

A Common Mechanism Underlying the E1A Repression and the cAMP Stimulation of the H Ferritin Transcription*

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Transcription of the H ferritin gene *in vivo* is stimulated by cAMP and repressed by the E1A oncoprotein. We report here the identification of the cis-element in the human promoter responsive to both cAMP- and E1A-mediated signals. This promoter region is included between positions -62 to -45 and binds a approximate 120-kDa transcription factor called Bbf. Bbf forms a complex *in vivo* with the coactivator molecules p300 and CBP. Recombinant E1A protein reduces the formation of these complexes. *In vivo* overexpression of p300 in HeLa cells reverses the E1A-mediated inhibition of the ferritin promoter transcription driven by Bbf. These data suggest the existence of a common mechanism for the cAMP activation and the E1A-mediated repression of H ferritin transcription.

The genes coding for the heavy and light ferritin subunits are expressed in a wide variety of organisms and, within an organism, in all cell types (1). Their expression is modulated by several factors, such as iron (2), hormones (3), drugs (4), and cytokines (5), which lead to the synthesis of multiple ferritin isoforms with different H to L ratio, called isoferritins.

Regulation of ferritin gene expression is exerted at translational and transcriptional levels. Iron interferes with the binding of the translational repressor (iron responsive element-binding protein) with the target sequence (iron responsive element) in the 5'-untranslated region of ferritin transcripts (for review, see Ref. 6). Hormones and drugs modulate transcription of ferritin genes. Transactivation of the H promoter has been reported in C6 glioma cells treated with insulin-like growth factor-I (7), in Friend erythroleukemia cells treated with hemin (8), in differentiating Caco-2 cells (9), in CL3 neuroepithelial cells exposed to G418 (4), and in FRTL5 rat thyroid cells treated with cAMP (10). Conversely, the gene product of the adenovirus, E1A, inhibits ferritin expression (11).

The molecular dissection of H ferritin promoter indicates

that the relevant regulatory sequence elements are located in a 200-base pair segment at 5' end of the transcription start site of the human gene (12). Recently we have mapped a DNA sequence, the B-site, located in the -62/-45 region of the human H gene, which acts as a positive regulatory element. Deletion analysis of this region has shown that a transacting factor, which binds the B site, is required for the transcription of ferritin gene (12). Cells undergoing *in vitro* differentiation or treated with translation inhibitors, actively transcribe ferritin gene (9, 4). Under these conditions, the activity of the B site binding factor (Bbf),¹ assayed by transient transfection with target promoter or by DNA binding assays, specifically increases in the nucleus.

Here we show that Bbf, a protein of approximately 120 kDa, is required for cAMP induction of the ferritin promoter. Bbf does not bind the consensus CRE sequence and does not react with antibodies *versus* CREB or ATF1. Furthermore, Bbf binds *in vivo* the transcriptional adaptors CBP and p300. The product of adenovirus E1A specifically competes the formation of the p300-Bbf complex. Transient or stable expression of E1A inhibits the transcription driven from the ferritin promoter. In the same cells, overexpression of p300 completely reverses E1A inhibition of ferritin transcription.

We propose that Bbf mediates cAMP induction of ferritin promoter and that the mechanism of transcriptional repression of the H ferritin gene by E1A is caused by the competition of E1A with Bbf for p300 binding, resulting in the disruption of the active transcriptional complex on the ferritin promoter.

MATERIALS AND METHODS

Cell Culture—HeLa cells were cultured as monolayers in Dulbecco's modified Eagle's medium (Life Technologies, Inc.), supplemented with 10% (v/v) fetal calf serum (Sigma), 100 units/liter penicillin (Hyclone). Cells were grown at 37 °C in a 5% CO₂ atmosphere.

DNA Transfections and CAT Assays—The H promoter/CAT constructs 5'-H A/M, 5'-H H/A, and 5'-H H/M have been previously described (12). The Bbf-TATAA construct contains a double-stranded B-site oligonucleotide (5'-CGGCGCTGATTGGCCGGG-GCGGGC-3'), in the correct 5' to 3' orientation, cloned in the *Sma*I site of the 5'-H H/M construct upstream the ferritin TATAA box. The Bbf_{mut}-TATAA construct was obtained by inserting a mutated B-site oligonucleotide (5'-CGGCTCTTACAGGCCGTTGCGAGC-3'), in the correct 5' to 3' orientation, in the *Sma*I site of the 5'-H H/M construct. The pSVEK and pSVEKmut are expression vectors carrying PKA-specific inhibitor tagged with a nuclear localization signal under the control of SV40

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¹ The abbreviations used are: Bbf, B box-binding factor; ATF, activating transcription factor; BMV, tobacco mosaic virus; CAT, chloramphenicol acetyltransferase; CBP, CREB-binding protein; CRE, cAMP-responsive element; CREB, CRE-binding protein; EMSA, electrophoretic mobility shift assay; H, heavy; L, light; PKA, protein kinase A.

promoter.² The CRE-CAT construct was a gift of Dr. P. Sassone-Corsi (13). For each experiment, 5 pmol of plasmid DNA were transfected into cultured HeLa cells by the calcium phosphate coprecipitation method (14). To correct for variations in DNA uptake and transfection efficiency, 5 μ g of a plasmid carrying the luciferase gene under the control of the cytomegalovirus enhancer were included. For the cAMP treatment, 10⁻⁶ M 8-bromoadenosine 3':5'-cyclic monophosphate (Sigma) was added directly for the indicated times. CAT and luciferase assays were performed as described (4, 15). The values reported in the figures are the mean of three independent transfection experiments.

Nuclear Extracts and EMSA—Nuclear extracts were prepared from HeLa cells as previously described (4). The oligonucleotides used for EMSAs were: CTF-site 5'-TTATTTTGGATTGAAGCCAATATGATAA-3'; CRE-site 5'-GTTCCGCCAGT-GACGTAGGAAGTC-3'. To study DNA-protein interaction on the B-site, the -100 to +1 region from the human H promoter, terminally labeled, was used. EMSAs and competition assays were performed as described previously (9). Anti-CREB antibody (Santa Cruz, X-12) was assayed as supershifting reagent; the antibody was added to the nuclear extract and incubated for 1 h on ice before the addition of the labeled probes.

UV Cross-linking—0.5 ng of terminally labeled B-site oligo (5'-CGCGCTGATTGGCCGGGCGGGC-3') were incubated in a final volume of 20 μ l, with 25 or 50 μ g of HeLa nuclear extracts in the presence of 3 μ g of poly(dI-dC) (Pharmacia) for 30 min at room temperature. After incubation, the samples were UV irradiated for 2 min at a wavelength of 254 nm and then loaded on a 5% polyacrylamide gel. The retarded bands corresponding to the DNA-protein complexes were excised from the gel and loaded on a 8% SDS-polyacrylamide gel electrophoresis.

Immunoprecipitations—Whole cell extracts were prepared as described (16). Immunoprecipitations, deoxycholate elutions, and gel retardation assays were performed as described in Ref. 17 with modifications. Briefly, 300 μ g of HeLa whole cell extracts were incubated with anti-p300 (05-222 UBI) antibody or with non-immune antisera for 1 h at 4 °C in a buffer containing 10 mM Hepes (pH 7.6), 10 mM KCl, 1.5 mM MgCl₂, 1 mM dithiothreitol, 1 mM phenylmethylsulfonyl fluoride, 0.5 mM sodium fluoride, 0.5 mM sodium orthovanadate (Na₃VO₄), 1 mg/ml leupeptine, 1 mg/ml aprotinin. The samples were further incubated for 1 h at 4 °C with protein A-Sepharose. The beads were collected by centrifugation, washed four times with the incubation buffer, and finally incubated 20 min on ice with 10 μ l of incubation buffer, 1% deoxycholate. Immunoprecipitates were collected and assayed by EMSA with the radioactive probes.

Alternatively, whole cell extracts from wild type HeLa cells were mixed with recombinant E1A protein or with recombinant BMV (tobacco mosaic virus, TNT Promega) protein 30 min at 4 °C prior to immunoprecipitation with anti-p300 antibodies.

RESULTS

Induction of Ferritin Promoter by cAMP: Identification of the Target Site—cAMP stimulates the transcription of H ferritin gene (3, 10). To analyze the promoter elements responsive to cAMP, we used several plasmid fusions carrying segments of ferritin promoter upstream the chloramphenicol acetyltransferase gene (CAT) (12). The 5'-H A/M construct (-160 to +1) contains the positive cis-elements A and B necessary for the expression of H ferritin *in vivo*. The A element is the target for the transcription factor Sp1, while the B element is recognized by a nuclear protein, which we named Bbf. The 5'-H H/A construct (-100 to +1) retains only the B element; the 5'-H H/M construct contains only the TATAA box and the start of transcription of the H gene (-50 to +1).

HeLa cells were transiently transfected with these plasmids and stimulated with cAMP. CAT activity was measured and normalized for the transfection efficiency by including in each transfection the luciferase gene driven by the cytomegalovirus promoter (CMV-Luc). Fig. 1 (panel A) shows that the segment of the promoter between -100 and -50 from the transcription start site was essential for cAMP-induced transcription. The presence of the Sp1 site in the 5'-H A/M construct increased the basal activity of the promoter (4, 12), and partly masked the

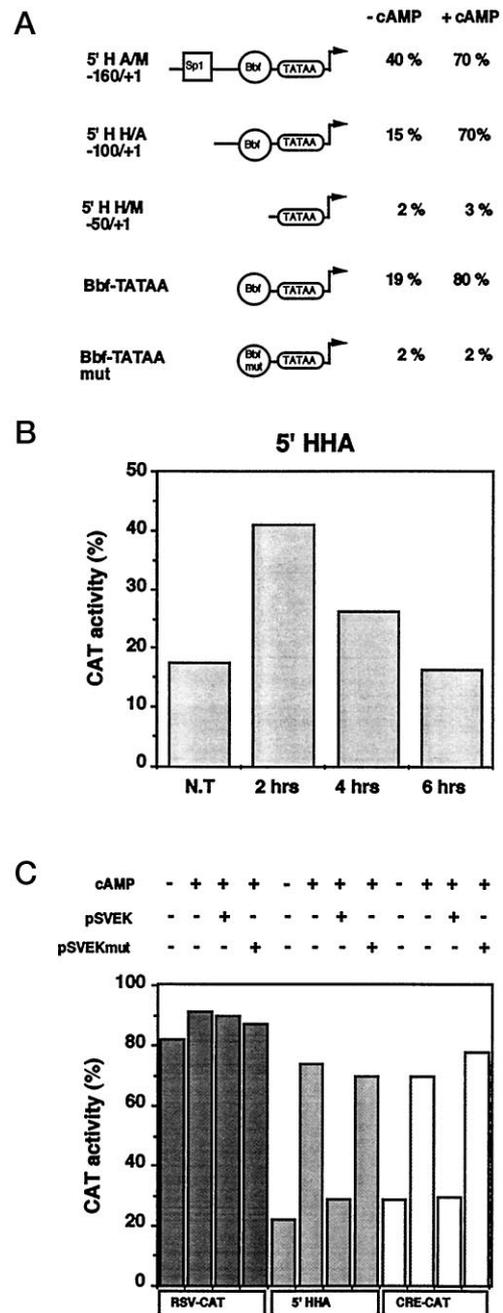
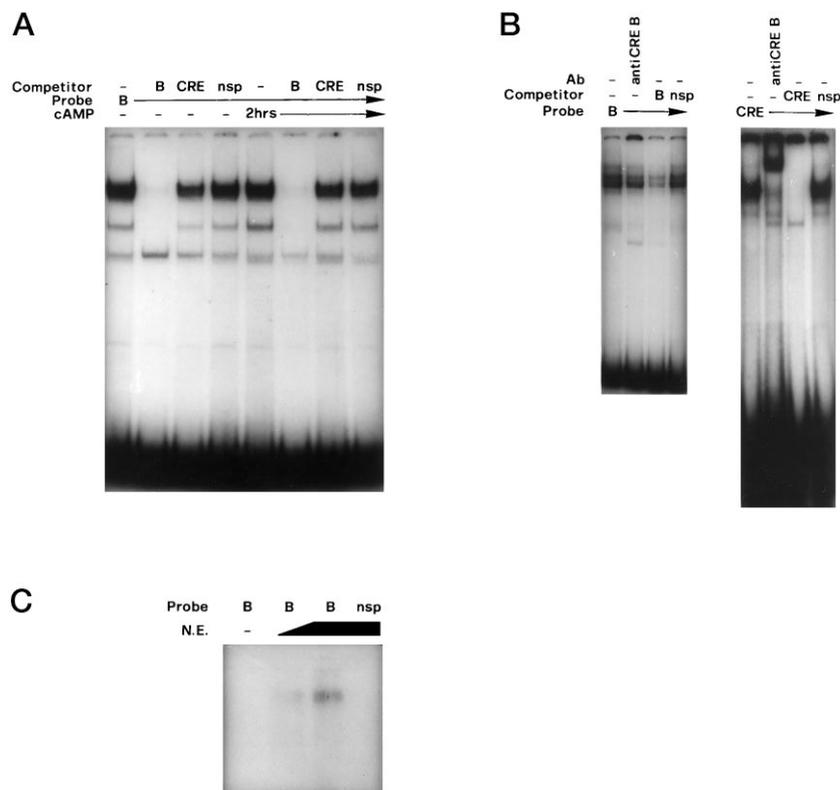


FIG. 1. The B site of the ferritin promoter mediates cAMP induction of transcription. Panel A, CAT activity driven by H ferritin promoter/CAT constructs, transiently transfected in HeLa cells stimulated or not with 10⁻⁶ M Bt₂cAMP for 4 h. On the left is shown the structure of the constructs (+1 is the transcription start site). The sequence of the B-site oligonucleotide (construct Bbf-TATAA) is: 5'-CGGCGCTGATTGGCCGGGCGGGC-3'. The sequence of the mutated B site oligonucleotide (construct Bbf_{mut}-TATAA) is: 5'-CGGCTCT-TACAGGCCGTTGCGAGC-3'. Panel B, CAT activity driven by the -100/+1 region of the H promoter (5'-H HA) in HeLa cells treated for the indicated times with dBcAMP. Panel C, CAT activity driven by the long terminal repeat of the Rous sarcoma virus (RSV-CAT); by the -100/+1 region of the H promoter (5'-H HA) and by a CRE-element/TK promoter construct (CRE-CAT) in HeLa cells treated with Bt₂cAMP and co-transfected with a PKA inhibitor (pSVEK) or with its inactive version (pSVEKmut). The values are the mean of three independent transfection experiments, and are expressed as percent of the acetylated form of the [¹⁴C]chloroamphenicol.

cAMP-induced transcription. Deletion of the -100 to -50 region inhibited significantly both the basal and cAMP induced transcription of the ferritin promoter. The region -100 to -50 contains the B site, which by footprint and EMSA appears to

² A. Porcellini, unpublished results.

FIG. 2. Bbf-DNA complex is not competed by a CRE site oligonucleotide, nor is it supershifted by an anti-CREB antibody. *Panel A*, 4 μ g of nuclear extracts from untreated HeLa cells and HeLa cells treated for 2 h with Bt₂cAMP were challenged with the region of the H promoter from -100 to +1 (B probe) terminally labeled, as described under "Materials and Methods." Competitions were performed by preincubating the nuclear extract with a 100-fold molar excess of the unlabeled CRE oligonucleotide (CRE) or with unspecific oligonucleotide (nsp). *Panel B*, the anti-CREB antibody was incubated with nuclear extract for 1 h on ice prior to addition of the -100 to +1 region (B probe) terminally labeled or the labeled CRE oligonucleotide. *Panel C*, 0.5 ng of terminally labeled B-site oligonucleotide (B probe) or unspecific oligonucleotide (nsp probe) were incubated with 25 μ g or with 50 μ g of HeLa nuclear extracts before UV irradiation, as described under "Materials and Methods."



bind only the Bbf complex. To determine if this site is responsible for cAMP induction of ferritin promoter, we synthesized a 20-base pair oligonucleotide spanning the wild-type Bbf binding sequence and an equivalent mutated version of this site, and fused these oligonucleotides to the minimal promoter element 5'-H H/M. Note that the mutated version of the oligonucleotide, tested by EMSA, did not bind Bbf (data not shown). The resulting constructs have been named Bbf-TATAA and Bbf_{mut}-TATAA, respectively. *Panel A* of Fig. 1 shows that the B oligonucleotide fused to the ferritin TATAA box transactivates the basal transcriptional activity of the resulting construct (compare the activities of constructs 5'-H H/A, 5'-H H/M, and Bbf-TATAA) and confers induction of transcription by cAMP. The Bbf_{mut}-TATAA construct shows a basal activity comparable to that given by the TATAA box only and does not respond to cAMP stimulation. Time course of the cAMP-induced transcription in cells expressing the -100/+1 promoter indicated that the maximal response was achieved within 1–2 h of continuous stimulation with cAMP (Fig. 1, *panel B*). The specificity of cAMP-induced transcription was tested by including in the transfection mixture a plasmid expressing the PKA-specific inhibitor tagged with a nuclear localization signal derived from SV40 LT antigen (pSVEK). cAMP-induced CAT expression driven by the ferritin promoter was completely inhibited by PKA-specific inhibitor, but not by its mutated version (pSVEK-mut) (Fig. 1, *panel C*). As positive control a CAT fusion driven by the CRE element derived from the somatostatin promoter was included (Fig. 1, *panel C*). The kinetics of induction of CAT activity was very similar to that of ferritin mRNA accumulation stimulated *in vivo* by cAMP (data not shown). These data indicate that the region of ferritin promoter between -100 to -50, where Bbf binds, contains the sequence element responsible for cAMP induction of transcription.

Bbf Binding Was Not Stimulated by cAMP—Inspection of the DNA sequence in the -100 to -50 region of ferritin promoter did not indicate the presence of a classical or spurious CRE site or a palindrome resembling the target of ATF/CREB family (for

review, see Ref. 18). DNase I footprinting analysis of this region with nuclear extracts from different cell types (4, 12) demonstrated that a single cis-element (B box) was recognized by the transcription factor Bbf. We determined the specificity of Bbf binding to its DNA element in cells stimulated with cAMP. *Panel A* of Fig. 2 shows that Bbf binding was not stimulated by cAMP and was not competed by a canonical CRE oligonucleotide. We have also analyzed the DNA-protein complexes at the CRE and Bbf sites in the presence of anti-CREB or anti-ATF1 antibodies. Bbf binding was not affected by the inclusion of the antibody (CREB) in the binding reaction; under the same conditions CRE-binding complexes were supershifted by the specific CREB antibody (Fig. 2, *panel B*). The same results have been obtained with an anti-ATF1 antibody (data not shown).

To identify more precisely Bbf, we cross-linked protein-DNA complexes to labeled B oligonucleotide and visualized the labeled band by SDS-polyacrylamide gel electrophoresis (Fig. 2, *panel C*). A single specific band of approximately 120 kDa was detected. This band was specifically removed by excess of specific unlabeled oligonucleotide (Fig. 2, *panel C*, and data not shown).

Bbf Interacts with the Coactivator p300—Recently, it has been shown that the nuclear proteins CBP and p300 interact with CREB to mediate cAMP induction of transcription. CBP and p300 are highly homologous (92%) and are thought to target CREB and other trans-acting factors to RNA polymerase initiation complex, by binding TFIIB (19).

We have explored the possibility that Bbf might use the same mechanism. To this end, we have immunoprecipitated HeLa total proteins with specific anti-p300 antibodies, eluted the immunoprecipitated proteins with deoxycholate, and analyzed the presence of Bbf by EMSA. *Panel A* of Fig. 3 shows that Bbf is present in the p300 immunocomplexes; CREB also was in the same immunocomplexes (Fig. 3, *panel B*). We have tested whether other transacting factors, such as CTF1 (Fig. 3, *panel C*) or NF κ B (not shown), were also forming complexes with p300. The results indicate that neither of the aforementioned

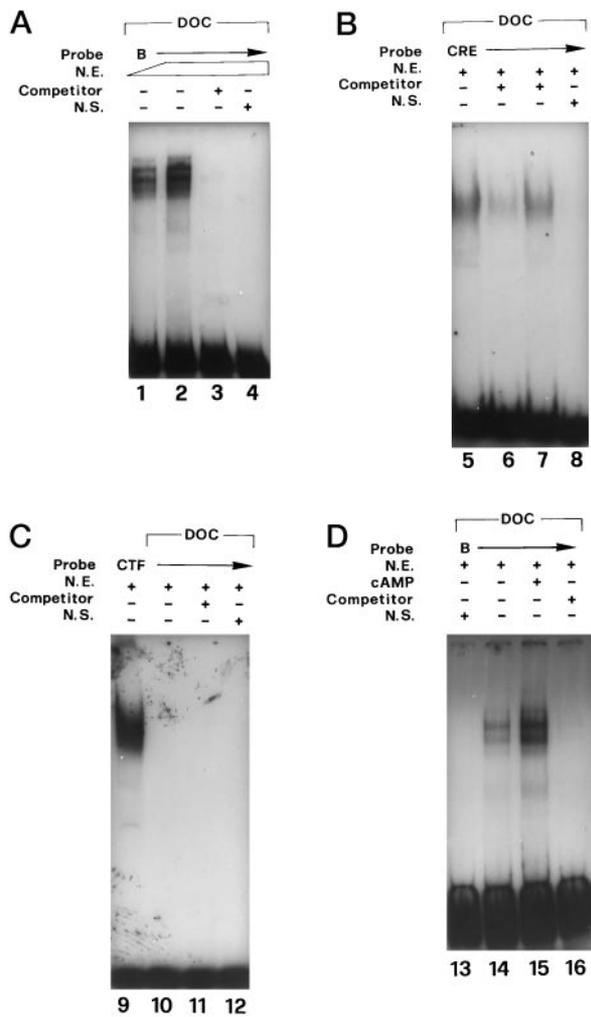


FIG. 3. Bbf is found in a DNA-bound complex with p300. Panel A, HeLa cell extracts were immunoprecipitated with anti-p300 antibody (lanes 1–3) or with non-immune antisera (N. S., lane 4) as indicated under “Materials and Methods.” Immunoprecipitates were eluted with deoxycholate (DOC) and two different concentrations were assayed by EMSA with the $-100/+1$ region from H promoter (B) as probe (lanes 1 and 2). Competition was performed by preincubating the eluted proteins with a 100-fold molar excess of the unlabeled B probe prior to addition of the labeled oligonucleotide (lane 3). Panel B, the same immunoprecipitates used for the experiment in panel A were assayed by EMSA with a CRE site oligonucleotide (CRE) as probe (lanes 5–7) or with non-immune antisera (N. S., lane 8). Competition were performed by preincubating the eluted proteins with a 100-fold molar excess of the unlabeled CRE-site probe (lane 6) or with a 100-fold molar excess of unrelated oligonucleotide (lane 7) prior to addition of the labeled probe. Panel C, lane 9, shows the EMSA of HeLa nuclear extract (4 μ g), challenged with a radiolabeled CTF site probe. The other lanes (DOC) show the same immunoprecipitates used for the experiment in panels A and B assayed by EMSA with the CTF site oligonucleotide (CTF) as probe. The specific competitions (lane 11) were as described for panels A and B. Panel D, extracts from HeLa cells stimulated or not with 10^{-6} M Bt_2 cAMP were immunoprecipitated with anti-p300 antibody (lanes 14 and 15) or with non-immune antisera (N. S., lane 13). Immunoprecipitates were eluted with deoxycholate (DOC) and assayed by EMSA with the $-100/+1$ region from the H promoter (B) as probe. Competition was performed by preincubating the eluted proteins with a 100-fold molar excess of the unlabeled B probe prior to addition of the labeled oligonucleotide (lane 16). The HeLa extracts used for these experiments were normalized for p300 content, as tested by Western blot analyses performed with the anti-p300 antibody (data not shown). The experiment presented has been repeated three times with similar results.

factors interacted with p300. Since cAMP does not stimulate the binding of Bbf to the promoter, we have tested whether Bbf binding to p300 was stimulated by cAMP. Fig. 3, panel D, shows that the binding of Bbf to p300 is markedly stimulated

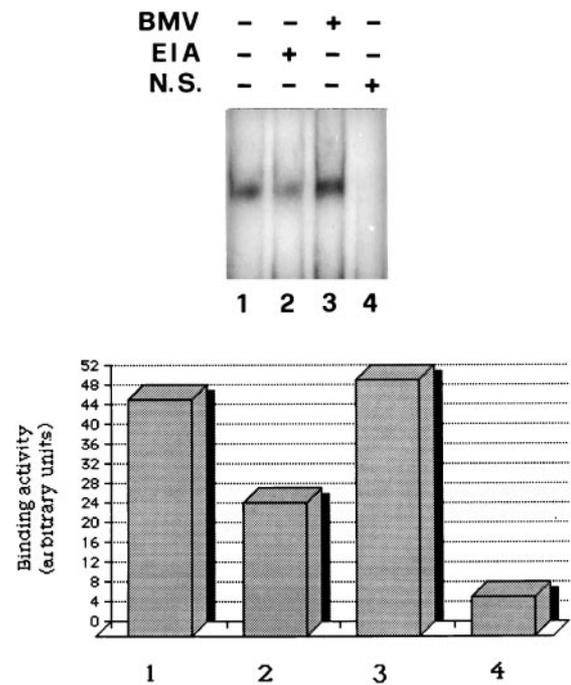


FIG. 4. E1A interferes with the p300-Bbf complex. Upper part, HeLa cells extracts were mixed with recombinant E1A protein or with recombinant BMV (tobacco mosaic virus) protein for 30 min at 4 °C prior to immunoprecipitation with anti-p300 antibodies or with non-immune antisera (N. S.) as indicated under “Materials and Methods.” Immunoprecipitates were eluted with deoxycholate and assayed by EMSA with the $-100/+1$ region from H promoter as probe. Lower part, the results of the experiment shown in the upper panel were quantitated by densitometric scanning of the x-ray film and expressed as arbitrary units.

in cAMP-treated extracts. At present we cannot determine if the phosphorylation of p300, Bbf1, or both stimulates the formation of the complex.

p300 is highly homologous to the protein CBP in the CREB-binding region (19). It is possible that Bbf could react with CBP as well. We have immunoprecipitated the HeLa whole cell extracts with specific anti-CBP antibody, and tested for the presence of Bbf. Similarly to p300, CBP immunoprecipitates contained Bbf (data not shown). These data indicate that Bbf interacts with p300 and CBP *in vivo*.

E1A Interacts with p300 and Reduces the Formation of the Complex Bbf-p300—p300 has been isolated as E1A binding nuclear protein (20). Since E1A represses ferritin transcription (11), we wished to determine whether E1A competed with the formation of Bbf-p300 complex. To this end, total extracts from HeLa cells were mixed with recombinant E1A protein prior to immunoprecipitation with anti-p300 antibodies. The immunoprecipitate was analyzed for Bbf binding activity by EMSA, as described above. Bbf-DNA retarded complex was inhibited when recombinant E1A was added to the extracts (Fig. 4). This inhibition was specific for E1A protein, since another recombinant protein (BMV) did not significantly reduce Bbf concentration in the p300 immunoprecipitates (Fig. 4). This result indicates that E1A competes with Bbf for the binding to p300.

p300 Rescues E1A-inhibited Ferritin Transcription—The mechanism of the E1A inhibition of ferritin transcription might be caused by competition of E1A with Bbf for p300 binding, as suggested by Fig. 4. To explore this possibility, we transiently expressed E1A carrying plasmids (12 S and 13 S) in several cell lines (HeLa, Chinese hamster ovary, and thyroid cells) with the $-100/+1$ ferritin-CAT construct (5'-H/HA). Fig. 5 shows that the transcription of the CAT gene, driven by the ferritin pro-

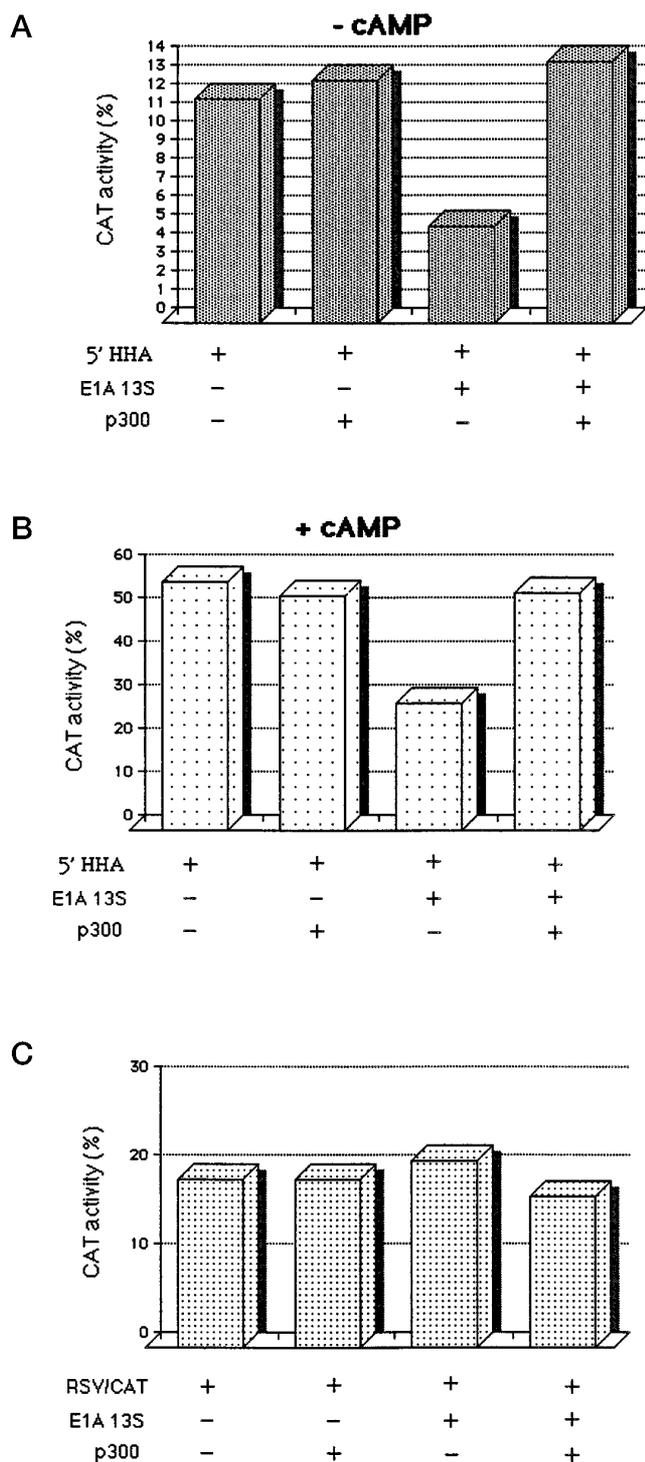


FIG. 5. p300 counteracts the E1A inhibition of ferritin transcription. Panel A, CAT activity driven by the $-100/+1$ region of the H promoter (construct 5'-H HA) in HeLa cells untreated (panel A) or treated with 10^{-6} M Bt₂cAMP (panel B) co-transfected with 13S E1A-expressing plasmid (E1A 13S) and/or with a p300-expressing plasmid (p300) (a gift of Dr. R. Eckner). Panel C, CAT activity driven by the long terminal repeat of the Rous sarcoma virus (RSV-CAT) in HeLa cells co-transfected with a 13S E1A-expressing plasmid (E1A 13S), and/or with a p300-expressing plasmid (p300). The values are the mean of three independent transfection experiments, and are expressed as percent of the acetylated form of the [¹⁴C]chloroamphenicol.

motor, is markedly inhibited by coexpression of E1A gene. Bbf appears to be the target of E1A repression, since the minimal ferritin promoter construct (5'-H H/M) is not affected by E1A expression.

Transient transfection of a p300-expressing plasmid in normal or in cAMP-treated HeLa cells slightly stimulated ferritin/CAT expression. Conversely, when p300 was co-transfected with E1A, the ferritin/CAT expression was restored at normal levels. Under the same conditions, p300 expression completely reversed the E1A inhibition of CAT transcription driven by CRE promoter (data not shown). The same results have been obtained with cells stably expressing E1A or transformed by adenovirus (data not shown). These data suggest that E1A reduces ferritin transcription by titrating p300 and inhibiting the assembly of productive p300 complexes with Bbf.

DISCUSSION

Expression of ferritin is controlled by intracellular (such as iron) and by extracellular (hormones, growth factors) signals (2, 3, 7). The expression of the protein is controlled at transcriptional, post-transcriptional, and translational levels (1). The multiplicity of the stimuli and the mechanisms governing the expression of the gene explain the variety of ferritin phenotypes exploited by the cell under different conditions.

Mechanism of cAMP-PKA Stimulation of Ferritin Gene Transcription—H ferritin mRNA levels and transcription of the gene *in vivo* are stimulated by cAMP (3, 10, 21, 22). The identification of the primary target sequences responsive to cAMP signaling in the H ferritin promoter has not been carried out extensively. The human H ferritin promoter is a compact element, where the basic and the upstream control elements are contained in about 170 nucleotides upstream the transcription start site (12). In the mouse promoter it has been also described as an enhancer element located at -4.1 kilobases from the start of transcription (23). We have restricted our analysis to the main promoter element, previously mapped by mutagenesis (12). Two sites have been mapped in this region, recognized by Sp1 (at -132) and Bbf (at -62), respectively. Bbf is a factor not yet cloned, which by competition analysis and size does not appear to be a known nuclear transacting protein (4, 12). The deletion of the element recognized by Bbf completely abolishes both the basal and the cAMP-induced transcription driven by the ferritin promoter. Moreover, fusion of a B-site oligonucleotide to the ferritin minimal promoter element confers cAMP stimulation of transcription. Bbf does not seem to be a CREB or ATF1 factor, since it is not competed by the specific oligonucleotides and its binding is not affected by anti-ATF1 or anti-CREB antibodies (Fig. 2). Note that we have used anti-CREB antibodies which recognize the KID domain, a common structural motif present in all members of the CREB family (18). However, Bbf shows all the functional properties of a CREB protein (Fig. 2): its binding is not sensitive to cAMP (Fig. 2), it is phosphorylated (data not shown), and it binds the nuclear adaptor molecules, CBP and p300 (Fig. 3). Definite answer to the question of Bbf identity has to await the molecular cloning of the protein. We have noted that a sequence similar to the Bbf-binding site (5'-CGGCGCTGATTGGC-CGGGGCGGGC-3') is present in the promoter of the human tryptophan hydroxylase gene and in the promoter of the rat fatty acid synthase gene. This sequence has been shown to be the target of cAMP induced transcription (24, 25). We suggest that this DNA element identify a new class of transacting factor(s) which mediate(s) cAMP-induction of transcription.

p300 Binds Bbf and Mediates cAMP Induction of Transcription—p300 and CBP are two high molecular weight proteins, resident in the nucleus, which have an intrinsic transcription activation function (26, 27). They bind TFIIB (19), as well as other transacting factors, such as CREB, Jun, and YY1 (27–29). The interaction with CREB and Jun is facilitated by phosphorylation of these proteins (19, 30). We have shown that also Bbf interacts *in vivo* with p300 and CBP (Fig. 3). We suggest that

p300 and CBP function as molecular adaptors which bring together Bbf and the RNA polymerase transcription initiation complex. Since the DNA binding of Bbf is not stimulated by cAMP or by treatment with okadaic acid (data not shown), we suggest that Bbf is permanently located on the DNA and that cAMP signaling stimulates the assembly of the complex Bbf-p300. This is consistent with our finding (Fig. 3, panel D) that BBF-p300 complex is stimulated by cAMP. Preliminary data indicate that both p300 and Bbf are phosphorylated by PKA. At present we cannot determine whether cAMP phosphorylation of Bbf directly stimulates the formation of the complex.

E1A Represses Ferritin Transcription by Interfering with the Formation of the Complex Bbf-p300—Ferritin composition is altered in some tumors (31, 32). The mechanisms underlying the ferritin phenotypes remains unknown. Adenovirus E1A expression in mouse cells induces preferential down-regulation of H ferritin transcription and results in the production of ferritin molecules with altered H-L ratio (11, 23). The data we have shown indicate that E1A protein reduces the formation of p300-Bbf complex *in vitro* (Fig. 4). This reduction parallels the inhibition of transcription *in vivo* by E1A (Fig. 5). Overexpression of p300 completely reverses the transcription block and restores ferritin expression (Fig. 5). In our conditions, we did not detect any significant stimulation of ferritin promoter, at variance with the CREB-mediated activation shown by others (26, 27). Note, that in the latter cases CREB was overexpressed (26, 27). This finding suggests that BBF1 is limiting *in vivo* and that p300 alone cannot stimulate ferritin transcription. E1A expression possibly titrates p300 and reduces the formation of the active complex; under these conditions, expression of exogenous p300 shifts the equilibrium from E1A-p300 to Bbf-p300 and reactivates ferritin transcription. At present we do not know if cAMP might alter the equilibrium between these complexes. We have evidence that ferritin transcription is not efficiently induced by cAMP in the presence of E1A. It is likely that the equilibrium between p300/Bbf and p300/E1A depends on the relative concentrations of active Bbf and E1A proteins.

Recently, it has been reported that an enhancer element, found at -4.1 kilobase from the start of transcription of the mouse H ferritin gene, is the target of E1A that mediates the repression of ferritin transcription (23). This sequence element located far upstream from the promoter, relative to the region target of Bbf, contains an AP1-like site. This site probably positively controls the ferritin transcription induced by other extracellular stimuli. At this site we predict p300 or CBP to play an essential role, since they can form complexes with Jun and other components of the AP1 complex (28). E1A expression in principle should disrupt both AP1 and Bbf complexes, re-

sulting in the complete inhibition of ferritin transcription.

We propose that the -4.1 kilobase enhancer is sensitive to signal-targeted transcriptional activators (30), while the Bbf site is induced by cAMP signaling. The interplay between cAMP and tyrosine kinases signals *in vivo* regulates the transcription of H ferritin gene.

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A Common Mechanism Underlying the E1A Repression and the cAMP Stimulation of the H Ferritin Transcription

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