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Repression of the β-amyloid gene in a *Hox-3.1*-producing cell line

(homeobox/Alzheimer disease/transcriptional regulation/transfection)

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ABSTRACT **Mammalian homeobox genes are widely expressed in the developing central nervous system and are postulated to control developmental processes by regulating gene expression at the transcriptional level. *In vitro* studies have identified consensus DNA sequences that contain an ATTA core as sites for interaction with homeodomain proteins. Such elements have been found in the upstream regulatory region of the gene encoding β-amyloid precursor protein, which is associated with the neurological disorder Alzheimer disease. As the β-amyloid precursor protein gene is also expressed in the developing central nervous system and appears to play a role in cellular regulatory processes, we have examined the possibility that a homeobox gene product can regulate its transcription. We demonstrate by Northern blot analyses and transfection experiments that the expression of the β-amyloid precursor protein gene is decreased in cultured cells expressing the mouse homeobox gene *Hox-3.1*.**

Studies on the regulation of gene expression underlying embryonic development and cellular differentiation have been facilitated by the discovery of genes containing homeobox sequences. These sequences were first identified as a conserved region shared by a number of genes involved in the determination of body pattern in *Drosophila*. Subsequently, homeobox genes have been isolated from various organisms, including mice and humans; recent results are consistent with the hypothesis that mammalian homeobox genes specify positional information during embryonic development (1, 2). Homeobox genes are believed to coordinate these events by regulating the expression of a panoply of genes enmeshed in an auto- and trans-regulatory network (2–4). In support of this view, homeobox gene products have been shown to be transcription factors containing a highly conserved helix–turn–helix motif (homeodomain) that enables the protein to bind to specific DNA sequences that contain an ATTA core (3, 4). These sequences have been found in the regulatory regions of several homeobox genes, and it has been demonstrated that the interaction of homeodomain proteins with these elements affects the transcriptional activity of the target gene (1, 3, 4). These studies provide evidence for a cross-regulatory network among *Drosophila* homeobox genes. However, limited information is available regarding the transcriptional regulation of non-homeobox genes by homeodomain proteins. Discovering such target genes will contribute significantly to a further understanding of how the homeobox gene network exercises control over developmental processes and cellular differentiation.

The β-amyloid precursor protein (APP) gene may be a target of the homeobox gene network. Twenty ATTA core sequences are present within a 2.2-kilobase (kb) region upstream of its transcription start sites. Seven sequences centering on the ATTA core share at least 8 out of 10 nucleotides with the consensus binding sequence for the Hox-1.3 protein (5, 6). What is particularly striking is that six of these elements are clustered within an 800-base-pair (bp) region (see Fig. 3*A*). Their presence in such numbers suggests a possible regulatory function. An understanding of the transcriptional regulation of the APP gene is of further interest because of its involvement in the neuropathology of the human disorders Alzheimer disease and Down syndrome (7–9). Mutations in this gene have been linked to familial Alzheimer disease (10–12). In patients with Alzheimer disease and Down syndrome, β-amyloid plaques are deposited in specific regions of the brain (8, 9). In addition to its association with neurological disorders, APP is also proposed to be a growth regulatory autocrine factor, a protease inhibitor, a cell surface receptor, and/or a cell surface adhesion molecule (18–22). By considering the number of putative homeodomain binding sites, the expression of APP in the central nervous system, and the multifarious activities attributed to the APP gene, we decided to investigate the possibility that a homeodomain protein can regulate its transcription.

MATERIALS AND METHODS

Plasmid DNAs. *hsp68/Hox-3.1* was derived by ligating a *Hox-3.1* minigene (23) containing a polyadenylylation signal from the mammalian expression vector pMSG (Pharmacia) into a pUC19 vector containing the 800-bp *Bam*HI–*Nco* I *hsp68* promoter derived from phspPTlacZpA (24). *hsp68/Hox-3.1Rev.* was derived in the same manner except the *Hox-3.1* cDNA and polyadenylylation signals were subcloned in the reverse orientation. *hsp68/Hox-3.1ΔBox* was derived from *hsp68/Hox-3.1* by deleting the 270-bp *Sal* I fragment that contains the homeobox. APP–*lacZ* constructs were derived from DNA containing 2.2 kb of the 5′ flanking region of the human APP gene ligated to the *lacZ* reporter gene from *Escherichia coli* and a simian virus 40-derived polyadenylylation signal, digested with exonuclease III/mung bean nuclease (5).

Heat Shock. Cells were heat-shocked in tissue culture dishes at 42°C in 7% CO_2O/93% air for 30–60 min and allowed to recover for 4–12 h at 37°C in 7% CO_2/93% air.

Transfection. Transfections were carried out with calcium phosphate-precipitated DNA (25). Stably transfected cell lines were derived by clonal selection in G418 at 400 μg/ml (25). For transient transfections, 2×10^5 cells were plated into 60-mm dishes, cultured for 16 h, and then transfected with DNA. After 16 h of culture in 3% CO_2/97% air, the DNA precipitate was washed off and the cells were refed with fresh Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum. Twenty-four hours later, the cells

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Abbreviations: APP, β-amyloid precursor protein; CAT, chloramphenicol acetyltransferase.

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were extracted and assayed for β-galactosidase and chloramphenicol acetyltransferase (CAT) activity (26, 27). All experiments were carried out using at least two preparations of CsCl-purified circular plasmid DNAs.

Immunoprecipitation. Exponentially growing cells were radiolabeled with [^{35}S]methionine (Tran^{35}S-label, ICN) at 0.5 mCi/ml (1 Ci = 37 GBq) in methionine-free DMEM containing 10% dialyzed fetal bovine serum for 2 h. Cells were washed with phosphate-buffered saline and lysed in 0.5 ml of RIPA buffer (0.15 M NaCl/0.05 M Tris·HCl, pH 7.5/1% Nonidet P-40/0.5% sodium deoxycholate/0.1% SDS) containing chymostatin (1 μg/ml), leupeptin (1 μg/ml), pepstatin (1 μg/ml), aprotinin (1 μg/ml), and phenylmethylsulfonyl fluoride (1.0 mM). Protein extracts were centrifuged at 100,000 × *g* for 10 min at 4°C. Supernatants were collected and precleared with 10 μl of rabbit preimmune serum for 45 min at 4°C followed by the addition of 10% (wt/vol) protein A-Sepharose (Pharmacia). Protein A-Sepharose was removed by centrifugation in a microcentrifuge at 15,000 rpm for 30 sec. Hox-3.1 protein was then immunoprecipitated with 10 μl of Hox-3.1-specific mouse antiserum diluted 1:10 for 60 min at 4°C followed by the addition of 10% protein A-Sepharose. After centrifuging in a microcentrifuge at 15,000 rpm for 30 sec, the precipitate was washed four times with RIPA buffer. Protein samples were electrophoresed on a SDS/10% polyacrylamide gel by the method of Laemmli (28) and visualized by fluorography.

Preparation of Nuclear Extract. Cells were collected by trypsinization, suspended in buffer A (10 mM Tris·HCl, pH 7.5/2 mM $MgCl_2$/10 mM KCl/0.5 mM dithiothreitol/1.0 mM phenylmethylsulfonyl fluoride) for 10 min on ice, and then lysed in a Dounce homogenizer. Cell lysis was monitored microscopically. Lysed cells were centrifuged at 750 × *g* for 10 min at 4°C, and the nuclear pellet was resuspended in buffer A and recentrifuged. The nuclear pellet was resuspended in buffer B (50 mM Tris·HCl, pH 7.5/250 mM sucrose/2 mM $MgCl_2$/25 mM KCl/0.5 mM dithiothreitol/1 mM phenylmethylsulfonyl fluoride), and then NaCl and Triton X-100 were added to a final concentration of 0.5 M and 0.5%, respectively. The suspension was incubated on ice for 30 min with intermittent mixing, passed repeatedly through a 21-gauge needle, and then centrifuged at 15,000 × *g* for 30 min at 4°C. The supernatant was dialyzed against buffer B overnight and centrifuged at 15,000 × *g* for 30 min at 4°C, and then the resulting nuclear extract was stored at −70°C. Protein concentrations were determined by a modification of Lowry's method (13).

Northern Blot Analysis. Total cellular RNA was obtained by guanidine thiocyanate solubilization and cesium chloride centrifugation (29). Thirty μg of total RNA was electrophoresed on a 1% agarose/formaldehyde gel and blotted onto nitrocellulose by capillary diffusion. Northern blots were hybridized with a ^{32}P-radiolabeled M13 single-stranded DNA probe (30) containing the 216-bp *Sal* I–*Alu* I restriction fragment of the first exon of the *Hox-3.1* gene (23) in 50% (vol/vol) formamide/5× standard saline citrate (SSC)/10% (wt/vol) dextran sulfate/5× Denhardt's solution at 45°C. Blots were also hybridized with a 1.1-kb *Eco*RI fragment of the APP cDNA (21) and a *Pst* I fragment of PA1 chicken β-actin cDNA (31), ^{32}P-radiolabeled by random priming in 50% formamide/5× SSC/10% dextran sulfate/5× Denhardt's solution/0.1% SDS/0.05% sodium phosphate/salmon sperm DNA (100 μg/ml) at 42°C. Filters were washed under stringent conditions and then exposed to Kodak XAR-5 film at −70°C for 12–24 h.

DNA-Mobility-Shift Analysis. Oligonucleotides were ^{32}P-radiolabeled using the Klenow fragment of DNA polymerase I. Binding reaction mixtures contained 20,000 cpm of ^{32}P-radiolabeled oligonucleotide, 2 μg of poly(dI-dC)·poly(dI-dC), bovine serum albumin (0.6 mg/ml) in buffer B, and the indicated amount of protein. Reaction mixtures were incubated on ice for 30 min and then electrophoresed on a 6% polyacrylamide gel in 0.5× TBE buffer (1× TBE = 89 mM Tris, pH 8.3/89 mM boric acid/2 mM EDTA). Protein–DNA complexes were visualized by exposing gels to Kodak XAR-5 film at −70°C for 12–24 h.

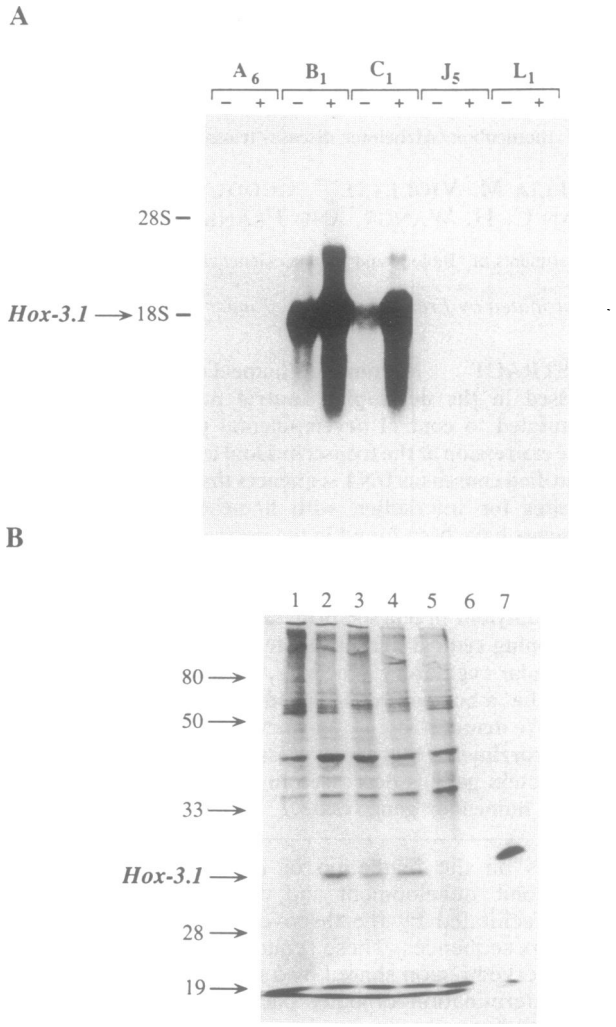

FIG. 1. (*A*) Northern blot analysis of Hox-3.1 mRNA in HeLa-derived clonal cell lines. Each clonal line is represented by the following two lanes: −, RNA from cells cultured at 37°C; +, RNA from cells heat-shocked at 42°C for 1 h. The negative control was a clonal cell line (J_5) that was transfected with hsp68/lacZ (24) and selected under the same conditions. (*B*) Immunoprecipitation of Hox-3.1 protein. Lanes 1–7 represent protein prepared from parent HeLa, B_1, C_1, C_2, K_1, wild-type Sf9, and Hox-3.1 recombinant-baculovirus-infected Sf9 cells, respectively. Parent HeLa and HeLa-derived cell lines were heat-shocked for 30 min followed by a 4-h recovery at 37°C prior to radiolabeling. Molecular masses (in kDa) are indicated.

RESULTS AND DISCUSSION

We have chosen mouse *Hox-3.1* as a representative member of the Antennapedia class of homeobox genes because of our previous studies to determine its embryonic patterns of expression, genomic structure, and cis-regulatory elements (23, 32, 33). To examine the effects of mouse *Hox-3.1* on the expression of other genes, we overexpressed its gene product in a cellular system in which it is not normally present. HeLa cells were cotransfected with the inducible mouse heat shock promoter (24) ligated to a mouse *Hox-3.1* minigene (23)

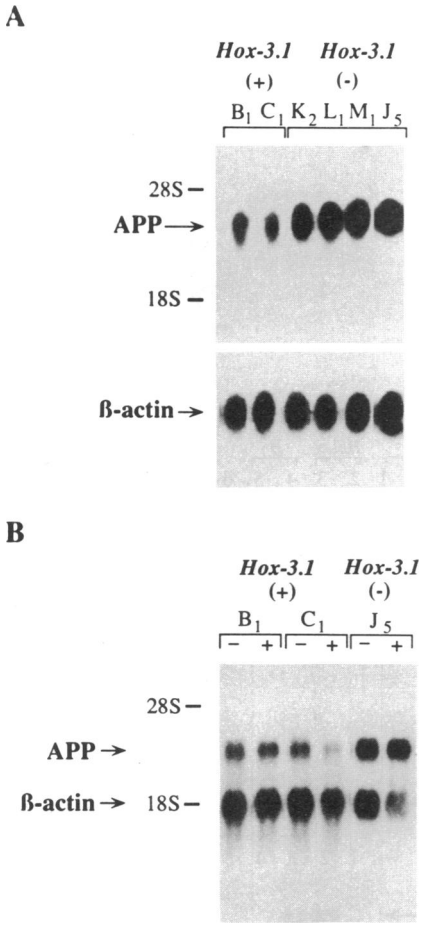

FIG. 2. Northern blot analysis of APP mRNA in HeLa-derived clonal cell lines. (*A*) RNA isolated from cells cultured at 37°C. As shown in Fig. 1, clonal lines B_1 and C_1 express Hox-3.1 mRNA, and L_1 and J_5 do not. K_2 and M_1 were also found to be negative for *Hox-3.1* expression (data not shown). The same filter was also hybridized with a probe for chicken β-actin as indicated. (*B*) Total RNA isolated from B_1, C_1, and J_5 cells cultured without (−) or with (+) a 1-h heat shock.

(*hsp68/Hox-3.1*) and a neomycin-resistance gene (pwLNeo) (34). After selection with the gentamycin analog G418, several clonal cell lines were isolated and analyzed for the expression of *Hox-3.1* under both normal and heat shock conditions. Northern blot analyses showed that two clonal lines, B_1 and C_1, expressed the expected 2.2-kb Hox-3.1 transcript (23); several other clones did not express *Hox-3.1* and served as controls in further experiments (Fig. 1*A*). Clonal lines B_1 and C_1 differed in their basal level of *Hox-3.1* expression. B_1 cells constitutively expressed 4- to 5-fold more *Hox-3.1* than non-heat-shocked C_1 cells, in which expression was detected in a Northern blot only after a longer exposure to film. In both these cell lines, *Hox-3.1* expression was increased 3- to 5-fold when the cells were heat-shocked. These cell lines were also analyzed for the presence of Hox-3.1 protein by immunoprecipitation with Hox-3.1-specific antiserum. Both B_1 and C_1 cells contain a 29- to 30-kDa immunoprecipitable protein, which is of the expected size compared to Hox-3.1 protein produced in a baculovirus expression system (Fig. 1*B*). No Hox-3.1 protein was detected in parental HeLa or control K_1 cells (Fig. 1*B*).

To determine whether the expression of *Hox-3.1* in HeLa-derived clonal cells affects the transcription of the endogenous APP gene, Northern blot analyses were performed on RNA isolated from the HeLa-derived cell lines. HeLa cells have been previously shown to express the APP gene (35). The level of the APP transcript was decreased at least 50% in both B_1 and C_1 cells when compared to the control cells that did not express *Hox-3.1* (Fig. 2*A*). Furthermore, when C_1 cells were subjected to heat shock, the level of the APP transcript was decreased by 75%, whereas there was a concomitant increase in the accumulation of Hox-3.1 mRNA (Fig. 2*B*). No further decrease in the amount of APP mRNA was observed in heat-shocked B_1 cells. The expression of β-actin was unaltered in all of these cells under both normal and heat shock conditions. We have also examined the expression of other genes, such as retinoic acid receptor γ, and found no change in their level of expression (S.M.V., unpublished data). These results suggest that the decreased expression of APP is specifically related to *Hox-3.1* expression and is not due to a generalized decrease in the transcriptional activity of these clonal cell lines.

The results of Northern blot analyses were further substantiated by transfection studies. Constructs that contained the upstream region of the APP gene ligated to a β-galactosidase (*lacZ*) reporter gene were transfected into HeLa-derived cell lines. In particular, the expression of two such constructs was examined: (*i*) 2.0-APP–*lacZ*, which contains 2.0 kb of the 5′ flanking region of the APP gene, and thus several of the putative homeodomain binding sites, and (*ii*)

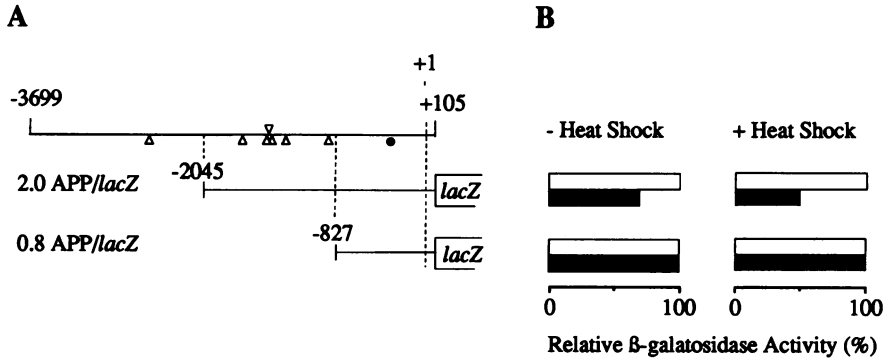

FIG. 3. (*A*) Schematic of APP–*lacZ* constructs. The location of ATTA core sequences (△) that contain at least an 8 out of 10 nucleotide similarity to the *Hox-1.3* consensus binding site (6) and of the heat shock element (●) are indicated. (*B*) Relative activity of *APP–lacZ* constructs in Hox-3.1-producing cells. C_1 and K_1 cells were cotransfected with 6.5 μg of 2.0-APP–*lacZ* or 0.8-APP–*lacZ* and 1 μg of an internal control construct, pPOH14 (36), encoding the CAT gene. Heat-shocked cells were cultured for 30 min at 42°C 12 h prior to measuring β-galactosidase and CAT activities. β-Galactosidase activity measured in C_1 cells (solid bars) is expressed as the mean of the percentage detected relative to transfected control K_1 cells (open bars). Values are derived from at least four experiments normalized to the internal control CAT activity. Experiments were not included if the CAT activity (transfection efficiency) for the different cell lines varied by >3-fold. Variations in the mean percentage of control activity were <15%.

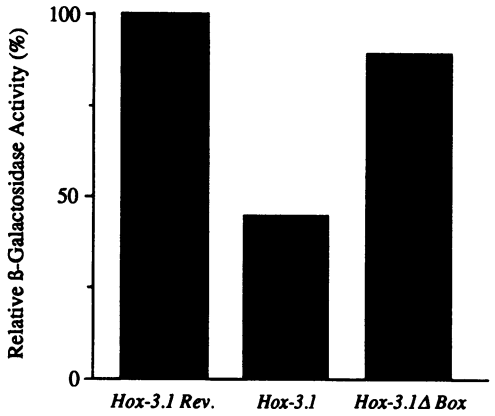

FIG. 4. Transient cotransfection of parent HeLa cells with 0.3 μg of hsp68/Hox-3.1Rev., hsp68/Hox-3.1, or hsp68/Hox-3.1ΔBox, 6.5 μg of 2.0-APP–*lacZ* or pMTI-2402 (contains 2.8 kb of the 5′ flanking sequence of the APP gene) (37), and 1 μg of pPOH14 or SV2CAT internal control plasmid DNA. Twelve hours before measuring β-galactosidase and CAT activities, cells were heat-shocked for 30 min. β-Galactosidase activity was normalized to an internal control CAT construct and is represented as the mean of the percentage detected relative to cells cotransfected with a control construct (hsp68/Hox-3.1Rev). Mean values are derived from at least four experiments. A sample was eliminated if the CAT activity (transfection efficiency) differed by >2 SD from the mean activity within an experiment. Variations in the mean percentage of control activity were <20%.

0.8-APP–*lacZ*, which contains the minimal promoter of the APP gene but lacks any putative homeodomain binding sites (Fig. 3*A*). Transfection of 2.0-APP–*lacZ* into *Hox-3.1*-expressing C_1 cells produced only 69% of the β-galactosidase activity measured in transfected control K_1 cells (Fig. 3*B*). Activation of *Hox-3.1* expression in the C_1 cells by heat shocking led to a further decrease in β-galactosidase activity. The activity of 2.0-APP–*lacZ* in C_1 cells after heat shocking was only 50% of that measured in heat-shocked control K_1 cells. Alternatively, 0.8-APP–*lacZ* had essentially equivalent activity when transfected into K_1 or C_1 cells under both normal and heat-shocked conditions. This suggests that the repression of APP–*lacZ* activity by *Hox-3.1* is dependent on the presence of putative homeodomain binding sites located upstream of this 0.8-kb region.

In these experiments, it was noticed that the APP promoter itself was heat-shock-responsive. A 2- to 3-fold increase in β-galactosidase activity was measured when control K_1 cells transfected with 2.0-APP–*lacZ* were heat-shocked. This activation may be mediated by a heat shock element previously identified in the APP promoter region (5). Despite this activation, transfection of 2.0-APP–*lacZ* into C_1 cells that were subsequently heat-shocked resulted in a decrease in its activity compared to the activity of this same construct in heat-shocked control K_1 cells. Thus, the presence of the *Hox-3.1* gene lowered the activity of 2.0-APP–*lacZ* whether or not the cells were heat-shocked.

In all the cases studied so far, the effect of a homeobox gene product on a target promoter has been shown to be mediated by the homeodomain (1). To test whether this is true for the *Hox-3.1*-elicited repression of the APP gene, the second exon containing the homeobox was deleted from hsp68/Hox-3.1; this construct was designated hsp68/Hox-3.1ΔBox. Transient cotransfections performed in parent HeLa cells showed that hsp68/Hox-3.1 repressed the expression of APP–*lacZ* reporter constructs by 55% (Fig. 4). No such effect was detected with the cotransfection of hsp68/Hox-3.1ΔBox. These studies also indicate that the effect of *Hox-3.1* on the expression of the APP gene was not specific to the HeLa-derived clonal cell lines because this effect was

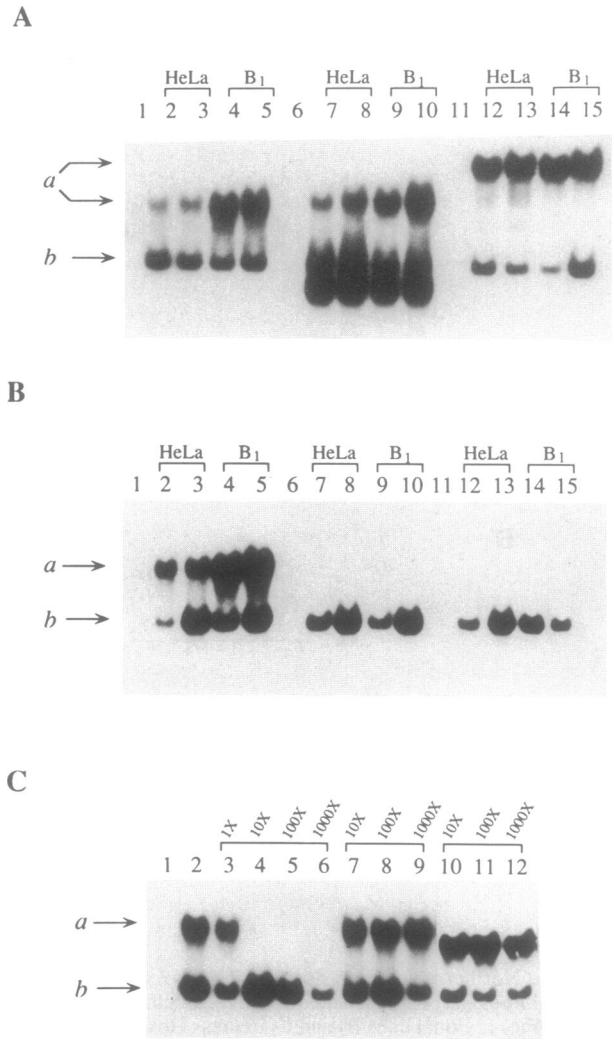

FIG. 5. DNA-mobility-shift analyses of nuclear proteins from parent HeLa and Hox-3.1-producing HeLa cells binding to putative homeodomain binding sites. (*A*) Binding of parent HeLa or B_1 nuclear extracts to the following synthetic oligonucleotides: APP1, AATTCGGAAGATGATTATCGCTG (lanes 1–5); APP2, AATTCTACAAAAAATTAGCCGGG (lanes 6–10); APP3, AATTCATGCAAAAATTAGCCGAG (lanes 11–15). Except for lanes 1, 6, and 11, in which no protein was added, even-numbered lanes contained 10 μg of protein and odd-numbered lanes contained 20 μg of protein in the binding reaction mixtures. (*B*) Binding of HeLa and B_1 nuclear extracts to the following normal and mutant APP1 oligonucleotides: APP1 (lanes 1–5); mAPP1a, AATTCGGAAGATGAggATCGCTG (lanes 6–10); mAPP1b, AATTCGGAAGATGcTTcTCGCTG (lanes 11–15), where lowercase letters refer to nucleotide substitutions. Protein concentrations were the same as in *A*. (*C*) Competition of APP1 binding to B_1 nuclear extract. Binding reaction mixtures contained 1 ng of ^{32}P-radiolabeled APP1 and 5 μg of B_1 nuclear extract. Lanes: 1, free probe; 2, no competitor APP oligonucleotide added; 3–6, 1, 10, 100, or 1000 ng of unlabeled APP1, respectively; 7–9, 10, 100, or 1000 ng of unlabeled mAPP1b, respectively; 10–12, 10, 100, or 1000 ng of unlabeled APP4 (AGCTTCATTGTGTCTGTCCTGAATTATAGAAATGAA), respectively. Nuclear extracts were isolated from HeLa and B_1 cells that were heat-shocked for 60 min followed by 8 h of recovery at 37°C.

detected in transiently transfected parent HeLa cells. To ascertain whether the same effect could be measured in a different cell type, these experiments were also carried out in mouse F9 embryonal carcinoma cells, and similar results were obtained (S.M.V., unpublished data). This indicates that the repression of the APP gene by *Hox-3.1* is not a cell-type-specific response.

The results of these cotransfection studies support the conclusion that the ability of *Hox-3.1* to repress the transcription of the APP gene requires the presence of the homeodomain. We have examined whether Hox-3.1-producing cells express proteins that will bind specifically to the putative homeodomain binding sites. By employing DNA-mobility-shift analyses, several oligonucleotides were examined for their ability to bind to parent HeLa and Hox-3.1-producing HeLa nuclear proteins (Fig. 5*A*). In most cases, two protein–DNA complexes were detected. Mutational and competition studies indicated that the formation of the slower mobility complex (complex a) is dependent on an intact ATTA core sequence, whereas the faster mobility complex (complex b) is not (Fig. 5 *B* and *C*). Thus, the formation of complex a is consistent with the binding properties of homeodomain proteins (1, 3, 4). The patterns of the protein–DNA interactions in B_1 and parental HeLa binding reactions were comparable except for two oligonucleotides tested, APP1 and APP2 (Fig. 5*A*). In these cases, the amount of protein present in complex a was greater using B_1 nuclear extract compared to that from the parental cells. The quantitative differences that were detected were not simply due to more protein being present in the B_1 binding reaction mixtures since there was an equal amount of complex b formed in both cases. Additionally, this difference was not observed with all of the oligonucleotides examined. The quantitative difference in complex a may result from the induced expression of Hox-3.1 protein in B_1 cells. Alternative explanations include the overproduction of a protein endogenous to the parental cells or synthesis of a protein other than Hox-3.1 in B_1 cells. Decreased expression of the APP gene in the Hox-3.1-producing cells may be a result of competition between endogenous DNA binding proteins and the mouse Hox-3.1 protein for some of the putative homeodomain binding sites.

In this report, we have presented evidence implicating Hox-3.1 in the regulation of the β-amyloid gene. This has wide significance since the β-amyloid protein is involved in both normal development and disease (7–9, 14, 18–22, 38). When the conserved nature of the homeodomain proteins, their multiplicity, and similarity of binding sites are considered, it is possible that other homeodomain proteins are also involved in the regulation of the APP gene. These may act singly or in combination as regulators of the APP promoter in different developmental contexts. Both the APP gene and the homeobox genes are expressed in the central nervous system during ontogeny. Recent evidence suggests that many homeobox gene products are expressed in the anterior regions of the central nervous system (15–17), where the accumulation of APP has been implicated in the etiology of Alzheimer disease. This paper describes the transcriptional regulation of the APP gene by a member of the homeobox gene family. An understanding of these interactions may provide crucial information as to how such a regulatory network functions to promote normal development or disease.

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1. Shashikant, C. S., Utset, M. F., Violette, S. M., Wise, T. L., Einat, P., Einat, M., Pendleton, J. W., Schughart, K. & Ruddle, F. H. (1991) *Crit. Rev. Eukaryot. Gene Exp.* **1**, 207–245.
2. Kessel, M. & Gruss, P. (1990) *Science* **249**, 374–379.
3. Levine, M. & Hoey, T. (1988) *Cell* **55**, 537–540.
4. Hayashi, S. & Scott, M. P. (1990) *Cell* **63**, 883–894.
5. Salbaum, J. M., Lemaire, H. G., Weidemann, A., Masters, C. L. & Beyreuther, K. (1988) *EMBO J.* **7**, 2809–2813.
6. Odenwald, W. F., Garbern, J., Arnheiter, H., Tournier-Lasserve, E. & Lazzarini, R. A. (1989) *Genes Dev.* **3**, 158–172.
7. Glenner, G. G. & Wong, C. W. (1984) *Biochem. Biophys. Res. Commun.* **120**, 885–890.
8. Masters, C. L., Simms, G., Weinman, N. A., Multhaup, G., McDonald, B. L. & Beyreuther, K. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 4245–4249.
9. Selkoe, D. J. (1990) *Science* **248**, 1058–1060.
10. Goate, A., Chartier-Harlin, M.-C., Mullan, M., Brown, J., Crawford, F., Fidani, L., Giuffra, L., Haynes, A., Irving, N., James, L., Mant, R., Newton, P., Rooke, K., Roques, P., Talbot, C., Pericak-Vance, M., Roses, A., Williamson, R., Rossor, M., Owen, M. & Hardy, J. (1991) *Nature (London)* **349**, 704–706.
11. Murrell, J., Farlow, M., Ghetti, B. & Benson, M. D. (1991) *Science* **254**, 97–99.
12. Chartier-Harlin, M.-C., Crawford, F., Houlden, H., Warren, A., Hughes, D., Fidani, L., Goate, A., Rossor, M., Roques, P., Hardy, J. & Mullan, M. (1991) *Nature (London)* **353**, 844–846.
13. Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275.
14. Fisher, S., Gearhart, J. D. & Oster-Granite, M. L. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 1779–1782.
15. Murtha, M. T., Leckman, J. F. & Ruddle, F. H. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 10711–10715.
16. Price, M., Lemaistre, M., Pischetola, M., Di Lauro, R. & Duboule, D. (1991) *Nature (London)* **351**, 748–751.
17. Porteus, M. H., Bulfone, A., Ciaranello, R. D. & Rubenstein, J. L. R. (1991) *Neuron* **7**, 221–229.
18. Saitoh, T., Sundsmo, M., Roch, J.-M., Kimura, N., Cole, G., Schubert, D., Oltersdorf, T. & Schenk, D. B. (1989) *Cell* **58**, 615–622.
19. Yankner, B. A., Duffy, L. K. & Kirschner, D. A. (1990) *Science* **250**, 279–282.
20. Oltersdorf, T., Fritz, L. C., Schenk, D. B., Lieberburg, I., Johnson-Wood, K. L., Beattie, E. C., Ward, P. J., Blacher, R. W., Dovey, H. F. & Sinha, S. (1989) *Nature (London)* **341**, 144–147.
21. Kang, J., Lemaire, H.-G., Unterbeck, A., Salbaum, J. M., Masters, C. L., Grzeschik, K.-H., Multhaup, G., Beyreuther, K. & Müller-Hill, B. (1987) *Nature (London)* **325**, 733–736.
22. Shivers, B. D., Hilbich, C., Multhaup, G., Salbaum, M., Beyreuther, K. & Seeburg, P. H. (1988) *EMBO J.* **7**, 1365–1370.
23. Awgulewitsch, A., Bieberich, C., Bogarad, L., Shashikant, C. & Ruddle, F. H. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 6428–6432.
24. Kothary, R., Clapoff, S., Darling, S., Perry, M. D., Moran, L. A. & Rossant, J. (1989) *Development* **105**, 707–714.
25. Chen, C. & Okayama, H. (1987) *Mol. Cell Biol.* **7**, 2745–2752.
26. Gorman, C. M., Moffat, L. F. & Howard, B. H. (1982) *Mol. Cell Biol.* **2**, 1044–1051.
27. An, G., Hidaka, K. & Siminovitch, L. (1982) *Mol. Cell. Biol.* **2**, 1628–1632.
28. Laemmli, U. K. (1970) *Nature (London)* **227**, 680–685.
29. Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982) in *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab., Cold Spring Harbor, NY), pp. 187–206.
30. Bentley, K. L., Bieberich, C. J. & Ruddle, F. H. (1991) in *Methods in Nucleic Acids Research* (CRC, Boca Raton, FL), pp. 131–156.
31. Cleveland, D. W., Lopata, M. A., MacDonald, R. J., Cowan, N. J., Rutter, W. J. & Kirschner, M. W. (1980) *Cell* **20**, 95–105.
32. Awgulewitsch, A., Utset, M. F., Hart, C. P., McGinnis, W. & Ruddle, F. H. (1986) *Nature (London)* **320**, 328–335.
33. Bieberich, C. J., Utset, M. F., Awgulewitsch, A. & Ruddle, F. H. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 8462–8466.
34. Thomas, K. R. & Capecchi, M. R. (1987) *Cell* **51**, 503–512.
35. Weidemann, A., König, G., Bunke, D., Fischer, P., Salbaum, M. J., Masters, C. L. & Beyreuther, K. (1989) *Cell* **57**, 115–126.
36. Byrne, G. W. & Ruddle, F. H. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 5473–5477.
37. Wirak, D. O., Bayney, R., Kundel, C. A., Lee, A., Scangos, G. A., Trapp, B. D. & Unterbeck, A. J. (1991) *EMBO J.* **10**, 289–296.
38. Ohyagi, Y., Takahashi, K., Kamegai, M. & Tabira, T. (1990) *Biochem. Biophys. Res. Commun.* **167**, 54–60.