MOLECULAR AND CELLULAR BIOLOGY, Nov. 1989, p. 5248–5253
0270-7306/89/115248-06$02.00/0

Vol. 9, No. 11

E1A-Induced Enhancer Activity of the Poly(dG-dT) · Poly(dA-dC) Element (GT Element) and Interactions with a GT-Specific Nuclear Factor

DAVID T. BERG, JENNA D. WALLS, ANNE E. REIFEL-MILLER, AND BRIAN W. GRINNELL*

Department of Molecular Biology, Lilly Research Laboratories, Lilly Corporate Center, Indianapolis, Indiana 46285

Received 22 February 1989/Accepted 31 July 1989

The alternating sequence poly(dG-dT) · poly(dA-dC) is a highly repeated sequence in the eucaryotic genome. We have examined the effect of *trans*-acting early viral proteins on the ability of the GT element to stimulate transcription of the adenovirus major late promoter (MLP). We find that the GT element alone does not activate expression from the MLP in either the presence or absence of another enhancer element. However, in the presence of the E1A gene products of either adenovirus type 5 or 2, the GT element activated expression from the MLP. The stimulatory activity of the GT element in the presence of E1A had the properties of an enhancer element, and the *trans*-activating effect on the GT element was additive in conjunction with the E1A-responsive BK virus enhancer. We also have demonstrated that a specific nuclear factor(s) binds to the GT element. However, the E1A protein(s) do not affect the initial factor interaction(s) with the GT element. Overall, our data demonstrate that *trans* modulation of promoter activity can be mediated through the GT element.

The eucaryotic genome contains approximately 100,000 copies of a widely dispersed copolymer, poly(dG-dT) · poly(dA-dC) (see reference 33). These GT elements have been found in the nontranslated regions and introns of a number of cloned genes, present as 20- to 60-base-pair tracts (14, 15). A number of studies have suggested that the GT element may be a site of recombination (32–34) as well as have an involvement in gene conversion events (8). In addition, the mammalian GT element has been shown to have weak transcriptional enhancer activity with the simian virus 40 (SV40) early promoter (16). The GT element is capable of forming left-handed, or Z-form, DNA (13, 17, 36), which has been suggested to play a role in the regulation of transcriptional enhancers (27), and potentially to be a negative regulatory sequence (28, 30). In addition, non-B-DNA and S1-sensitive structures have been suggested to be involved in transcriptional control (see reference 39).

In the present study, we have examined the effect of the GT element on transcription from the adenovirus major late promoter (MLP) in the presence and absence of *trans*-acting viral proteins. Our data demonstrate the presence of a host cell-independent GT-binding protein and the ability to modulate a promoter through the *trans* induction of enhancer activity from the GT element.

Effect of the GT element on expression from the MLP. To determine the effect of the GT element on the activity of the MLP, we utilized a transient expression system with the chloramphenicol acetyltransferase (CAT) gene. Shown in Fig. 1 are the CAT expression plasmids used in the study. Each plasmid in Fig. 1A is the same except for the regulatory region driving expression of the CAT gene.

In Fig. 2A, the effect of the GT element on MLP activity in the monkey kidney cell line, MK2, is shown. The GT sequence had no stimulatory effect on the MLP; the levels of CAT produced from pLP-CAT (lane 3) and pLP(GT)-CAT (lane 1) were approximately the same. In addition, there were no differences in the levels of CAT expression from GT-containing plasmids in the HeLa and COS-1 cell lines (Table 1). This is in contrast to previous results demonstrating that a GT (TG) element stimulated activity of the SV40 early promoter in HeLa, COS-1, and CV1 cells (16). These data suggest a possible promoter dependence for GT enhancer activity.

As shown in Fig. 2A, lane 2, the level of CAT activity from pLP(GT)-CAT increased approximately 10-fold following cotransfection with the adenovirus type 2 E1A expression plasmid pSVE1A. The levels of CAT from pLP-CAT in the presence and absence of E1A (lanes 5 and 4, respectively) were not significantly different. Thus, the GT element appeared to activate transcription from the MLP in the presence of E1A. In support of these data, we determined the relative promoter strength of pLP-CAT and pLP(GT)-CAT in the 293 cell line, which constitutively expresses the E1A gene products. Whereas the GT element alone had no effect on the MLP in MK2 cells, a five- to seven-fold increase in activity was observed with the GT element in this E1A-producing cell line (Fig. 2B). The ability of the E1A protein(s) to stimulate activity in the presence of the GT element was host cell specific. As shown in Fig. 2C, the level of CAT produced from pLP(GT)-CAT (lane 1) was not increased by E1A (lane 2) in the HeLa cell line. In fact, the presence of the E1A proteins resulted in a slight decrease in CAT activity.

Previously, we demonstrated that the T antigen of SV40 activated the MLP in *trans* (10). To determine whether this *trans*-acting protein also activated the GT element, both pLP-CAT and pLP(GT)-CAT were cotransfected with the T antigen expression vector pSV3gpt (ATCC 37144). The SV40 T antigen activated expression from both plasmids approximately sevenfold; MLP expression from pLP(GT)-CAT was not higher than that from pLP-CAT (data not shown). In addition, the levels of CAT with or without the GT element were approximately the same in the T-antigen-producing COS-1 cell line (Table 1).

Effect of the GT element on enhancer-activated expression

* Corresponding author.

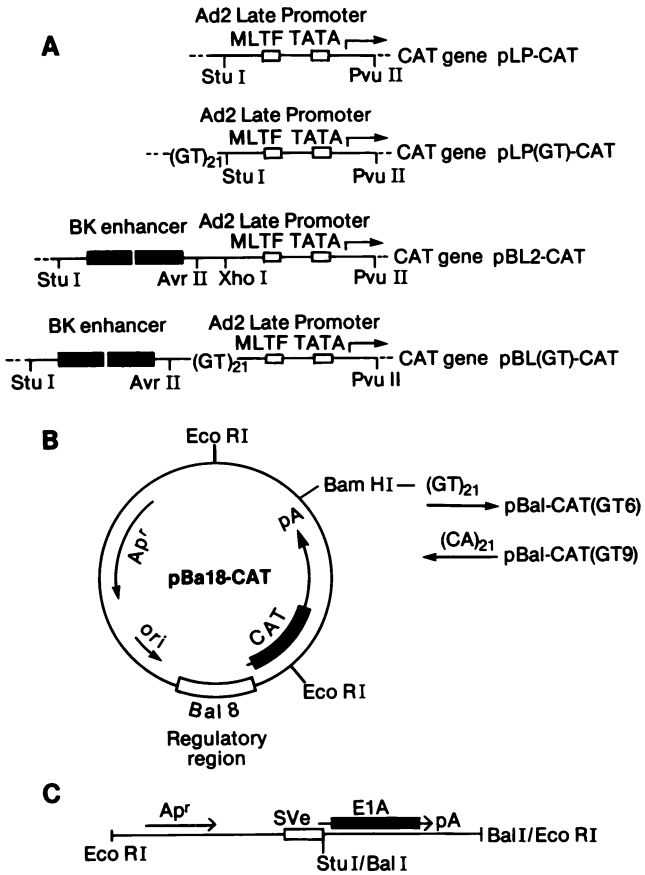

FIG. 1. CAT expression plasmids used to study the effect of the GT element on transcription of the MLP of adenovirus. (A) The construction of plasmids pLP-CAT (10), pBL2-CAT (11), and pBa18-CAT (10) has been described previously. A synthetic GT element, consisting of GT repeated 21 times and flanked by *Xho*I and *Bam*HI sites [GATCCTCGA$(GT)_{21}$GAATTCTCGAG], was inserted into the CAT expression plasmids described above. The *Eco*RI site in the synthetic element was used to orient the insert. pBL(GT)-CAT was constructed by inserting the GT element between the BK virus P2 (1) enhancer and the MLP at the *Xho*I site (adenovirus 2 [AD2], nucleotide 5778). pLP(GT)-CAT was constructed from pBL(GT)-CAT by deleting the BK enhancer from the *Mst*I (nucleotide 188) to the *Bgl*II (BK; nucleotide 4654) site. (B) Plasmids pBa18-CAT(GT6) and pBa18-CAT(GT9) were derived from pBa18-CAT by insertion of the GT element at the *Bam*HI site 3′ to the CAT gene and SV40 polyadenylation site (SV40, nucleotide 2533) in both orientations (GT6, sense; GT9, antisense). ori, Origin. (C) The adenovirus E1A expression plasmid, pSVE1A, was constructed with the *Bal*I restriction fragment (Ad2, nucleotides 270 to 2065) containing the E1A coding sequence, inserted downstream of the SV40 early promoter-enhancer (at the *Stu*1 site at nucleotide 5190). pSVE1A also contains the *Eco*RI to *Nde*I restriction fragment of pBR322, including the ampicillin resistance gene and origin of replication.

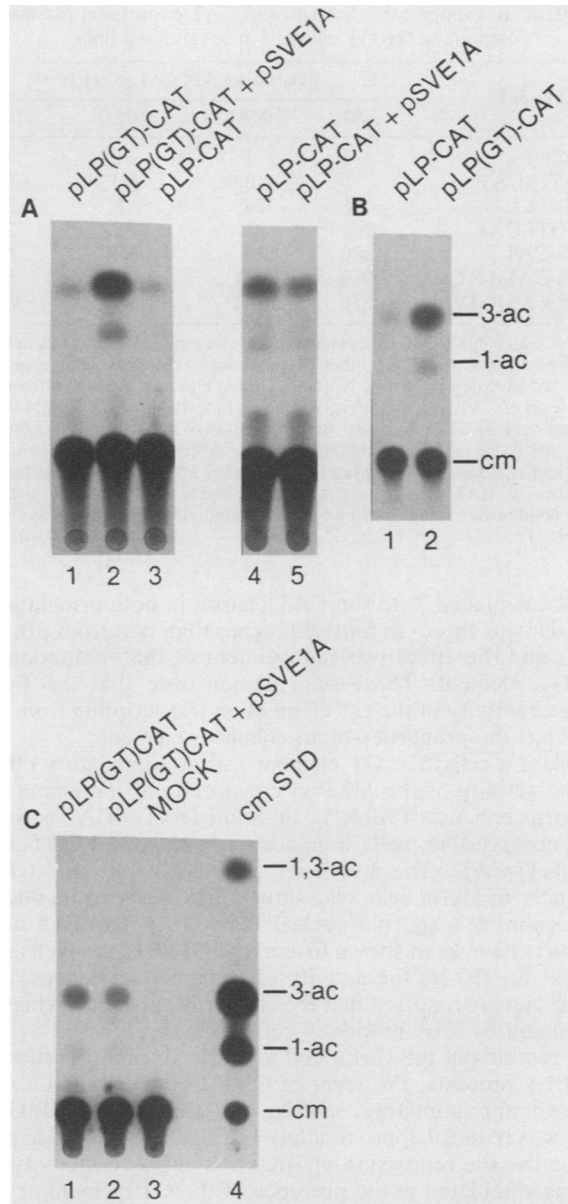

FIG. 2. Effect of poly(GT) element on the expression of the MLP alone and in the presence of the adenovirus E1A gene products in the monkey kidney cell line MK2 (ATCC CCL7) (A), the adenovirus-transformed human kidney cell line 293 (ATCC CRL 1573) (B), and the human HeLa cell line (ATCC CRL 2.1) (C). Cells were transfected, and after 48 h, a cell extract was prepared and assayed for CAT activity as described previously (10). Unreacted [^{14}C]chloramphenicol (cm) and its acetylated forms, chloramphenicol 1-acetate (1-ac) and chloramphenicol 3-acetate (3-ac), were separated by thin-layer chromatography and detected by autoradiography. The cm STD lane is the control, showing acetylated chloramphenicol forms using purified CAT (Pharmacia). In cotransfection experiments, the E1A gene was introduced by using pSVE1A, and E1A-dependent repression of pSL-CAT (10) was used as a control for E1A activity for each transfected cell line.

of the MLP. In a previous report, we demonstrated that the activity of the BK virus enhancer also could be stimulated by E1A (10). As shown in Table 1, expression of CAT from the BK virus enhancer-stimulated MLP was increased approximately fivefold by the GT element in the 293 cell line but was unchanged in the MK2 and COS-1 cell lines. In addition, the stimulatory effects of E1A on the BK virus enhancer and the GT elements appeared to be additive. In MK2 cells, the level of CAT activity from pBL(GT)-CAT also was approximately sevenfold higher than that from pBL2-CAT following cotransfection of both plasmids with pSVE1A (data not shown). Thus, the GT element could activate expression from the MLP in the presence of another E1A-responsive element. As shown in Table 1, the level of CAT expression from the pBa18(GT)-CAT plasmids, in which the GT ele-

TABLE 1. Comparative activities of CAT expression plasmids containing the GT element in several cell lines

Plasmid	Relative level of CAT in cell line[a]:			
	293	MK2	COS 1	HeLa
pLP-CAT	1	1	1	1
pLP(GT)-CAT	5	0.9	NT	0.8
pBL2-CAT	66	28	8	2.1
pBL(GT)-CAT	263	21	8	2.2
pBa18-CAT	280	20	NT	NT
pBa18-CAT(GT6)	960	NT	11	NT
pBa18-CAT(GT9)	830	NT	NT	NT

[a] The level of CAT activity from pLP-CAT was assigned a value of 1 in each cell line, and the level from other plasmids was expressed relative to that value. We have determined by Northern (RNA) blot analysis that differences in the level of CAT activity reflect differences in the level of CAT mRNA and by primer extension analysis that all transcription of the CAT mRNA initiates at the MLP cap site. In addition, we have determined by Southern blot analysis that differences in plasmid copy number are not responsible for the differences in CAT activity or CAT mRNA levels (data not shown). Each datum point is the average of from two to eight individual determinations. NT, Not tested.

ment was placed 3′ to the CAT cistron in both orientations (Fig. 1), was three- to fourfold higher than that from pBa18-CAT, and the effect was independent of the orientation of the GT element. These data demonstrate that the E1A-induced activity of the GT element on transcription from the MLP has the properties of an enhancer element.

In HeLa cells, the GT element had no stimulatory effect on the activity of the MLP in constructions containing the BK virus enhancer (Table 1); the same level of CAT activity was observed in cells transfected with pBL2-CAT and pBL(GT)-CAT. The low-level activity of the BK virus enhancer in HeLa cells was shown previously to be due to repression by a specific nuclear factor (11). The E1A gene products have been shown to activate (3, 10, 22) as well as to repress (2, 19, 36) the activity of enhancer sequences, and we previously reported that the BK virus enhancer, which is stimulated by E1A in kidney cells (10), is repressed by the E1A proteins in the HeLa cell line (11). In the presence of the E1A proteins, the level of CAT from pBL2-CAT was reduced approximately 3.5-fold and that from pBL(GT)-CAT was reduced approximately 11-fold. These data suggest that either the repression of BK virus enhancer activity by E1A is stimulated in the presence of the GT element or the repressive effect of E1A on both elements is additive. Overall, our data show that E1A affects the BK virus enhancer and the GT element in the same way, i.e., stimulating enhancer activity in MK2 cells and repressing the activity in the HeLa cell line. These data suggested that both the BK virus enhancer and the GT element may be interacting with the same factor(s) that specify their host cell-dependent responses to E1A.

Interaction of nuclear factors with the GT element. Experiments were performed to determine whether a specific nuclear factor(s) interacted with the GT element and, if so, whether or not the E1A protein(s) affected the interaction(s). Nuclear extracts were prepared, and specific binding of protein factors to the poly(GT)$_{21}$ element was determined by DNase footprint and gel migration retardation analyses by using conditions described previously (11). To assure that nuclear extracts from each cell line were as equivalent as possible, extracts were prepared at the same time and under identical conditions and were shown to be transcriptionally active, as measured by runoff transcription from the MLP (12). As an additional control for the consistency of extracts, we determined by gel shift analysis that extracts prepared at the same time gave the same level of binding to the major late transcription factor (data not shown). The DNase footprint analysis was performed by using an end-labeled restriction fragment of pGTC, a plasmid in which the GT element is upstream of the BK virus enhancer (Fig. 3A). With increasing concentrations of 293 cell nuclear extract (lanes 1 to 3), we observed a protected region corresponding to the GT element. Approximately fourfold less nuclear extract was needed to obtain an equivalent level of protection of the BK virus enhancer sites I and III (11), suggesting that the activity or concentration of the GT binding factor is less than that of the BK virus enhancer-binding factor (data not shown). In gel retardation experiments using a ^{32}P-labeled synthetic poly(GT) element (Fig. 3B), the addition of increasing amounts of nuclear extract from either 293, MK2, or HeLa cells resulted in the appearance of distinct bands of slower mobility. In competition studies, the shifted bands, B1 and B2, were shown to be specific whereas the minor band migrating between the GT probe and B1 could not be specifically competed with unlabeled GT element (data not shown). As shown in the example in Fig. 3C, the level of B1 appeared to plateau with increasing nuclear extract, while the level of B2 increased. These data suggested that B2 was derived from B1, which was confirmed in the experiments described below. As shown in Fig. 3D, the amount of nuclear extract required to obtain an equivalent level of binding to the GT element was the same for each cell line. Thus, we could not demonstrate a significant difference in the level of binding of nuclear proteins to the GT element between cells expressing E1A (293) and cells not expressing the E1A proteins (HeLa, MK2). We also examined the level of GT-binding activity in nuclear extracts prepared from cells 7 h postinfection with adenovirus type 5 or dl312, an adenovirus mutant with a defective E1A gene. There was no significant difference in the amount of extract required to obtain the same level of B1 and B2 (Fig. 3E). Thus, the host cell-dependent effects of E1A do not appear to be mediated through the factor(s) involved in the initial interaction(s) with the GT element.

To more fully characterize the GT binding factor(s), the dose-response parameters for the formation of the B1 and B2 complexes were determined. As shown in Fig. 4A and B, the B1 complex appeared at low concentrations of extract and began to plateau as the B2 complex began to increase. With further increases in nuclear protein concentration, B1 disappeared as B2 continued to increase and ultimately plateau. In competition experiments, starting with conditions of equal B1 and B2 complex, increases in the level of cold GT resulted first in a reduction of B2, followed by reduction in the level of B1 (Fig. 4C). In fact, no competition of B1 was observed until the level of B2 had been reduced by 80 to 85%. The dose-response and competition data suggest that the B2 complex is derived from the preformed B1 complex. Similar dose-response patterns have been observed in the dimer formation of the truncated glucocorticoid receptor to the steroid response element (35). Additional experiments will be needed to determine whether the B2 shift is due to dimer formation of the factor responsible for the B1 shift or is the next factor in the assembly of a transcription complex. As shown in Fig. 3D, E1A did not affect either B1 or B2 formation.

We have demonstrated that binding of a nuclear factor to an NF1 homology (BK-NF1 site) was involved in the positive activity of the BK virus enhancer (11). Markowitz and Dynan (25) have shown that a factor that binds to the

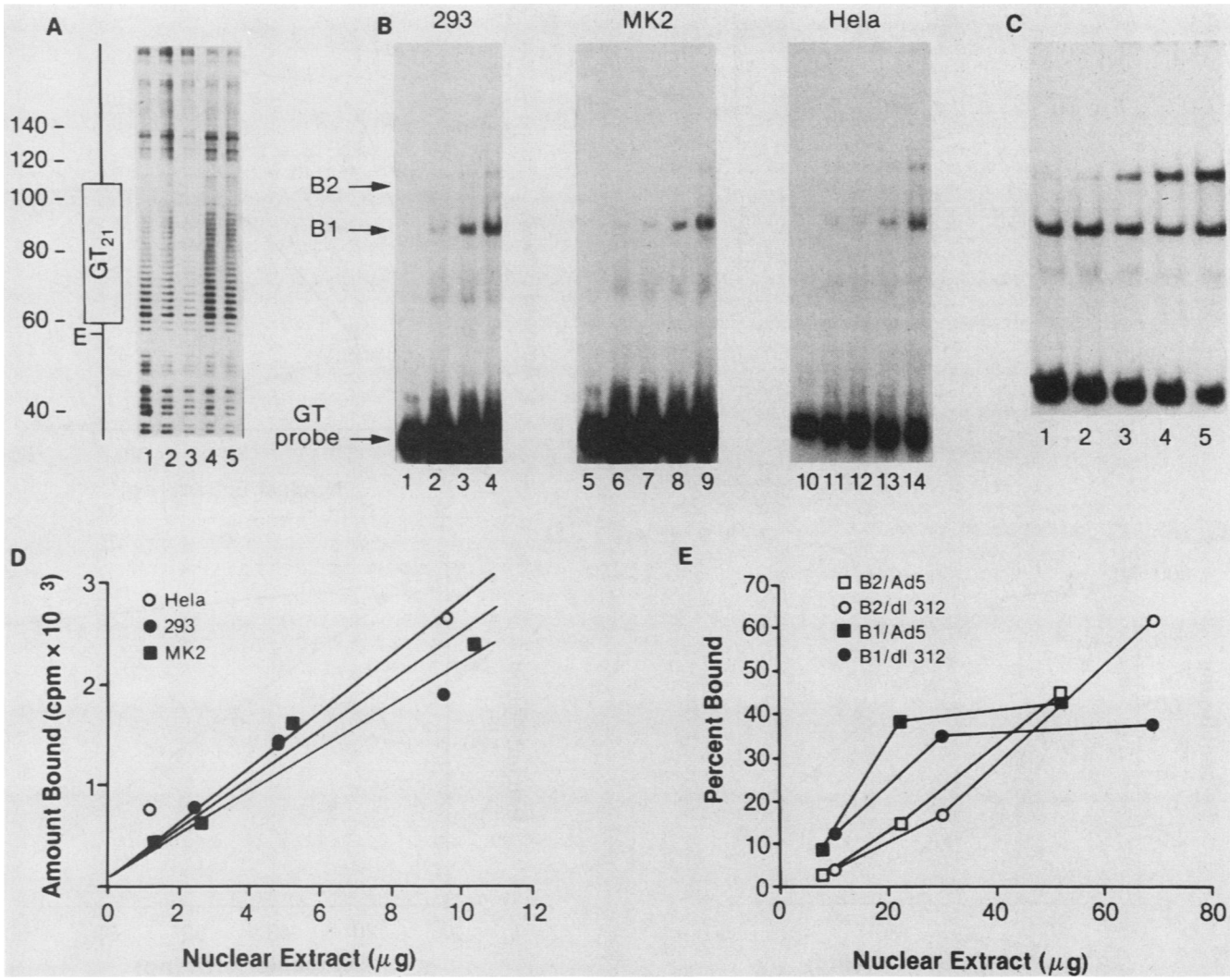

FIG. 3. Binding of 293, MK2, and HeLa cell nuclear proteins to the poly(GT) element. Nuclear extracts were prepared from HeLa, 293, and MK2 cells as described by Dignam et al. (6). (A) The DNA fragment for footprint analysis was prepared from pGTC by restriction digestion with *Hin*dIII (BK, nucleotide 5076) and end labeling with polynucleotide kinase and [γ-^{32}P]ATP, followed by digestion with *Avr*II (BK, nucleotide 366). The plasmid pGTC contains the synthetic poly(GT) element (Fig. 1) upstream of the BK virus enhancer at the *Stu*I site (BK, nucleotide 5122). Approximately 10 μg of labeled DNA was incubated with increasing amounts of 293 cell nuclear extract under conditions described previously (11): lane 1, 5 μg; lane 2, 10 μg; lane 3, 20 μg; lanes 4 and 5, no extract. The position of the GT element relative to the *Hin*dIII site is shown. E, Position of the *Eco*RI site in the synthetic GT element. (B) Approximately 5 ng of GT probe, end labeled with polynucleotide kinase and [γ-^{32}P]ATP, was incubated with various amounts of a nuclear extract prepared from either 293, MK2, or HeLa cells, under conditions similar to those described by Carthew et al. (4), modified as described previously (11); the protein-bound probe was separated from the free probe on a low-ionic-strength 4% polyacrylamide gel as described by Fried and Crothers (9), and after the gels were dried, the protein-DNA complexes were visualized by autoradiography. Lanes 1, 5, and 10, No extract; lanes 6 and 11, 1.25 μg of extract; lanes 2, 7, and 12, 2.5 μg of extract; lanes 3, 8, and 13, 5 μg of extract; lanes 4, 9, and 14, 10 μg of extract. B1 and B2, Locations of bound probe. (C) GT probe incubated with 1.65, 5, 8, 16.5, and 24.5 μg of HeLa cell nuclear extract in lanes 1 to 5, respectively. (D and E) Amount and percent of nuclear extract bound; for details, see text.

positive site also interacts with additional sequences in BK virus MM. These same sequences in the BK virus P2 enhancer were shown to be involved in both the repression of enhancer activity (11) and positive response to E1A (10). As shown in Fig. 4D, the synthetic BK-NF1 site (11) did not compete for binding with the GT binding factor. The synthetic GT element also could not compete for the nuclear factor binding to the BK-NF1 site (data not shown). Therefore, the same factor is not binding to these two E1A-responsive elements even though both respond to E1A in a host cell-specific manner.

In this paper, we have demonstrated that the highly repeated GT element can affect transcription of an adjacent promoter in the presence of E1A, a *trans*-acting factor. In combination with the BK virus enhancer, the GT element still functioned to enhance transcription in the presence of E1A. In fact, the combination of these two E1A-responsive elements resulted in approximately a 1,000-fold enhancement of transcription from the MLP in the E1A-producing cell line 293. Such strong transcriptional signals may be generally useful for the overexpression of recombinant genes. In contrast, E1A repressed MLP activity in the presence of the GT element in HeLa cells. A similar host cell-dependent effect of E1A on enhancer activity has been observed for the immunoglobulin heavy-chain enhancer (3, 19). Our data also suggest that the effect of E1A does not

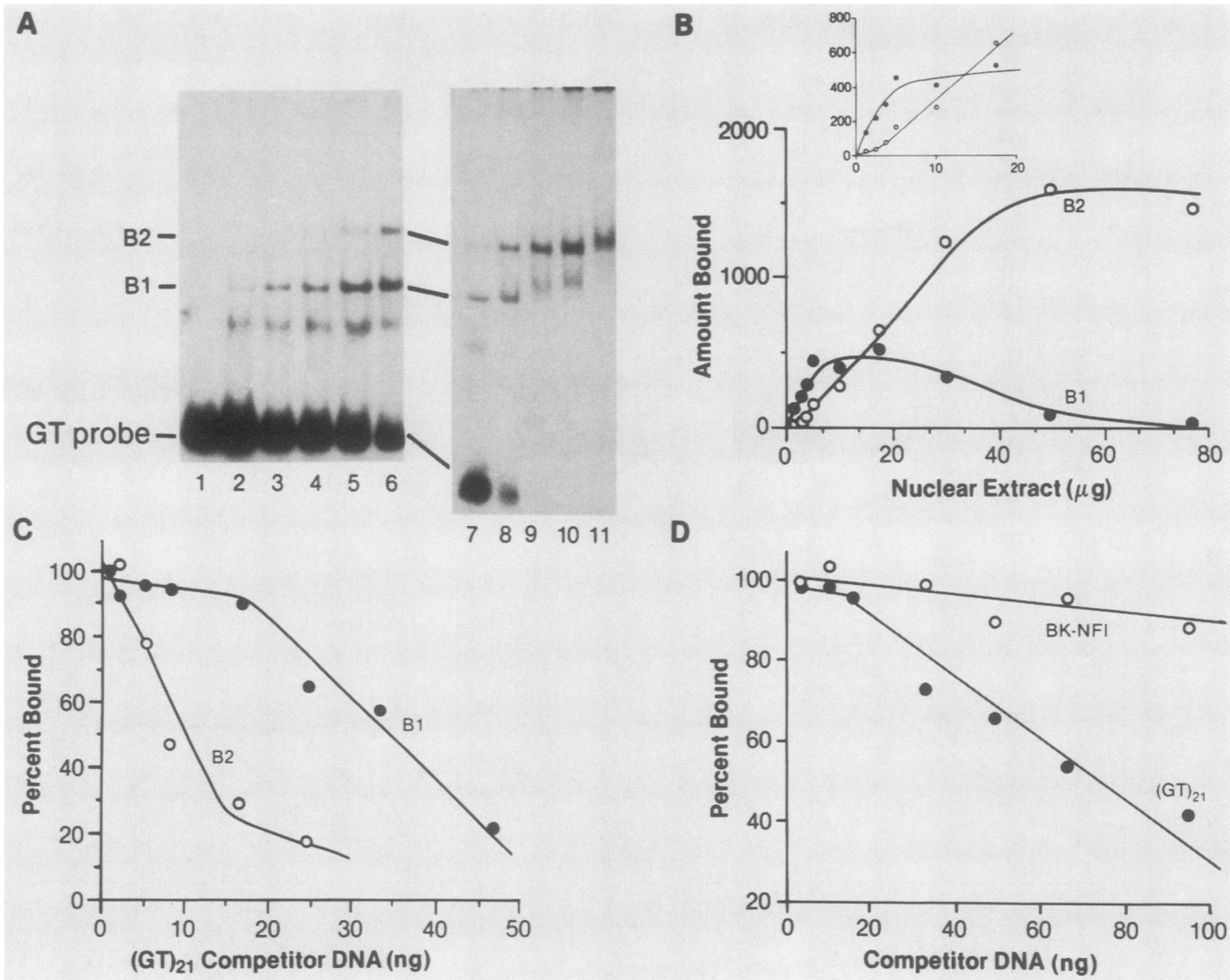

FIG. 4. Dose-response results for the binding and competition of the GT-binding factor. (A) Effect of increasing levels of HeLa cell nuclear protein on the formation of the B1 and B2 complexes. Lane 1, No extract; lane 2 through 11, 1.25, 2.5, 3.75, 5, 10, 10, 17.5, 29, 29.5, and 77 μg of extract, respectively. (B) Summary of B1 and B2 complex formation with increasing levels of nuclear protein. Amount of probe bound (in counts per minute) was determined from four individual experiments. (C) Competition for B1 and B2 complex formation with increasing levels of the cold GT element determined by gel shift analysis. Reactions contained approximately 5 ng of labeled GT probe, 10 μg of nuclear extract, and increasing levels of cold GT probe. The level of binding with no competitor was taken as 100% for each complex. (D) Competition for B1 complex formation with increasing levels of the GT element and BK-NF1 site (11). Conditions were as described for panel C.

involve initial interactions with the GT element. The E1A gene product(s) has been shown to interact with cellular factors (7, 19) and actually increase the binding activity of factors for sequences upstream of the E2 promoter (23, 24) and TATA binding factor (37) and also the active concentration of TFIIIC (20, 38). In other cases, there has been no observable effect of E1A on the interaction of nuclear factors with E1A-responsive elements (5, 21, 31).

While it is reasonable to suggest that the binding of a GT-specific factor(s) is involved in the function of the GT element, it is possible that the function of the GT element is to alter local DNA structure. The formation of Z-form DNA is stabilized by negative supercoiling and has been amply demonstrated in vitro (17, 26). However, there is disagreement in the literature as to the stability of Z-form DNA in vivo (see reference 29). While it has been suggested that Z-DNA structure could be stabilized by Z-DNA-binding proteins (29), it is unlikely that the GT-binding factor described here is a Z-DNA-binding protein because the probe was not used in conditions conducive to Z-DNA formation in vitro. Whether or not altered DNA structure is involved in the function of the GT element, the GT-binding protein described here may be important in the function of the GT element and in its ability to regulate expression through *trans* activation or repression.

We thank R. Belagaji for synthesizing the poly(GT) element used in these studies and R. Schneider for kindly supplying the adenovirus mutant dl312.

LITERATURE CITED

1. **Berg, D. T., J. D. Walls, and B. W. Grinnell.** 1988. A variant enhancer/regulatory region from a cloned human prototype BK virus genome. Nucleic Acids Res. **16**:9057.
2. **Borrelli, E., R. Hen, and P. Chambon.** 1984. Adenovirus-2 E1A products repress enhancer-induced stimulation of transcription. Nature (London) **312**:608–612.

3. **Borrelli, E., R. Hen, C. Wasylyk, B. Wasylyk, and P. Chambon.** 1986. The immunoglobulin heavy chain enhancer is stimulated by the adenovirus type 2 E1A products in mouse fibroblasts. Proc. Natl. Acad. Sci. USA **83**:2846–2849.
4. **Carthew, R. W., L. A. Chodosh, and P. A. Sharp.** 1985. An RNA polymerase II transcription factor binds to an upstream element in the adenovirus major late promoter. Cell **43**:439–448.
5. **Devaux, B., G. Albrecht, and C. Kedinger.** 1987. Identical genomic footprints of the adenovirus EIIa promoter are detected before and after EIa induction. Mol. Cell. Biol. **7**: 4560–4563.
6. **Dignam, J. O., R. M. Lebovitz, and R. G. Roeder.** 1983. Accurate transcription initiation by RNA polymerase II in a soluble extract from isolated mammalian nuclei. Nucleic Acids Res. **11**:1475–1489.
7. **Egan, C., S. B. Yee, B. Ferguson, M. Rosenberg, and P. E. Branton.** 1987. Binding of cellular polypeptides to human adenovirus type 5 E1A proteins produced in *Escherichia coli*. Virology **160**:292–296.
8. **Flanagan, J. C., M. P. LeFranc, and T. H. Rabbitts.** 1984. Mechanisms of divergence and convergence of the immunoglobulin $\alpha 1$ and $\alpha 2$ constant region gene sequences. Cell **36**:681–688.
9. **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.
10. **Grinnell, B. W., D. T. Berg, and J. Walls.** 1986. Activation of the adenovirus and BK virus late promoters: effects of the BK virus enhancer and *trans*-acting viral early proteins. Mol. Cell. Biol. **6**:3596–3605.
11. **Grinnell, B. W., D. T. Berg, and J. D. Walls.** 1988. Negative regulation of the human polyomavirus BK enhancer involves cell-specific interaction with a nuclear repressor. Mol. Cell. Biol. **8**:3448–3457.
12. **Grinnell, B. W., and R. R. Wagner.** 1984. Nucleotide sequence and secondary structure of VSV leader RNA and homologous DNA involved in inhibition of DNA-dependent transcription. Cell **36**:533–543.
13. **Gross, D. S., S. Y. Huang, and W. T. Garrard.** 1985. Chromatin structure of the potential Z-forming sequence $(dT\text{-}dG)_n \cdot (dC\text{-}dA)_n$: evidence for an "alternating-B" conformation. J. Mol. Biol. **183**:251–256.
14. **Hamada, H., and T. Kakunaga.** 1982. Potential Z-DNA forming sequences are highly dispersed in the human genome. Nature (London) **298**:396–398.
15. **Hamada, H., M. G. Petrino, and T. Kakunaga.** 1982. A novel repeated element with Z-DNA-forming potential is widely found in evolutionarily diverse eukaryotic genomes. Proc. Natl. Acad. Sci. USA **79**:6465–6469.
16. **Hamada, H., M. Seidman, B. H. Howard, and C. M. Gorman.** 1984. Enhanced gene expression by the poly (dT-dG) · poly(dC-dA) sequence. Mol. Cell. Biol. **4**:2622–2630.
17. **Haniford, D. B., and D. E. Pullyblank.** 1983. Facile transition of poly[d(TG) · d(CA)] into a left-handed helix in physiological conditions. Nature (London) **302**:632–634.
18. **Harlow, E., P. Whyte, B. R. Franza, Jr., and C. Schley.** 1986. Association of adenovirus early-region 1A proteins with cellular polypeptides. Mol. Cell. Biol. **6**:1579–1589.
19. **Hen, R., E. Borrelli, and P. Chambon.** 1985. Repression of the immunoglobulin heavy chain enhancer by the adenovirus-2 E1A products. Science **230**:1391–1394.
20. **Hoeffler, W. K., and R. G. Roeder.** 1985. Enhancement of RNA polymerase III transcription by the E1A gene product of adenovirus. Cell **41**:955–963.
21. **Hurst, H. C., and N. C. Jones.** 1987. Identification of factors that interact with the E1A-inducible adenovirus E3 promoter. Genes & Dev. **1**:1132–1146.
22. **Jalinot, P., and C. Kedinger.** 1986. Negative regulatory sequences in the E1a-inducible enhancer of the adenvirus-2 early EIIa promoter. Nucleic Acids Res. **14**:2651–2669.
23. **Kovesdi, I., R. Reichel, and J. R. Nevins.** 1986. E1A transcription induction: enhanced binding of a factor to upstream promoter sequences. Science **231**:719–722.
24. **Kovesdi, I., R. Reichel, and J. R. Nevins.** 1986. Identification of a cellular transcription factor involved in E1A *trans*-activation. Cell **45**:219–228.
25. **Markowitz, R.-B., and W. S. Dynan.** 1988. Binding of cellular proteins to the regulatory region of BK virus DNA. J. Virol. **62**:3388–3398.
26. **Nordheim, A., and A. Rich.** 1983. The sequence $(dC\text{-}dA)_n\text{-}(dG\text{-}dT)_n$ forms left-handed Z-DNA in negatively supercoiled plasmids. Proc. Natl. Acad. Sci. USA **80**:1821–1825.
27. **Nordheim, A., and A. Rich.** 1983. Negatively supercoiled simian virus 40 DNA contains Z-DNA segments within transcriptional enhancer sequences. Nature (London) **303**:674–679.
28. **Peck, L. J., and J. C. Wang.** 1985. Transcriptional block caused by a negative supercoiling induced structural change in an alternating CG sequence. Cell **40**:129–137.
29. **Rodriguez-Campos, A., M. J. Ellison, L. Pe'rez-Graw, and F. Azorin.** 1986. DNA conformation and chromatin organization of a $d(CA/GT)_{30}$ sequence cloned in SV40 minichromosomes. EMBO J. **5**:1727–1734.
30. **Santoro, C., F. Costanzo, and G. Ciliberto.** 1984. Inhibition of eukaryotic transcription by potential Z-DNA sequences. EMBO J. **3**:1553–1559.
31. **Siva Raman, L., S. Subramanian, and B. Thimmappaya.** 1986. Identification of a factor in HeLa cells specific for an upstream transcriptional control sequence of an E1A-inducible adenovirus promoter and its relative abundance in infected and uninfected cells. Proc. Natl. Acad. Sci. USA **83**:5914–5918.
32. **Stringer, J. R.** 1982. DNA sequence homology and chromosomal deletion at a site of SV40 DNA integration. Nature (London) **296**:363–366.
33. **Stringer, J. R.** 1985. Recombination between poly [d(GT) · d(CA)] sequences in simian virus 40-infected cultured cells. Mol. Cell. Biol. **5**:1247–1259.
34. **Swanstrom, R., R. C. Parker, H. E. Varmus, and J. M. Bishop.** 1983. Transduction of a cellular oncogene: the genesis of Rous sarcoma virus. Proc. Natl. Acad. Sci. USA **80**:2519–2523.
35. **Tsai, S. Y., J. Carlstedt-Duke, N. L. Weigel, K. Dahlman, J.-A. Gustafsson, M.-J. Tsai, and B. W. O'Malley.** 1988. Molecular interactions of steroid hormone receptor with its enhancer element: evidence for receptor dimer formation. Cell **55**:361–369.
36. **Velcich, A., and E. Ziff.** 1985. Adenovirus E1a proteins repress transcription from the SV40 early promoter. Cell **40**:705–716.
37. **Wu, L., D. S. E. Rosser, M. C. Schmidt, and A. J. Berk.** 1987. A TATA box implicated in E1A transcriptional activation of a simple adenovirus 2 promoter. Nature (London) **326**:512–515.
38. **Yoshinaga, S., N. Dean, M. Han, and A. J. Berk.** 1986. Adenovirus stimulation of transcription by RNA polymerase III: evidence for an E1A dependent increase in transcription factor IIIC concentration. EMBO J. **5**:343–354.
39. **Yu, Y.-T., and J. L. Manley.** 1986. Structure and function of the S1 nuclease-sensitive site in the adenovirus late promoter. Cell **45**:743–751.