Promoter Activity and Distance Constraints of One Versus Two Sp1 Binding Sites*

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In the simple adenovirus 2 early 1B (E1B) promoter, moving the single Sp1 transcription factor binding site (GC box) from its wild-type position eight base pairs (bp) from the TATA box to 30 bp upstream is equivalent to its deletion (Wu, L., and Berk, A. (1988) Genes & Dev. 2, 403–411). In more complex promoters, multiple upstream elements regulate transcription over greater distances. To understand these spacing constraints, we placed two GC boxes in phase at various distances from the E1B TATA box. Whereas one GC box at eight bp from TATA increases transcription in vivo 5-fold compared with TATA alone (Wu, L., Rosser, D. S. E., Schmidt, M. C., and Berk, A. (1987) Nature 326, 512–515), two GC boxes increased in vivo transcription 25–30-fold. Transcriptional stimulation by two GC boxes fell off rapidly in vivo at 30 bp from TATA, remained approximately constant through 50 bp, and then decreased again at 70 bp. Consequently, both GC boxes have a multiplicative effect at eight bp from TATA and continue to stimulate transcription at a greater distance from TATA than a single Sp1 site. Quantitatively different results were observed for in vitro transcription using a nuclear extract; a more linear fall off with increasing distance from TATA was observed. Separating the two GC boxes progressively decreased transcription. E1A stimulation of these promoters in vivo indicates that Sp1 transcription control is independent of E1A transactivation.

In eukaryotes, the initiation and regulation of transcription is dependent on the interaction between specific and general transcription factors, the multi-subunit RNA polymerase II, and transcriptional control sequences in DNA (reviewed in Refs. 3–6). In order to understand the complexities of transcriptional regulation, utilization of a relatively simple promoter such as the adenovirus type 2 (Ad2) early region 1B (E1B) promoter (1, 2) is desirable. The E1B promoter region contains two cis-acting promoter elements, a GC box (−48 to −39) (7), and a TATA box (−30 to −25) (8), which are required both in vivo and in vitro for wild-type transcriptional activity (1, 2, 9). These elements, respectively, constitute binding sites for the Sp1 (2, 10–13) and TFIID (9, 14, 15) transcription factors. Further upstream elements (−250 to −125) only modestly effect transcription in vivo (2, 16, 17) and have no effect in vitro in the presence of the GC box (9, 16).

Insertion of additional sequence between the single GC box and the TATA box of the wild-type E1B promoter results in a rapid fall off in transcription (1). Twenty-four and 30-base pair (bp) separations reduce transcription to a level comparable to that observed with deletion of this Sp1 binding site. As observed, for more complex promoters (18, 19), a modest phasic effect is observed in the E1B promoter when the GC box is in close proximity to the TATA box (1). However, the rigid spacing constraints observed with the E1B promoter (1) are not apparent with more complex promoters (18–21). For example, in the herpes simplex virus type 1 thymidine kinase promoter, which contains two GC boxes and a CAAT box (21), separating the distal elements and the TATA box by 50 bp has no effect on the rate of transcription when compared with the wild type, where the proximal GC box is separated from the TATA box by 19 bp (20). This contrast between the E1B and thymidine kinase promoters suggests that the interaction between the additional upstream factors in the thymidine kinase promoter and proteins bound to the TATA box allow for regulation of transcription over a greater distance than observed for the single Sp1 transcription factor in the E1B promoter.

In order to understand the distinction between the rigid spacing constraints of the E1B promoter and the flexibility of more complex promoters in which regulation can occur from promoter elements over greater distances, we examined the effect on transcription of two GC boxes (i.e., an additional Sp1 transcription factor binding site) at various distances from the TATA box in the E1B promoter both in vivo and in vitro. We also analyzed adenovirus E1A transactivation (22) of these two GC box promoters.

**MATERIALS AND METHODS**

*Cells and Viruses—* HeLa and 293 cell (23) suspension cultures were grown in minimal essential medium (GIBCO) supplemented with 5% newborn calf serum (NCS). Monolayers of 293 cells were grown in Dulbecco's modified Eagle's medium (GIBCO) containing 10% NCS. Adenoviruses with the E1B promoter mutations were propagated in 293 suspension cultures grown in suspension minimal essential medium containing 2% NCS. Viral stocks harvested from 293 cells were assayed by plaque titration on 293 monolayers in Dulbecco's modified Eagle's medium with 0.7% agarose, 2 mM L-glutamine, minimal essential medium nonessential amino acids (GIBCO), 25 mM MgCl₂, and 2% NCS.

*Construction of Mutant E1B Promoter Plasmids and Virus—* The pBR322-derived plasmids (tetracycline-resistant) were propagated in Escherichia coli HB101, and plasmid DNA purified by the alkaline-
sodium dodecyl sulphate method (24). Enzymes for cloning were used per manufacturers' recommendations. Construction of mutant E1B promoter containing plasmids with multiple GC boxes (Sp1 transcription factor binding sites) was performed using standard cloning protocols (25). The plasmid construction was based on a pBR derivative with Ad2 E1B promoter sequence (pBE1500/IN16) (1). The proximal EcoRI restriction site which separates the GC box from the TATA box and a distal XhoI site were utilized. Synthesized oligonucleotides (Applied Biosystems) containing the two GC boxes (Fig. 1) were first ligated into a pUC vector (pAd1B) (9) and then the 321-bp XhoI-EcoRI fragment was cloned between the XhoI and EcoRI sites of pHIF1500/IN16/RI to generate mutant 2Sp150. For mutants 2Sp140, 2Sp150, and 2Sp170 (see Fig. 3) a 10-mer Smal-EcoRI adaptor (5′-AATTTCCGGG-3′) (New England Biolabs 1102) was phosphorylated with T4 polynucleotide kinase and then ligated into the EcoRI site of 2Sp150 in single or multiple copies. 2Sp134 was generated by filling in the EcoRI site of 2Sp150 with deoxyribonucleotides using Klenow fragment followed by blunt end ligation. The 2Sp1wt mutant was generated by utilizing oligonucleotide-directed in vitro mutagenesis (26) (modifications am Peramesses kit, catalogue no. RPN2322). The intervening sequence was deleted to bring the two GC boxes to the wild-type position eight bp upstream of the TATA box. The oligonucleotide sequence was 5′-CATTATTACCCTTTTAAGCCCCGCCCCCTCTT-3′. Thus the sequence separating the proximal Sp1 site and the TATA box was identical to that of the wild-type promoter.

Oligonucleotide-directed in vitro mutagenesis (26) was also used to generate a set of mutants in which the two GC boxes were separated by 21, 31, and 51 bp, designated Ap21, Ap31, and Ap51. The sequence used to prime mutagenesis of 2Sp130 was 5′-AGCCCCGCCCTTACCGCTTACACCGCCC-3′, which created a novel MluI site between the two GC boxes. One or more copies of a 10-mer, self-hybridizing oligonucleotide, (5′-CCGACATACT-3′) was ligated into the MluI site to generate mutants Ap21, Ap31, and Ap51. All mutant sequences were confirmed by deoxyribonucleotide sequencing (27) (Sequenase Version II kit, U. S. Biochemical Corp. catalog no. 70770).

The selected E1B promoter adenoviral mutants that were examined in vivo were generated by in vitro recombination after transfection of viral DNA and isolated mutant plasmids (28). One microgram of Xbal/ClaI-digested dl309 (29) viral DNA and 5 pg of BamHI-digested pBE1500-derived mutant plasmid DNA were cotransfected. Virus from isolated plaques was propagated, viral DNA isolated by Hirt lysis, and the desired mutants identified by restriction digest mapping with XhoI and EcoRI. Two rounds of plaque purification were performed on 293 cells for each mutant virus to ensure purity. Further confirmation that viral mutants containing multimer linkers were stably propagated was obtained by restriction digestion of mutant viral DNA.

**Experimental Single-stranded 3′ Probe—Twenty pmol of 20-mer oligonucleotide B1820 (5′-GCTTGGAGAGGAGCCCACAG-3′), identical to the bottom strand of adenovirus type 2 sequence nucleotides 1820–1800, was phosphorylated with 32P using 5 units of cloned T4 polynucleotide kinase (U. S. Biochemical Corp.) at 37 °C for 30 min, in a 20-μl reaction volume with 20 pmol of [γ-32P]ATP (7000 Ci/mmol). The reaction was stopped by incubating at 68 °C for 10 min and ethanol-precipitated. Equimolar concentrations (1 pmol each) of the 5′-32P-labeled oligonucleotide, B1820, and the limiting unlabeled upstream oligonucleotide T1551 (sequence: 5′-CCCCGCAGGGGACAGGAGCCTCTGAG-3′) identical to the top strand Ad2 sequence nucleotides 1551–1568, were used in a standard polymerase chain reaction mix (Cetus). The templates used were linearized E1B or wild-type BE1500 plasmids. The amplification was taken through 20 cycles (92 °C for 30 s, 59 °C for 30 s, and elongation at 72 °C for 10 s). A 10-fold excess of the labeled B1820 oligonucleotide (10 pmol) was then added at the 20th cycle and asymmetric amplification continued for an additional 15 cycles. The amplified products (single- and double-stranded) were separated on a native polyacrylamide gel (6%) and visualized by autoradiography or ethidium bromide staining. (The double-stranded amplified product migrated ahead of the 400-nucleotide single-stranded product.) The single-stranded fragment was then cut out of the gel and electro-eluted into 1 ml of TE buffer (10 mm Tris, pH 8.0, 1 mm EDTA) in an Schleicher & Schuell electro-elution device (100 V for 1 h, then reversal of polarity for 20 s at 150 V). The probe was used directly for S1 analysis (see below) or reprecipitated with 0.3 M sodium acetate/ethanol and resuspended in 1 ml of TE buffer (10 mm Tris, pH 7.9, 0.5 mm EDTA) to be used. Based on the known specific activity of the [γ-32P]ATP, approximately 1–3 pmol of 32P-labeled probe was generated as determined by direct counting. Thirty-five to 50 fmol of probe was used per S1 reaction.

Quantitative S1 Analysis of E1B RNA—HeLa suspension cells were infected with mutant viruses at 20 plaque-forming units/cell. The transcriptional activation effect of E1A on the E1B promoter mutants was determined by coinfection with helper adenovirus 413B (1, 2) which provided functional E1A protein in trans. The 413B E1B gene contains a small deletion that distinguishes the truncated 413B E1B RNA from the E1B RNA expressed from the E1B promoter and thus served as an internal control in the majority of experiments (2). To commence infection, absorption of adenovirus was performed by adding virus stocks to suspension cultures of exponentially growing HeLa cells, concentration to 4 × 106 cells/ml for 1 h. Cells were then diluted with SMEM, 2% NCS to 4 × 106 cells/ml, harvested 6 h later, and cytoplasmic RNA was extracted as described (30). Hybridization was performed in 50 ml of 0.5 M NaCl, 10 ml Tris-HCl, pH 7.1, 0.1 mm EDTA containing 50 μg of cytoplasmic RNA and 35–50 fmol of single-stranded probe. The reaction mix was first placed in a 90 °C water bath for 5 min and subsequent hybridization performed at 68 °C for 2 h. (In order to confirm the sensitivity and specificity of the polymerase chain reaction-generated (Cetus) single-stranded probe, a BstEI, 32P-labeled, double-stranded probe as previously described (1, 2) was used in initial experiments.)

Subsequent S1 digestion was performed at 22 °C for 60 min by adding 450 μl of ice-cold 0.25 M NaCl, 0.03 M sodium acetate (pH 4.5), 1 mM ZnCl2, 5% glycerol, and 400–500 units of nuclease S1 (BRL) to the hybridization mix. Protected probe fragments (see Fig. 2) were separated by electrophoresis on an 8% denaturing polyacrylamide gel. Gels were subjected to direct counting of B emissions by an Ambis counter and data analyzed by accompanying software data analysis programs. Alternatively, preflashed-intensified autoradiograms were analyzed with a Hoefer densitometer, and peak integration was performed by computer (data acquisition program for Acomn BBC) on gels that were not directly counted.

In Vitro Transcription Analysis—In vitro transcription assays were performed as previously described (9). Briefly, primer extension was used to quantify transcripts from a 60-min incubation using 0.4 μg of pBE1500 wild-type DNA and 10 pmol of unfraccionated nuclear extract protein (31) in a total volume of 50 μl. The incubation buffer was modified to include ZnCl2 (final concentration 1 μM).

**RESULTS**

One possible explanation for the strict spacing constraints between transcription control elements in the E1B promoter is that there is only a single transcription factor binding site upstream of the TATA box. In most earlier studies of distance constraints between the TATA box and upstream promoter elements, promoters with several upstream transcription factor-binding sites were studied (18–21). Moreover, where synthetic transcription factor binding sites have been tested for their ability to control transcription in vitro, it has generally been necessary to insert multiple binding sites in order to obtain an effect (32–34). Therefore it seemed possible that the relaxed spacing constraint observed for other binding sites compared with the E1B promoter might be due to the fact that nearly all other promoters examined contain binding sites for multiple transcription factors upstream of the TATA box. To test this hypothesis in the simplest possible way, we analyzed the spacing constraints for derivatives of the E1B promoter containing two GC boxes rather than one. Derivatives of the E1B promoter region were constructed (see Fig.
3) which contained two GC boxes positioned such that the proximal GC box was separated from the TATA box by the same distance as for the single GC box in the wild-type promoter (2SP150) and at increasing distance from the TATA box (2SP130, 34, 40, 50, and 70). In each case the second GC box was separated from the proximal GC box by 11 bp so that the centers of the promoter elements were separated by 21 bp, i.e. two turns of the DNA helix. This choice was based on the 21-bp separation of the centers of the two highest affinity Sp1 sites in the SV40 early promoter (35). The two Sp1 binding sites are thus in phase on the same side of the DNA helix.

To analyze transcription in vivo from these derivatives of the E1B promoter, each was reconstituted into the genome of an infectious adenovirus recombinant, substituting them for the wild-type E1B promoter region. HeLa cells were co-infected with these recombinant adenoviruses plus the E1B deletion mutant S13B (described in Ref. 2) which acted as an internal control for transcription from the wild-type E1B promoter. Cytoplasmic RNA was isolated 6 h post-infection and the concentration of E1B RNAs was analyzed by S1 analysis with 5'-end-labeled single-stranded DNA probe as diagrammed in Fig. 2. RNA expressed from the S13B internal control generated a S1-protected fragment of 47 nucleotides, while RNA expressed from wild-type adenovirus 2 and recombinants with two GC boxes in the E1B promoter region generated S1-protected fragments of 121 nucleotides (Fig. 3).

As shown previously (2), substitution of the single GC box in the E1B promoter by a sequence which does not bind transcription factor Sp1 reduced transcription to approximately 20% of the level observed for the wild-type promoter. Fig. 3 shows results of a representative experiment, whereas Fig. 6 summarizes the results of three separate experiments with two or more S1 analyses for each experiment. As observed earlier (1), a 30-bp separation between the single GC box and the TATA box of the wild-type promoter resulted in a similar decrease in transcription (Figs. 3 and 6). When an additional GC box was inserted upstream of the E1B promoter, transcription increased approximately 5-6-fold relative to the wild-type promoter (Figs. 3 and 6). In this case, when 30 bp were inserted between the proximal GC box and the TATA box, transcription fell off considerably. However, unlike the situation with a single GC box where transcription was diminished to a level equivalent to that of the TATA box alone (mutant LS-48(−GC)), the two GC boxes continued to stimulate transcription, resulting in 3-4-fold more transcription than observed with the TATA box alone. The two GC boxes stimulated transcription to a slightly greater extent when separated from the TATA box by 34, 40, and 50 bp (Figs. 3 and 6). Transcription fell off with an insertion of 70 bp between the TATA box and the first GC box, to a level two times that observed for the TATA box alone.

These results show that two GC boxes stimulate transcription to a greater extent than a single GC box when placed very close to the TATA box as in the wild-type E1B promoter. Addition of the second GC box stimulated transcription 5-6-fold, an effect similar to the stimulation of transcription observed for a single GC box compared with the TATA box alone (i.e. comparing the wild-type promoter with mutant LS-48(−GC)). Two GC boxes also influenced transcription at a much greater distance from the TATA box than a single GC box. This can explain the unusual constraint on spacing between the promoter elements of the E1B promoter compared with other eukaryotic promoters such as the thymidine kinase promoter where much more flexibility is allowed in the distance between promoter elements. Binding sites for multiple factors permit transcription control over greater distances as seen in the simple example of GC boxes studied here. In contrast, the single GC box of the wild-type E1B promoter region must be located quite close to the TATA box in order to stimulate transcription significantly in vivo.

We also examined the effect of insertions between the two GC boxes and the E1B TATA box on in vitro transcription using a HeLa cell nuclear extract (Fig. 4, A and B). As observed in vivo, two Sp1 sites resulted in an increase in transcription in vitro compared to a single Sp1 site (Fig. 4, A and B). However, addition of the second Sp1 site increased in vitro transcription only approximately 3-fold, whereas in vivo transcription was increased 5-6-fold by addition of the second GC box (compare Figs. 3 and 4A). Moreover, a more linear fall off was observed at increasing distances from TATA for in vitro transcription compared with in vivo transcription. In particular, unlike the situation observed in vivo, the fall off in transcription comparing a separation of eight bp from TATA with a separation of 30 bp from TATA was not as sharp.

We also examined the influence of separating the two GC boxes on in vitro transcription. The proximal GC box was maintained at a distance of 30 bp from TATA while 21, 31, or 51 bp were inserted between the distal and proximal GC boxes (mutants Ap21, Ap31, and Ap51, respectively). Constructs in which the centers of the two GC boxes were separated by two (2SP130) or three (Ap21) turns of the DNA helix were transcribed to a similar extent (Fig. 4, A and B). Inser-
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FIG. 5. S1 nuclease analysis of transcription and E1A inducibility of selected 2Sp1 mutants. The presence (+) or absence (−) of E1A from S13B virus coinfection is indicated at the top of the lanes. In A the DNA probe used was the double-stranded 32P-end-labeled BstEII fragment (1, 2) (see "Materials and Methods") from the 2Sp130 mutant. In B the single-stranded polymerase chain reaction-generated probe from wild-type template was used. E1A-E1B fusion product present is schematically detailed in Fig. 2.

DISCUSSION

The interaction of Sp1 and the general transcription factors is complex and appears to involve at least one bridging factor or co-activator (3, 37-39). In the simple E1B promoter, the single Sp1 transcription factor must bind to its cognate recognition sequence in close proximity to the TATA box in order to stimulate transcription (1, 2). Separating the single GC box from the TATA box of the wild-type promoter by 30 base pairs results in a decrease in transcription comparable with that observed for deletion of this Sp1 binding site (1). Such rigid spacing constraints required for upstream regulation are not observed in more complex promoters, which contain binding sites for several transcription factors (18-21). In particular, in the herpes simplex virus type 1 thymidine kinase promoter, which contains two GC boxes and a CAAT box (21), separating the distal elements and the TATA box level of E1A transactivation was observed for mutant E1B with wild-type, where the proximal GC box is separated from the TATA box by 19 bp (20). This contrast between the E1B and thymidine kinase promoters suggests that the interaction between the additional upstream factors by which Sp1 is activated is not observed.

During the early phase of infection (2,17,29,30,36). A similar level of E1A transactivation was observed for wild-type E1B promoters with a deletion or substitution of the single GC box (2) or insertions between the single GC box and the TATA box (1). These results suggested that the mechanism by which E1A stimulates transcription from the E1B promoter is independent of the action of Sp1 (1, 2). To test this idea further, we measured the stimulation of transcription by E1A for selected mutant E1B promoters with two GC boxes. The recombinant adenoviruses described above with two GC boxes in the E1B promoter region all contain the d1500 deletion in the E1A region, which prevents expression of the large E1A protein (36). Expression of RNA from the wild-type and mutant E1B promoters, 2Sp130 and 2Sp1Wt, in the absence of E1A transactivation was measured following infection of HeLa cells with these recombinants alone (lanes designated − in Fig. 5, A and B). To measure transcription from these promoters in the presence of the large E1A protein, HeLa cells were co-infected with the two GC box mutants plus mutant S13B which has a wild-type E1A region (lanes designated + in Fig. 5, A and B). The large E1A protein stimulated transcription from the mutant 2Sp130 4-6-fold in three independent experiments. Similarly, in single experi-
6. Whereas insertion of additional sequence between the single GC box and the wild-type TATA box results in a rapid fall off in transcription (1), the two Sp1 binding sites allow for regulation over a greater distance. The rigid spacing constraint of a single GC box is thus overcome, and the two GC boxes exert an effect even at 70 bp from TATA. In vivo, the influence of the two GC boxes at 30 bp from the TATA box fell off sharply compared with that at eight bp from the TATA box (Figs. 3 and 6). Nonetheless, in comparison to the loss of activity of a single GC box at this position (equal to that of a GC box substitution mutant, LS-48/−39), two Sp1 binding sites were clearly exerting an effect. At greater distances, the level of transcription of the 2Sp1 constructs remained approximately constant from 30 to 50 bp. At a 70-bp separation it decreased again by 50% to a level two times that observed for the TATA box alone.

For in vitro transcription in a HeLa cell nuclear extract, two Sp1 sites also stimulated transcription to a much greater extent than a single Sp1 site at 30 bp from the TATA box (Fig. 4, A and B). However, in vitro transcription, increasing the distance between the two Sp1 sites and the TATA box caused a more linear decrease in transcription than observed in vivo. It is not clear why the in vitro transcription results differ from the in vivo results in this way. The contrast indicates that one cannot extrapolate directly from in vitro to in vivo results, even for simple promoters. Nonetheless, moving the two GC boxes further upstream from TATA clearly resulted in a decrease in transcription. In addition, the influence of the upstream Sp1 site also fell off with increasing distance from the TATA box when the position of the proximal Sp1 site was fixed at 30 bp from TATA (Fig. 4, A and B). This result suggests that the synergistic activation of transcription by two bound Sp1 factors requires an interaction between the two proteins. This could be a direct interaction between DNA-bound Sp1 molecules, or it could involve other protein factors which mediate the interaction.

The mechanism by which Sp1 stimulates transcription is not presently well understood. Recent results suggest that one or more proteins termed co-activators may mediate an interaction between Sp1 and TFID (3, 37–39). The results presented here emphasize the cooperativity of transcriptional stimulation by two Sp1 factors. These strong cooperative effects of two Sp1 molecules on transcription stimulation are unlikely to be due to cooperative binding by the two Sp1 molecules; no cooperativity of Sp1 binding was observed in the early SV40 promoter where there are six closely spaced binding sites (35).

Carter et al. (40) recently reported that derivatives of the yeast GALA transcription factor also function cooperatively in vivo and in vitro to stimulate transcription when multiple binding sites are placed upstream from a TATA box. These authors suggested that the strong synergism they observed may be due to multiple interactions between the GAL4 derivatives and a single general transcription factor bound at the TATA box. However, the nature of these interactions was not defined. Our results for two Sp1 molecules bound very close to the TATA box are consistent with this type of model.

Another model recently suggested for activation by transcription factors is that they prevent nonspecific repression by nucleosomes of TFID binding to the TATA box (41, 42). However, it is difficult to envision how this model can account for the influence of distance to the TATA box on Sp1 activation as described in our study. The magnitude of Sp1 activation changed greatly over a distance of 8–30 bp from TATA, a much smaller distance than the 146 bp tightly bound to a nucleosome octamer. It is also difficult to see how this model can explain the stimulation observed for two Sp1 factors bound at 30–50 bp from TATA. Rather, our results suggest a more complex mechanism of Sp1 activation than simply excluding nucleosomes from the TATA box.

We also analyzed the ability of the adenovirus large E1A protein to stimulate transcription in vivo from the E1B constructs with two Sp1 sites. Each of the derivatives of the E1B promoter that were separated from the TATA box at a distance were stimulated to a similar extent as the wild-type E1B promoter. These results are consistent with our conclusion that E1A transactivation of the E1B promoter depends only on the TATA box and is independent of the action of Sp1 (2). Interestingly, the 2Sp1wt mutant was stimulated only 2-fold by E1A. This may indicate that transcription from this promoter in the absence of E1A is close to some maximal rate. Further activation by E1A may only increase transcription to this maximal level.

These studies emphasize the importance of the extremely close spacing between the GC box and the TATA box in the wild-type E1B promoter. Small icosahedral DNA viruses are constrained to use their sequence with high efficiency, since only a fixed length of DNA can be packaged into the virion particle. Adenovirus type 2 evolved an extremely simple E1B promoter. These results are consistent with our conclusion that E1A transactivation of the E1B promoter depends only on the TATA box and is independent of the action of Sp1 (2). Interestingly, the 2Sp1wt mutant was stimulated only 2-fold by E1A. This may indicate that transcription from this promoter in the absence of E1A is close to some maximal rate. Further activation by E1A may only increase transcription to this maximal level.

These studies emphasize the importance of the extremely close spacing between the GC box and the TATA box in the wild-type E1B promoter. Small icosahedral DNA viruses are constrained to use their sequence with high efficiency, since only a fixed length of DNA can be packaged into the virion particle. Adenovirus type 2 evolved an extremely simple E1B promoter region with only a single GC binding site upstream of the TATA box. This single Sp1 site is able to stimulate transcription significantly because it is positioned so close to the TATA box. More complex promoters demonstrate regulation from promoter elements at much greater distances from the TATA box. This depends on the strong cooperativity of transcriptional stimulation produced by two or more transcription factors bound at adjacent sites along the DNA.

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