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DNA sequence required for initiation of transcription *in vitro* from the major late promoter of adenovirus 2

(*in vitro* mutagenesis/RNA polymerase II/RNA cap site/eukaryotic promoter)

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ABSTRACT **We have identified a region of the viral genome required for the initiation of transcription *in vitro* from the major late promoter of adenovirus 2. A fragment of the adenovirus genome containing the cap site of the major late transcripts was inserted into plasmid pBR322 and cloned. Deletions were then generated *in vitro* in and around the T-A-T-A-A-A-A sequence located 25–31 nucleotides (positions −25 to −31) upstream from the cap site. DNAs with these deletions were tested for their ability to initiate transcription *in vitro* by the method of Manley *et al.* [Manley, J. L., Fire, A., Cano, A., Sharp, P. A. & Gefter, M. L. (1980) *Proc. Natl. Acad. Sci. USA* 77, 3855–3859]. Whereas removal of sequences upstream from position −47 or downstream from position −12 did not abolish transcription, deletions extending into, or beyond, the T-A-T-A-A-A-A sequence reduced transcription to less than 1/10th. Removal of the normal cap site slightly reduced, but did not abolish, transcription. These results indicate that the region of the genome upstream of the cap site, with boundaries within 15–17 nucleotides to either side of the T-A-T-A-A-A-A sequence, is required for the initiation of transcription *in vitro* from the major late promoter of adenovirus 2.**

Control of transcription constitutes an important step in the regulation of gene expression. Studies of prokaryotic systems indicate that the initiation of mRNA synthesis requires interactions between RNA polymerase and a specific region of DNA upstream from the gene to be transcribed, called the promoter (1). Similar attempts to identify promoters for eukaryotic genes have been handicapped by the lack of suitable genetic and biochemical systems. Recently, however, developments in recombinant DNA technology and the advent of *in vitro* transcription systems offer an approach to the study of the molecular mechanisms of transcriptional controls in eukaryotic systems (2–5).

The major late transcription unit of adenovirus type 2 (Ad2) is one of the best characterized in eukaryotes. During the late stage of Ad2 infection, the major viral transcript is initiated at a site around coordinate 16.5 and elongated toward the right end of the genome (6, 7). This primary transcript is believed to be processed into several families of cytoplasmic messenger RNAs that all have the same capped 5′ end as the primary transcript (7, 8). Twenty-five to 31 nucleotides upstream from the RNA cap site, there is a stretch of A+T-rich sequence (T-A-T-A-A-A-A) that appears to be conserved in a number of eukaryotic genes transcribed by RNA polymerase II (9). The similarity of this A+T-rich sequence and the "Pribnow box" of prokaryotic promoters has been noted (10). We report here experiments designed to determine the role of this A+T-rich sequence in the initiation of transcription *in vitro* from the major late promoter of Ad2.

MATERIALS AND METHODS

Enzymes and Reagents. Phage T4 DNA ligase and restriction endonucleases *Alu* I and *Ava* I were purchased from New England BioLabs. Other restriction enzymes and nuclease BAL 31 were obtained from Bethesda Research Laboratories (Rockville, MD). T4 polynucleotide kinase was obtained from P-L Biochemicals and calf intestine alkaline phosphatase from Boehringer Mannheim. Synthetic *Eco*RI and *Hin*dIII linkers were obtained from Collaborative Research (Waltham, MA). [γ-^{32}P]ATP (>2000 Ci/mmol; 1 Ci = 3.7×10^{10} becquerels) was purchased from Amersham and [α-^{32}P]UTP from ICN. All enzymatic reactions, unless otherwise stated, took place under conditions specified by the manufacturer.

Construction of Late Promoter Clones. Ad2 DNA was isolated from purified virions grown in HeLa cells (11). One microgram of Ad2 DNA was digested to completion with *Hin*dIII and the fragments produced were ligated (15°C, 16 hr) to 1 μg of *Hin*dIII-cut DNA of plasmid pBR322. Transformation of *Escherichia coli* χ1776 was performed according to unpublished procedures of D. Hanahan. Ampicillin-resistant colonies were picked and screened for their plasmid inserts (12). Clones of all internal *Hin*dIII fragments of Ad2 DNA were obtained. The *Hin*dIII C clone (pAd6) contains adenovirus sequences from coordinate 8 to 17. The cap site of the major late transcripts is located 193 nucleotides from the *Hin*dIII site at 17. This clone was used as one of the late promoter clones for the construction of deletion mutants. Another late promoter clone, called pϕ4, was constructed by ligating the 430-base-pair (bp) *Alu* I fragment of pAd6 to the large fragment of *Eco*RI–*Hin*dIII-cut pBR322 DNA by using *Eco*RI and *Hin*dIII linkers (see Fig. 1). This clone contains adenovirus sequence complementary to the first 33 nucleotides of the major late transcript and about 400 bp upstream of the cap site. The structures of these clones were determined by restriction endonuclease analysis and, in the case of pϕ4, by DNA sequencing.

Construction of Deletion Mutant Clones. Both pAd6 and pϕ4 were used as starting materials for the construction of deletions upstream from the cap site. Three micrograms of pAd6 or pϕ4 DNA was linearized with *Xho* I and then digested with nuclease BAL 31 (0.5 unit in a 50-μl reaction mixture) at 30°C for 3–5 min. Nuclease reactions were stopped by dilution with 10 mM Tris·HCl, pH 7.9/1 mM EDTA followed by extractions with phenol and chloroform and precipitation with ethanol. The resulting fragments were ligated to 20-fold molar excess of

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Abbreviations: Ad2, adenovirus type 2; bp, base pair(s); kb, kilobase(s).

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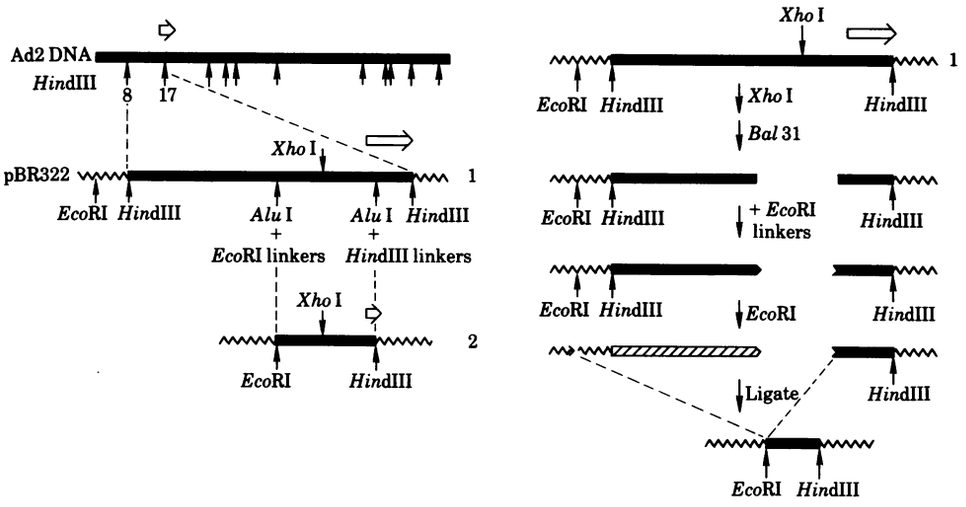

FIG. 1. Cloning of the Ad2 major late promoter and the construction of deletion clones. The left side of the figure illustrates the cloning of the region of Ad2 genome containing the major late promoter. Structure 1 represents the parental clone, pAd6, used to construct upstream deletion clones. Structure 2 represents pϕ4, an *Alu* I subclone of Ad6, used to construct both upstream and downstream deletion clones. The scheme used to construct upstream deletion clones from pAd6 is illustrated on the right side of the figure. See text for details. Heavy solid lines represent adenovirus genome. Wavy lines represent pBR322 sequences. Restriction endonuclease sites are indicated by upward or downward arrows. The relevant positions of *Hin*dIII cleavage sites on Ad2 DNA are indicated in % Ad2 map units. The position of the cap site and the direction of major late transcripts are indicated by open arrows.

polynucleotide kinase-treated *Eco*RI linkers and then redigested with *Eco*RI. The DNA fragments were separated from unligated linkers by passage through a Sepharose 4B column equilibrated with 0.15 M NaCl/10 mM Tris·HCl, pH 7.9/1 mM EDTA. Excluded fractions from the column were pooled and the DNA was precipitated by ethanol. This DNA was religated and used for the transformation of *E. coli*. Ampicillin-resistant clones were selected and the clones with plasmids of appropriate sizes were identified (12). The extent of deletion was estimated by the size of fragments produced by *Hae* III digestion and resolved by electrophoresis in 7 M urea/8% acrylamide gels. Exact endpoints of the deletions were located by the DNA sequence determination method of Maxam and Gilbert (13). The procedure is summarized in Fig. 1. Plasmid pϕ4 was used as the starting material for the construction of deletions downstream from the cap site. The experimental design was analogous to that for the construction of 5′ deletions, except that *Hin*dIII was used for the initial linearization of pϕ4 and that, after a brief digestion (0.5–1.5 min) with nuclease BAL 31, *Hin*dIII linkers were used to recircularize the plasmid. Because these deletions had different endpoints not only in the Ad2 sequences but also in pBR322, the Ad2 fragments were released by digestion with *Eco*RI and *Hin*dIII and reinserted into pBR322 at the corresponding sites. Both the original and the recloned DNAs were used in the experiments described below. Methods of Tanaka and Weisblum (14) were used to prepare plasmid DNA. All procedures were in accordance with the National Institutes of Health guidelines (P2/EK2).

***In Vitro* Transcription Assay.** Plasmid DNA was digested with restriction enzymes. After extractions with phenol and chloroform, and two precipitations with ethanol, resulting fragments were used as templates in the *in vitro* transcription systems of Manley *et al.* (3). Conditions for the preparation of cell extracts, the *in vitro* transcription reaction, and the analysis of RNA products by gel electrophoresis or RNA fingerprinting were as described (3), except that transcription reaction mixtures contained 4 mM creatine phosphate.

RESULTS

To determine whether the Ad2 sequences contained in parental clone pϕ4 were sufficient to bring about accurate transcription initiation, samples of pϕ4 DNA were first digested by restriction enzymes that cut at various sites in the pBR322 sequence. Run-off RNAs were then synthesized *in vitro* from these DNA templates and analyzed on a 1.4% agarose gel (Fig. 2, lanes 3–7). Transcripts of lengths 4.36, 3.7, 1.43, 0.65, and 0.37 kilobases (kb) were observed for pϕ4 DNA cut by *Eco*RI, *Pst* I, *Ava* I, *Hin*cII, and *Bam*HI enzymes, respectively. These are the sizes expected for transcripts that initiated at the Ad2 cap site, continued into pBR322 sequences, and terminated at the end of the DNA fragment. These results indicate that transcription initiates accurately from the Ad2 major late promoter lo-

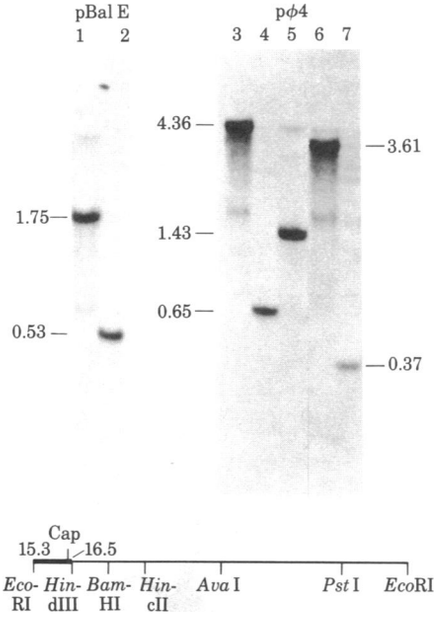

FIG. 2. *In vitro* transcription from the Ad2 major late promoter in pϕ4. Restriction fragments of plasmids pBal E (3) and pϕ4 containing the Ad2 major late promoter were used as templates for *in vitro* transcription reactions. RNAs synthesized were extracted, denatured with glyoxal, and resolved by electrophoresis in a 1.4% agarose gel. Sizes are expressed in kb. The top of the panel corresponds to the origin of electrophoresis. Lanes 1 and 2: pBal E DNA digested with *Bam*HI and *Sma* I, respectively. Lanes 3–7: pϕ4 DNA digested with *Eco*RI, *Hin*dIII, *Ava* I, *Pst* I, and *Bam*HI, respectively. The structure of pϕ4 and the cleavage sites of the restriction enzymes used are shown at the bottom. The coordinates of the Ad2 sequence present in pϕ4 are expressed in map units. Note the absence of any transcript from the *IVa2* promoter, which, if active, would have given rise to RNAs of 0.94 and 4.2 kb in lanes 6 and 7, respectively.

cated within the pϕ4 template. Comparison between similar-sized transcriptional products of pϕ4 and another late promoter clone, pBal E (13), which contains Ad2 sequences from 14.7 to 21.5 map units, indicates that both clones initiate transcription with comparable efficiencies (Fig. 2, lanes 1–2).

A sample of *in vitro* synthesized RNA made from pϕ4 template cut with *Hin*cII was purified, digested with RNase T1, and passed through a column of dihydroxylboryl cellulose as described (8). The bound material was eluted and subjected to two-dimensional chromatographic analysis (Fig. 3). The arrow in the figure points to the predicted capped T1 undecanucleotide that is found at the 5′ ends of Ad2 major late mRNAs. The identity of this spot was confirmed by analysis of the digestion products obtained after treatment of the oligonucleotide with RNase A, RNase T2, or RNase U2 (results not shown). Furthermore, the amount of the capped undecanucleotide obtained from reaction mixtures that contained pϕ4 DNA as template was approximately the same as the amount obtained when pBal E DNA was used as template (results not shown). Thus, we conclude that no Ad2 specific sequences further downstream than 33 nucleotides from the presumptive transcription start site are required for either efficient initiation or capping.

We mapped the 5′ boundary of the region required for accurate transcription initiation simply by testing whether pBal E DNA that had been digested with the restriction enzyme *Hpa* II could still serve as a template for transcription *in vitro*. This enzyme cuts the Ad2 sequences at positions −52 and +507. Fig. 4, lanes 1–3, shows that this DNA functions as a transcription template, as does a pBal E template that contains 680 bp of Ad2 DNA upstream from the cap site. These data thus suggest that all the sequence information required to obtain transcription initiation *in vitro* is contained within a region bounded by nucleotides −52 and +33 from the RNA cap site.

To define the boundary of this region more precisely, we tested the ability of several of the deletion mutant DNAs to serve as templates for transcription initiation. All DNAs were digested with *Ava* I. Transcriptionally active deletion DNAs constructed from pAd6 should give rise to transcripts of approximately 1.59 kb (193 nucleotides encoded by Ad2 DNA plus 1395 nucleotides encoded by pBR322). Transcriptionally active deletion DNAs derived from pϕ4 should give rise to transcripts approximately 1.43 kb in length (33 Ad2 nucleotides plus 1395 pBR322 nucleotides, and, in the case of downstream deletions, minus whatever nucleotides were deleted). The results of *in vitro* transcription analysis of these deletion mutant DNAs are shown in Fig. 5. As expected from the results shown above, a mutant DNA with a deletion removing sequences upstream of nucleotide −66 (Fig. 5*A*, lane 5) initiated transcription as efficiently as the parental DNA, pAd6 (Fig. 5*A*, lane 1). Transcription was reduced by as much as one-half for mutant DNAs lacking sequences upstream of nucleotide −47 (Fig. 5*B*, lanes 2–4). However, mutant DNAs with deletions ending at positions −16, −17, or −22, which removed the T-A-T-A-A-A-A sequence, reduced transcription to 1/10th (Fig. 5*B*, lane 5; Fig. 5*A*, lanes 3 and 4); DNAs with deletions removing both the T-A-T-A-A-A-A sequence and the cap site were also inactive (Fig. 5*A*, lane 2; Fig. 5*B*, lane 6). These results indicate that the upstream boundary of the region required for the initiation of transcription is located between positions −47 and −22.

To locate the downstream boundary of the region required for initiating transcription, we tested the ability of mutant DNAs with downstream deletions to serve as templates for transcription *in vitro*. As shown in Fig. 5*C*, lane 2, a deletion removing sequences downstream of nucleotide +7 does not affect the ability of that DNA to initiate transcription. Mutant DNAs with deletion endpoints at +5 or at +2 not only are capable of ini-

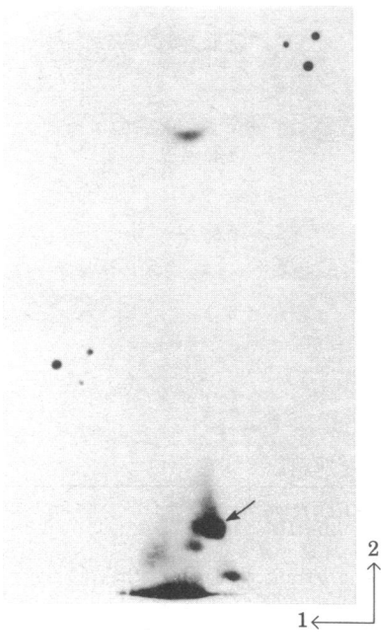

FIG. 3. Two-dimensional chromatographic analysis of the RNA synthesized *in vitro* from pϕ4 templates. Plasmid pϕ4 was digested with *Hin*cII and the resulting fragments were used for an *in vitro* transcription reaction as described (3). RNA synthesized was purified and then digested with RNase T1. Capped oligonucleotides were selected on a dihydroxyboryl cellulose column, purified, and analyzed (8). The first dimension was electrophoresis from right to left, and the second, homochromatography from bottom to top. The arrow shows the capped T1 undecanucleotide as found at the 5′ end of Ad2 major late transcripts.

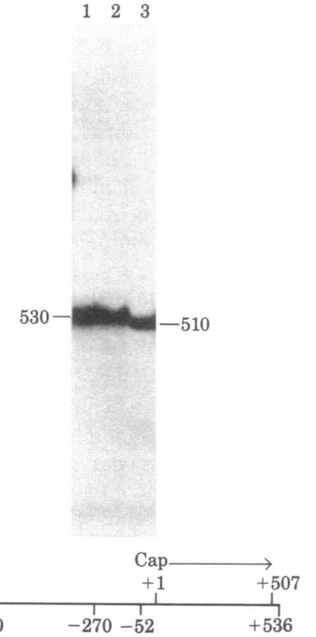

FIG. 4. Localization of the upstream boundary of the region required for the initiation of transcription *in vitro*. RNA was synthesized in standard reaction mixtures that contained as templates pBal E DNA digested by restriction enzymes *Bam*HI + *Sma* I (lane 1), *Xho* I + *Xma* I (lane 2), or *Hpa* II (lane 3). Run-off transcripts were analyzed as described in the legend to Fig. 2. The top of the panel corresponds to the origin of electrophoresis. Sizes of transcripts are expressed in nucleotides. Digestion of pBal E with *Bam*HI and *Sma* I produces a fragment from −680 to +530. *Xho* I cuts at position −270. *Hpa* II cuts at −52 and +507.

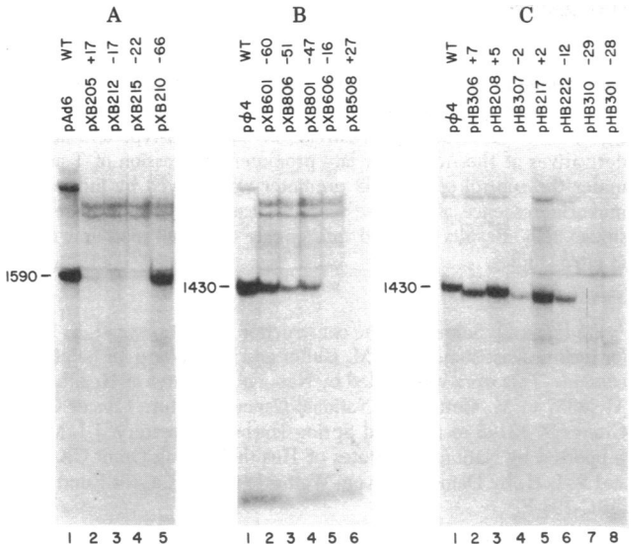

FIG. 5. *In vitro* transcription of deletion clones. DNA was digested with *Ava* I and was used as templates for *in vitro* transcription as described (3). Run-off transcripts synthesized were extracted, denatured with glyoxal, and resolved on a 1.4% agarose gel. An autoradiogram of the dried gel was developed in the linear range of exposure without using intensifying screens. *A* and *B* show results of upstream deletions and *C*, downstream deletions. Lane 1 in each panel shows the RNA synthesized on the parental wild-type clone. The top of each panel corresponds to the origin of electrophoresis. The lengths of the transcripts from the major late promoter present in pAd6 and pφ4 are indicated in numbers of nucleotides, to the nearest 10 nucleotides. The designations of the deletion clones and their deletion endpoints are indicated above the panels. The locations of deletion endpoints, as determined by the methods of Maxam and Gilbert (13), are indicated by the number of nucleotides upstream (− numbers) or downstream (+ numbers) from the cap site, which is +1.

tiating transcription but do so at a 2-fold higher level than does parental clone pφ4 (Fig. 5*C*, lanes 3 and 5, compared to lane 1). Mutant DNAs with a deletion removing the cap site and up to 12 nucleotides upstream of that site are still able to initiate transcription, but at a rate half that of the parental DNA (Fig. 5*C*, lanes 4 and 6, compared to lane 1). Deletions removing sequences downstream of nucleotide −29 reduced transcription to 1/15th (Fig. 5*C*, lane 7). These results indicate that the downstream boundary of the region required for initiation of transcription is located between position −29 and −12. The normal mRNA cap site is not required for the initiation of transcription *in vitro*. However, this site does appear to be required to obtain wild-type levels of initiation. All the downstream deletions tested above had different deletion endpoints in the pBR322 sequences as well as in the Ad2 sequence. To test the possibility that the different levels of transcription were partly due to the particular sequences of pBR322 present at the deletion junctions, we recloned all the downstream deletion DNAs in pBR322 as described in *Materials and Methods*. All the mutant DNAs now had identical endpoints in the pBR322 sequence (i.e., the *Hin*dIII site). When we tested the ability of these DNAs to serve as templates for transcription *in vitro*, we obtained results (not shown) identical to those obtained with the original clones, eliminating the possibility that the pBR322 sequence at the deletion junction would affect the rate of transcription. Accurate initiation of transcription *in vitro* is dependent on a precise optimum of template concentration (3). We have repeated each transcription reaction at least twice with carefully quantitated template and obtained reproducible levels of transcription for each DNA tested. We also showed that none of the DNA preparations contained nonspecific stimulator or inhibitor of transcription by carrying out mixing experiments with other promoter-containing DNAs.

Fig. 6 summarizes the results of the *in vitro* transcription analysis of deletion DNAs.

DISCUSSION

The main conclusion drawn from this work is that a region of the adenovirus genome upstream from the cap site, 15–17 nucleotides to either side of the T-A-T-A-A-A-A sequence, is required for the initiation of transcription *in vitro* from the major late promoter of the virus. A similar conclusion was recently reached by Corden *et al.* (15). These authors have shown that DNAs with

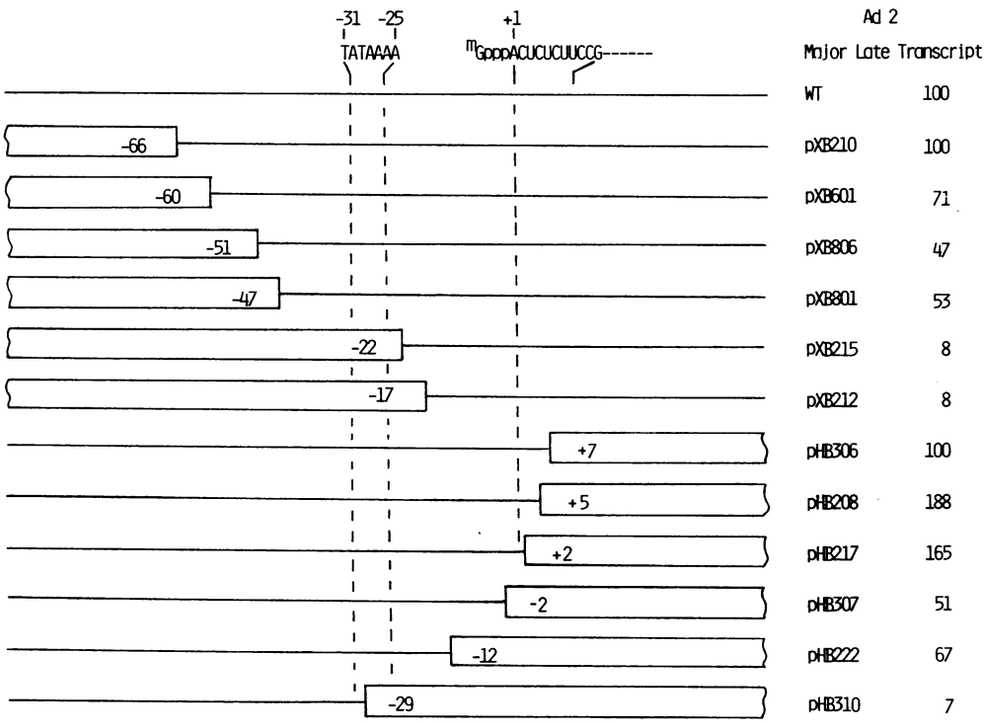

FIG. 6. Localization of the upstream and downstream boundaries of the region required for the initiation of transcription *in vitro*. The top line represents the wild-type Ad2 genome with the positions of the cap site of major late transcripts and the A+T-rich region as indicated. Sequences removed in deletion clones are indicated by open bars. The number within each open bar indicates the position of the deletion endpoint. The level of transcription from each deletion clone was estimated by first scanning the autoradiograms shown in Fig. 5 with an Optronics microdensitometer and then integrating the intensity of the band corresponding to the position of the run-off transcript. The numbers obtained were normalized to the background level in each lane and were expressed as percentages of the value obtained with the parental clone pAd6 or pφ4. The variability of these numbers is ≤10%.

upstream deletions removing sequences up to position −32 are transcriptionally active, whereas DNAs with deletions removing sequences up to −29 are not. This result defines the 5′ boundary of the region required for the initiation of transcription *in vitro* between nucleotides −32 and −29. Analyses of the chicken conalbumin gene (16), the rabbit β-globin gene (G. Grosveld, C. Shewmaker, and R. A. Flavell, personal communication) and the human β-globin gene (V. Parker, N. J. Proudfoot, M. H. M. Shander, and T. Maniatis, personal communication) have also shown that an A+T-rich sequence 25–31 nucleotides upstream from the mRNA cap site is required for the normal *in vitro* transcription of these genes. Concordantly, the promoters for the *IVa2* and the *72K* genes of Ad2 (ref. 17; C. C. Baker and E. Ziff, personal communication), which do not have recognizable A+T-rich sequences upstream from the mRNA cap sites, generally do not initiate specific transcripts *in vitro* (Fig. 2; unpublished data). However, the late region of simian virus 40 DNA, which lacks any recognizable A+T-rich sequence upstream from the mRNA cap sites (18, 19) is transcribed efficiently *in vitro* (20). It is interesting to note that simian virus 40 late mRNAs have extremely heterogeneous 5′ ends (21), whereas the major late transcripts of Ad2 (7), the rabbit β-globin mRNA (22), the human β-globin messengers (23), and the mRNAs of the chicken conalbumin gene (24) all have predominantly one 5′ end. It is therefore possible that promoters that direct the synthesis of transcripts with unique 5′ ends have a more stringent requirement for the A+T-rich sequence to function *in vitro* than those that give rise to transcripts with heterogeneous 5′ ends.

The role of the A+T-rich sequence has also been investigated *in vivo* in two different systems, the simian virus 40 early region (25, 26) and the sea urchin histone genes (27). Local deletions of the A+T-rich sequences do not abolish the expression of these genes, although more extensive deletions that remove sequences further upstream eliminate the expression entirely. Results reported here indicate that no sequence upstream of position −47 is required for the *in vitro* transcription from the major late promoter of Ad2 (Fig. 6). Whether any such sequence is required for the expression *in vivo* from this promoter is not yet determined.

It has been proposed that the cap site and the promoter for the major late transcription unit of Ad2 are coincident (7). Our present data show that the normal cap site is not required for the initiation of transcription *in vitro* and therefore indicate that the cap site is functionally separate from the promoter. This notion is supported by similar findings in other systems in which deletion of cap site does not abolish the synthesis of RNA (15, 26, 27). It is therefore not clear what role, if any, the sequence around the cap site plays in the transcription process. However, it is interesting to note that different levels of transcription were obtained for plasmids that differ only by several nucleotides in their extents of Ad2 sequences around the cap site (Fig. 5*C*, lanes 2–6). Analysis of the 5′ ends of the transcripts synthesized by these plasmids should be informative.

It is generally assumed that the mRNA cap site corresponds to the site where transcription initiates (i.e., the start site). Although this notion is supported by indirect evidence (e.g., ref. 7), it has never been shown directly. We have demonstrated that a fragment of Ad2 DNA spanning the major late promoter region from −52 to +507 is sufficient for the accurate initiation of transcription *in vitro* (Fig. 3). Our result provides direct evidence that the transcriptional start site must be located within 52 nucleotides upstream from the cap site. This corroborates the idea that the mRNA cap site and the transcriptional start site are coincident.

Note Added in Proof. M. A. Anderson and S.-L.H. have recently constructed plasmids containing the T (tumor) antigen coding region of simian virus 40 inserted downstream of both wild-type and deletion derivatives of the Ad2 major late promoter. Expression of T antigen under the control of the Ad2 promoter was assayed by indirect immunofluorescence after these plasmids had been microinjected into simian cells. Results obtained substantiate the conclusion drawn from *in vitro* studies.

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1. Rosenberg, M. & Court, D. (1979) *Annu. Rev. Genet.* **13**, 319–353.
2. Weil, P. A., Luse, D. S., Segall, J. & Roeder, R. G. (1979) *Cell* **18**, 469–484.
3. Manley, J. L., Fire, A., Cano, A., Sharp, P. A. & Gefter, M. L. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 3855–3859.
4. Sakonju, S., Bogenhagen, D. F. & Brown, D. D. (1980) *Cell* **19**, 13–25.
5. Bogenhagen, D. F., Sakonju, S. & Brown, D. D. (1980) *Cell* **19**, 27–35.
6. Evans, R. M., Fraser, N., Ziff, E., Weber, J., Wilson, M. & Darnell, J. E. (1977) *Cell* **12**, 733–739.
7. Ziff, E. & Evans, R. M. (1978) *Cell* **15**, 1463–1475.
8. Gelinas, R. E. & Roberts, R. J. (1977) *Cell* **11**, 533–544.
9. Goldberg, M. (1979) Dissertation (Stanford Univ., Stanford, CA).
10. Gannon, F., O'Hare, K., Perrin, F., LePennec, J. P., Benoist, C., Cochet, M., Breathnach, R., Royal, A., Garapin, A., Cami, B. & Chambon, P. (1979) *Nature (London)* **278**, 428–434.
11. Petterson, U. & Sambrook, J. (1973) *J. Mol. Biol.* **73**, 125–130.
12. Davis, R., Roth, J. & Botstein, D. (1980) *Advanced Bacterial Genetics* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY), p. 33.
13. Maxam, A. & Gilbert, W. (1980) *Methods Enzymol.* **65**, 499–560.
14. Tanaka, T. & Weisblum, B. (1975) *J. Bacteriol.* **121**, 354–362.
15. Corden, J., Wasylyk, B., Buchwalder, A., Sassone-Corsi, P., Kedinger, C. & Chambon, P. (1980) *Science* **209**, 1406–1413.
16. Wasylyk, B., Kedinger, C., Corden, J., Brison, O. & Chambon, P. (1980) *Nature (London)* **285**, 367–373.
17. Baker, C., Herisse, J., Courtois, G., Galibert, F. & Ziff, E. (1979) *Cell* **18**, 569–580.
18. Reddy, V., Thimmappaya, B., Dhar, R., Subramanian, K., Zain, S., Pan, J., Ghosh, P., Celma, M. & Weissman, S. (1978) *Science* **200**, 494–502.
19. Fiers, W., Contreras, R., Haegeman, G., Rogiers, R., van de Voorde, A., van Heuverswyn, H., van Herreweghe, J., Volckaert, G. & Ysebaert, M. (1978) *Nature (London)* **273**, 113–120.
20. Handa, H., Kaufman, R. J., Manley, J., Gefter, M. & Sharp, P. A. (1981) *J. Biol. Chem.* **256**, in press.
21. Ghosh, R. K., Reddy, V. B., Swinscoe, J., Lebowitz, P. & Weissman, S. M. (1978) *J. Mol. Biol.* **126**, 813–846.
22. Hardison, R. C., Butler, E. T., Lacy, E., Maniatis, T., Rosenthal, N. & Efstratiadis, A. (1979) *Cell* **18**, 1285–1297.
23. Baralle, F. E. (1977) *Cell* **12**, 1085–1095.
24. Cochet, M., Gannon, F., Hen, R., Maroteaux, L., Perrin, F. & Chambon, P. (1979) *Nature (London)* **282**, 567–574.
25. Benoist, C. & Chambon, P. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 3865–3869.
26. Gluzman, Y., Sambrook, J. F. & Frisque, R. J. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 3898–3902.
27. Grosschedl, R. & Birnstiel, M. L. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 1432–1436.