the manufacturer's instructions.

Overlap extension site-directed mutagenesis was used to introduce a CAA instead of an ATG initiation codon into the DNA sequence comprising three upstream ORFs (uORFs). First, two individual fragments were amplified from the target gene. For the first one the primers MuPrP31, MuPrPD1, MuPrPD3, and MuPrPD5 were used. The primers MuPrPD1, MuPrPD3 and MuPrPD5 introduced a CAA at the 3'-end of the PCR product. The second fragment was amplified with the primers MuPrP24, MuPrPD2, MuPrPD4, and MuPrPD6 introducing the same mutation but at the 5' end of the PCR product. The primers MuPrP24 and MuPrP31 also contained a *Xho*I restriction site at the 5'-end of the sequence. The following primers were used for the PCR amplification (the mutated sequences as well as the *Xho*I restriction site of the primers MuPrP24 and MuPrP31 are underlined):

MuPrPΔ1: 5'-GAGCGAGGAAGCTTGCGTTGAACTTT-3'
MuPrPΔ2: 5'-AAAGTTCAACGCAAGCTTCCTCGCTC-3'
MuPrPΔ3: 5'-ATGCTCCCGTTATTGAATGGGTAGTTG-3'
MuPrPΔ4: 5'-CAACTACCCATTCAATAACGGGAGCAT-3'
MuPrPΔ5: 5'-ACTATAAAAATTTGCTTTAATGGAAG-3'
MuPrPΔ6: 5'-CTTCCATTAAAGCAAATTTTTATAGT-3'
MuPrP24: 5'-GCACTCGAGGCTGCGTCGCATCGGTGGCAG GTAA-3'
MuPrP31: 5'-GCACTCGAGCAGGAGATTGAGGGAGAAG CATTGCTCT-3'

pBLCAT 2-XhoI vector construction and the CAT reporter gene assay. The presence of the Herpes simplex virus 1 thymidine kinase (HSV-1 tk) promoter in the pBLCAT2 vector (Luckow and Schütz, 1987) allowed analysis of the influence of putative regulatory elements in this heterologous viral promoter. *Bam*HI and *Bgl*II fragments from the HSV-1 tk linker scanning mutant LS-115/-105 spanning the promoter from nt –105 to nt +51 was inserted into the corresponding restriction sites of pBLCAT3, thereby generating pBLCAT2 (Luckow and Schütz, 1987). pBLCAT2 was digested with *Xho*I. The restriction site was located between the tk promoter and the CAT reporter gene of pBLCAT2. All DNA fragments generated by the overlap extension site-directed mutagenesis contained an *Xho*I restriction site within the primer sequence and were inserted between the tk promoter and the CAT reporter gene. Consequently, the tk promoter was located immediately 5' of the inserts whereas the uORF sequences in the PRNP leader were 5' of the CAT gene. The newly generated vector was designated pBLCAT2-XhoI.

Cell lines and transfection. The cell lines N_2A and ScN_2A that permanently produce PrP^{res} isoforms, were kindly provided by Dr. M. Groschup, (Institut für Neue und Neuartige Tierseuchenerreger, Island Riems, Germany). The cell lines were tested mycoplasma-negative. Plasmids used for transfection were prepared using the EndoFree System (Qiagen). The transfection was carried out by use of LipofectAMINE (Life Technologies). pBLCAT3 and pBLCAT2-transfections were performed in 6-well-plates using plasmid DNA (1 μg) and a pSV-ß-galactosidase control vector (Promega). The cells (5 x 10^5 per well) were lysed with 300 μl of the passive lysis buffer (Promega) at room temperature for 10 mins, centrifuged at 14, 000 rpm for 1 min. at RT and supernatants were stored at -20°C. The lysates were assayed for CAT activity using [C^{14}] chloramphenicol as substrate. Acetylated and non-acetylated forms were measured following the manufacturer's instructions. The ß-galactosidase activity was measured at 420 nm after incubation of lysates at 37°C for 5 hrs using ONPG as a substrate. All values of CAT activity in cell extracts were normalized to the ß-galactosidase activity. All constructs were compared with the 648 bp full-length construct (clone 3124) (100% activity).

Statistical analysis. Each cell line and vector transfection was carried out in triplicate. To normalize the CAT activity on the basis of the internal control activity (pSV β-galactosidase) and to minimize the experimental variability, the values of internal controls were set at 1 and the CAT activities were calculated. For each triplicate the mean values were determined. The CAT activity of the wild type (wt) sequence was set at 100% and the CAT activities of the mutated sequences were calculated accordingly.

The combined in vitro transcription and translation assay for the uORFs of murine PRNP. The *in vitro* translation procedure was performed following the recommendations of the supplier (TnT Quick coupled Transcription/translation systems, Promega). To demonstrate the *in vitro* translated products PAGE and [^{35}S]methionine autoradiography were performed. The *in vitro* transcription reaction mixture (20 μl) contained the 5X buffer (4 μl, Promega), 100 mmol/l DTT (2 μl), RNasin, an RNase inhibitor (20 U, 1 μl), dXTP (2.5 mmol/l each, 4 μl), dCTP (100 μmol/l, 2.4 μl), linearized DNA template (1 μg), [P^{32}]CTP (50 μCi, 5 μl), and 20 U of T7 polymerase (Promega). The mixture was incubated at 37°C for 2 hrs and stopped at 65°C for 10 mins. To demonstrate the *in vitro* transcribed products PAGE and autoradiography were used.

RNA preparation and Northern blot analysis. Total RNA was prepared using the TRIzol reagent according to the manufacturer's instructions. mRNA was purified from total RNA using the QIAGEN Oligotex II Direct mRNA Kit. The sequence located upstream of the highly conserved sequences and at the end of the exon

1 sequence was used as a probe for Northern blot analysis. Briefly, 20 µg of total RNA from normal as well as permanently infected neuronal ScN_2A cells was denatured with glyoxal at 50°C for 1 hr and loaded onto 1% agarose gel in 10 mmol/l sodium phosphate buffer pH 6.8. After electrophoresis, RNA was blotted onto a positively charged nylon membrane. Hybridization and detection were performed using the ECL DNA Labeling and Detection System (Amersham), followed by densitometric analysis using a GS-700 Imaging Densitometer (BioRad).

Results and Discussion

Translational regulation of the CAT reporter gene as assessed by transfection into N2A cells

In contrast to the transcriptional analyses (data not shown) we found significant differences in the amount of CAT activity generated after introducing the respective sequences within the *XhoI* site of the pBLCAT2 vector (Figs 2 and 3A), thereby generating the pBLCAT2-XhoI vector. This construct allowed us to investigate whether the inserted sequences are transcribed into mRNA and translational regulation mechanisms might operate.

The pBLCAT2-XhoI vector was generated to demonstrate the influence of a putative enhancer (tk) of transcription of uORFs. Using the two other vectors, pBLCAT 2 and 3, we did not expect and could not demonstrate any expression of uORFs because of lack of a potential enhancer sequence. With the vectors pBLCAT 2 and 3 we could only investigate the influence of the introduced sequences on the CAT expression and activity. The interesting result was, that neither pBLCAT 3 nor pBLCAT2 vectors showed any differences in CAT activity. This is in contrast to that what we found with the pBLCAT 2-XhoI vector. Thus, the major question arose, what kind of RNA was expressed and whether this expressed mRNA influenced the CAT activity. With the CAT activity of the wt clone uORFwt set at 100% the CAT activity of other clones showed that each unique mutation conferred its own specific influence on the CAT activity (Fig. 3A). No significant difference between infected and uninfected N_2A cells on the CAT expression from the vector constructs was observed (data not shown).

Northern blot analysis experiments were performed to prove the translational regulation of protein synthesis (Fig. 3B). CAT-specific probes were generated to determine the amount of CAT mRNA in the pBLCAT2-XhoI-transfected N_2A-cells. The expression of CAT mRNA and activity had to correspond to the GAPDH mRNA in order to ensure that similar numbers of cells were analyzed. The CAT transcription level of the wt sequence was set to 100% and compared with that in individual vector constructs. All but one experiment demonstrated that there were no significant differences in the transcription level between the wt and individual mutated cloned *PRNP* sequences. The only significant difference was observed with clone uORF1-3 (ATG-CAA). In this case, an increased level of CAT mRNA by 45% was detected. The experiment performed with comparable number of cells showed that transfection efficacy monitored by *PRNP* mRNA did not vary in all but one transfection.

The discrepancy between the amount of CAT mRNA and the CAT activity of the pBLCAT2 and pBLCAT2-XhoI constructs (Fig. 3A and B) could be explained by the lack of a reinitiating event. The tk promoter, which is still a part of pBLCAT2-XhoI constructs, enables transcription of both the uORF- and the CAT encoding mRNA. Therefore, we observed a significant amount of RNA. But the RNA containing the authentic uORF prevented the translation of the CAT mRNA into protein since the ribosomal entry was affected.

The lack of the prion promoter in the promoter deletion constructs (Figs 3A and B) decreased the amount of the generated CAT protein, although the tk promoter was present. Therefore, we observed only a 50% activity of CAT with this construct. In addition, one will also recognize an increased level of GAPDH RNA, which indicated that we introduced an increased number of cells into the respective transfection experiment.

Translational regulation of the CAT reporter gene as assessed by in vitro transcription and translation

All inserts used for the pBLCAT2-XhoI transfections were introduced adjacent to the mouse prion protein ORF. These constructs were cloned into Promega's pGEM-3Z expression vector under control of the T7 promoter. Fig. 4 demonstrates that the amount of cellular PrP generated depends on the mutation introduced into the authentic sequence. The construct consisting of the PrP ORF only and the Δ1-3AUG sequence led to the highest rate of translated PrP^C. When compared to all other clones, no differences in the amount of synthesized cellular PrP was observed. All constructs containing at least the original third uORF (Fig. 4A, lanes 4, 5, 6, and 7) generated a minimum (lane 4) or no detectable level of PrP (Fig. 4A, lanes 5, 6, and 7). The wt mRNA sequence containing the authentic three AUGs (Fig. 4A, lane 7) did not mediate the synthesis of a detectable amount of PrP. Plasmid constructs containing the CAA sequence within the third uORF (Fig. 4A, lanes 2 and 3) mediated a higher amount of translated prion protein than the original counterpart. The level was lower as compared to the PrP construct (Fig. 4A, lane 9) or the Δ1-3AUG clone (Fig. 4A, lane 8). Similar amounts of transcripts were synthesized regardless of the constructs used (Fig. 4 B).

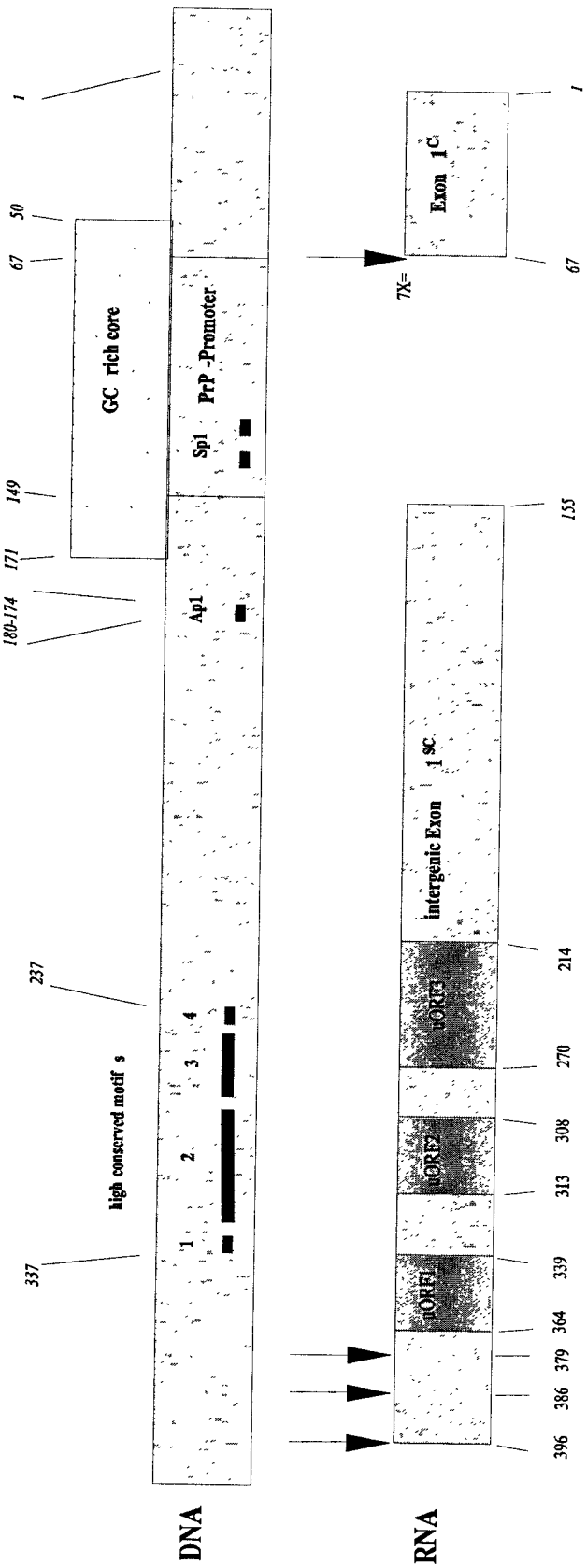


Fig. 1

Organization of the 648 bp fragment clone 34124 of the mouse strain C57516 genome and its transcription

The arrows point to the transcription initiation sites of the exon 1^{SC}. The transcribed RNA comprises 3 translational start codons preceding two uORFs for 6 and 8 amino acids and a sequence containing a start and stop codon. HCM3 and HCM4 coincide with the short uORFs. uORF2 is almost identical with the HCM2 sequence. The small uORF1 is not a part of HCM and HCM1 does not coincide with any of short uORFs.

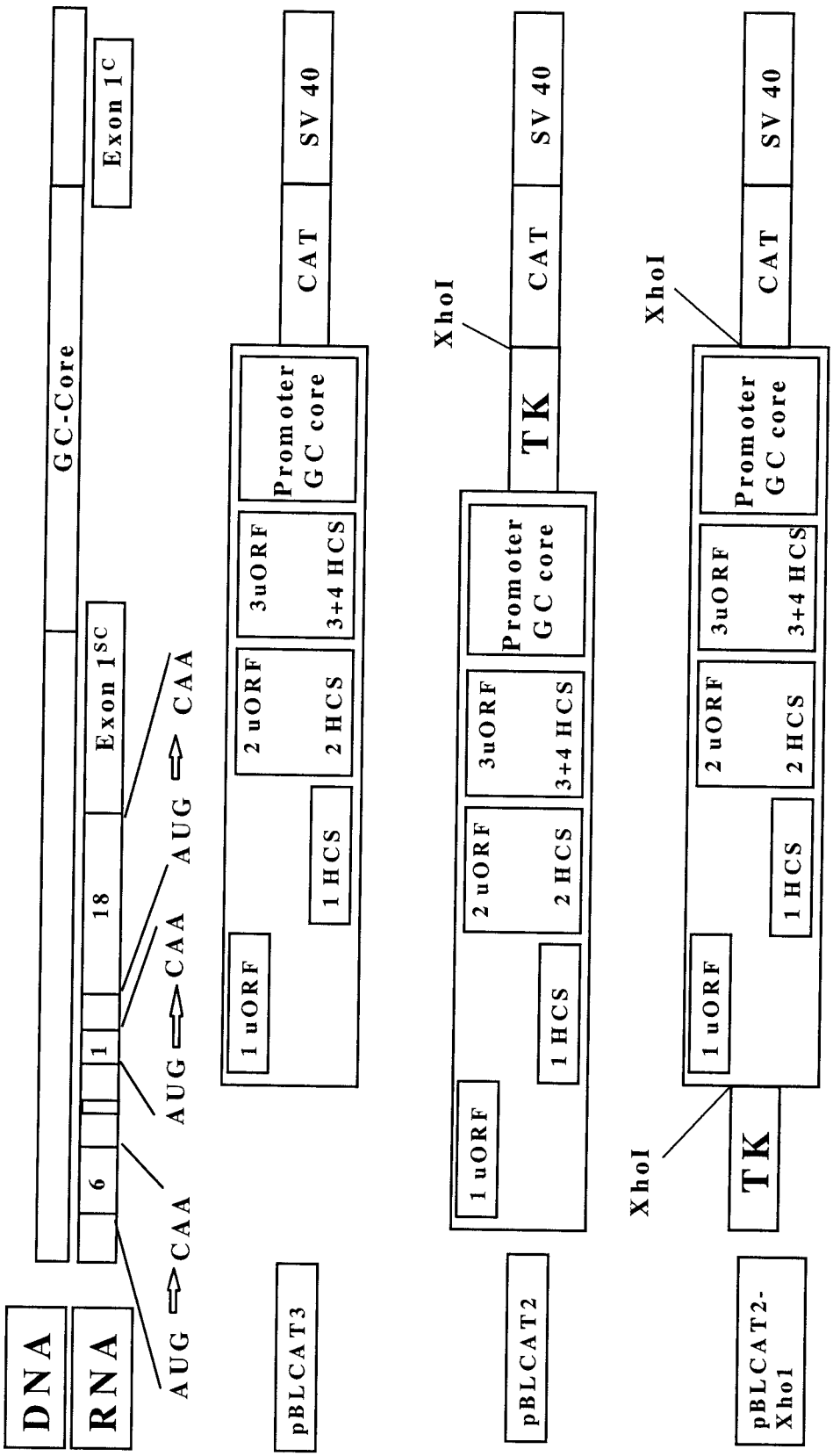
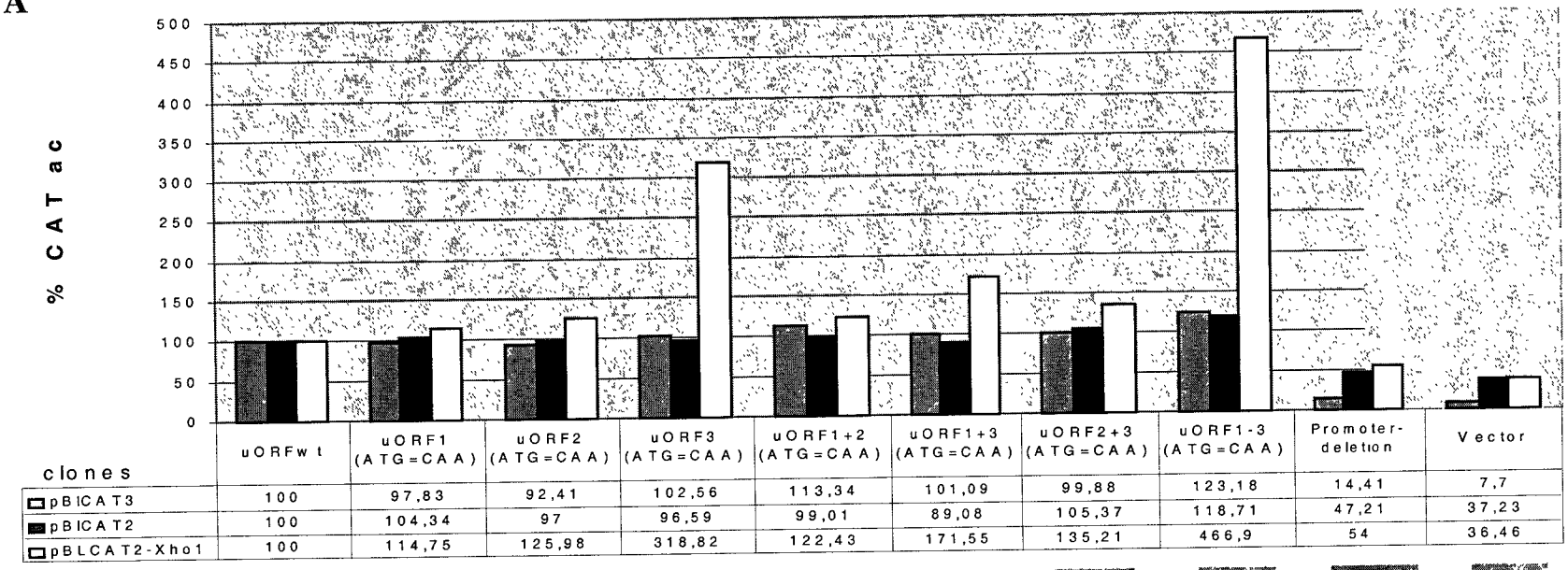


Fig. 2

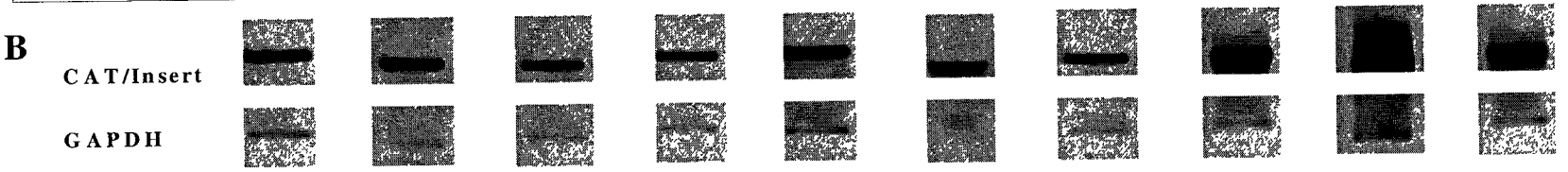
Construction and properties of CAT expression vectors pBLCAT3, pBLCAT2 and pBLCAT2-XhoI

pBLCAT3: no tk promoter, no complete PRMP promoter, only a leader (5'-uORFs and short promoter GC core). pBLCAT2: tk promoter downstream of the putative enhancer sequence of the PRNP region., no complete PRMP promoter, only a leader (5'-uORFs and short promoter GC core).
pBLCAT2-XhoI: it uses tk as a promoter and allows translational regulation by 5'-uORFs and the residual promoter GC-core.

A



B



Density OD/mm²	35.24	34.10	31.18	29.24	42.58	32.21	42.93	51.24	61.07	53.08
Density in %	100	96.78	88.49	82,30	120.8	91.4	121.8	145.4	173.3	150.64

Fig. 3

CAT activity of various CAT expression vector constructs (A) and CAT mRNA level in various pBLCAT2-XhoI constructs as determined by Northern blot analysis (B)

A. Constructs containing a wt sequence (the 1st columns) and various mutated sequences (the 2nd = 8th columns), PrP promoter deletion constructs (the 9th columns) and original vector (the 10th columns).

B. Northern blot analysis of CAT mRNA with various pBLCAT-XhoI constructs. A wt sequence (lane 1), various deleted sequences (lanes 28) and original vector (lane 10)

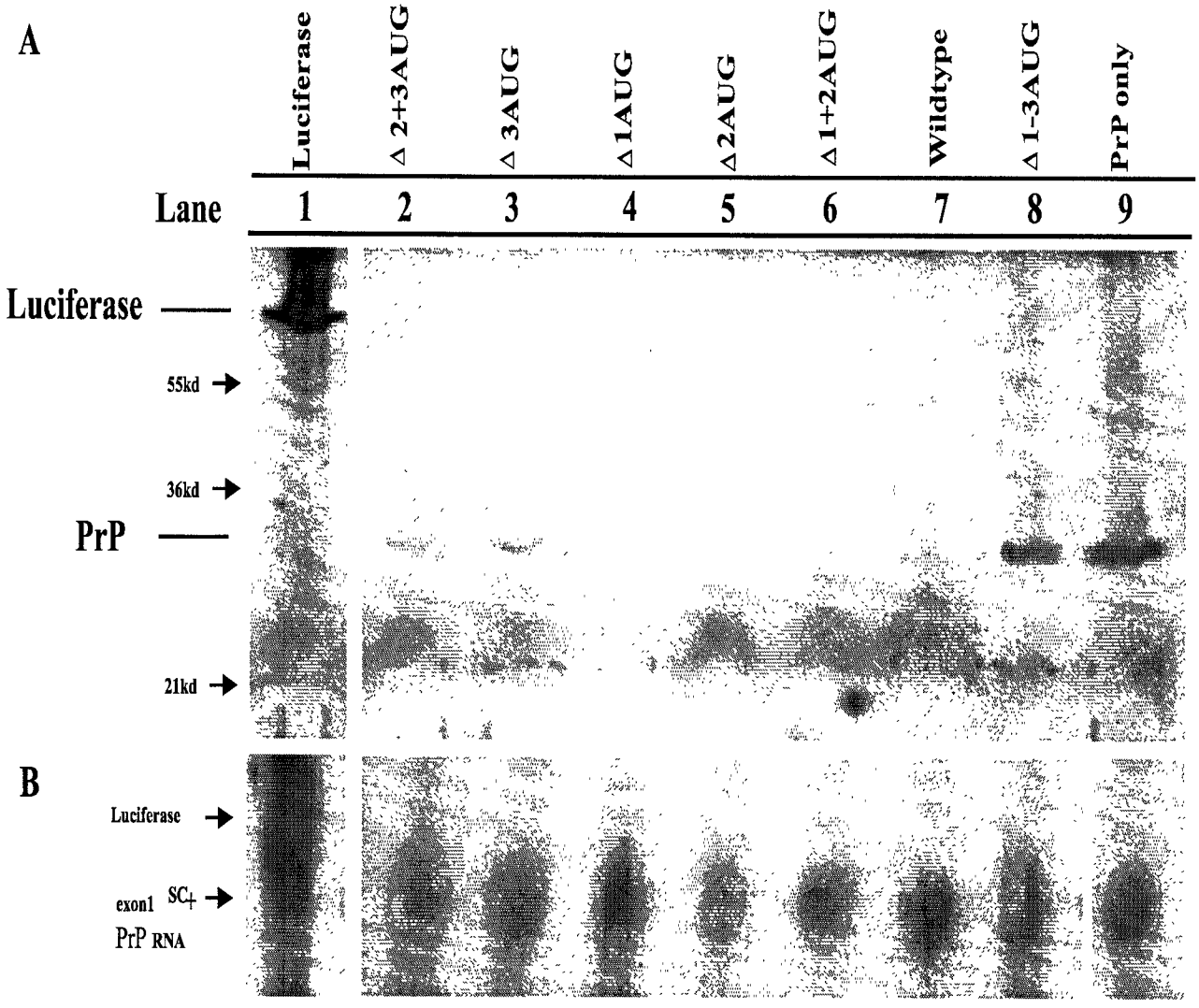


Fig. 4

***In vitro* translation (A) and transcription (B) of short uORFs containing varioue mutated sequences**

The inserts containing wt or mutated sequences were located proximal to the mouse prion protein ORF. Both translation (A) and transcription (B) products were detected by PAGE and autoradiography. The luciferase construct served as positive control (B, lane 1).

Translational regulation by the uORF encoding murine PRNP in transfection assays

Previously we have observed in brain material from scrapie-infected mice that a region 5' of the GC-rich core, immediately upstream of the transcription start site, can be transcribed into mRNA (Schröder *et al.*, 1997, 1998). The observed mRNA species comprised the four highly conserved motifs including three putative translational start codons coupled to preceding uORFs with 6 and 18 aa, respectively, and a sequence consisting of a start and stop codon. Using primer extension, the RT-PCR and RNA arbitrary primed PCR (RAP-PCR) methods and *in vitro* transcription experiments we have identified a mRNA species, which is expressed in prion-infected mouse brains but not in uninfected ones.

In this study we investigated the putative function of uORFs in relation to translational regulation of the CAT reporter gene *in vitro*. We found that the complete insert was transcribed into mRNA (Fig. 3B) and distinct mutations influenced the amount of CAT activity. The mutations had no influence on the amount of synthesized mRNA (Fig. 3B) but dramatically altered the amount of translated protein as detected by its enzymatic activity (Fig. 3A).

Although mRNA signals in Northern blot analysis indicating amounts of mRNA were comparable, CAT activities were significantly different. These results were confirmed by the *in vitro* transcription and translation studies (Fig. 4) and also pointed to a translational regulation. This result can be explained by reinitiation of protein synthesis by ribosomes (Morris and Geballe, 2000). Interestingly, Ford *et al.* (2002) could recently demonstrate a marked disparity between the mRNA level and the amount of expressed prion protein in different neuron types of the CNS. The authors concluded that this disparity reflects a primary post-transcriptional control.

Different amounts of CAT could be synthesized due to reinitiation of protein synthesis, a post-termination phenomenon in eukaryotic ribosomes. This is not only of great significance in terms of diversity of mRNAs that are affected, but it also reflects important mechanic properties of the eukaryotic translation apparatus. Cellular eukaryotic mRNAs are generally monocistronic. However, there are likely hundreds of genes with so called upstream ORFs (uORFs), which could regulate gene expression by reinitiation (MacCarthy, 1998; Morris and Geballe, 2000). An obvious consequence of existence of short length uORFs is that they cannot allow simultaneous initiation, elongation, and termination but can only be translated by a single ribosome at a time. However, apparently not all uORFs are essential for the regulation of protein synthesis. In the case of the yeast GCN4 gene only the first and fourth uORF are essential for the regulation, whereas the second and third uORF are not.

The previously reported new prion gene sequence encoding mRNA (Schröder *et al.*, 1998) contains two small uORFs starting with an almost identical Kozak sequence. Interestingly, the first HCM [CTTTCATTTTC], which does not coincide with one of the uORF sequences, has a high homology to the cap site signal for transcription initiation following weight matrix description of unrelated promoter sequences (Bucher, 1990).

Introducing a mutation within the AUGs of the first and/or second uORF did not cause any significant change of CAT activity. In contrast, a three-fold increase of CAT activity was observed when the third uORF had been mutated. A more than four-fold increase of CAT activity was obtained when each of the three AUGs had been changed. In all cases the level of CAT mRNA remained unchanged (Fig. 3B). The authentic sequence with the AUG of the third uORF strongly suppressed the CAT activity. Both observations with different amounts of a translated product in the presence of the same level of CAT mRNA, implicated a translational regulation of CAT synthesis under these experimental conditions.

In the *in vitro* transcription and translation experiments, we introduced wt sequences of the clone 3124 or mutated sequences upstream of the ORF of the mouse *PRNP* gene. The coupled transcription/translation assay was performed to prove that any other unexpected post-translational mechanisms could not be responsible for our observations. Although the RNA expression level of the insert containing the exon 1^{SC} and the mouse *PRNP* ORF are highly comparable (Fig. 4B), the amount of the translated prion protein depended on the introduced mutation (Fig. 4A). The clone with the prion ORF sequence only and the Δ1-3ATG/PrP clone mediated the highest synthesis of the prion protein. In contrast, no translation product was observed using the wt PrP vector clone containing the authentic three ATGs or the native third ATG only (clone Δ1+2ATG/PrP and Δ2ATG/PrP), and only a very low level, at most, using the clone Δ1ATG/PrP. A mutated third ATG alone (clone Δ3ATG/PrP) resulted in about half the amount of prion protein as compared to the Δ1-3ATG/PrP clone. These results support our assumption that a translational mechanism is responsible for our observations.

Acknowledgement. We thank Dr. M. Groschup, Institut für Neue und Neuartige Tierseuchenerreger, Island Riems, Germany for the kind gift of the permanently infected N_2A cells. This work was supported by the Bundesministerium für Bildung und Forschung (BMBF), Grant-No. DLR01KJ97593.

References

Alperovitch A, Zerr I, Pocchiari M, Mitrova E, de Pedro Cuesta J, Hegyi I, Collins S, Kretzschmar H, van Duijn C, Will RG (1999): Codon 129 prion protein genotype and sporadic Creutzfeldt-Jakob disease. *Lancet* **353**, 1673–1674.

Baybutt H, Manson J (1997): Characterisation of two promoters for prion protein (PrP) gene expression in neuronal cells. *Gene* **184**, 125–131.

Bucher P (1990): Weight matrix description of four eukaryotic RNA Polymerase II promoter elements derived from 502 unrelated promoter sequences. *J. Mol. Biol.* **212**, 563–578.

Collinge J, Palmer MS, Dryden A (1991): Genetic predisposition to iatrogenic Creutzfeldt-Jakob disease. *Lancet* **337**,1441–1442.

Ford MJ, Burton LJ, Li H, Graham CH, Frobert Y, Grassi J, Hall SM, Morris (2002): A marked disparity between the expression of prion protein and its message by neurons of the CNC. *Neuroscience* **111**, 533–551.

Kozak M (1986): Point mutations define a sequence flanking the AUG initiator codon that modulates translation by eukaryotic ribosomes. *Cell* **44**, 283–292.

Luckow B, Schütz G (1987): CAT constructions with multiple unique restriction sites for the functional analysis of eukaryotic promoters and regulatory elements. *Nucleic Acids Res.* **15**, 5490.

Lemaire-Vieille C, Schulze T, Podevin-Dimster V, Follet J, Bailly Y, Blanquet-Grossard F, Decavel JP, Heinen E, Cesbron JY (2000): Epithelial and endothelial expresen of the green flourescent protein reporter gene under the control

of bovine prion protein (PrP) gene regulatory sequences in transgenic mice. *Proc. Natl. Acad. Sci. USA* **97, 10,** 5422–5427.

McCarthy JEG (1998): Posttranscriptional control of gene expression in yeast. *Microbiol. Mol. Biol. Rev.* **62,** 1492–1553.

Morris DR Geballe AP (2000): Upstream open reading frames as regulators of mRNA translation. *Mol. Cell Biol.* **20,** 8635–8642.

Outram GW (1976): The pathogenesis of scrapie in mice. *Front Biol.* **44,** 325–357.

Brown DR (1998): Prion protein-overexpressing cells show altered response to a neurotoxic prion protein peptide. *J. Neurosci Res.* **54,** 331–340.

Schröder B, Groschup M, Hunsmann G, Bodemer W (1997): Differentially expressed prion protein mRNA associated with progression of the prion disease. Abstract in *J. Mol. Med.* **75,** B175/84.

Schröder B, Groschup M, Hunsmann G, Bodemer W (1998): A differentially expressed prion gene mRNA is found in prion-infected mouse brains and in N2A cells but not in uninfected mice. *Biochem. Biophys. Res. Commun.* **251,** 423–428.

Scott M, Foster D, Mirenda C, Serban D, Coufal F, Walchli M, Torchia M, Groth D, Carlson G, DeArmond SJ *et al.* (1989): Transgenic mice expressing hamster prion protein produce species-specific scrapie infectivity and amyloid plaques. *Cell* **59,** 847–857.

Westaway D, Cooper C, Turner S, Da Costa M, Carlson GA, Prusiner SB (1994): Structure and polymorphism of the mouse prion protein gene. *Proc. Natl. Acad. Sci. USA* **91,** 6418–6422.