DEVELOPMENTAL BIOLOGY 121, 105–110 (1987)

# Developmental Expression of Prion Protein Gene in Brain

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*Received May 16, 1986; accepted in revised form October 13, 1986*

Synthesis of the cellular isoform of the prion protein ($PrP^C$) was found to be regulated during development of the hamster brain. PrP poly A(+) RNA was readily detectable 10 days postpartum; after 20 days of age, no change in its level could be detected through 13 months of age. Low levels of PrP poly A(+) RNA were detectable 1 day after birth. By contrast, myelin basic protein poly A(+) RNA was found at high levels in brain at 30 days of age and thereafter declined steadily. Using monospecific PrP antisera, immunoprecipitable cell-free translation products were detected at low levels 2 days after birth and increased progressively through 10 days of age. How the levels of PrP mRNA participate in brain development and function remains to be established. 

## INTRODUCTION

The scrapie agent displays unusual molecular properties (Alper *et al.*, 1966, 1967; Gajdusek, 1977, 1986; Diener *et al.*, 1982; Prusiner, 1982; McKinley *et al.*, 1983a). These unique properties of the scrapie agent prompted introduction of the term "prion" to describe this class of infectious pathogens (Prusiner, 1982). Purification of scrapie prions led to the identification of a protease-resistant sialoglycoprotein, PrP 27–30, which was subsequently found to be a component of the prion (Bolton *et al.*, 1982; Prusiner *et al.*, 1982; McKinley *et al.*, 1983b; Bolton *et al.*, 1985). Molecular cloning of a cDNA encoding PrP 27–30 led to the identification of a 2.1-kb PrP mRNA in both scrapie-infected and uninfected hamster brains (Oesch *et al.*, 1985). With antibodies raised against either PrP 27–30 or a synthetic peptide corresponding to the amino terminus of PrP 27–30 (Bendheim *et al.*, 1984; Barry *et al.*, 1986), the cellular and scrapie isoforms of the prion protein were identified (Oesch *et al.*, 1985). Both isoforms have an apparent $M_r$ of 33–35 kDa as determined by immunoblotting (Oesch *et al.*, 1985; Barry *et al.*, 1986). These proteins were designated $PrP^C$ or PrP $33–35^C$ for the cellular isoform and $PrP^{Sc}$ or PrP $33–35^{Sc}$ for the scrapie isoform. Proteinase K digestion destroys $PrP^C$, whereas $PrP^{Sc}$ is hydrolyzed only partially to form PrP 27–30.

Identification of the cellular isoform ($PrP^C$) suggested that studies on this protein might provide insight into the structure and function of the scrapie PrP isoform. Since PrP mRNA was found at constant levels in both scrapie-infected and uninfected adult hamster brains (Oesch *et al.*, 1985; Kretzschmar *et al.*, 1986a), we chose to investigate the regulation of PrP mRNA levels in developing hamster brain. PrP mRNA is undetectable until 1 day after birth and it remains at very low levels until about 10 days of age. Between 10 and 20 days of age PrP mRNA attains maximal levels which appear to be maintained throughout life.

## MATERIALS AND METHODS

Timed pregnant female Syrian golden hamsters (LVG/LAK) random bred in a closed colony were purchased from Charles River Laboratories (Lakeview, NJ). Male and female animals were not separated in this study. Total RNA was isolated from the brains of uninfected hamsters using the guanidinium/hot phenol method (Feramisco *et al.*, 1982). Poly A(+) RNA was recovered by one cycle of oligo(dT) chromatography (Aviv and Leder, 1972). RNA samples (5 μg) were ethanol precipitated, washed once with absolute ethanol, and dissolved in 50% formamide, 2.2 *M* formaldehyde. After the addition of one-fifth vol 10% Ficoll, 1% bromophenol blue, the samples were electrophoresed through a 1% agarose gel. The running buffer was 20 m*M* MOPS[3] (pH 7.0), 1 m*M* EDTA, 2.2 *M* formaldehyde. After electrophoresis, the gel was soaked in 20 × SSC, the RNA

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[3] Abbreviations used: MOPS, [3-(*N*-morpholino)-propane sulfonic acid], sodium salt; EDTA, ethylenediaminetetraacetate; SSC, salvine sodium citrate; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; DTT, dithiothreitol; MBP, myelin basic protein; RFLPs, restriction fragment length polymorphisms.



0012-1606/87 $3.00

transferred to nitrocellulose (Thomas, 1980) and the filters were prehybridized and hybridized essentially as described by Shank *et al.* (1978). The Sau 1 to Taq 1 fragment representing primarily the open reading frame of the pHaPrPcDNA-1 insert (Oesch *et al.*, 1985; Westaway and Prusiner, 1986) was labeled to a specific activity of $10^8$ dpm/$\mu$g using random priming with oligonucleotides from calf thymus DNA (Taylor *et al.*, 1976) as modified by Payne *et al.* (1981). Hybridizations were performed at 42°C in 3 × SSC, 50% formamide, 0.05 *M* Hepes (pH 7.4), 0.2 mg/ml salmon sperm DNA, 0.15 mg/ml yeast RNA, 0.02% bovine serum albumin, 0.02% polyvinyl purrolidone. Filters were washed in 0.1 × SSC, 0.1% $NaDodSO_4$, at 60°C and autoradiographed for 5 days at −70°C using Dupont Cronex intensifying screens and Kodak XAR-5 film.

Cell-free translations were performed on RNA extracted as described above from freshly dissected brains of hamsters of various ages. One $A_{260}$ unit of RNA was diluted into a translation reaction premix with a final volume of 100 $\mu$l and final concentrations of 20 m*M* Hepes, pH 7.5, 140 m*M* K-OAc, 3 m*M* DTT, 2.2 m*M* Mg-OAc, 10 m*M* Tris-HCl, pH 7.5, 0.4 m*M* spermidine, 1 m*M* ATP and GTP, 10 m*M* creatine phosphate, 40 m*M* of each of the 19 L-amino acids minus methionine, 1 mCi/ml [$^{35}$S]methionine (1200 Ci/mmole), 0.1 mg/ml calf liver tRNA, 20 $\mu$g/ml creatine phosphokinase, 1 U/ml ribonuclease inhibitor (RNasin), and 20% by volume wheat germ extract prepared according to the method of Erickson and Blobel (1983) at a concentration of 2.5 $A_{280}$ units/ml. Translations were incubated for 1 hr at 24°C. Samples (2 $\mu$l) to be analyzed directly for total products were dissolved directly in 15 $\mu$l $NaDodSO_4$ loading buffer (10% $NaDodSO_4$, 0.1 *M* Tris-OAc, pH 8.9, 0.5 *M* DTT) and boiled for 2 min before $NaDodSO_4$ polyacrylamide gel electrophoresis (Laemmli, 1970) and fluorography by a modification of the method of Bonner and Laskey (1974). Samples to be immunoprecipitated (98 $\mu$l) were precipitated with trichloroacetic acid, washed with ethanol:ether (1:1), and solubilized in 100 $\mu$l of 1% $NaDodSO_4$, 0.1 *M* Tris-OAc, pH 8, 0.1 *M* NaCl, 10 m*M* EDTA, 1 m*M* phenylmethylsulfonylfloride before addition of antisera (Barry *et al.*, 1986). Antigen-antibody complexes were pelleted with protein A Sepharose (Kessler, 1975), washed, and resuspended in polyacrylamide gel electrophoresis sample buffer as above.

The presence of MBP mRNA in hamster brain samples was detected using a cDNA encoding rat MBP (Roach *et al.*, 1983). MBP cDNA was labeled using random priming as described above. Hybridizations were performed as described except that formamide was at 40% concentration. Filters were washed in 1.0 × SSC, 0.1% $NaDodSO_4$, at 55°C prior to being autoradiographed as above.

## RESULTS

Having discovered the presence of a prion protein cellular isoform and its mRNA in adult animals (Oesch *et al.*, 1985), we investigated the possibility that the expression of PrP mRNA is developmentally regulated. Poly A(+) RNA was prepared from the brains of uninfected hamsters at 1 day before birth, as well as at 1, 2, 4, 6, 8, 10, and 20 days postnatally. Ethidium bromide profiles revealed that RNA was not degraded (not shown). Northern blotting with a PrP cDNA derived principally from the open reading frame identified a 2.1-kb transcript in samples prepared from animals at 10 and 20 days of age (Fig. 1, lanes 7 and 8). In other studies described below, the level of the PrP transcript remained unchanged throughout the lifetime of the animal. No difference was found between the levels of PrP mRNA at 20 days of age and at 13 months.

Cell-free translation studies were undertaken using total RNA isolated from hamster brains at various times before and after birth. No PrP-related translation prod-

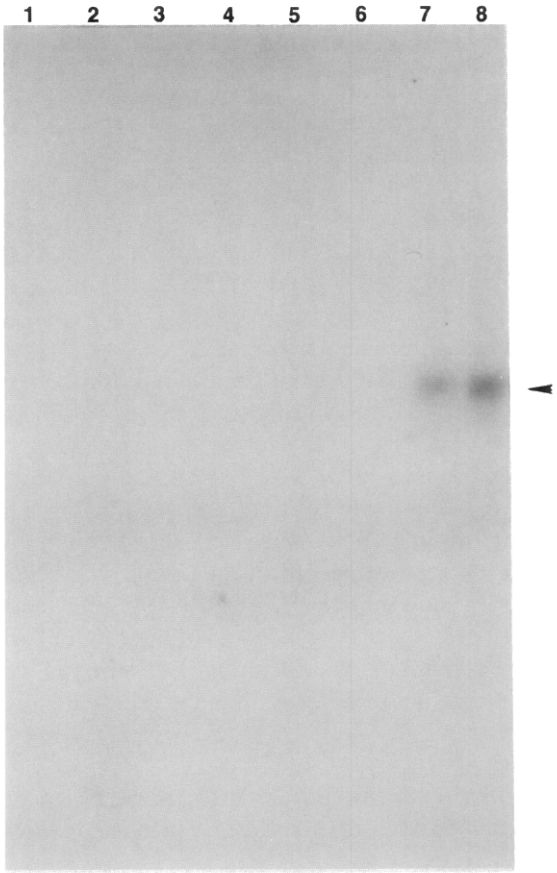


FIG. 1. Northern analysis of PrP mRNA during early development of the hamster brain. Lane 1 contains poly A(+) RNA from uninfected hamster brains at 1 day before birth. Lanes 2-8 contain poly A(+) RNA from hamster brains at 1, 2, 4, 6, 8, 10, and 20 days after birth, respectively. The arrow indicates 2.1 kb.

ucts were seen with the prenatal RNA isolated 1 day before birth. The translation products were precipitated using a monospecific antiserum raised against a synthetic peptide corresponding to the N-terminal 13 amino acids of PrP 27–30 (Barry *et al.*, 1986). At 2 and 4 days after birth a faint PrP-related translation product of approximately 23 kDa was found (Fig. 2, lanes B and D). By 6 days of age this product was readily discerned and continued to increase at 8 and 10 days of age (Fig. 2, lanes F, H, and J). The lanes with preimmune serum showed no immunoprecipitable products from the translation mixture. When the total RNA used in these cell-free translation studies was blotted for Northern analysis without prior purification on an oligo(dT) cellulose column, an RNA of 2.1 kb was identified at 8 days after birth by hybridization with a PrP cDNA (data not shown). Again, the level of PrP mRNA did not change throughout the next 13 months.

When 5 μg of poly A(+) RNA was analyzed by Northern blots from animals 1 day of age, no PrP transcript could be detected (Fig. 1, lane 2 and Fig. 3A, lane 1). Increasing the poly A(+) RNA fivefold from 5 to 25 μg per lane resulted in a faint but detectable signal (Fig. 3A, lane 2). These results are consistent with those observed with the cell-free translation described in Fig. 2.

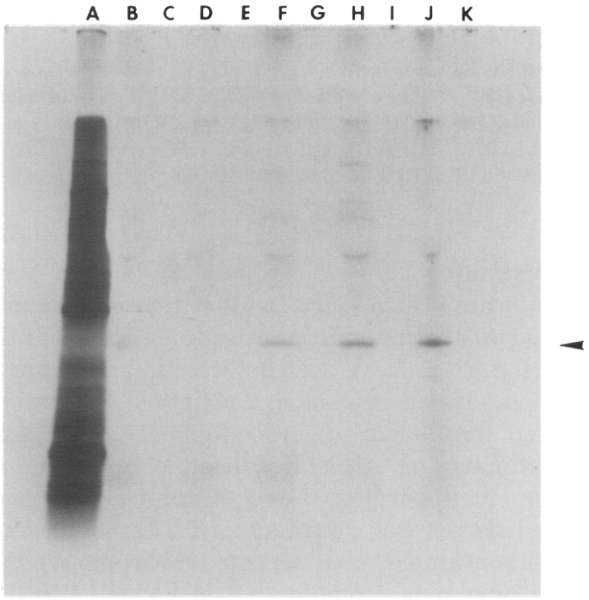


FIG. 2. Developmental profile of RNA encoding PrP immunoreactive products. Autoradiography revealed a very faint PrP immunoreactive product in the 2- and 4-day immune lanes (B, D). However, the immunoreactive product in the immune lanes for Days F, H, and J is readily apparent (lanes F, H, J). Lanes: (A) control for total translation products, (B) 2-day, immune, (C) 2-day, preimmune, (D) 4-day, immune, (E) 4-day, preimmune, (F) 6-day, immune, (G) 6-day, preimmune, (H) 8-day, immune, (I) 8-day, preimmune, (J) 10-day, immune, (K) 10-day, preimmune. Arrowhead is 23-kDa product.

As shown in Fig. 3A (lanes 3–6), between 1 and 4 months of age there was no change in the level of the PrP mRNA. As noted above, the level of PrP mRNA was the same at both 1 and 13 months.

In contrast to PrP mRNA, which is detected at 1 day after birth when five times as much poly A(+) RNA was loaded on the gel, no detectable signal is found with a hybridization probe which encodes rat MBP. As shown in Fig. 3B, lane 3, there is an intense MBP mRNA signal for 30-day-old animals, which continues to decrease progressively as the animals age. This is in accord with the developmental regulation of MBP and its mRNA levels in the rodent brain (Norton, 1977; Zeller *et al.*, 1984).

## DISCUSSION

The role of $PrP^C$ in cellular metabolism is unknown. The highest concentrations of $PrP^C$ mRNA are found in brain, with other organs showing significant but lower levels of the transcript (Oesch *et al.*, 1985). It is unknown whether the PrP mRNA detected in organs other than brain reflects the presence of the transcript either in the stroma of these organs or in the peripheral nerves which innervate the organs. In the brain, $PrP^C$ is synthesized largely in neurons while relatively little $PrP^C$ mRNA is detected in glial cells by *in situ* hybridization (Kretzschmar *et al.*, 1986a). Both $PrP^C$ and $PrP^{Sc}$ are integral membrane proteins; upon detergent extraction $PrP^{Sc}$ polymerizes into amyloid rods and $PrP^C$ is solubilized. Late during scrapie infection a small portion of prion proteins, presumably $PrP^{Sc}$, are detected within the extracellular space of brain as amyloid filaments (DeArmond *et al.*, 1985). The molecular basis for the differences between $PrP^C$ and $PrP^{Sc}$ remains to be established, but molecular cloning studies of the PrP gene suggest that these differences are most likely due to post-translational modifications (Basler *et al.*, 1986). $PrP^{Sc}$ is protease-resistant and is found only in infected animals, while $PrP^C$ is protease-sensitive and is found in both uninfected controls and infected animals (Oesch *et al.*, 1985; Meyer *et al.*, 1986). Interestingly, the only PrP transcript identified in scrapie-infected hamster brain is a 2.1-kb mRNA apparently identical in both size and concentration to that present in uninfected control brain. While PrP poly A(+) RNA increases during the early development of the brain, it does not change following scrapie infection even though the level of $PrP^{Sc}$ increases along with the titer of prions.

The marked morphological and physiological changes which occur during mammalian brain development are known to be accompanied by or to result from changes in gene expression. The temporal correlation between the synthesis of specific proteins and neuronal differ-

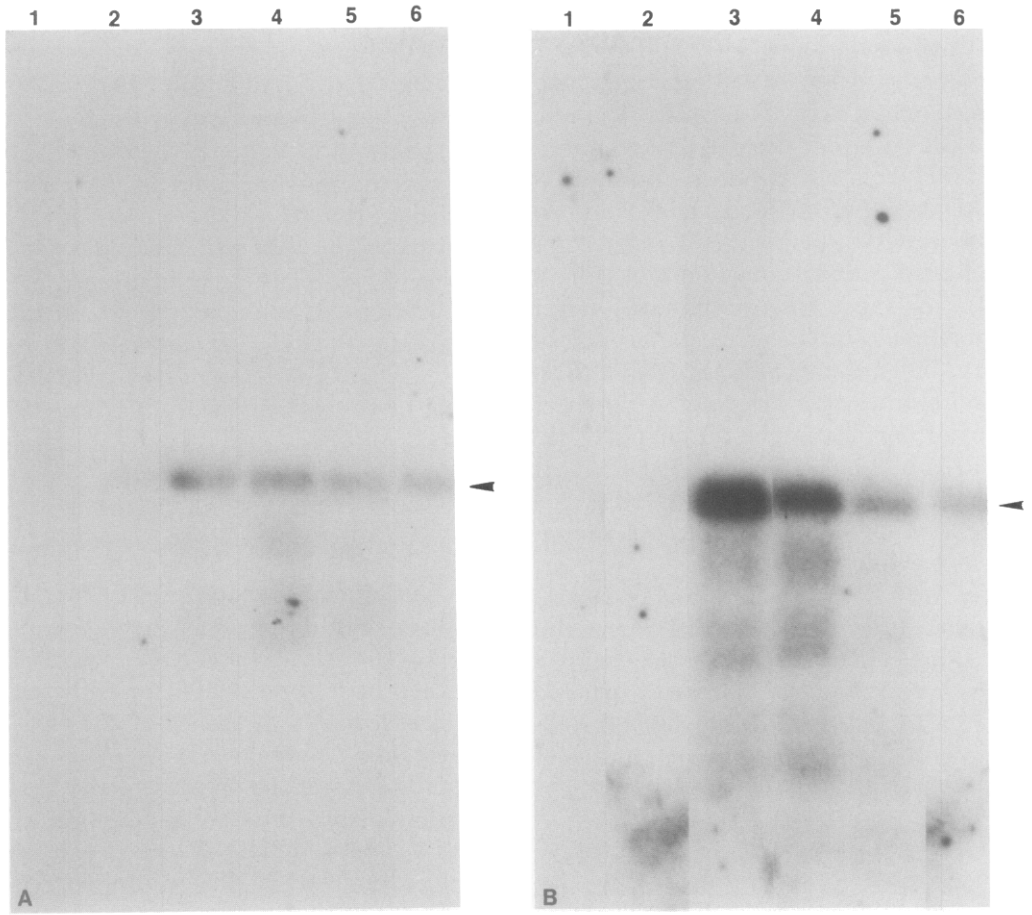


FIG. 3. Northern analysis of PrP and MBP poly A(+) RNA during development of the hamster brain. (A) PrP poly A(+) RNA. Preparation of probe and hybridization conditions as described under Materials and Methods. Lane 1 is 5 μg of poly A(+) RNA from 1-day-old hamster brain. Lane 2 is 25 μg of poly A(+) RNA from 1-day-old hamster brain. Lanes 3-6 contain 5 μg of poly A(+) RNA from 30-, 50-, 90-, and 120-day-old hamster brains, respectively. Arrowhead indicates 2.1 kb. (B) MBP poly A(+) RNA. MBP cDNA probe preparation and hybridization conditions as described under Materials and Methods. Lanes 1-6 contained samples identical to those described in (A). Arrowhead indicates 2.0 kb.

entiation strongly suggests that brain-specific functions are accompanied by precisely regulated steady-state levels of specific brain mRNAs (Morrison *et al.*, 1981). As neurons differentiate in rodents, after postnatal Day 6, the rates of synaptogenesis and myelination of neurons increase (Morrison *et al.*, 1981).

The adult mouse brain contains both poly A(+) and poly A(−) RNAs which exhibit similar degrees of complexity. It has been suggested that virtually all adult brain poly A(+) RNAs are present at birth, whereas most poly A(−) RNAs are absent (Chaudhari and Hahn, 1983). Interestingly, poly A(−) RNAs begin to appear soon after birth, suggesting that they specify proteins required for the biological capabilities of brain that emerge during the course of postnatal development (Chaudhari and Hahn, 1983). Although the levels are low, PrP poly A(+) RNA is clearly present at 1 day of age. There is no evidence for a poly A(−) RNA encoding PrP.

Approximately 35% of the poly A(+) RNA species found in brain is also found in other tissues, suggesting "housekeeping" functions for the encoded proteins. Poly A(+) PrP mRNAs have been detected in all nonnervous tissues examined as well as in a variety of cultured cells including fibroblasts, neurobastomas, and gliomas (Kretzschmar *et al.*, 1986b; D. Butler, M. Scott, and S. B. Prusiner, in preparation). Recent studies have shown that the hamster PrP gene lacks a TATA box promoter but does contain GC-rich repeats which are typical of housekeeping gene promoters (Basler *et al.*, 1986). The structure of the PrP gene promoter is of particular interest since we clearly demonstrate in the studies reported here that PrP gene expression is developmentally regulated.

The low level of PrP mRNA at birth in hamsters is an interesting observation with respect to the development of scrapie infection. When newborn mice were inoculated peripherally with scrapie prions during the first

6 hr after birth, the incubation period for scrapie was increased nearly threefold (Hotchin and Buckley, 1977). Whether this delay in scrapie infection is related to the expression or regulation of PrP mRNA remains to be established. Intracerebral inoculation of newborn mice abolished the delay in scrapie infection, suggesting that systemic transport or processing of injected prions, and not the level of intraneuronal PrP mRNA, may be responsible for this delay (Hotchin *et al.*, 1983). It is noteworthy that mice have gestation periods of 19 to 20 days while hamsters are born after a gestation period of ~16 days. How this difference in gestation period for these two species affects the comparative levels of PrP mRNA during development remains to be determined.

Whether the regulation of PrP mRNA expression is coordinated with other neuronal proteins remains to be determined. The mechanism by which the levels of PrP transcripts are modulated is unknown. Transcriptional regulation of the gene for intermediate filament proteins during development is well documented (Capoetanaki *et al.*, 1984), and although $PrP^{C}$ does not assemble into filaments, $PrP^{Sc}$ does.

Recent experiments demonstrate that one of the murine genes controlling the length of the scrapie incubation period (*Prn-i*) is linked to the gene (*Prn-p*) encoding PrP. RFLPs were found in the PrP genes of inbred strains of mice exhibiting different scrapie incubation periods (Carlson *et al.*, 1986). Analysis of incubation periods and RFLPs in backcross mice established that the incubation time (*Prn-i*) and prion protein (*Prn-p*) genes are linked. Thus, the regulation of PrP mRNA expression is of great interest with respect to scrapie infection. Although genetic factors are known to modulate the length of the scrapie incubation period in both sheep and mice (Gordon, 1964; Dickinson *et al.*, 1968; Kingsbury *et al.*, 1983), the molecular basis for this control is uncertain.

The developmental regulation of the mammalian brain is certainly the most complex and most highly programmed phase of differentiation of all living organisms. The large number of cell types and their extensive connections in the CNS present a fascinating problem in deciphering the controlling elements in brain development. Our finding that PrP mRNA transcription in brain is under developmental control poses many interesting questions with respect to neuronal maturation, the control of synaptogenesis, and the susceptibility to scrapie prion infection.

We thank Dr. David Westaway for advice on the RNA analysis and for critical review of the manuscript, Dr. Karen Hsiao for helpful discussions, and Lorraine Gallagher for manuscript preparation. This work was supported by research grants from the National Institutes of Health (AG02132 and NS14069) and a Senator Jacob Javits Center of Excellence in Neuroscience, as well as by gifts from RJR-Nabisco, Inc., and Sherman Fairchild Foundation.

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