BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS **234,** 59–63 (1997)
ARTICLE NO. RC976575

Expression of PrP mRNA Is Regulated by a Fragment of MRP8 in Human Fibroblasts

Marina Kniazeva,[1] Rena Orman,[2] and Victor P. Terranova[3,4]
Laboratory of Tumor Biology and Connective Tissue Research, Bronx VAMC, 130 West Kingsbridge Road, Bronx, New York, 10468

Received March 18, 1997

Prion protein (PrP^c) is expressed in many tissues, both in human and animals. The scrapie isoform of PrP^c has been shown to cause neurodegeneration. In other studies it has been demonstrated that overexpression of the PrP gene can result in nonneuronal tissue degradation. Little is known, however, about the normal function of PrP^c and prion protein gene regulation. Using cultured periodontal ligament cells as an experimental model, we have demonstrated the stimulation of PrP mRNA expression by MRP8 (migration inhibitory factor-related protein). Additionally, we have shown that PDGF has an opposite effect acting as a suppresser. We propose that a correlation exists between PrP^c mRNA expression and cell growth arrest and differentiation.

Prion neurodegenerative diseases are accompanied by the appearance of pathological structures, namely amyloid plaques. The major component of these infectious formations is a proteinase K-resistant peptide of 27-30 kD, termed prion protein (PrP^{Sc}). PrP^{Sc} represents an infectious isoform of the normal (or cellular) protein PrP^c, and has only been detected in animals and humans with prion diseases (1,2,3,4,5). Normal prion protein (PrP^c) does not cause neurodegeneration. PrP mRNA is detectable in a large number of nonneuronal tissues throughout the body (6,7,8). However, little is known about its biological function, and the mechanisms controlling PrP gene expression remain unknown. The purpose of this study was to investigate some of the factors that affect PrP gene expression in periodontal ligament (PDL) cells and to correlate PrP^c function with cellular function. There is a significant shortage of information about the PrP^c regulatory pathway, the potential ability of PrP^c in nonneuronal tissues to contribute to the prion protein level in brain (9), and the ability of PrP^c to cause degeneration of peripheral tissues in experimental conditions (10). An understanding of the meaning and mechanisms leading to the accumulation of prion messenger and possible accumulation of prion protein is crucial for defining the role of PrP^c in prion diseases. The importance of peripheral tissues as a potential source of prion replication with subsequent transport to the brain was previously suggested by Bendheim (9). Unlike many other connective tissues of the body, the PDL derives from neural crest. Therefore, it is possibly that brain tissue and PDL may have certain similarities during development. In this respect PDL cells could possibly be considered as a model system in which to study PrP^c expression in extraneuronal tissues. We have observed that prion protein mRNA is highly expressed in PDL cells. The present study demonstrates that cell starvation followed by MRP8 fragment stimulation has resulted in an increase of PrP mRNA and protein levels. The inhibitory effect of PDGFab stimulation on PrP mRNA level was also demonstrated. This report represents new information on the regulation of PrP mRNA expression and raises the possibility that PrP^c may play a role in cell growth arrest and differentiation.

MATERIAL AND METHODS

Cell culture. Primary cultures of periodontal ligament cells were prepared according to the method of Terranova (11). For the induction experiments PDL cells were grown to 70% confluency (passage number 7) in DMEM supplemented with 10% serum. The cells were then incubated in DMEM devoid of serum for 5 days. Each flask contained approximately 2×10^6 cells. The MRP8 fragment (amino acids 21 to 45) (10pg/ml) and PDGFab (50 ng/ml) were added to the corresponding flasks. Total RNA and protein extracts were obtained from matched pairs of flasks at 0 min., 30 min., 60 min., 4, 2 days,

[1] Present address: MCDB, Colorado University at Boulder, CO.
[2] Present address: New York University, New York, NY.
[3] Present address: Edward Hines Jr., Hospital, Veterans Affairs Medical Center, Hines, IL 60141.
[4] To whom correspondence should be addressed. Fax: (708) 343-9676.

0006-291X/97 $25.00

and 24 hours after withdrawal of the stimulator. All experiments were performed 3 times for the PDL cell lines that originated from different patients.

RNA isolation. Total RNA was isolated by TRI REAGENT (MRC, INC.). Poly(A $^{+}$) RNA was isolated using the FAST-TRACK kit (Invitrogen). The concentration and purity RNA samples were determine by spectrophotometry.

Western blot analysis. Protein extracts were obtained from PDL cells using TRI REAGENT (MRC, INC). 30 mg of all protein extracts were analyzed by electrophoresis on 12% polyacrylamide minigels in the presence of 0.1% SDS according to Laemmli (12). From the SDS-PAG, the samples were transferred to nitrocellulose membranes using a Western Transfer Apparatus (NOVEX) and then immunostained using the 3F4 MAb by the ECL Method (ECL Western Blotting Detection Reagent, Amersham). The 3F4 monoclonal anti prion protein antibody and the control hamster prion protein were kindly provided by Dr. Kascsak, Institute of Basic Research, Staten Island, New York.

PCR analysis. 5 mg of total RNA was reverse transcribed with avian myeloblastosis virus reverse transcriptase using an oligo dT-primer (cDNA Cycle Kit) (Invitrogen). 50 ng of reverse transcribed total RNA was used to amplify PrP^{c} mRNA by PCR with prion-specific synthesized primers designed by MacVector v 4.0 (International Biotechnolgies Inc.). The forward and backward primers used were: F2:5′-ttcttagctcttgggatgcagg-3′ and B6: 5′-ggacaaaggagatttgccttcag-3′. All reactions were subjected to 24 cycles: 94°C for 1 min., 60°C for 1 min., and 72°C for 2 min. 4 ul of the reaction mixtures were electrophoresed in 3% MetaPhor (FMC Bioproducts) agarose (1 × TBE buffer, 5.5 V/cm, 1 h). The PCR product that was generated with the F2-B6 primers was 364 base pairs. An internal standard was prepared and quantitative PCR was performed as described in the protocol of Förster (13). Internal mediate primer for the standard synthesis was: 5′-tttgccttcagcatctaaaatgggaggttgcctc-3′. The specificity of the PCR products were verified by Southern Hybridization with PrP cDNA. The quantitating PCR was repeated with total RNAs collected from the different cell lines.

Proliferation assay. PDL cells were plated and incubated in 12-well plates at a density 1× 10^{4} cells/well in DMEM supplemented with 10% fetal bovine serum for 4 hours. The cells were next washed with PBS and the medium was changed to fresh DMEM without serum and incubated for a period of 24 h. After the aspiration of medium, DMEM supplemented with 10% bovine serum, DMEM containing 1% platelet-poor plasma and DMEM alone with and without 10 pg/ml MRP8 fragment were added. The concentration of MRP8 fragment in these experiments matched the concentration used for PrP stimulation in starved PDL cells. At time periods 0, 3, 7 and 10 days, the cells were removed from culture by trypsinization and counted electronically. Each assay was performed in triplicate, with every well counted three times. A mean number of cells was determined.

RESULTS

MRP8 Increases PrP mRNA Concentration in PDL Cells

We have examined the effect of an addition of the synthetic fragment of MRP8 at physiological concentration (10 pg/ml) to PDL cells growing in vitro. Total RNAs were collected at different time intervals and analyzed by quantitative PCR, with an internal standard. As shown in Fig.1, PrP mRNA concentration increased 2 fold after 5 days of serum starvation and approximately 10 fold at 30 min. after addition of the MRP8 fragment. This increase was maintained as long as the MRP8 fragment was present. The PrP^{c} protein level was also elevated during the experiment, and was barely detectable after 24 hours of incubation, following MRP8 fragment withdrawal (Fig.2). These results suggest that the PrP mRNA and prion protein expression may be regulated by MRP8 in PDL cells.

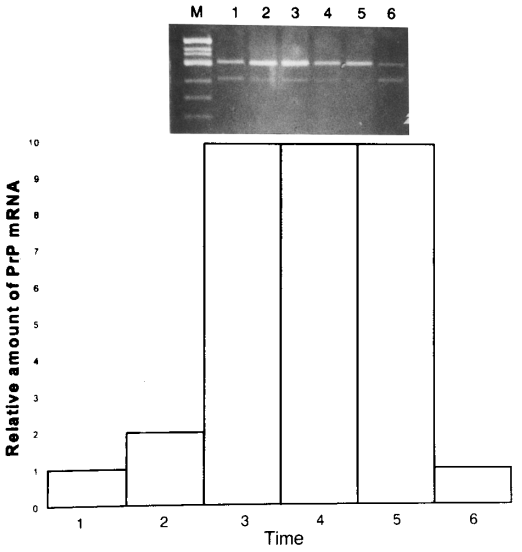

FIG. 1. Modulation of PrP mRNA expression in PDL cells in response to serum starvation followed by MRP8 fragment stimulation (10 pg/ml) and MRP8 withdrawal. The figure represents the results of titration of the PrP mRNA PCR fragments by coamplification with the internal standard. 50 ng of reverse transcribed total RNAs of PDL cells corresponding to 1. conventionally growing cells; 2. 5 days of cell starvation; 3. 30 min.; 4. 4 hours; 5. 2 days of MRP8 fragment stimulation; and 6. 24 hours after MRP8 fragment withdrawal, were coamplified with a dilution series of the competitive standard and F2B6 primers.Relative amounts of PrP mRNA were determine by separation PCR fragments on 3% agarose gel, and normalized to the control sample value. Separation of the PCR products on 3% agarose gel (*above*). Competitive standard at the control concentration was added to a series of PCR reactions, containing a constant amount of reverse transcribed total RNA collected from 1. conventionally growing cells; and after 3. 30 min.; 4. 4 hours; 5. 2 days of MRP8 fragment stimulation; and 6. 24 hours after MRP8 fragment withdrawal.

PDGFab Does Not Stimulate PrP mRNA Expression in PDL Cells

Since both PDGF and MRP8 have a strong chemotactic effect on PDL cells (14, 15), we investigated whether or not PDGFab will also elevate PrP mRNA level in PDL cells. As shown in Fig.3, PDGFab at a concentration 50 ng/ml does not induce an increase in PrP mRNA level. At 30 min. of incubation with PDGFab, a 3 fold decrease of prion mRNA level was observed when com-

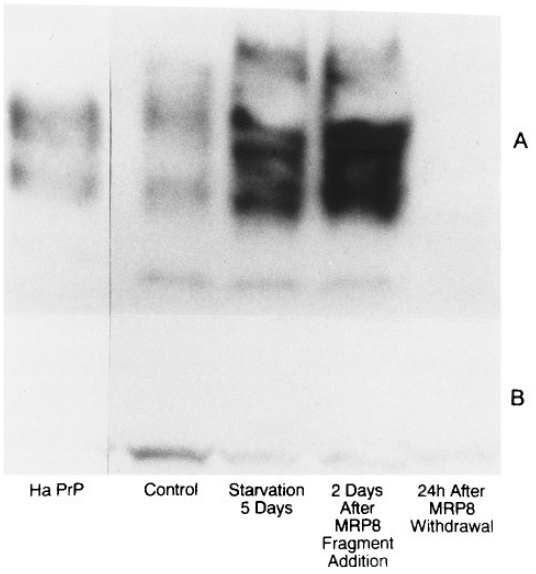

FIG. 2. Western blot analysis of PrP in PDL cells stimulated by MRP8 fragment. Protein extracts were obtained from PDL cells using TRIREAGENT. 12% denaturing PAGE was used for separation of the proteins. After electroblotting, the membrane was stained with 3F4 MAb to PrP. Section A, lane 1. 30 ng of hamster PrP (standard); 30 ug of protein extracts from PDL cells growing in vitro: lane 2: conventional growth; 3. 5 days of serum starvation; lanes 3,4: 4 h, 2 days after MRP8 fragment addition, lane 5: 24 h after MRP8 withdrawal. Section B, control staining with the polyclonal antibody to beta-actin.

pared to the level in serum starved cells. After 4h of incubation PrP mRNA concentration was equal to the control value (normally growing cells), and remained unchanged for up to 2 days of incubation. Thus, MRP8 and PDGFab appear to have common and different effects on PDL cells. PDGFab shows the opposite effects on the modulation of PrP mRNA levels in PDL cells in vitro. Prion protein level is not affected by PDGFab addition (Fig.4).

DISCUSSION

This communication reports an increase of PrP^c mRNA levels in response to serum starvation and MRP8 stimulation in PDL cells, as well as MRP8-dependent prion protein expression. The control experiments demonstrate an inhibitory effect of PDGFab on PrP mRNA expression. The results of the studies to suggest that PrP^c may be involved in cell growth arrest and differentiation.

In the present studies we have observed that PrP^c mRNA and protein is expressed at a relatively high level in PDL cells. A question was raised as to what factors are responsible for the regulation of PrP mRNA level in PDL cells. As was previously demonstrated in this laboratory, serum-deprived PDL cells express and secrete an chemotactic protein factor of low molecular weight (PDL-CTX) (14). A partial sequence of the polypeptide exhibits 100% homology to the 21 to 45 amino acid fragment of MRP8 (13). We have asked if MRP8 is responsible for the increase of PrP^c mRNA level in the starved PDL cells. In this report we show that a synthetic fragment of MRP8 elicits a pronounced increase in prion protein messenger when added to PDL cells growing in a serum-free medium. Prion protein is barely detectable by Western analysis after the withdrawal of MRP8 fragment in these experiments. Taken together, the data suggest that there is a correlation between PrP gene expression and MRP8 fragment stimulation. In order to investigate what cellular function prion mRNA elevation is associated with, we performed a proliferation assay. The data indicate the addition of the that MRP8 fragment does not cause any changes in the PDL cell proliferation rate under the conditions we used (data not shown). Consequently, PrP^c mRNA up-regulation has occurred in the absence of the stimulation of proliferation. In order to elucidate

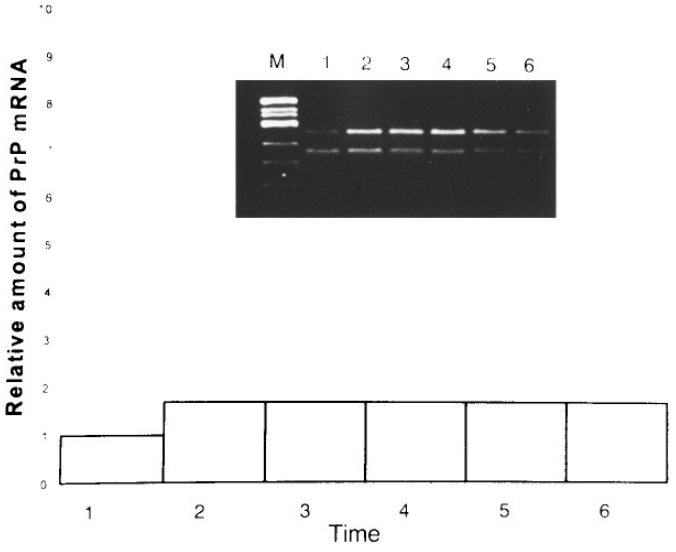

FIG. 3. Modulation of PrP mRNA expression in PDL cells in response to serum starvation followed by PDGFab stimulation (50 ng/ml) and PDGF withdrawal. The figure represents the results of titration of the PrP mRNA PCR fragments by coamplification with the internal standard. 50 ng of reverse transcribed total RNAs of PDL cells corresponding to 1. conventionally growing cells; 2. 5 days of cell starvation; 3. 30 min.; 4. 4 hours; 5. 2 days of PDGFab stimulation; and 6. 24 hours after PDGF withdrawal, were coamplified with a dilution series of the competitive standard and F2B6 primers. Relative amounts of PrP mRNA were determine by separation PCR fragments on 3% agarose gel, and normalized to the control sample value. Separation of the PCR products on 3% agarose gel (*above*). Competitive standard at the control concentration was added to a series of PCR reactions, containing a constant amount of reverse transcribed total RNA collected from 1. conventionally growing cells; and after 3. 30 min.; 4. 4 hours; 5. 2 days of PDGFab stimulation; and 6. 24 hours after PDGFab withdrawal.

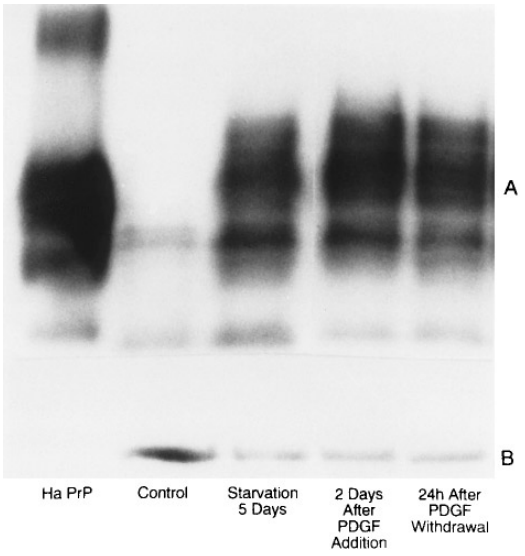

FIG. 4. Western blot analysis of PrP in PDL cells stimulated by PDGFab. Protein extracts were obtained from PDL cells using TRIREAGENT. 12% denaturing PAGE was used for separation of the proteins. After electroblotting, the membrane was stained with 3F4 MAb to PrP. Section A, lane 1. 30 ng of hamster PrP (standard); 30 ug of protein extracts from PDL cells growing in vitro: lane 2: conventional growth; 3. 5 days of serum starvation; lanes 3,4: 4 h, 2 days after PDGFab addition, lane 5: 24 h after MRP8 withdrawal. Section B, control staining with the polyclonal antibody to beta-actin.

if there is a linkage between chemotactic activity and overproduction of PrP mRNA, we performed similar experiments: a MRP8 fragment (a potent chemoattractant for PDL cells (14)) was substituted with PDGFab. Despite the fact that PDGFab is also chemoattractant for PDL cells (16), no elevation of the PrP mRNA level was observed in the growth factor treated cells. Thus, it seems unlikely that the overexpression of PrP is a necessary condition for the cell migration. Alternatevely, knowing that PDGFab causes a proliferation of PDL cells, one can assume that a mitogenic signal may suppress stimulation of PrP mRNA production.

As was previously shown, PrP gene expression can be modulated by different factors. PrP mRNA level elevates in response to NGF in developing hamster brain (15), elevates with IL-6 in PC12 pheochromocytoma cells (16), human growth hormone (hGH) and insulin-like growth factor I (IGF-I) in PC12 cells (17), and elevates with hGH and dexamethasone in pancreatic endocrine cells (18). Despite the intensive accumulation of data on the modulation of PrP gene expression little is understood about PrP regulatory pathway and the function of PrP^c. In this connection, it is interesting to notice that most known stimulators of PrP mRNA expression in distinct tissues are implicated in growth arrest and differentiation in corresponding cell lines. PC12 cells stimulated by NGF stop division and differentiate (19, 20). IL-6 mimics the effect observed with NGF in PC12 cells (21). hGH and IGF-I have an additive effect with NGF on PC12 cells (22). hGH and dexamethasone are implicated in beta cell maturation (18). Finally, resting human lymphocytes express more PrP mRNA than the cells activated to proliferation by Con A in vitro (23). As we have shown, MRP8, which is expressed during an early stage of monocyte/macrophage differentiation (24, 25), and may be a part of the mechanism of differentiation (26), is also responsible for the increase of PrP mRNA expression in PDL cells. PDGF, in contrast to MRP8, is a strong mitogenic factor for PDL cells (16), and as we have demonstrated, has an inhibitory effect on PrP mRNA expression. Thus, we cautiously suggest that PrP^c plays a role in cell growth arrest and differentiation.

In conclusion, the data indicate that PrP mRNA and protein expression is regulated by MRP8 in PDL cells. The interrelation between MRP8 and prion protein in our model may reveal a functional linkage between the proteins. In the similar experiments we have shown that the mitogenic factor, PDGFab, decreases PrP messenger concentration in PDL cells. We have suggested that the up-regulation of PrP mRNA may be relevant to cell growth arrest and differentiation.

REFERENCES

1. Meyer, R. K., McKinley, M. P., Bowman, K. A., Braunfeld, M. B., Barry, R. A., and Prusiner, S. B. (1986) *Proc. Natl. Acad. Sci. USA.* **35,** 57–62.
2. Oesch, B., Westaway, D., Waichli, M., McKinley, M. P., Kent, S. B. H., Aebersold, R., Barry, R. A., Tempst, P., Teplow, D. B., Hood, L. E., Prusiner, S. B., and Weissmann, C. (1985) *Cell* **40,** 735–746.
3. Basler, K., Oesch, B., Scott, M., Westaway, D., Walchli, M., Groth, D. F., McKinley, M. P., Prusiner, S. B., and Weissmann, C. (1986) *Cell* **46,** 417–428.
4. Prusiner, S. B. (1991) *Science* **252,** 1515–1522.
5. Prusiner, S. B. (1992) *Biochemistry* **31,** 12278–12288.
6. Harris, D. A., Lele, P., and Snider, W. D. (1993) *Proc. Natl. Acad. Sci. USA.* **90,** 4309–4313.
7. Manson, J., West, J. D., Thompson, V., McBride, P., Kaufman, M. H., and Hope, J. (1992) *Development* **115,** 117–122.
8. Meiner, Z., Halimi, M., Polakiewicz, R. D., Prusiner, S. B., and Gabizon, R. (1992) *Neurology* **42,** 1355–1360.
9. Terranova, V. P., Odziemiec, C., Tweden, K. S., and Spadone, D. P. (1989) *J. Periodont* **60,** 293–301.
10. Laemmli, V. K. (1970) *Nature* **227,** 680–685.
11. Förster, E. (1994) *BioTechniques* **16,** 18–20.
12. Boyan, L. A., Bhargava, G., Nishimura, F., Orman, R., Price, R., and Terranova, V. P. (1994). *J. Dent. Res.* **73**(10): 1593–1600.
13. Ye, J., Nishimura, F., Orman, R., And Terranova, V. P. (1995) *J. Dent Res.* **74,** 1303–1309.
14. Mobley, W. C., Neve, R. L., Prusiner, S. B., and McKinley, M. P. (1988) *Proc. Natl. Acad. Sci. USA.* **85,** 9811–9815.
15. Lazarini, F., Bastelnov, P., Chermann, J.-F., Deslys, J.-P., and Dormont, D. (1994) *Mol. Brain Res.* **22,** 268–274.
16. Bendheim, P. E., Brown, H. R., Rudelli, R. D., Scala, L. J., Goller,

N. L., Wen, G. Y., Kascsak, R. J., Cashman, N. R., and Bolton, D. C. (1992) *Neurobiology* **42,** 140–156.

17. Odnik, K., Cerletti, N., Bruggen, J., Clerc, R. G., Tarcsay, L., Zwadlo, G., Gerhards, G., Schlegel, R., and Sorg, C. (1987) *Nature* **330,** 80–82.
18. Westaway, D., DeArmond, S. J., Cayetano-Canlas, J., Groth, D., Foster, D., Yang, S.-L., Torchia, M., Carlson, G. A., and Prusiner, S. B. (1994) *Cell* **76,** 117–129.
19. Zwadlo, G., Bruggen, J., Gerhards, G., and Sorg, C. (1988) *Clin. Exp. Immunol.* **72,** 510–515.
20. Wistow, G. J., Shaughnessy, M. P., Lee, D. C., Hodin, J., and Zelenka, P. S. (1993). *Proc Natl. Acad. Sci. USA.* **90,** 1272–1275.
21. Cashman, N. R., Loertscher, R., Nalbantoglu, J., Show, I., Kascsak, R. J., Bolton, D. C., and Bendheim, P. E. (1990) *Cell* **61,** 185–192.
22. Green, L. A., and Tischler, A. (1976) *Proc. Natl. Acad. Sci. USA,* **73,** 2424–2428.
23. Wion, D., Le Bert, M., and Brachet, P. (1988) *Int. J. Dev. Neurosci.* **6,** 387–393.
24. Lasmezas, C., Deslys, J-P., and Dormont, D. (1993) *Biochemical and Biophisical Research Communications.* **196,** 1163–1169.
25. Atouf, F., Scharfmann, R., Lasmezas, C., and Czernichow, P. (1994) *Biochemical and Biophisical Research Communications.* **201,** 1220–1226.
26. Satoh, T., Nakamura, S., Taga, T., Matsuda, T., Hirano, T., Kashimoto, T., and Kaziro, Y. (1988) *Mol Cell Biol* **8,** 3546–3549.