Regulation of the Cellular Prion Protein Gene Expression Depends on Chromatin Conformation*

Ana Lucia B. Cabral‡§, Kil S. Lee‡§, and Vilma R. Martins¶¶

From the §Ludwig Institute for Cancer Research, Departamento de Bioquímica, Instituto de Química da Universidade de São Paulo, São Paulo 01509-900 and the ¶Centro de Tratamento e Pesquisa Hospital do Câncer, Rua Prof. Antônio Prudente 109/4A, 01509-010, São Paulo, SP, Brazil

Conversion of the normal cellular prion protein (PrPc), whose physiological function is still under investigation, to an infectious form called prion is the cause of some neurodegenerative diseases. Therefore, the elucidation of PrPc gene regulation is important both to define a strategy to control the infection and to better understand PrPc function. We cloned the rat PrPc gene promoter region into a luciferase reporter vector, transfected C6 and PC-12 cells, and isolated clones with stable enzyme expression. The dependence of chromatin conformation on PrPc promoter activity was evaluated using the histone deacetylase inhibitor, trichostatin A, which was able to highly increase not only promoter activity but also PrPc mRNA and protein levels. The phorbol ester (12-O-tetradecanoylphorbol-13-acetate) and cAMP poorly induced promoter activity; retinoic acid decreased it by 50%, whereas nerve growth factor and dexamethasone had no effect. When 12-O-tetradecanoylphorbol-13-acetate or cAMP but not retinoic acid was associated with trichostatin A, a potentiation of the primary effects was observed. These new data indicate that PrPc gene regulation is highly dependent on disruption of chromatin fiber assembly, which allows some ubiquitous transcription factors accession to specific DNA elements.

Prions cause a variety of neurodegenerative disorders both in animals and humans (1). A wealth of data supports the contention that scrapie prions (PrPsc)³ are devoid of nucleic acid and seem to be composed exclusively of a modified isoform of the cellular prion protein (PrPc) (2).

Pan and co-workers (3) postulated that the PrPc is converted into PrPsc through a process whereby a portion of its α-helical and coil structure is refolded into a β-sheet, which is insoluble and partially resistant to protease digestion (4). Thus, an autocatalytic mechanism initiated by PrPsc-PrPc interaction is the main explanation for prion infectivity (5). The rate of infection is dependent on the levels of PrPc (6), but a precise understanding of the conversion mechanism, its prerequisites, and immediate consequences are hampered because the role of the cellular prion protein is still under investigation.

The physiological functions of PrPc were recently described (reviewed in Ref. 7); among them is the involvement in anti-apoptotic pathways (8) and in neuritogenesis mediated by its association with laminin (9, 10). Moreover, this protein is also able to bind copper (11), but the antioxidant activity of PrPc-copper complex is still under discussion (12–14).

PrPc is considered to be a housekeeping gene because its mRNA is constitutively expressed in tissues from adult animals (4, 15). However, PrPc is highly regulated during development (16), and nerve growth factor (NGF) up-regulates PrPc mRNA expression (17). In fact, we observed that PC-12 treatment with NGF increases the levels of PrPc protein, which potentiates neurite formation and maintenance, mediated by PrPc interaction with laminin (9, 10).

Saeki and co-workers (18) demonstrated the presence of a promoter region upstream of multiple initiation sites of the rat PrPc gene. This promoter has an inverted CCAAT motif, AP-1 and AP-2-binding sites, and within the region that is critical for promoter activity three Sp-1-binding sites are found (18). In particular, members of Sp-1 family of transcription factors can act as both positive and negative regulators of gene expression with the latter function being mainly modulated by Sp-1 interaction with histone deacetylase 1 (19).

One of the hallmarks of the regulation of gene transcription is local chromatin decondensation mediated by histone acetylation, which leads to a reduced attraction between chromosomal DNA and histones and increases the accession of high molecular weight protein complexes of the transcription machinery. Conversely, histone deacetylase can repress transcription by increasing histone-DNA attraction (reviewed in Ref. 20).

The presence of AP-1 sites on the PrPc gene promoter (18) suggests that transcription factors like those belonging to the Jun and Fos family, which are induced by phorbol ester (TPA) (21), could be effectors of PrPc gene regulation. Furthermore, it has been extensively demonstrated in the literature that a large number of transcription factors like CREB (cAMP-regulated protein) and glucocorticoid receptors can associate with Jun and Fos proteins to modulate gene expression (22). In addition, PrPc mRNA levels can be inhibited by retinoic acid (RA) (23) whose receptor (RAR) could mediate gene expression via AP-1 and AP-2 transcriptional factors (22, 24) and AP-1 element (25).

We observed that trichostatin A (TSA), a potent histone deacetylase (HDAC) inhibitor (26), highly increases the PrPc promoter activity in PC-12 (rat pheochromocytoma) and C6 (rat glioma) clones stably transfected with a luciferase reporter vector under control of this promoter. TPA and cAMP up-

---

*This work was supported in part by Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) Grant 99/07124-8. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1754 solely to indicate this fact.

‡ Supported by FAPESP fellowships.

¶To whom correspondence should be addressed. Tel.: 55-11-3207-4922; Fax: 55-11-3207-7001; E-mail: vmartins@ludwig.org.br.

³ The abbreviations used are: PrPsc, scrapie prions; PrPc, cellular prion protein; TPA, 12-O-tetradecanoylphorbol-13-acetate; RA, retinoic acid; RAR, RA receptor; TSA, trichostatin A; PBS, phosphate-buffered saline; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; CRE, CAMP-response element; CREB, CRE-binding protein; NGF, nerve growth factor; HDAC, histone deacetylase.
regulate promoter activity when associated with TSA, whereas RA down-modulates it. NGF and glucocorticoid hormone, known to regulate both NGF and its receptor in glia and neuron (27), were unable to regulate PrPc gene promoter activity. Indeed, PrPc mRNA levels are consistent with promoter activity for most the treatments, and induction of PrPc protein levels is detected in TSA-treated cells.

These results indicate that PrPc promoter activity is extremely dependent on chromatin fiber disassembly, which permits the accessibility of specific elements in the DNA to the respective transcription factors.

**EXPERIMENTAL PROCEDURES**

**Cell Culture**—PC-12 and C6 cells were maintained at 37 °C in 5% CO₂ atmosphere in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal bovine serum. The concentration of each drug is as follows: NGF (Sigma), 50 ng/ml; dexamethasone (Sigma), 10⁻⁶ M; TPA (Sigma), 1.6 x 10⁻⁶ M; cAMP (Sigma) 5 x 10⁻⁴ M; RA (Biomol) 10⁻⁶ M; and TSA (Calbiochem), 2.6 x 10⁻¹¹ M.

**Construction of PrPc Promoter Luciferase Vector**—Two oligonucleotides were used as primers (5’-TTAAGTAACGTTTAAGGCACCCCTCTG-3’ and 5’-TGGCAAGGTTGCCCACCGACACGCGACG-3’). Long-PCR amplify base pairs –2831 to +47 of the rat PrPc gene, as described previously by Sacki et al. (18). These primers have HindIII restrictions sites at their 5’ and 3’ ends, and the DNA fragment was amplified using *Thermus thermophilus* Amersham Biosciences amplification kit. PCR products were digested with HindIII and cloned into the luciferase reporter vector, pGUL-3 basic (Promega). Sequencing analysis was performed to check the integrity of the amplified region and the appropriate orientation of the insert.

**Stable Transfection**—PC-12 and C6 cells were stably transfected using the calcium phosphate precipitation method (28), with the PrPc promoter luciferase vector and pSV2neo (29) at a ratio of 10:1, respectively. After selection in medium containing 0.8 mg/ml amphotericin G418 (Sigma), clones were isolated.

**Luciferase Assay**—PC-12 and C6 clones stably transfected with the PrPc promoter luciferase vector were grown on 24-well plates and after each treatment were washed with PBS and harvested in 100 μl of lysis buffer (25 mM Tris, pH 7.5, 10 mM EDTA, 15% sucrose, 2 mg/ml lysozyme). Luciferase activity was measured using the Promega Luciferase Assay System, and the relative light unit was quantified with a fluorometer (Amersham Biosciences). The non-immune IgG presented no background for the reaction and was omitted in the final figure. Flow cytometry assays were performed using C6 and PC-12 parental cells treated or not with TSA for 18 h. Cells were washed with PBS and incubated with polyclonal anti-PrPc serum (10) or non-immune serum followed by incubation with fluorescein isothiocyanate-conjugated anti mouse IgG (Dako) for 1 h at 4 °C. Cells were then washed three times, incubated with a fluorescein isothiocyanate-conjugated anti mouse IgG (Dako) for 1 h at 4 °C, and washed again three times with PBS. Fluorescence was measured with a flow cytometer FACSCAN (Becton Dickinson).

**Statistical Analysis**—Each experiment was done in triplicate, and mean values represent at least three independent experiments. Statistical significance of luciferase assays using just one drug was tested by single mean Student’s t test, and when TSA and other drugs were combined, results were evaluated by the Mann-Whitney test for unpaired samples. In all comparisons, p < 0.05 was considered statistically significant.

**RESULTS**

**Basal Activity of PrPc Promoter Luciferase in C6 and PC-12 Clones**—In an attempt to analyze PrPc promoter activity, 12 PC-12 and 6 C6 clones stably transfected with PrPc promoter luciferase vector were generated. Basal luciferase activity of each clone was measured (Table I). The large variation in promoter activity between clones was probably due to the plasmid copy number incorporated or to the place of the genome where they were randomly inserted. Clones with different basal levels were chosen to perform the experiments (PC-12.6, PC-12.8, PC-12.9, and PC-12.11 and C6.1, C6.2, and C6.4), because it is necessary to check if the basal promoter activity levels would not alter the response to different treatments.

**PrPc Promoter Activity Regulation**—The presence of three Sp-1 sites in the proximal PrPc gene promoter (18) and the ability of histone deacetylase to repress gene transcription by binding to Sp-1 transcriptional factors (19) lead us to investigate the role of chromatin remodeling in its activity. PC-12 and C6 clones were treated for 8–48 h with TSA, a potent histone deacetylase inhibitor (26). A large increase on promoter activity from 10 to 4000 times was observed in all clones analyzed (Fig. 1). Thus, these data suggest that chromatin structure has to be disrupted to allow the accession of endogenous transcription factors to specific PrPc promoter-binding sites.

In an attempt to test this hypothesis, potential regulators of endogenous transcription factors related with the binding ele-

### Table I

| PC-12 clones | Relative values |
|--------------|----------------|
| 1            | 1              |
| 2            | 130            |
| 3            | 81             |
| 4            | 5              |
| 5            | 6              |
| 6            | 36             |
| 7            | 1              |
| 8            | 41             |
| 9            | 19             |
| 10           | 1              |
| 11           | 4              |
| 12           | 5              |

* Clones selected for study.

**Biosciences**, or non-immune mouse IgG followed by incubation with goat anti-mouse IgG peroxidase labeled and developed using the ECL technique (Amersham Biosciences). The non-immune IgG presented no background for the reaction and was omitted in the final figure. Flow cytometry assays were performed using C6 and PC-12 parental cells treated or not with TSA for 18 h. Cells were washed with PBS and incubated with polyclonal anti-PrPc serum (10) or non-immune serum for 1 h at 4 °C. Cells were then washed three times, incubated with a fluorescein isothiocyanate-conjugated anti mouse IgG (Dako) for 1 h at 4 °C, and washed again three times with PBS. Fluorescence was measured with a flow cytometer FACSCAN (Becton Dickinson).

**Stable Transfection**—PC-12 and C6 clones stably transfected with the PrPc promoter luciferase vector were generated. Basal luciferase activity of each clone was measured (Table I). The large variation in promoter activity between clones was probably due to the plasmid copy number incorporated or to the place of the genome where they were randomly inserted. Clones with different basal levels were chosen to perform the experiments (PC-12.6, PC-12.8, PC-12.9, and PC-12.11 and C6.1, C6.2, and C6.4), because it is necessary to check if the basal promoter activity levels would not alter the response to different treatments.

**PrPc Promoter Activity Regulation**—The presence of three Sp-1 sites in the proximal PrPc gene promoter (18) and the ability of histone deacetylase to repress gene transcription by binding to Sp-1 transcriptional factors (19) lead us to investi-
ments within the PrPc promoter were used alone or in combination with TSA to treat PC-12 and C6 clones. The phorbol ester TPA, a potent inducer of both expression and phosphorylation of the AP-1 family members (21), and cAMP are potential candidates to control PrPc gene transcription. Treatment with TPA increased luciferase activity in all PC-12 clones reaching a maximum of 3-fold induction between 2 and 8 h. The effect was less prominent in C6 clones (Fig. 2A). Indeed, it is interesting to note that in all C6 clones, promoter activity decreased to values lower than basal after 18 h of treatment. This effect was not observed in PC-12 clones, suggesting that this pattern may represent a cell type-specific modulation.

The effect of cAMP on the PrPc gene promoter was slighter than that mediated by TPA, with 1.5–2 times induction between 8 and 24 h in all PC-12 and one out three C6 clones (Fig. 2B). Regulation of promoter activity by this compound was also checked for shorter times (data not shown), but no changes were observed.

RA was also able to modulate PrPc gene promoter activity (Fig. 2C). With the exception of the C6.4 clone, all others underwent a 50% down-regulation of the PrPc promoter activity after 24 or 72 h of treatment. These results are in agreement with previous data (23) showing that RA decreases PrPc mRNA levels in HL-60 cells.

Dexamethasone (data not shown), as well as the neurotrophin NGF (Table III), two potential candidates for PrPc gene transcriptional regulation, had no effect on the promoter activity.

TPA, cAMP, RA, NGF, and dexamethasone were combined with TSA for different times (chosen based on the better response to each treatment alone). When TPA was combined with TSA for 8 h (Table II), the induction of PrPc promoter activity was potentiated in PC-12, which suggests that disassembly of PrPc promoter chromatin increased the accessibility of transcription factors induced by TPA. On the other hand, TPA association did not change the initial TSA induction in C6 clones. In fact, the different responses observed between PC-12 and C6 cells could be due to their distinct transcription factor composition (32).

cAMP had a small influence on PrPc promoter regulation (Fig. 2B). When combined with TSA for 24 h, it generated distinct results as follows: potentiation of TSA effect in four clones (PC-12.8, PC-12.11, C6.2, and C6.4), a tendency to induce in one (PC-12.6), and a decrease in two (PC-12.9 and C6.1) (Table II). One can argue that the inductive effect is the most prevalent and should represent the real effect, whereas the promoter inhibition seems to be a clonal event.

As described previously (Fig. 2C), retinoic acid treatment down-regulated PrPc promoter activity, and this effect was maintained when TSA was associated (Table II). After 24 h of co-treatment, PC-12.6, PC-12.8, PC-12.9, and C6.1 showed a decrease in the TSA-inductive effect. RA tended to down-regulate the TSA effect in clones C6.2 and C6.4, and it had no activity on clone PC-12.11.

NGF did not affect PrPc gene promoter activity but potentiated the induction mediated by TSA treatment for 48 h in all PC-12 clones (Table III). This event seemed to occur earlier (24 h) in two of the C6 clones (C6.2 and C6.4), whereas a different pattern was observed for C6.1. These results suggest that NGF is unable to regulate PrPc promoter activity unless a chromatin disassembly is present.

Dexamethasone did not affect promoter activity even after TSA treatment (data not shown), indicating its inability to modulate PrPc transcription regulation.

PrPc mRNA Expression—In an attempt to evaluate whether modifications of the PrPc promoter activity are reflected at the mRNA levels, Northern blot assays were performed. PC-12 (Fig. 3, A and B) or C6 (Fig. 3, C and D) parental cells were treated with TSA, TPA, cAMP, RA, and NGF as indicated.

TSA has a strong positive effect on PrPc mRNA levels, in agreement with our data on promoter activity shown in Fig. 1. The effect was more prominent after 8 than after 24 h of treatment on PC-12 cells (Fig. 3A). These data confirm the importance of chromatin structure for PrPc gene expression.
Cell treatment with TPA increased PrPc mRNA levels and its association with TSA potentiated the effect on PC-12 and C6 cells (Fig. 3, A and C). These data correspond to what was observed before for PC-12 cells regarding promoter activity (Figs. 1 and 2 and Table II). However, different results were observed for C6 cells because TPA was unable to increase TSA

**Fig. 2.** TPA and cAMP cause a slight up-regulation, and RA decreases PrPc promoter activity. PC-12 and C6 clones stably transfected with a construction containing the luciferase gene under control of the PrPc promoter were treated with $1.6 \times 10^{-6} \text{ M}$ TPA (A), $5 \times 10^{-4} \text{ M}$ cAMP (B), and $10^{-6} \text{ M}$ RA (C) for the indicated time (hours). Cells were lysed after each treatment and the extracts assayed for luciferase activity as described under “Experimental Procedures.” Luciferase activity of each clone after treatment is expressed as relative levels compared with the values obtained for the equivalent clone without treatment. Values are reported as the mean ± S.D. of at least three independent experiments carried out in triplicate. *, $p < 0.002$ for PC-12 cells treated with TPA and PC-12 and C6 treated with RA; $p < 0.02$ and $< 0.04$ for C6 treated with TPA, PC-12, and C6 treated with cAMP according to the single mean Student’s $t$ test.
**TABLE II**

Effect of TPA, cAMP, and RA association with TSA on PrPc promoter activity

The values shown are mean ± S.D. relative luciferase activity (compared with the equivalent clone without treatment) from at least three independent experiments.

| Clone  | 8 h TSA | 8 h TSA + TPA | 24 h TSA | 24 h TSA + cAMP | 24 h TSA + RA |
|--------|---------|--------------|---------|----------------|--------------|
| PC12.6 | 6.5 ± 5.8 | 87.8 ± 65.3   | 184.7 ± 97.7 | 247.9 ± 78.4 | 50.3 ± 15.6   |
| PC12.8 | 23.5 ± 17.5 | 108.1 ± 27.1  | 260.7 ± 106 | 761.2 ± 200.1 | 131.9 ± 30.7  |
| PC12.9 | 15.6 ± 10.6 | 84.9 ± 44.4   | 246.8 ± 85.5 | 133.1 ± 54.8 | 159.8 ± 67.6  |
| PC12.11| 7.4 ± 4.4  | 28 ± 7.1      | 134.2 ± 66.6 | 774 ± 423.8  | 221 ± 71.4    |
| C6.1   | 209.2 ± 87.4 | 270.7 ± 112.4 | 3000.4 ± 855.7 | 1635.1 ± 749.9 | 1715.1 ± 937.5 |
| C6.2   | 2 ± 0.9    | 1.9 ± 0.8     | 2.4 ± 0.8  | 3.6 ± 1.6    | 1.9 ± 0.4     |
| C6.4   | 2.5 ± 0.8  | 2.5 ± 0.8     | 7 ± 4.2    | 21.7 ± 6.5   | 3.3 ± 1.8     |

* *p < 0.05 versus TSA in the equivalent time treatment.

**TABLE III**

Effect of NGF association with TSA on PrPc promoter activity

The values shown are mean ± S.D. relative luciferase activity (compared with the equivalent clone without treatment) from at least three independent experiments.

| Clone   | 24 h NGF | 24 h TSA | 24 h TSA + NGF | 48 h NGF | 48 h TSA | 48 h TSA + NGF |
|---------|----------|---------|----------------|----------|---------|----------------|
| PC12.6  | 1.3 ± 0.4 | 184.7 ± 97.7 | 187.1 ± 94.4 | 1.1 ± 0.2 | 48.6 ± 24.9 | 318 ± 157.1   |
| PC12.8  | 1.1 ± 0.6 | 260.7 ± 106 | 274.2 ± 182.2 | 1.2 ± 0.3 | 701.9 ± 96.6 | 2539 ± 938.2  |
| PC12.9  | 1.2 ± 0.3 | 246.8 ± 85.5 | 361.9 ± 232  | 1.5 ± 0.5 | 274.6 ± 93.7 | 1994.4 ± 768.5 |
| PC12.11 | 1.1 ± 0.3 | 134.2 ± 66.6 | 414.5 ± 185  | 1.4 ± 0.2 | 64.9 ± 29.1  | 1659.8 ± 906.2 |
| C6.1    | 0.8 ± 0.2 | 3000.4 ± 855.7 | 3350.5 ± 1997.4 | 1.5 ± 0.1 | 1362.7 ± 516.2 | 676.2 ± 262.2  |
| C6.2    | 1.5 ± 0.4 | 2.4 ± 0.8   | 3.3 ± 1     | 1.2 ± 0.2 | 1.8 ± 0.7    | 2.4 ± 1.5     |
| C6.4    | 0.5 ± 0.1 | 7 ± 4.2     | 25.8 ± 10   | 0.8 ± 0.3 | 2.5 ± 0.8    | 1.9 ± 0.7     |

* *p < 0.05 versus TSA in the equivalent time treatment.

Fig. 3. PrPc mRNA expression regulation. Northern blots were performed using 10 μg of total RNA purified from PC-12 (A and B) or C6 (C and D) parental cells after the indicated treatments. The blot was probed with [α-32P]dCTP-labeled PrPc and GAPDH cDNAs. PC-12 (A and B) and C6 cells (C and D) were treated with TSA, TPA, or RA (A and C) and TSA, cAMP, or NGF (B and D) at the concentrations indicated under “Experimental Procedures.” The 1st column in each panel corresponds to the untreated cells. TSA treatment itself serves as control for the association between this drug and cAMP, TPA, RA, or NGF, as indicated.

RA treatment for 24 h had no apparent effect on PrPc mRNA expression in either cell line (Fig. 3, A and C), which diverges with its respective effect on promoter activity (Fig. 2C). These results might be due to Northern blot sensitivity in detecting repression in an already low basal level of PrPc mRNA (Fig. 3, A and C). However, the inhibition was easily detected in PC-12 and C6 cells previously treated with TSA (Fig. 3, A and C), thus confirming that RA represses PrPc gene expression.

PrPc mRNA levels were apparently not affected by cAMP in either cell line (Fig. 3, B and D), in agreement with the slight induction (around 1.5-fold) of promoter activity in most clones evaluated (Fig. 2B). cAMP potentiated the PrPc mRNA induction mediated by TSA in PC-12 but did not affect TSA activity in C6 cells (Fig. 3, B and D). These data agree with what was observed regarding PrPc promoter activity in 3 of 4 PC-12
clones, but no correlation was found with C6 clones (Table II).

NGF treatment for 24 h had no effect on PrPc mRNA levels in PC-12 or C6 cells, but after 120 h this neurotrophin was able to increase PrPc mRNA levels in PC-12 cells (Fig. 3, B and D). This result confirms previous data showing PrPc mRNA (17) and protein level (10) up-regulation after similar treatment. NGF association with TSA did not affect PrPc mRNA induction mediated by the latter, in contrast to the data shown above indicating that the neurotrophin potentiates the PrPc promoter activity induced by chromatin disassembly (Table III). We would argue that mRNA stability might account for this result.

**PrPc Protein Levels**—The detection of PrPc expression in cell lines by Western blots is particularly hard, probably because of the small amount of this protein in established lineages despite their neural origin (33). However, PrPc expression is easily detected, for example in different tissues (34) or transfected overexpressing lineages (33).

As expected, the basal levels of PrPc in both PC-12 and C6 cell lines were almost undetectable by Western blot assays (Fig. 4, A and B, left panels respectively). However, it was possible to visualize them either in PC-12 or C6 by flow cytometry analysis (Fig. 4, A and B, right panels). TSA treatment mediated a strong induction of PrPc protein levels as clearly seen in both procedures employed (Western blot and flow cytometry). It is also interesting to note that C6 cells seem to express different glycosylated isoforms of PrPc when compared with PC-12 (Fig. 4, A and B, left panels). These results were probably due to cell type-specific post-translational mechanisms rather than to any TSA effect on protein glycosylation. PrPc protein levels were not altered when cells were treated with TPA, cAMP, or RA. However, NGF induced PrPc levels after 5 days of treatment, as described previously by our group (10). The association of TPA, cAMP, or RA with TSA did not induce any detectable change in protein levels when compared with TSA alone (data not shown).

**DISCUSSION**

The PrPc gene promoter has been identified in rats (18), mice (35), hamsters (36), cattle (37), and humans (38), and the major region of transcriptional control was found upstream of the initiation site. However, other regulatory regions were also identified (35, 37). All reported PrPc gene promoters lack a TATA box and contain GC-rich features, which are potential binding sites for Sp-1 transcriptional factors. Specific sites for AP-1 and AP-2 were also identified (18, 35, 38). Thus, the PrPc gene promoter conservation among species and its content of
transcriptional elements for ubiquitous factors suggest that it may be similarly regulated.

The family of Sp-1 transcription factors can act both as positive and negative regulators of gene expression. This mechanism is dependent on the competition between the transcription repressor, HDAC1, and the transactivating factor E2F1 that has histone acetylase activity (19). The importance of histone acetylation/deacetylation in chromatin structure and transcription has been extensively discussed (39, 40). It has been postulated that histone acetylation reduces its affinity for DNA and thus leads to an increased access of transcription factors to the repressed chromatin template (40).

Our results show that cell treatment with TSA produced a marked induction of PrPc promoter activity in PC-12 and C6 clones, which was followed by mRNA and protein up-regulation (Figs. 1, 3, and 4) in parental cells. Indeed, all PC-12 and C6 clones transfected with the reporter plasmid containing the luciferase gene under control of the rat PrPc promoter are sensitive to TSA. Therefore, we may speculate that, independently of DNA organization around the site of insertion, the transfected PrPc promoter is able to recruit histones, acquiring a conformation comparable with that of resident promoter.

It is important to note that the effect of TSA is not generalized to all cellular gene promoters. HDAC inhibitors such as TSA and sodium butyrate have been described to increase Gα conformation comparable with that of resident promoter.

transfected PrPc promoter is able to recruit histones, acquiring a conformation comparable with that of resident promoter.

Our data show that AP-1 activation by TPA mediates a discrete induction of promoter activity that is followed by an increase in mRNA levels. Moreover, an impressive up-regulation was observed when TPA was associated with TSA, suggesting that chromatin remodeling around the AP-1 element in the PrPc gene promoter is important for Jun/Fos family access. This is in agreement with data showing that Jun/Fos binding activity to acetylated DNA is 4–5-fold higher than the non-acetylated one (58).

cAMP induces phosphorylation of the transcription factor CREB (59), but despite the fact that the PrPc promoter does not have a consensus CREB-binding element (CRE), CREB in some systems is also able to interact with AP-1 (60) and AP-2 (61) elements, both present in this promoter. Nevertheless, cAMP treatment generated a poor induction in promoter activity and mRNA levels, suggesting that its relative concentrations in both cell lines are not sufficient to compete with other transcription factors with higher affinity for AP-1 and AP-2 DNA elements (60, 61). However, the association of cAMP with TSA potentiates PrPc promoter activity in most PC-12 and C6 clones, an event followed by increased mRNA levels in PC-12. These results support the notion that chromatin remodeling is important for accession of transcriptional factors. Moreover, because the promoter induction by TSA association with cAMP is not followed by an increase in mRNA levels in C6, we would predict that lower message stability might be present in this case.

Activated RAR (by retinoic acid treatment) is able to regulate AP-2 transcription factor (24) to interact with AP-1 family members (22), to bind AP-1 elements (25), and to repress c-fos mRNA induction by serum (62). Our data show that RAR activation in the absence or presence of TSA generated the same level of down-regulation of promoter activity, which was followed by decreased mRNA levels. These data indicate that RAR regulates the promoter activity by a mechanism that is independent of chromatin structure. It is tempting to speculate that RAR acts throughout a DNA element that is far from the CpG-rich region, like the AP-2, which could be in a region where chromatin is less condensed.

NGF was unable to regulate PrPc promoter activity, but increased PrPc mRNA levels were observed after 5 days of neurotrophin treatment. The data correspond to what has been described before (17) and suggest that NGF mediates an increase in PrPc mRNA stability, which is followed by an increase in PrPc protein levels in PC-12 cells (10). NGF acts in synergism with TSA at the promoter activity level when cells are treated for long periods (around 48 h), indicating that PrPc promoter regulation by NGF depends on chromatin disassembly and expression/repression of other transcriptional factors. The synergistic effect is not observed at mRNA level, and therefore, we assume that attenuation on mRNA stability is also present.

PrPc expression is highly regulated during development with transcripts detected in embryos by 13.5 days in neuronal and non-neuronal cells (16). Indeed, all adult tissues analyzed expressed PrPc, with highest levels found in neuronal cells (16), intermediate levels in heart and lung, and low levels in spleen (4). Therefore, we believe that tissue-specific transcription factors may not account for this regulation. Our data strongly reinforce this idea, because assembly of tissue-specific chromatin fibers around the PrPc gene would expose the PrPc gene promoter to ubiquitous transcriptional factors like Sp1, AP-1, and RAR and determine the level of transcription.

These results are important to evaluate the involvement of PrPc in cell growth and differentiation and may allow the development of strategies helping the treatment/control of prion diseases. Indeed, we can approach this aspect in at least two ways. Drugs like RA or compounds that up-regulate histone deacetylase activity can be used to decrease PrPc levels and delay infection. Conversely, agents that rescue PrPc expression, for example histone deacetylase inhibitors, may bypass a possible loss-of-function component of these diseases.

Acknowledgments—We thank Dr. Luisa L. Villa, (Ludwig Institute for Cancer Research, São Paulo, Brazil) for primer synthesis; Regina Nomizo (Fundação Antônio Prudente) for assistance with flow cytometry analysis; Elisangela Monteiro (Fundação Antônio Prudente) for DNA sequencing; and Drs. Luiz Fernando L. Reis (Ludwig Institute for Cancer Research) and Ricardo R. Brentani (Ludwig Institute for Cancer Research) for critical reading of the manuscript.

REFERENCES

1. Prusiner, S. B. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 13363–13363
2. Stahl, N., Baldwin, M. A., Teplow, D. B., Hood, L., Gibson, B. W., Burlingame,
31. Fort, P., Marty, L., Piechaczyk, M., El Sabrouty, S., Dani, C., Jeanteur, P., Martin, M. (1997) Biochim. Biophys. Acta 1344, 143–144

32. Glaus, S., Stengena, S. L., and Hirayama, K. (1996) J. Biol. Chem. 271, 9547–9557

33. Scott, M., Butler, D. A., Bredesen, D. E., Walchli, M., Hsiao, K. K., and Prusiner, S. B. (1998) Protein Eng. 2, 69–76

34. Zanussi, G., Liu, D., Ferrari, S., Ilijina, I., Yin, S., Aguzzi, A., Horvath, S., Brinckerhoff, C., Liemann, S., Blank, H., Tschammler, R., Nishio, J., Bloom, M., Aksenov, I., Lukasch, K., Kubo, S., Matsuzawa, Y., Saeki, K., Matsumoto, Y., Yoyokuma, T., Iiura, S., and Onodera, T. (1999) Nature 400, 225–226

35. Schonefeld, J. F., Schlegel, J. W., Herms, J. W., Madlung, A., Manson, J., Strome, R., Dinsmore, S., Kornberg, R. D., and Lorch, Y. (1999) J. Biol. Chem. 294, 10962–10966