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Negative Transcriptional Regulatory Element That Functions in Embryonal Carcinoma Cells

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We have cloned the polyomavirus mutant fPyF9, which persists in an episomal state in F9 embryonal carcinoma cells (K. Ariizumi and H. Ariga, Mol. Cell. Biol. 6:3920–3927, 1986). fPyF9 carries three copies of exogenous sequences, the prototype of which is a 21-base-pair repeat (box DNA), in the region of the enhancer B domain of wild-type polyomavirus DNA. The consensus sequence, GCATTCCATTGTT, is 13 base pairs long. The box DNA inserted into fPyF9 appeared to come from a cellular sequence and was present in many kinds of DNAs, including F9 chromosomal DNA. The biological function of box DNA was analyzed by chloramphenicol acetyltransferase expression assays, using chimeric plasmids containing box DNA conjugated with simian virus 40 promoter elements. The results showed that box DNA repressed the activities both of the simian virus 40 promoter and enhancer only in transfected undifferentiated F9 cells and not in differentiated LTK$^-$ cells. Box DNA functioned independently of orientation and position with respect to the promoter in an enhancerlike manner, although the effect of box DNA was opposite that of the enhancer. The *Xho*I linker insertion into the consensus sequences of box DNA abolished the repression activity, and the protein(s) recognizing the consensus sequences was identified only in F9 cells, not in L cells. These analyses suggest that box DNA may be a negative regulatory element that functions in undifferentiated cells.

Eucaryotic enhancer sequences play important roles in the regulation of a variety of viral and cellular genes (23). An enhancer is defined as an element that stimulates the level of transcription of a linked gene independently of position and orientation with respect to the promoter (23). It has recently been shown that many enhancers function in specific cells or tissues or in response to an inducing signal; the immunoglobulin heavy-chain gene enhancer functions only in B lymphocytes (4, 14), and the expression of the cytochrome P_1-450 gene is induced by dioxin (20).

On the other hand, a negative enhancer has recently been discovered. This regulatory element functions in *cis*, and its mode of action is similar to that of a positive enhancer. This element, now called a silencer or dehancer, was initially discovered in the *MAT* locus of *Saccharomyces cerevisiae*, the locus that determines mating type (8). Furthermore, elements with similar properties have been shown to be located in upstream regions of the c-*myc* (26), p53 (7), and human β-interferon (15) genes and in the long terminal repeat of human T-cell lymphotropic virus type III (27). Enhancers in the long terminal repeat (32, 33) and β-interferon gene (15, 40), however, are also present close to the negative regulatory sequence. Both sequences appear to play a key role in transcriptional control of the gene linked to the 3′ end. Cellular genes are generally thought to be cooperatively regulated by negative and positive enhancers, with the exception of a housekeeping gene such as the actin gene (34). To understand the mechanism of gene regulation, the properties of both negative and positive regulatory sequences should be analyzed.

We have previously isolated a new class of polyomavirus mutant (fPyF9) that can replicate in mouse embryonal carcinoma (EC) cells (2). fPyF9 differs from other polyomavirus EC mutants in that it can persist episomally in F9 cells and its copy number in F9 cells is amplified at 4 days after induction of differentiation. fPyF9 contains mutations in the enhancer B domain of wild-type polyomavirus DNA, located in the noncoding regulatory region, and the sequences of the enhancer B domain of fPyF9 are extremely rearranged, as has been observed in many polyomavirus EC mutants (1). Furthermore, three exogenous sequences have been inserted into the enhancer B domain of fPyF9. These sequences are homologous to each other, and the sequence GCATTCCAT TGTTGTCAAAAG (box) is considered the prototype. The unique structure in the enhancer region of fPyF9 appears to confer the unique biological properties described above.

In this study, we describe the characterization of box DNA. The results show that box DNA decreases activities of the simian virus 40 (SV40) promoter and enhancer in undifferentiated F9 cells. Thus, this box DNA appears to be a negative regulatory sequence specific to undifferentiated cells.

MATERIALS AND METHODS

Constructions of plasmid DNAs. The plasmid DNAs were constructed by the following procedures. The chloramphenicol acetyltransferase (CAT)-coding region from pSVO-CAT (17) was recloned into the *Hin*dIII site of pUC19 (pUC19-O-CAT). After the *Bam*HI linker was added to the 5′ end of the *Nsi*I-*Hin*dIII fragment, which contains the SV40 promoter and replication origin, the resultant *Bam*HI-*Hin*dIII fragment was inserted into the *Bam*HI site of pUC19-O-CAT (pSVP-CAT). The *Pvu*II-*Bam*HI fragment of pSV_2CAT was end filled with Klenow fragment and cloned into the *Hin*cII

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site of pUC19 in either orientation [pSV$_2$CAT(+) and pSV$_2$CAT(−)]. Synthetic box DNA or box DNA containing the *Xho*I linker CTCGAG was introduced into the *Bam*HI site of pSVP-CAT, pSV$_2$CAT(+), and pSV$_2$CAT(−). The orientation and copy number of introduced box DNA sequences were determined by the dideoxy-chain teremination method (28, 29); plasmid designations are given in the legend to Fig. 2.

Cell culture and transfection procedure. The F9 EC and LTK$^-$ cells were grown in Dulbecco modified Eagle medium supplemented with 10% fetal calf serum (24). F9 EC cells were cultured in dishes coated with 0.1% gelatin. F9 and LTK$^-$ cells were transfected by the calcium phosphate method (18). For assays of LTK$^-$ cells, 10^6 cells were transfected with 5 μg of DNA in 1 ml of transfection buffer (0.1% dextrose, 0.14 M NaCl, 5 mM KCl, 1 mM Na_2HPO_4, 20 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid [HEPES; pH 7.05] 125 mM $CaCl_2$). The cells were washed twice in 5 ml of PBS(−), harvested with a rubber policeman at 48 h after transfection, suspended in 200 μl of Tris buffer (pH 7.8), and disrupted by three cycles of freeze-thawing, followed by sonication. For assays of F9 cells, 2×10^5 cells were transfected with 25 μg of DNA, harvested at 36 h after transfection, and disrupted as described above. The cell lysates were used for CAT assays.

Analysis of cell lysates for CAT assays. To quantitate CAT activity, the indicated volumes of cell lysates were used to acetylate 0.15 μCi of [^{14}C]chloramphenicol with 0.7 mM acetyl coenzyme A at 37°C (17, 31). The labeled chloramphenicol and acetylated derivatives were separated by ascending silica gel thin-layer chromatography (chloroform/methanol, 95:5) and visualized by autoradiography. To quantitate CAT expression, the radioactive spots were cut from the silica gel plates and counted in a liquid scintillation counter.

Gel shift assay. L or F9 cell nuclear extracts were prepared as described by Dignam et al. (10). In the band shift assay, the binding reactions were performed by incubating 2 to 4 fmol of end-labeled DNA (5,000 cpm) with 2 to 3 μg of nuclear protein and 0.5 μg of poly(dI-dC) in a buffer containing 15 mM HEPES (pH 7.9), 15% glycerol, 2% polyvinyl alcohol, 36 mM NaCl, and 0.4 mM dithiothreitol at room temperature for 10 min in a final volume of 15 μl. After incubation, the cells were placed in 0.25× TBE buffer and electrophoresed at 10 V/cm. For competition experiments, various amounts of box DNA or pBR322 fragments digested with *Hae*III were added to the reaction mixture before the addition of the extract.

RESULTS

Insertion of cellular sequences into the enhancer B domain of fPyF9. Sequencing of fPyF9 DNA showed that there were three exogenous sequences in the enhancer B domain (Fig. 1) (1). These sequences were homologous to each other, and the consensus sequence was GCATTCCATTGTT. Southern blot analysis showed that box DNA inserted into fPyF9 appeared to have come from cellular sequences dispersed throughout the chromosomal DNA and was not unique to F9 chromosomal DNA (data not shown).

Reduction by box DNA of SV40 promoter activity. To examine the effect of box DNA on transcription, box DNA was inserted into the 5′ end of the SV40 promoter linked to the CAT gene (Fig. 2), and transient expression of the CAT gene was analyzed after transfection of plasmid DNA into F9 and LTK$^-$ cells. Since the efficiency of transfection into F9 cells is about 100-fold less than that into LTK$^-$ cells, the procedures used for F9 cells differed from those used for LTK$^-$ cells in the following respects: the amount of DNA used was fivefold more, the incubation time for the CAT reaction was twofold longer, and the volume of cell lysate used for the CAT assay was two- to threefold greater (see legends to Fig. 3, 4, and 6). Results of the CAT assays are shown in Fig. 3A. The plasmid containing box DNA (pBoxSVP-CAT) gave 20 to 30% of the acetylated product in transfected F9 cells relative to the amount produced by pSVP-CAT, which contains the SV40 promoter, whereas

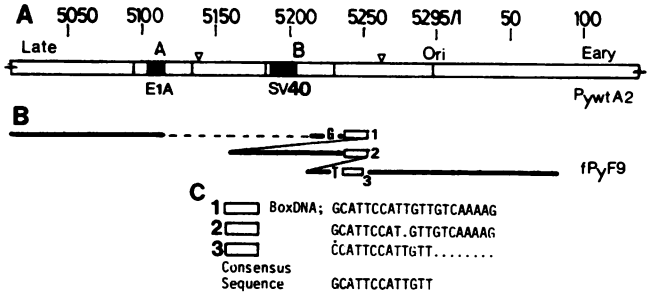

FIG. 1. Control region of polyomavirus F9 mutant fPyF9. (A and B) Comparison of the control regions of the polyomavirus wild-type strain A2 (A) and mutant fPyF9 (B). (C) Comparison of three exogenous sequences (▭) integrated into the enhancer B domain. Symbols for panel A: ▽, *Pvu*II site; ■, region in enhancer A or B homologous to the E1A and SV40, respectively. Symbols for panel B: ▬, point mutation (G or T) in the enhancer B domain of fPyF9 (4); – – –, deletion.

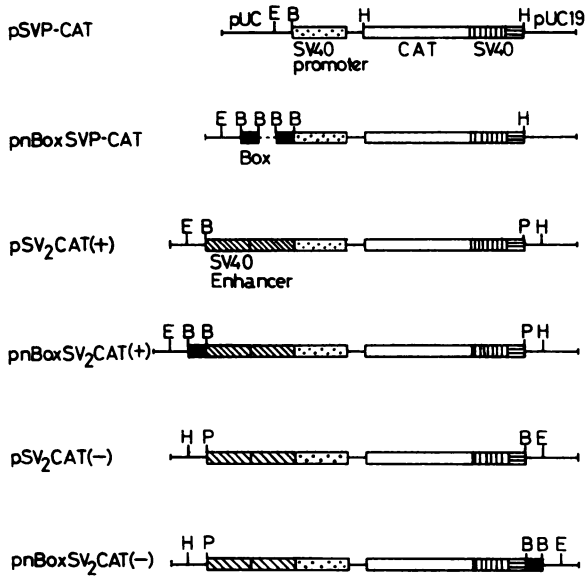

FIG. 2. Maps of box DNA-containing SV-40 CAT genes. All of the genes were cloned into the *Eco*RI-*Hin*dIII polylinker region of pUC19 as described in Materials and Methods. The maps are depicted on the 5′-to-3′ orientation except that for pSV$_2$CAT(−). The "n" in pnBoxSVP-CAT or pnBoxSV$_2$CAT denotes the copy number of box DNA inserted into the 5′ end of pSVP-CAT, and the (+) of pnBoxSV$_2$CAT(+) and (−) of pnBoxSV$_2$CAT(−) indicate the locations of box DNA (upstream and downstream, respectively), in pSV$_2$CAT. Symbols: ▨, ▩, ▥, ▤, and ▭, enhancer, promoter, splicing signal, poly(A) additional signal of SV40, the CAT gene, and box DNA, respectively. Restriction enzyme sites: B, *Bam*HI; E, *Eco*RI; H, *Hin*dIII; P, *Pst*I.

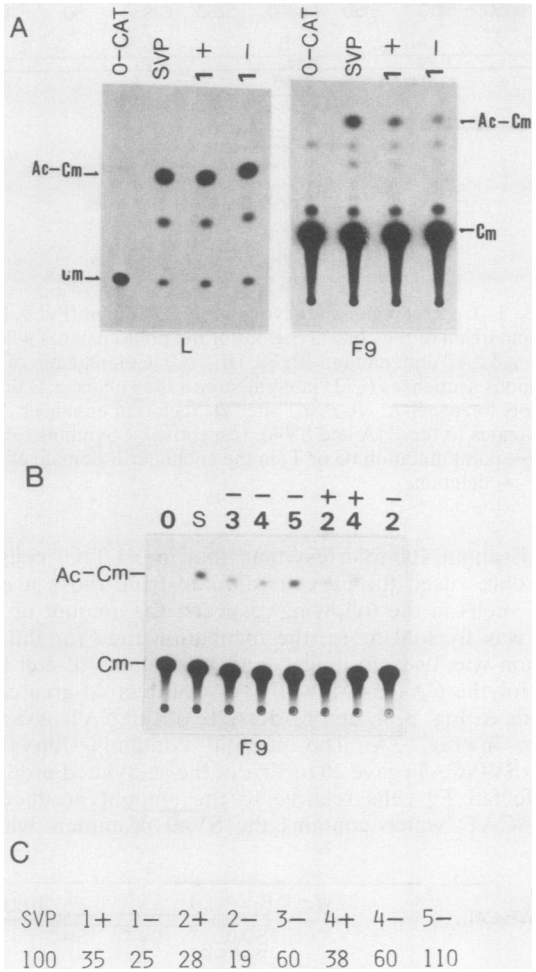

FIG. 3. CAT activities in F9 and LTK$^-$ cells transfected with pnBoxSVP-CAT. CAT enzymatic activity was measured in 100 μl of lysate prepared from p1BoxSVP-CAT(A)- or pnBoxSVP-CAT(B)-transfected cells. The lysate from L cells was incubated at 37°C for 3 h, and that from F9 cells was incubated for 6 h. Separation of chloramphenicol (Cm) and its acetylated derivatives (Ac-Cm) in ascending chromatography is shown. (A) CAT expression analyzed in F9 (right) and LTK$^-$ cells (left) cells. (B) Expression in F9 cells. (C) Relative values of CAT activity expressed by various plasmids. Values are averages from three independent experiments relative to that of the control plasmid, pSVP-CAT. Abbreviations for genes used: O, promoterless CAT; S, SVP-CAT; 1+, 2+, ---, copy number and orientation of box DNA in pnBoxSVP-CAT.

there was little difference among products in LTK$^-$ cells. pSVO-CAT, containing the promoterless CAT gene and used as a negative control, yielded undetectable products. These results indicated that SV40 promoter activity was inhibited by box DNA sequences in F9 cells but not in LTK$^-$ cells. Furthermore, either orientation of box DNA in pSVP-CAT produced an inhibitory effect (Fig. 3A, lanes 1+ and 1−), indicating that the repressive effect was independent of orientation with respect to the promoter. Basically, levels of enhancer activity parallel the number of enhancer units present in the gene of interest (19). Therefore, we examined the relationship between copy number and negative effect of box DNA on CAT expression. Of the plasmids containing various copies of box DNA (Fig. 3C), the plasmid containing the dimer of box DNA reduced SV40 promoter activity most effectively (Fig. 3B). The pentamer of box DNA had no

TABLE 1. Relative expression of box DNA-containing SV40-CAT genes in F9 cell lysates

Transfected plasmid DNA	Relative CAT activity[a]
(I)[b] pSVP-CAT	1
$pSV_2CAT(+)$	100
$pBox1+SV_2CAT$	13
$pBox3+SV_2CAT$	15
$pBox4+SV_2CAT$	35
$pSV_2CAT(-)$	100
$pBox1\text{-}SV_2CAT$	12
$pBox3\text{-}SV_2CAT$	14
$pBox4\text{-}SV_2CAT$	25
(II)[c] pSVP-CAT	0.9
$pSV_2CAT(+)$	100
$pBox1+SV_2CAT$	10
$pMB1+SV_2CAT$	91
$pMB2+SV_2CAT$	28
$pMB3+SV_2CAT$	10
$pSV_2CAT(-)$	100
$pBox1\text{-}SV_2CAT$	9
$pMB1\text{-}SV_2CAT$	80
$pMB2\text{-}SV_2CAT$	23
$pMB3\text{-}SV_2CAT$	8

[a] Cells were transfected and lysates were prepared, assayed, and counted as described in Materials and Methods and Fig. 4. The variance was approximately 20% of the sample mean in three transfections. All values are expressed relative to that of the control plasmid, $pSV_2CAT(+)$ or $pSV_2CAT(-)$.
[b] Summary of data from Fig. 4.
[c] Summary of data from Fig. 6.

effect on CAT expression. The effect of copy number of box DNA on CAT expression was quantitated by counting the radioactive spots containing acetylated products, and the results described above were confirmed (Fig. 3C). These results suggest that the optimal copy number may be considerable at the maximum repression level of box DNA. On the other hand, none of these copy number variants showed such a repressive effect in LTK$^-$ cells (data not shown).

Inhibition by box DNA of the activity of the SV40 enhancer. To determine whether box DNA also has a negative effect on enhancer activity in F9 cells, box DNA was inserted into the 5′ or 3′ end of pSV_2CAT, which contains both the SV40 enhancer and promoter linked to the CAT gene [$pnBoxSV_2CAT(+)$ and $pnBoxSV_2CAT(-)$, respectively] (Fig. 2). Enhancer activity was measured by determining CAT expression (Table 1). Box DNA repressed the activity to 12 to 35% of that of pSV_2CAT, which does not contain box DNA. Although the effect was observed with both plasmids containing box DNA inserted in either position, box DNA located at the 3′ side of the chimeric CAT gene repressed enhancer activity more effectively than did box DNA inserted at the 5′ side. This repression was observed in F9 cells but not in LTK$^-$ cells (Fig. 4), as was found for repression of promoter activity.

The relationship between copy number and negative effect of box DNA on enhancer activity was measured simultaneously, as was done in assays of the SV40 promoter. The increase in copy number of box DNA decreased the enhancer-repressive activity, similar to results obtained for pnBoxSVP-CAT.

Dose-response of the activity produced by box DNA. The CAT assay used is based on enzyme activity, and the results obtained should be evaluated by repeated transfection procedures. To do so, we examined the dose response of the enhancer-repressive activity of box DNA. F9 cells were transfected with 10 and 25 μg of $p1BoxSV_2CAT(+)$, and

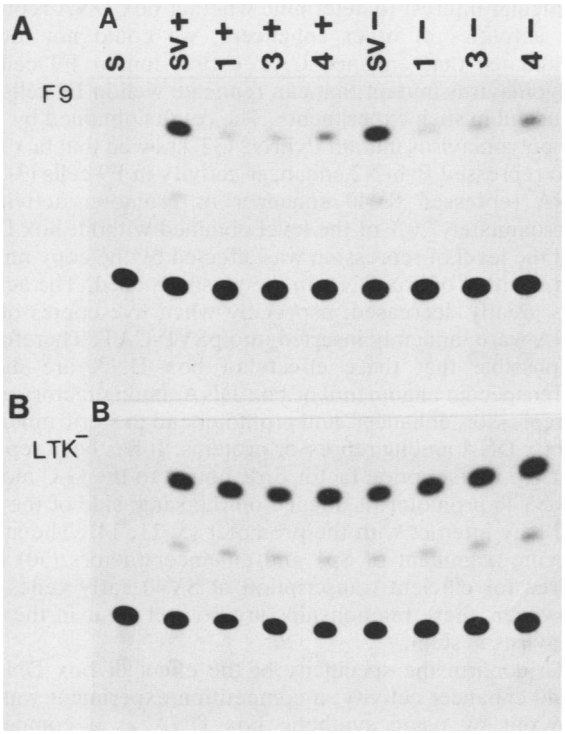

FIG. 4. CAT expression in cells transfected with pnBoxSV_2 CAT. LTK^- (A) and F9 (B) cells were transfected with pnBoxSV_2 CAT, and CAT activity was measured. F9 (100 μl) or LTK^- (30 μl) cell lysate was incubated with labeled chloramphenicol at 37°C for 2 h or 1 h, respectively. Relative values of CAT expression are given in Table 1. Numbers indicate copy number of box DNA; + and − indicate 5′ and 3′ positions, respectively, of SV_2CAT. For example, 1+ indicates p1BoxSV_2CAT(+), and SV+ and SV− are pSV_2 CAT(+) and pSV_2CAT(−) genes, respectively.

CAT activity was compared with that of the control plasmid, pSV_2CAT(+). The repression obtained at each point (Fig. 5) was the same as that shown in Fig. 4. Therefore, the results of the assay we used were reproducible, and repression paralleled the dose of transfected pBoxSV_2CAT.

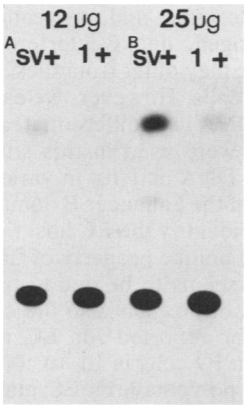

FIG. 5. Dose response of box-CAT genes in F9 cells. F9 cells were transfected with pSV_2CAT(+) (SV+) or p1BoxSV_2CAT(+) (1+), and CAT expression was analyzed as described in the legend to Fig. 4. The repressive activity of box DNA was compared in transfections using 12 (A) and 25 (B) μg of DNA. The appropriate amount of pBR322 was added in transfection buffer to a final concentration of 25 μg/ml.

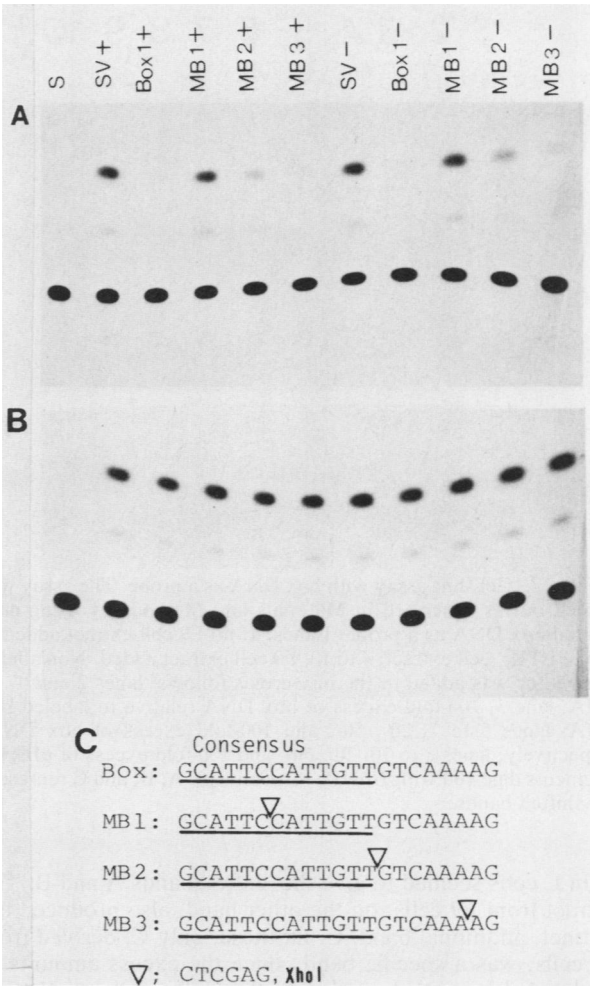

FIG. 6. Inhibition of box DNA activity by *Xho*I linker insertion mutation. Mutated box DNA-containing SV40-CAT was transfected into F9 cells (A) and LTK^- (B) cells. CAT activity was assayed as described in the legend to Fig. 4 and compared with that of control plasmid p1BoxSV_2CAT(+) or p1BoxSV_2CAT(−). Relative values of CAT expression are shown in Table 1. (C) Sequences of box DNA inserted with the *Xho*I linker (MB). The linker (▽) was inserted into the consensus sequence (underlined) (MB1), into the 3′ end of the consensus sequence (MB2), and into the 3′ end of box DNA (MB3). These sequences, containing *Bam*HI sites at both ends, were synthesized chemically and introduced into the *Bam*HI site of pSV_2CAT(+) or pSV_2CAT(−) (Fig. 2).

Abolishment by mutated box DNA sequences of repression activity. The *Xho*I linker CTCGAG was inserted in various positions in box DNA sequences (Fig. 2). F9 and LTK^- cells were transfected with 5 and 25 μg, respectively, of MB-SV40-CAT, and the CAT assay was performed. Mutation in the consensus sequence (MB1) produced a lethal effect on box DNA activity (Fig. 6), whereas mutation at the 3′ end of the consensus sequence (MB2) produced a weak inhibitory effect (Table 1). However, the MB3 sequence, containing a mutation near the 3′ end of box DNA, still showed repression activity. These results indicate that box DNA may be a form of negative transcriptional regulatory element functioning in F9 cells.

Gel shift assay. Nuclear extracts were prepared from F9 and L cells, and the mobility shift (gel shift) assay was carried out, using box DNA as a probe (Fig. 7). The extract

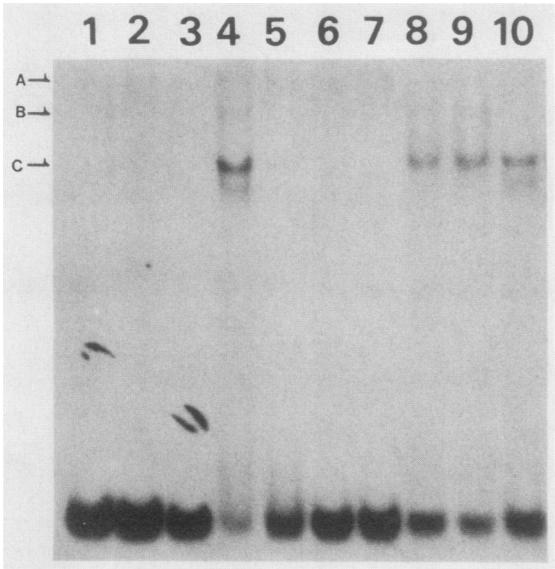

FIG. 7. Gel shift assay with box DNA as a probe. The assay was carried out as described in Materials and Methods by using end-labeled box DNA as a probe. Lanes: 1, no F9 cell extract added; 2 and 3, LTK$^-$ cell extract; 4 to 10, F9 cell extract added. Nonlabeled competitor was added to the mixture as follows: lanes 2 and 4, no DNA; lane 3, 100-fold excess of box DNA relative to labeled box DNA; lanes 5 to 7, 20-, 50-, and 100-fold excess of box DNA, respectively; lanes 8 to 10, 20-, 50-, and 100-fold excess of pBR322 fragments digested with *Hae*III, respectively. A, B, and C represent the shifted bands.

from L cells seemed to give the shifted bands A and B. The extract from F9 cells, on the other hand, also produced the distinct, additional band, C. Of these, only C, derived from F9 cells, was a specific band, since the excess amounts of nonlabeled box DNA abolished the shift, whereas *Hae*III-digested pBR322 fragments did not. Furthermore, linker insertion DNA did not inhibit binding in the reaction with the F9 cell extract (data not shown). This result indicates that a box DNA (silencer)-binding protein is present only in F9 cells.

DISCUSSION

Box DNA, GCATTCCATTGTTGTCAAAAG, was originally discovered in the enhancer B domain of polyomavirus mutant fPyF9, which persists extrachromosomally in F9 cells (2). Three copies of box DNA were inserted into the region controlling early transcription, and the sequences were found to be located at similar distances from the promoter motif CCACCC, which is similar to the SV40 CCGCCC motif (G-C motif) (27, 38). The results showed that box DNA acts as a negative regulatory element specifically in F9 cells and has properties similar to those of an enhancer in terms of direction and location with respect to the promoter.

To analyze the effect of box DNA on promoter or enhancer activity, the SV40 system was selected for the following reasons. (i) The regions of the polyomavirus promoter and enhancer cannot be clearly distinguished; in particular, the promoter has not been well characterized. (ii) The SV40 promoter and enhancer have been studied in detail, and their locations are well separated (39). (iii) SV40 enhancer and promoter activities are strong enough in F9 EC cells to be analyzed by the CAT assay. Although it was of particular interest to determine whether box DNA represses the activities of other enhancers, we could not identify candidates that enhance CAT expression in F9 cells. A polyomavirus mutant that can replicate well in F9 cells may be useful in such experiments. The results obtained by using the polyomavirus mutant PyhrN2 (32) showed that box DNA also repressed PyhrN2 enhancer activity in F9 cells (3). Box DNA repressed SV40 enhancer or promoter activity to approximately 10% of the level obtained without box DNA, and the level of repression was affected by the copy number and position of box DNA in the plasmids used. The activity was greatly decreased, especially when five copies of box DNA were tandemly inserted into pSVP-CAT. Therefore, it is possible that these effects of box DNA are due to stereospecific alignments of box DNA among factors acting as repressor, enhancer, and promoter and to steric hindrance of box DNA among repressor proteins. It has been reported that the transcription factor Sp1, bound to the G-C motif of the SV40 promoter, is aligned on the same side of the helix and may interact with the promoter (5, 11, 14). The stereospecific alignment of Sp1 and enhancer factors (30) is required for efficient transcription of SV40 early genes (35). However, these relationships are not yet clear in the polyomavirus system.

To confirm the specificity of the effect of box DNA on SV40 enhancer activity, a competition experiment was carried out by using synthetic box DNA as a competitor. However, satisfactory results were not obtained. Since CAT assays on F9 cells were performed with the lysate from cells transfected with 25 μg of DNA, a large amount of competitor box DNA was necessary. For example, 1.25 and 12.5 μg of box DNA had to be applied in 10- and 100-fold molecular excess, respectively. Also, the efficiency was less when cells were transfected with a small DNA fragment such as box DNA containing 26 base pairs. To overcome these difficulties, a mutation was introduced into box DNA. The results of mutation analysis reveal that the consensus sequence of box DNA is essential to repression. Box DNA must be derived from cellular chromosomal DNA. However, the sequences of box DNA cannot be found in any other cellular regulatory region reported thus far. Negative regulatory elements have recently been detected in the *MAT* locus of *S. cerevisiae*, in the long terminal repeat of human B-cell lymphotropic virus, and upstream of the c-*myc*, p53 cellular tumor antigen, and β-interferon genes. The box DNA reported here may differ from these elements by acting in undifferentiated cells. However, we cannot determine the specificity of box DNA in undifferentiated cells, since only L cells and F9 cells were used in this study. Therefore, we must examine box DNA activity in various cell lines.

Rearrangement of the enhancer B domain of polyomavirus may be required to identify the EC host range mutant (12, 21, 22, 25, 32, 37). The unique property of fPyF9, persistence in an episomal state, seems to be due to box DNA, since the effects of insertion of box DNA in the control region differ from those thus far reported for EC mutants. The copy number of fPyF9 in F9 cells is 10- to 100-fold lower than in cells infected with polyomavirus EC mutants. Therefore, it is possible that the lower copy number of fPyF9 results from the function of box DNA. However, the mechanism by which box DNA interacts with the polyomavirus enhancer and the determinant of the unique biological property of fPyF9 remain to be analyzed. Polyomavirus DNA replication requires an enhancer (36). Therefore, box DNA may repress not only enhancer activity but also DNA replication.

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LITERATURE CITED

1. **Amati, P.** 1985. Polyoma regulatory region: a potential probe for mouse cell differentiation. Cell **43**:561–562.
2. **Ariizumi, K., and H. Ariga.** 1986. New class of polyomavirus mutant that can persist as free copies in F9 embryonal carcinoma cells. Mol. Cell. Biol. **6**:3920–3927.
3. **Ariizumi, K., H. Takahashi, M. Nakamura, and H. Ariga.** 1989. Effect of silencer on polyomavirus DNA replication. Mol. Cell. Biol. **9**:4026–4031.
4. **Banerji, J., L. Olson, and W. Shaffner.** 1983. A lymphocyte-specific cellular enhancer is located downstream of the joining region in immunoglobulin heavy chain genes. Cell **33**:729–740.
5. **Barrera-Saldana, H., K. Takahashi, M. Vigneron, A. Wildeman, I. Davidson, and P. Chambon.** 1985. All six GC-motifs of the SV40 early upstream element contribute to promoter activity *in vivo* and *in vitro*. EMBO J. **4**:3839–3850.
6. **Benoist, C., and P. Chambon.** 1981. *In vitro* sequence requirements of the SV40 early promoter region. Nature (London) **290**:304–310.
7. **Bienz-Tadmor, B., R. Zakut-Houri, S. Libresco, D. Givol, and M. Oren.** 1985. The region of the p53 gene: evolutionary conservation and evidence for negative regulatory element. EMBO J. **4**:3209–3213.
8. **Brand, A. H., L. Breeden, J. Abraham, R. Sternglanz, and N. Nasmyth.** 1985. Characterization of a "silencer" in yeast: a DNA sequence with properties opposite to those of a transcriptional enhancer. Cell **41**:41–48.
9. **De Villiers, J., W. Schaffner, C. Tyndall, S. Lupton, and R. Kamen.** 1984. Polyoma virus DNA replication requires an enhancer. Nature (London) **312**:242–246.
10. **Dignam, J. D., R. M. Lebowitz, and R. G. Roeder.** 1983. Accurate transcription initiation by RNA polymerase II in a soluble extract from isolated mammalian nuclei. Nucleic Acids Res. **14**:1475–1489.
11. **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.
12. **Fujimura, F. K., P. L. Deininger, T. Friedmann, and E. Linney.** 1981. Mutation near the polyoma DNA replication origin permits productive infection of F9 embryonal carcinoma cells. Cell **23**:809–814.
13. **Gidoni, D., W. S. Dynan, and R. Tjian.** 1984. Multiple specific contacts between a mammalian transcription factor and its cognate promoters. Nature (London) **312**:409–413.
14. **Gillies, S., S. L. Morrison, U. T. Oi, and S. Tonegawa.** 1983. A tissue-specific transcription enhancer element is located in the major intron of a rearranged immunoglobulin heavy chain gene. Cell **33**:717–728.
15. **Goodbourn, S., H. Burstein, and T. Maniatis.** 1986. The human β-interferon gene enhancer is under negative control. Cell **45**:601–610.
16. **Goodbourn, S., K. Zinn, and T. Maniatis.** 1985. Human beta-interferon gene expression is regulated by an inducible enhancer element. Cell **41**:509–520.
17. **Gorman, C., L. F. Moffat, and B. H. Howard.** 1982. Recombinant genomes which express chloramphenicol acetyltransferase in mammalian cells. Mol. Cell. Biol. **2**:1044–1051.
18. **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.
19. **Herr, W., and Y. Gluzman.** 1985. Duplication of a mutated simian virus 40 enhancer restores its activity. Nature (London) **313**:711–713.
20. **Jones, P. B. C., D. R. Galeazzi, J. M. Fisher, and J. P. Whitlock, Jr.** 1985. Control of cytochrome P_1-450 gene expression by dioxin. Science **227**:1499–1502.
21. **Katinka, M., M. Vasseur, M. Montreu, M. Yaniv, and D. Blangy.** 1981. Polyoma DNA sequences involved in control of viral gene expression in murine embryonal carcinoma cells. Nature (London) **290**:720–722.
22. **Katinka, M., M. Yaniv, M. Vasseur, and D. Blangy.** 1980. Expression of polyoma early function in mouse embryonal carcinoma cells depends on sequence rearrangements in the beginning of the late region. Cell **20**:393–399.
23. **Khoury, G., and P. Gruss.** 1983. Enhancer elements. Cell **33**:313–314.
24. **Lehman, J. M., W. C. Speers, D. E. Swartsendruber, and G. B. Pierre.** 1974. Neoplastic differentiation: characterization of cell lines derived from a murine teratocarcinoma. J. Cell. Physiol. **84**:13–28.
25. **Melin, F., H. Pinon, C. Reiss, C. Kress, N. Montreau, and D. Blangy.** 1985. Common features of polyomavirus mutants selected on PCC4 embryonal carcinoma cells. EMBO J. **4**:1799–1803.
26. **Remmers, E. F., J.-G. Yang, and K. B. Marcu.** 1986. A negative transcriptional control element located upstream of the murine c-*myc* gene. EMBO J. **5**:899–904.
27. **Rosen, C. A., J. K. Sodroski, and W. A. Haseltine.** 1985. The location of *cis*-acting regulatory sequences in the human T cell lymphotropic virus type III (HTLV-III/LAV) long terminal repeat. Cell **41**:8113–8123.
28. **Sanger, F., A. R. Coulson, B. G. Barrel, A. S. H. Smith, and B. Roe.** 1980. Cloning in single-stranded bacteriophage as an aid to rapid DNA sequencing. J. Mol. Biol. **143**:161–165.
29. **Sanger, F., S. Nicklen, and A. R. Coulson.** 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA **74**:5463–5467.
30. **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.
31. **Scholer, H. R., and P. Gruss.** 1984. Specific interaction between enhancer-containing molecular and cellular components. Cell **36**:403–411.
32. **Sekikawa, K., and A. J. Levine.** 1981. Isolation and characterization of polyoma host range mutants that replicate in multipotential embryonal carcinoma cells. Proc. Natl. Acad. Sci. USA **78**:1100–1104.
33. **Sodroski, J. G., C. A. Rosen, and W. A. Haseltine.** 1984. *Trans*-acting transcriptional activation of the long terminal repeat of human T lymphotropic viruses in infected cells. Science **225**:381–384.
34. **Sodroski, J. G., C. A. Rosen, F. Wong-Staal, S. Salahuddin, M. Popovic, S. Arya, and R. C. Gallo.** 1985. *Trans*-acting transcriptional regulation of human T-cell leukemia virus type III. Science **227**:171–173.
35. **Takahashi, K., M. Vigneron, H. Matthes, A. Wildeman, M. Zenke, and P. Chambon.** 1986. Requirement of stereospecific alignments of initiation from SV40 early promoter. Nature (London) **319**:121–126.
36. **Tyndall, C., G. La Montia, C. M. Thacker, J. Favaloro, and R. Kamen.** 1981. A region of the polyoma virus genome between the replication origin and late protein coding sequences is required in *cis* for both early gene expression and viral DNA replication. Nucleic Acids Res. **9**:6231–6236.
37. **Vasseur, M., C. Kress, N. Montreau, and D. Blangy.** 1980. Isolation and characterization of polyoma virus mutants able to develop in embryonal carcinoma cells. Proc. Natl. Acad. Sci. USA **77**:1068–1072.
38. **Vigeron, M., H. A. Barrera-Saldana, D. Baty, R. Everett, and P. Chambon.** 1984. Effect of the 21-bp repeat upstream element on *in vitro* transcription from the early and late SV40 promoters. EMBO J. **3**:2373–2382.
39. **Wildeman, A. G., M. Zenka, C. Schatz, K. Takahashi, H. A. Barrera-Saldana, T. Grudstrom, M. Wintzerith, H. Mathes, M. G. Vigeron, and P. Chambon.** 1985. p. 19–26. *In* Y. Gluzman (ed.), Eukaryotic transcription. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
40. **Zinn, K., D. Dimaio, and T. Maniatis.** 1983. Identification of two distinct regulatory regions adjacent to the human β-interferon gene. Cell **34**:865–879.