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Identification of the Human β-Actin Enhancer and Its Binding Factor

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An enhancer of the human β-actin gene and a factor that specifically interacts with it were detected. A mobility shift assay showed that the factor bound to the 25-base-pair sequence (between +759 and +783 downstream from the cap site) with high specificity. This finding correlated with those of DNase I protection and exonuclease III digestion assays. This binding region of the β-actin enhancer contained a hyphenated dyad symmetry and an enhancer core-like sequence. In vitro competition experiments indicated that the factor did not bind to the simian virus 40 enhancer core region.

Transcription of many eucaryotic genes is regulated by enhancer elements, which are usually defined as *cis*-acting DNA sequences 100 to 200 base pairs (bp) long that act in either orientation and over a considerable distance to activate transcription from a linked homologous or heterologous promoter (25). While no single sequence motif is common to all enhancers, a so-called enhancer core consensus sequence has been reported for elements found in many enhancers (14, 25, 32). Recently, an in vitro transcription system or protein-DNA mobility shift assay has proved useful in identifying some *trans*-acting factors that interact with discrete upstream elements (5, 25). For example, a *trans*-acting factor that binds to a conserved sequence motif (ATGCAAAT) in the immunoglobulin heavy-chain enhancer, immunoglobulin promoters, the histone H2B promoter, human U2 and amphibian U1/U2 small nuclear RNA gene promoters, and the simian virus 40 (SV40) enhancer has been shown to be present in both B cells and HeLa cells (1, 27, 29, 30). By using these in vitro systems, some *trans*-acting factors have been identified.

β-Actin is one of the most abundant proteins in eucaryotic cells and is expressed in a variety of tissues, although there is only one functional β-actin gene per haploid genome (20, 21). Thus, it is of interest to know the regulatory mechanisms involved in expression of the β-actin gene. We and others reported that the human β-actin gene contains five introns (20, 21). A large intron was found in the 5′ untranslated region 6 nucleotides upstream from the ATG initiation codon. The other four introns were found within the coding region at codons 267 and between 41 and 42, 121 and 122, and 327 and 328. Recently, we found that the transcriptional activity of the 5′ untranslated region about 1.2 kilobases (kb) of the human β-actin gene was higher than that of SV40 and that its first intron sequences show enhancer activity (manuscript in preparation).

To determine the region required for enhancer activity and to search for transcription factors interacting with the β-actin enhancer, we constructed a series of deletion mutations in the enhancer region of the human β-actin gene and performed in vivo chloramphenicol acetyltransferase (CAT), mobility shift, DNase I protection, and exonuclease III digestion assays.

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MATERIALS AND METHODS

Construction of plasmids. The *Bgl*II-*Bam*HI DNA fragment which contains the *cat* gene with the SV40 early promoter lacking 72-bp repeats, was obtained from the plasmid pA10CAT2 (14) and inserted into the *Sal*I site of pUC18 by using a *Sal*I linker (pUA10CAT; see Fig. 3C). The human β-actin 84-bp fragment (+742 to +825 downstream from the cap site [21]) was obtained by double digestion of the human β-actin gene (20) with *Sac*II and *Nae*I (see Fig. 3A). This fragment was blunt-ended with T4 DNA polymerase and subcloned into the *Sma*I site of pUA10CAT (pUA10βAECAT825).

Deletion mutants were constructed as described by Hong (11).

Transfection and CAT assay. DNA transfection into NIH 3T3 cells was performed by the calcium phosphate-DNA coprecipitation method (10). CAT activities were assayed by the procedure described by Gorman et al. (9). Briefly, NIH 3T3 cells were transfected with *cat* plasmid DNAs, 24 h after being seeded at 5×10^5 cells in 100-mm-diameter dishes. At 48 h after transfection, the cells were harvested, and the cell extracts were incubated with [^{14}C]chloramphenicol and acetyl coenzyme A for 1 h at 37°C. The samples were analyzed on a silica gel thin-layer plate. Spots containing acetylated chloramphenicol were cut out and quantitated by liquid scintillation counting.

DNA probes. The β-actin enhancer fragment (+742 to +825) was excised from pUA10βAECAT825 by digestion with *Kpn*I and *Bam*HI and 5′-end labeled at the *Bam*HI site. The *Eco*RI-*Bam*HI fragment containing sequences from +742 to +793, which was excised from pUA10βAECAT793, was 5′-end labeled at the *Eco*RI site. These fragments were used for mobility shift, exonuclease III digestion, and DNase I protection assays.

Mobility shift assay. A HeLa cell extract was prepared as described by Manley et al. (16). Approximately 0.15 ng of the ^{32}P-labeled DNA fragments were mixed with 2.5 μg of poly[d(I-C)], 10 μg of protein, and various amounts of competitor DNAs in a total volume of 10 μl of a solution containing 10 mM Tris (pH 7.5), 50 mM NaCl, 1 mM dithiothreitol, 1 mM EDTA, and 5% glycerol. The samples were incubated for 10 min at 37°C and loaded onto a 4% polyacrylamide gel. The gel was electrophoresed at 120 V

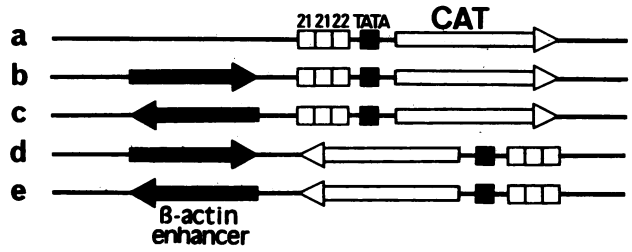

FIG. 1. Illustration of the position and the orientation of the inserted β-actin gene adjacent to the *cat* gene. The β-actin *Sac*II-*Nae*I fragment (+742 to +825) was inserted into the 5′ or 3′ side of the *cat* gene in both orientations, as described in the text. The transcriptional activities of the resulting plasmids are shown in Fig. 2. Open arrow, *cat* gene; solid arrow, β-actin enhancer; open and solid boxes, the SV40 early promoter lacking 72-bp repeats. Numbers above open boxes indicate number of base pairs of each repeated sequence.

with constant circulation of the electrophoresis buffer (6.7 mM Tris, pH 7.5, 3.3 mM sodium acetate, and 1 mM EDTA) (6, 8, 27). Labeled DNAs, free or bound form, were visualized by autoradiography.

Oligonucleotides. Three double-stranded 27-mer oligonucleotides were synthesized, one corresponding to the SV40 enhancer core region (CAGCAACCAGGTGTGGAAAG TCCCCAG) (14), one to the c-*fos* serum response element (CCAGGATGTCCATATTAGGACATCTGG) (31), and the third to the β-actin enhancer sequences from +759 to +783 (CGTGTTTGCCTTTTATGGTAATAACGG). These oligonucleotides were used to test for competition with the β-actin enhancer sequence for binding to factors present in a HeLa cell extract.

Exonuclease III digestion assay. The exonuclease III digestion assay was performed by the methods of Wu and Wu et al. (33, 34). DNA-factor binding reactions were carried out as described for the mobility shift assay except that the concentrations of labeled DNAs, protein, and poly[d(I-C)] were 0.3 ng, 20 μg, and 5 μg/20 μl, respectively. After incubation for complex formation, the concentration of $MgCl_2$ was adjusted to 10 mM, and 180 U of exonuclease III was added. Digestion proceeded at 30°C for 20 min and was stopped by the addition of phenol. The samples were extracted with phenol, precipitated with ethanol, and applied to an 8% polyacrylamide–urea sequencing gel.

Column chromatography. A HeLa cell extract was dialyzed in a buffer containing 12 mM Tris (pH 7.9), 7.5 mM $MgCl_2$, 0.06 mM EDTA, 1.2 mM dithiothreitol, and 10% glycerol. The extract was applied to a phosphocellulose column (Whatman P11) and eluted with a 40-ml linear gradient of NaCl (0 to 1 M). Fractions (1.4 ml each) were collected, and the DNase I protection assay was performed.

DNase I protection assay. The binding reaction mixture (30 μl) consisted of 0.7 μg of DNA in 10 mM Tris (pH 7.5), 50 mM NaCl, 1 mM dithiothreitol, 1 mM EDTA, 2% polyvinyl alcohol, and 5% glycerol, containing 5 μg of protein and 0.6 μg of poly[d(I-C)]. After incubation at 37°C for 15 min, the concentration of $MgCl_2$ was adjusted to 3.3 mM, and the samples were treated with DNase I for 5 min at 37°C. Digestion was stopped by the addition of phenol, and the samples were extracted with phenol and precipitated with ethanol. Recovered fragments were electrophoresed in an 8% polyacrylamide–urea sequencing gel and visualized autoradiographically (7, 12).

RESULTS

Enhancer activity of the first intron sequences of the human β-actin gene. The plasmid pA10CAT2, which has only a low level of CAT activity, was used to test for the enhancer activity of a DNA sequence (18). The *Bgl*II-*Bam*HI DNA fragment of the plasmid pA10CAT2, which contains the *cat* gene with the SV40 early promoter but lacks a functional enhancer, was inserted into the *Sal*I site of pUC18 in both orientations by using a *Sal*I linker. The *Sac*II-NaeI fragment (+742 to +825), which contained an enhancer core-like sequence, from the first intron of the β-actin gene was ligated in both orientations to the 5′ or 3′ side of the *cat* gene (Fig. 1). Insertion of this *Sac*II-*Nae*I fragment in either orientation resulted in a remarkable increase in the CAT activity in NIH 3T3 cells when assayed 48 h after transfection with plasmid DNAs (Fig. 2). The results indicate that the sequence between +742 and +825 in the first intron of the human β-actin gene has enhancer activity.

Enhancer sequences. To determine the 3′ border of the β-actin enhancer sequence, we constructed an extensive set of deletion mutants (Fig. 3B). We performed CAT assays several times to overcome the variability inherent in this type of experiment. Figure 4 shows the CAT activities obtained with 3′ deletion mutant plasmids. The CAT activity level of the wild type was retained in a +793 mutant (Fig. 4, lane 3). However, when sequences between +783 and +793 were removed, the CAT activity decreased to about 40% of the wild-type level (lane 4). Deletion from +770 to +783 further decreased the CAT activity to 5% of the wild-type level (lane 5). These results indicate that sequences between +770 and +783 are very important for enhancer activity and that sequences between +783 and +793 are also required for maximal activity.

An enhancer-binding factor. The mobility shift assay was used to detect factors that interacted specifically with the β-actin enhancer. When end-labeled β-actin *Kpn*I-*Bam*HI fragments containing sequences from +742 to +825 were mixed with a HeLa cell extract, factor-DNA complexes migrated more slowly than free DNA fragments (Fig. 5A, lane N). To determine whether the factor in the slowly migrating band was specifically bound to the β-actin enhancer, an excessive amount of unlabeled competitor DNAs

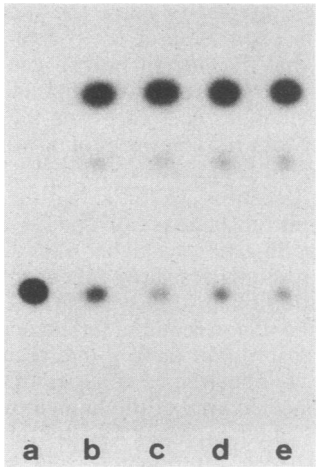

FIG. 2. Enhancer activity of the β-actin *Sac*II-*Nae*I fragment (+742 to +825) on the SV40 promoter. Plasmid construction is shown in Fig. 1; a, b, c, d, and e correspond to the constructs shown in Fig. 1. CAT assays are described in the text.

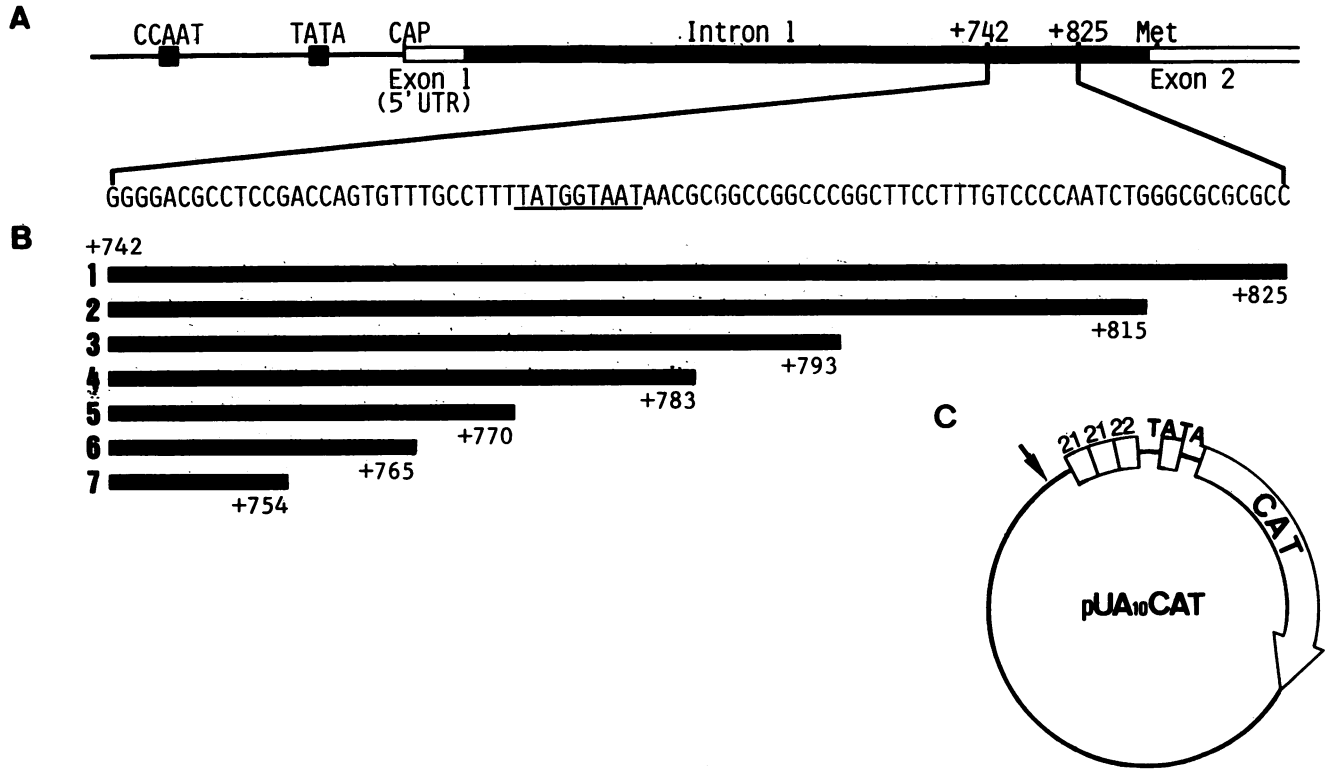

FIG. 3. (A) Map of a portion of the human β-actin gene, showing the promoter, exon 1, intron 1, and exon 2. The entire sequence of the 84-bp *Sac*II-*Nae*I fragment, with the enhancer core-like sequence underlined, is shown below the map. Solid bar, Intron; open bars, exons; solid boxes, CCAAT and TATA boxes; UTR, untranslated region; Met, translation initiation site. Numbers indicate nucleotide positions relative to the cap site (21). (B) 3′ deletions in the β-actin 84-bp fragment. The nucleotide positions of deletion endpoints are shown below the solid bars. These fragments were inserted into pUA10CAT (C), and their enhancer activities are shown in Fig. 4. (C) Outline of pUA10CAT used for testing enhancer activity of the deletion mutants. Open arrow, *cat* gene; open boxes, SV40 early promoter lacking 72-bp repeats; arrow, insertion site of β-actin enhancer deletion mutants.

was added. As shown in Fig. 5A, the intensity of the band corresponding to the factor-DNA complexes was reduced dose-dependently by the addition of β-actin enhancer fragments (lanes 1 to 3), but *Hin*fI-digested pUC9 fragments did not compete even when present in 250-fold molar excess (lanes 4 to 6). To determine the sequences required for factor binding, DNA fragments of various lengths (Fig. 3B) were examined for competition for the binding to the factor. While DNA fragments containing sequences between +742 and +783 competed for binding (Fig. 5B, lanes 1 to 4), the sequence deleted to +770 did not compete efficiently (Fig. 5B, lanes 5 to 7). These results indicate that the β-actin enhancer sequence contains a unique binding site for the factor and that this binding site at least partially overlaps sequences from +770 to +783. The sequences from +763 to +779 are completely conserved among human (20, 21), rat (22), and chicken (13) DNAs. Therefore, an oligonucleotide containing human β-actin gene sequences between +759 and +783 was synthesized to examine its binding affinity to the factor. The oligonucleotide competed efficiently, and the 25-bp sequence between +759 and +783 was sufficient for factor binding (Fig. 5C, lanes 1 and 2). To test whether the binding factor also bound to other elements, oligonucleotides of the SV40 enhancer core region and the c-*fos* serum response element were used as competitors. As seen in Fig. 5C, neither of these competitors affected complex formation by the β-actin DNA fragments. The more rapidly migrating bands detected in the gels were ascribed to nonspecific

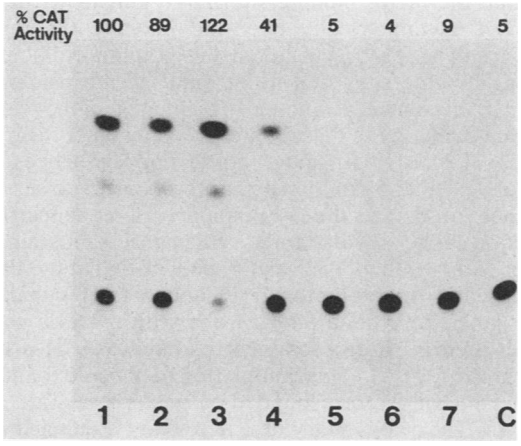

FIG. 4. Transient expression of CAT activity in NIH 3T3 cells after transfection with *cat* recombinant DNAs containing various lengths of the β-actin enhancer region. NIH 3T3 cells were transfected with the indicated recombinant DNAs, and CAT activities were determined as described in the text. Relative CAT activities are expressed as a percentage of the activity of the parent pUA10βAECAT825 plasmid. Lanes 1 to 7, Plasmid DNAs carrying various 3′-deleted β-actin enhancer regions; the relative positions of 3′ deletion endpoints and the recombinant plasmids are shown in Fig. 3B and C. Lane C, Control plasmid DNA without the β-actin enhancer region (pUA10CAT).

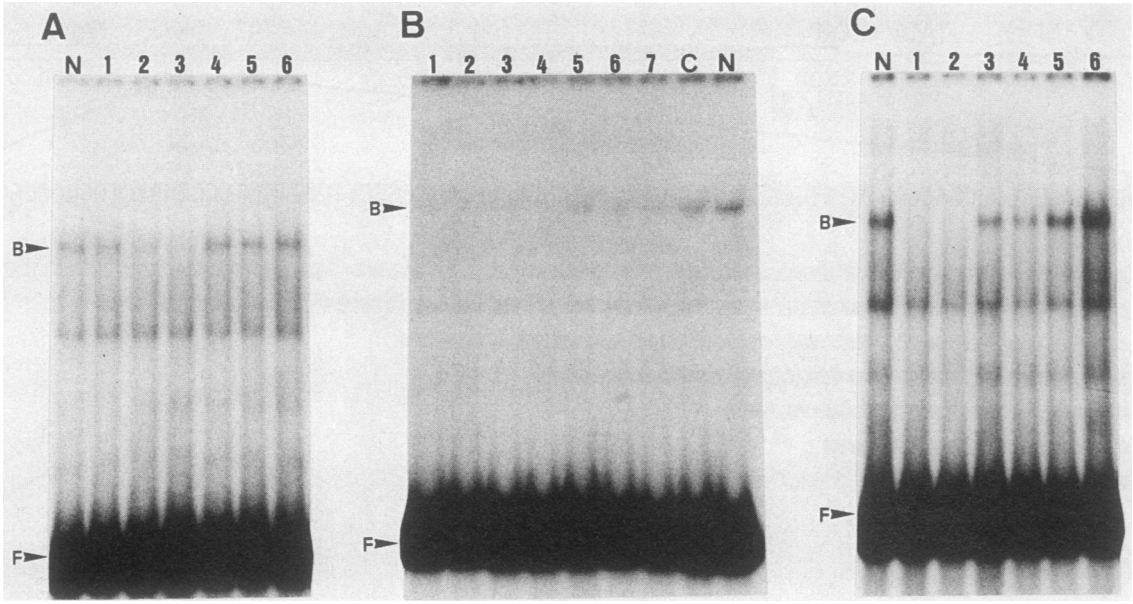

FIG. 5. Competition analysis of factor binding. The end-labeled β-actin enhancer fragments (+742 to +825) were incubated with a HeLa cell extract and electrophoresed on a 4% polyacrylamide gel. Bands representing β-actin enhancer DNA-factor complexes were identified. F and B indicate positions of free and bound DNA fragments, respectively. (A) Competition with the β-actin enhancer fragment and *Hin*fI-digested pUC9 fragment. Lane N, Control, no competitor; lanes 1 to 3, competition with the sequences of β-actin enhancer region from +742 to +783 (62 bp); lanes 4 to 6, competition with the *Hin*fI-digested pUC9 fragment (65 bp). Binding competition reaction mixes contained 10-fold (lanes 1 and 4), 50-fold (lanes 2 and 5), or 250-fold (lanes 3 and 6) molar excess of competitor DNA. (B) Competition with 3′ deletion mutants. To map the sequences required for factor binding, 100-fold molar excesses of unlabeled enhancer mutant DNAs (about 0.6 kb) with deletions originating from the 3′ side of the enhancer were added to the reaction mixture. Lanes 1 to 7, Competition with DNAs containing various 3′ deletion fragments; the deletion endpoints of competitor DNAs are indicated in Fig. 3B. Lane C, Control competition with DNAs without β-actin gene insert; lane N, control, no competitor. (C) Competition with synthetic oligonucleotides (27-mers). Lane N, Control, no competitor; lanes 1 and 2, competition with the β-actin enhancer; lanes 3 and 4, competition with the SV40 enhancer core region; lanes 5 and 6, competition with the c-*fos* serum response element. Binding reaction mixes contained 150-fold (lanes 1, 3, and 5) or 300-fold (lanes 2, 4, and 6) molar excess of competitor DNA.

DNA-protein binding, since their intensities were not substantially altered by the presence of any competitor.

An exonuclease III digestion assay was carried out to determine the factor-binding site. DNA fragments that are positioned asymmetrically over the putative binding site were chosen as probes to avoid the potential difficulty of detecting binding sites that lie beyond the intrinsic exonuclease III resistance at one-half fragment length, which is generated by nuclease digestion proceeding on both strands. The *Eco*RI-*Bam*HI fragment containing sequences from +742 to +793 and 5′-end labeled at the *Eco*RI site served as the probe to detect the 3′ boundary of enhancer-factor interaction. The *Kpn*I-*Bam*HI fragment, containing sequences from +742 to +825 and 5′-end labeled at the *Bam*HI site, served as the probe for the 5′ boundary. Incubation of the ^{32}P-labeled *Eco*RI-*Bam*HI probe with a HeLa cell extract resulted in three prominent exonuclease III-resistant fragments (Fig. 6A). The boundaries of these exonuclease III-resistant bands corresponded to positions +772, +774, and +775. The 5′ boundary detected with ^{32}P-labeled *Kpn*I-*Bam*HI probe corresponded to position +748 (Fig. 6B). These results indicate that factor binding to the β-actin enhancer protected the region between approximately +748 and +775 from exonuclease III digestion. To confirm these results, the binding of this factor was visualized by the DNase I protection assay. The factor was partially purified by phosphocellulose column chromatography (Whatman P11). The region between approximately +749 and +788 was protected from DNase I digestion by preincubation with the fractions of a HeLa cell extract eluted at approximately 0.35 M NaCl (Fig. 7). The data from exonuclease III digestion and DNase I protection assays are summarized in Fig. 8.

DISCUSSION

In this paper we have shown that sequences which bound to a factor present in a HeLa cell extract had enhancer activity. Competition assays with deletion mutant DNAs and synthetic oligonucleotides of the β-actin enhancer sequence indicated that the sequences from +759 to +783 were sufficient for factor binding. DNase I protection and exonuclease III digestion assays delineated the region of factor binding between approximately +748 and +788 downstream from the β-actin cap site. This binding site contained a region of dyad symmetry and an enhancer core-like sequence.

A so-called enhancer core sequence is found in many enhancers, and such a sequence may be the target for a general *trans*-acting factor. Sassone-Corsi et al. (26) showed that the factor that interacted with the SV40 enhancer also interacted with some other enhancer elements, such as the adenovirus type 2 E1A enhancer and the immunoglobulin heavy-chain enhancer. However, these interactions cannot be a universal feature of all enhancers. The SV40 enhancer did not efficiently compete with the polyomavirus B enhancer (24) or the insulin enhancer (23) for binding to the nuclear proteins, although all these enhancers contain one copy of the enhancer core sequence (23). Moreover, some upstream elements that contain corelike sequences do not show all the properties of enhancers. Thus, interactions

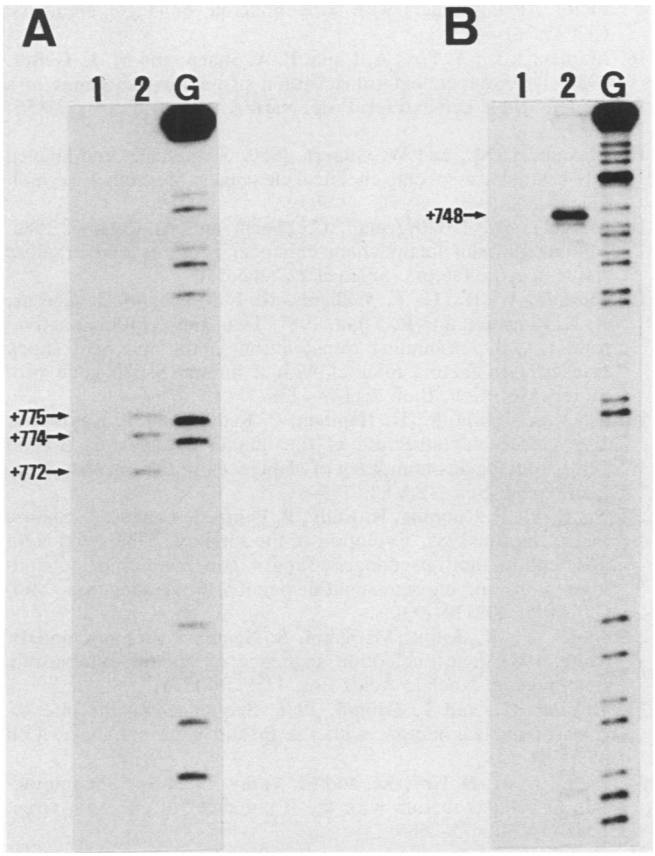

FIG. 6. Exonuclease III digestion assay. Panels A and B indicate the upper and lower strands of the β-actin enhancer region, respectively. (A) Binding and exonuclease III reactions were performed with the ^{32}P-labeled β-actin enhancer *Eco*RI-*Bam*HI fragment from +742 to +793. Lane 1, Reaction in the absence of extract; lane 2, reaction with a HeLa cell extract; lane G, Maxam and Gilbert G sequence of the labeled DNA fragment (17). Arrows point to exonuclease III-resistant fragments, whose corresponding positions on the β-actin enhancer region are given in Fig. 8. (B) Binding and exonuclease III reaction were performed with the ^{32}P-labeled β-actin enhancer *Kpn*I-*Bam*HI fragment from +742 to +825. Lane 1, Reaction in the absence of extract; lane 2, reaction with a HeLa cell extract; lane G, Maxam and Gilbert G sequence of the labeled DNA fragment.

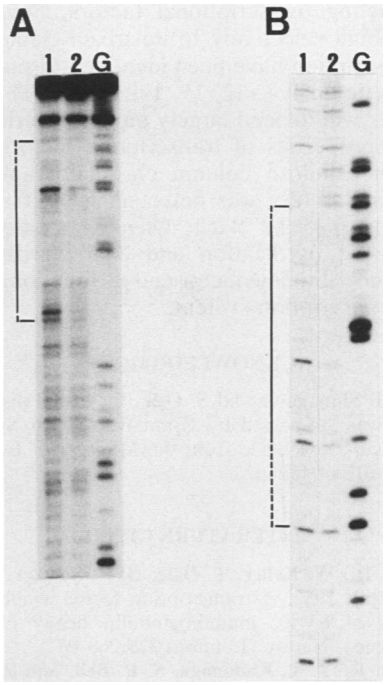

FIG. 7. DNase I protection assay with a partially purified binding factor on the β-actin enhancer upper strand (A) and lower strand (B). Lane 1, Control without extract; lane 2, reaction with extract; lane G, Maxam and Gilbert G sequence. Brackets show the region protected from DNase I cleavage. Dashed lines show the boundary area.

between *trans*-acting factors and enhancers are considerably specific and probably complicated.

In our studies the SV40 enhancer core region did not compete with the β-actin enhancer for factor binding, although the β-actin enhancer contains a corelike sequence. This result might indicate that the β-actin enhancer-binding factor does not bind to the core nucleotide but to the surrounding sequences, since the β-actin enhancer also includes a dyad symmetry sequence in which the enhancer core-like sequence is located. The region (+763 to +779) of dyad symmetry is completely conserved among human (20, 21), rat (22), and chicken (13) β-actin genes. Competition experiments with synthetic oligonucleotides indicated that the dyad symmetry sequence was sufficient for factor binding. Thus, the dyad symmetry could be the target for the β-actin-specific *trans*-acting factor.

It has become increasingly clear that transcriptional regulation in higher eucaryotes is dependent on the combined action of multiple specific DNA-binding factors interacting with distinct control elements (2, 3). At least some of the sequence-specific transcription factors are required for initiation at many promoters. Certain promoters require multiple distinct DNA-binding factors working in conjunction with each other to modulate the level of transcription. Enhancer elements have also been shown to be targets for interaction

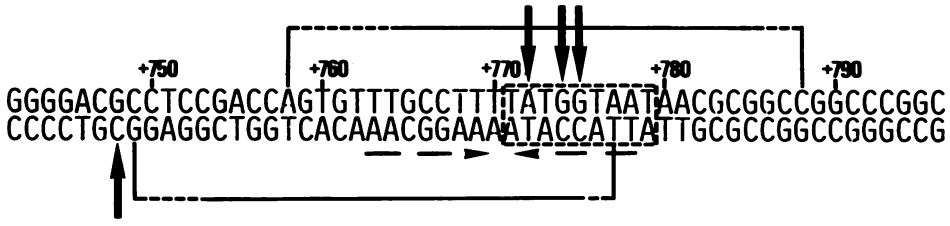

FIG. 8. Summary of DNase I protection and exonuclease III digestion assay results. Brackets, Regions protected from DNase I cleavage; vertical arrows, boundaries of factor binding derived by resistance to exonuclease III; dotted box, enhancer core-like sequence; horizontal arrows, inverted repeat.

with *trans*-acting transcriptional factors. Several cellular factors that bind selectively to upstream elements and potentiate transcription have been identified through biochemical and genetic analysis (2, 15, 19, 28, 31). The success of future studies will depend largely on characterization of the biochemical properties of transcription factors (2, 4). We used phosphocellulose column chromatography, and enhancer binding affinity was detected in fractions eluted at approximately 0.35 M NaCl. Ongoing studies are being directed toward purification and characterization of the β-actin enhancer-binding factor and its functional analysis in an in vitro transcription system.

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LITERATURE CITED

1. **Bohmann, D., W. Keller, T. Dale, H. R. Schöler, G. Tebb, and I. W. Mattaj.** 1987. A transcription factor which binds to the enhancers of SV40, immunoglobulin heavy chain and U2 snRNA genes. Nature (London) **325**:268–272.
2. **Briggs, M. R., J. T. Kadonaga, S. P. Bell, and R. Tjian.** 1986. Purification and biochemical characterization of the promoter-specific transcription factor, Sp1. Science **234**:47–52.
3. **Dailey, L., S. M. Hanly, R. G. Roeder, and N. Heintz.** 1986. Distinct transcription factors bind specifically to two regions of the human histone H4 promoter. Proc. Natl. Acad. Sci. USA **83**:7241–7245.
4. **Diffley, J. F. X., and B. Stillman.** 1986. Purification of a cellular, double-stranded DNA-binding protein required for initiation of adenovirus DNA replication by using a rapid filter-binding assay. Mol. Cell. Biol. **6**:1363–1373.
5. **Dynan, W. S., and R. Tjian.** 1983. Isolation of transcription factors that discriminate between different promoters recognized by RNA polymerase II. Cell **32**:669–680.
6. **Fried, M., and D. M. Crothers.** 1981. Equilibria and kinetics of lac repressor-operator interactions by polyacrylamide gel electrophoresis. Nucleic Acids Res. **9**:6505–6525.
7. **Galas, D. J., and A. Schmitz.** 1978. DNAase footprinting: a simple method for the detection of protein-DNA binding specificity. Nucleic Acids Res. **5**:3157–3170.
8. **Garner, M. M., and A. Revzin.** 1981. A gel electrophoresis method for quantifying the binding of proteins to specific DNA regions: application to components of *Escherichia coli* lactose operon regulatory system. Nucleic Acids Res. **9**:3047–3060.
9. **Gorman, C. M., L. F. Moffat, and B. H. Howard.** 1982. Recombinant genomes which express chloramphenicol acetyltransferase in mammalian cells. Mol. Cell. Biol. **2**:1044–1051.
10. **Graham, F. L., and A. J. van der Eb.** 1973. A new technique for the assay of infectivity of human adenovirus 5 DNA. Virology **52**:456–467.
11. **Hong, G. F.** 1982. A systemic DNA sequencing strategy. J. Mol. Biol. **158**:539–549.
12. **Jones, K. A., K. R. Yamamoto, and R. Tjian.** 1985. Two distinct transcription factors bind to the HSV thymidine kinase promoter in vitro. Cell **42**:559–572.
13. **Kost, T. A., N. Theodorakis, and S. H. Hughes.** 1983. The nucleotide sequence of the chick cytoplasmic β-actin gene. Nucleic Acids Res. **11**:8287–8301.
14. **Laimins, L. A., G. Khoury, C. Gorman, B. Howard, and P. Gruss.** 1982. Host-specific activation of transcription by tandem repeats from simian virus 40 and Moloney murine sarcoma virus. Proc. Natl. Acad. Sci. USA **79**:6453–6457.
15. **Lee, W., P. Mitchell, and R. Tjian.** 1987. Purified transcription factor AP-1 interacts with TPA-inducible enhancer elements. Cell **49**:741–752.
16. **Manley, J. L., A. Fire, A. Cano, P. A. Sharp, and M. L. Gefter.** 1980. DNA-dependent transcription of adenovirus genes in a soluble whole-cell extract. Proc. Natl. Acad. Sci. USA **77**:3855–3859.
17. **Maxam, A. M., and W. Gilbert.** 1980. Sequencing end-labeled DNA with base-specific chemical cleavages. Methods Enzymol. **65**:499–560.
18. **Mercola, M., J. Goverman, C. Mirell, and K. Calame.** 1985. Immunoglobulin heavy-chain enhancer requires one or more tissue-specific factors. Science **227**:266–270.
19. **Morgan, W. D., G. T. Williams, R. I. Morimoto, J. Greene, R. E. Kingston, and R. Tjian.** 1987. Two transcriptional activators, CAAT-box-binding transcription factor and heat shock transcription factor, interact with a human hsp70 gene promoter. Mol. Cell. Biol. **7**:1129–1138.
20. **Nakajima-Iijima, S., H. Hamada, P. Reddy, and T. Kakunaga.** 1985. Molecular structure of the human cytoplasmic β-actin gene: interspecies homology of sequences in the introns. Proc. Natl. Acad. Sci. USA **82**:6133–6137.
21. **Ng, S.-Y., P. Gunning, R. Eddy, P. Ponte, J. Leavitt, T. Shows, and L. Kedes.** 1985. Evolution of the functional human β-actin gene and its multi-pseudogene family: conservation of noncoding regions and chromosomal dispersion of pseudogenes. Mol. Cell. Biol. **5**:2720–2732.
22. **Nudel, U., R. Zakut, M. Shani, S. Neuman, Z. Levy, and D. Yaffe.** 1983. The nucleotide sequence of the rat cytoplasmic β-actin gene. Nucleic Acids Res. **11**:1759–1771.
23. **Ohlsson, H., and T. Edlund.** 1986. Sequence-specific interactions of nuclear factors with the insulin gene enhancer. Cell **45**:35–44.
24. **Piette, J., M.-H. Kryszke, and M. Yaniv.** 1985. Specific interaction of cellular factors with the B enhancer of polyoma virus. EMBO J. **4**:2675–2685.
25. **Sassone-Corsi, P., and E. Borrelli.** 1986. Transcriptional regulation by *trans*-acting factors. Trends Genet. **2**:215–219.
26. **Sassone-Corsi, P., A. Wildeman, and P. Chambon.** 1985. A *trans*-acting factor is responsible for the simian virus 40 enhancer activity in vitro. Nature (London) **313**:458–463.
27. **Singh, H., R. Sen, D. Baltimore, and P. A. Sharp.** 1986. A nuclear factor that binds to a conserved sequence motif in transcriptional control elements of immunoglobulin genes. Nature (London) **319**:154–158.
28. **SivaRaman, L., S. Subramanian, and B. Thimmappaya.** 1986. Identification of a factor in HeLa cells specific for an upstream transcriptional control sequence of an EIA-inducible adenovirus promoter and its relative abundance in infected and uninfected cells. Proc. Natl. Acad. Sci. USA **83**:5914–5918.
29. **Sive, H. L., and R. G. Roeder.** 1986. Interaction of a common factor with conserved promoter and enhancer sequences in histone H2B, immunoglobulin, and U2 small nuclear RNA (snRNA) genes. Proc. Natl. Acad. Sci. USA **83**:6382–6386.
30. **Staudt, L. M., H. Singh, R. Sen, T. Wirth, P. A. Sharp, and D. Baltimore.** 1986. A lymphoid-specific protein binding to the octamer motif of immunoglobulin genes. Nature (London) **323**:640–643.
31. **Treisman, R.** 1986. Identification of a protein-binding site that mediates transcriptional response of the c-*fos* gene to serum factors. Cell **46**:567–574.
32. **Weiher, H., M. König, and P. Gruss.** 1983. Multiple point mutations affecting the simian virus 40 enhancer. Science **219**:626–631.
33. **Wu, B. J., G. T. Williams, and R. I. Morimoto.** 1987. Detection of three protein binding sites in the serum-regulated promoter of the human gene encoding the 70-kDa heat shock protein. Proc. Natl. Acad. Sci. USA **84**:2203–2207.
34. **Wu, C.** 1985. An exonuclease protection assay reveals heat-shock element and TATA box DNA-binding proteins in crude nuclear extracts. Nature (London) **317**:84–87.