JOURNAL OF VIROLOGY, Feb. 1983, p. 594–599
0022-538X/83/020594-06$02.00/0

Vol. 45, No. 2

Far Upstream Initiation Sites for Adenovirus Early Region 1A Transcription Are Utilized After the Onset of Viral DNA Replication

TIMOTHY F. OSBORNE* AND ARNOLD J. BERK

Department of Microbiology and Molecular Biology Institute, University of California, Los Angeles, Los Angeles, California 90024

Received 19 August 1982/Accepted 13 October 1982

Adenovirus early region 1A (E1A) is the first transcription unit expressed after infection. It encodes a protein which controls the expression of all other early viral genes. The E1A mRNAs have one major capped 5′ terminus which maps 31 nucleotides downstream from a T-A-T-A sequence (C. Baker and E. Ziff, J. Mol. Biol. **149**:189–221, 1981). In addition, a minor set of E1A mRNAs are observed during the early phase of infection which have 5′ termini mapping at approximately −160, −185, and −230 relative to the major cap site (Osborne et al., Cell **29**:139–148, 1982). Here we report the occurrence of another set of minor E1A mRNAs which were observed exclusively after the initiation of viral DNA replication. These late specific E1A mRNAs had cap sites which mapped at approximately −300, −325, −360, and −375 relative to the major cap site. The appearance of these minor late E1A mRNAs was blocked by the DNA synthesis inhibitor cytosine arabinoside. These same late specific E1A mRNAs were synthesized from E1A-containing plasmids which replicate in monkey cells. This demonstrated that neither late specific adenovirus proteins nor adenovirus-specific chromatin structure was required for the production of the late specific E1A mRNAs. Adenovirus mutants in which the E1A T-A-T-A box region had been deleted also synthesized the corresponding deleted forms of the late specific mRNAs after initiation of DNA replication. These results indicate that the process of DNA replication alters the specificity of E1A transcription initiation in a promoter region which is at least 375 nucleotides in length.

The biosynthesis of mRNA from small mammalian DNA viruses has been studied extensively in the hope of elucidating control mechanisms which regulate gene expression in higher eucaryotes. The human adenoviruses have been particularly amenable to both genetic and biochemical manipulation and analysis (32). Adenovirus early region 1A (E1A) encodes a 289-amino acid phosphoprotein which induces the expression of all other early viral genes (2, 6, 14, 19, 25). Because of the crucial role of E1A expression in controlling the course of infection, we are engaged in a detailed study of these mRNAs and the regulation of their synthesis. Adenovirus E1A mRNA synthesis begins very early during a productive infection of HeLa cells and continues throughout the course of infection (21, 27). The steady-state level of E1A mRNA increases approximately five-fold after the onset of DNA replication; however, the number of viral DNA molecules increases by a factor of 10^4 to 10^5 (27), indicating that the level of E1A mRNA is regulated. The major start site for the E1A messages at both early and late times in infection maps at nucleotide 498 from the left end of the adenovirus type 2 (Ad2) genome (1; Fig. 1). In addition, there is a set of minor E1A mRNAs observed during the early phase of infection which have 5′ termini that map upstream from the major E1A cap site (23; Fig. 1). These minor wild-type species are the major 5′ ends of E1A mRNA transcribed from viral mutants in which the E1A T-A-T-A box region has been deleted (23). In this communication we report the observation of a late-phase-specific set of E1A mRNAs with 5′ termini that map still further upstream from those mentioned above. We show that the synthesis of these mRNAs occurs from templates which are replicating or were newly replicated and does not require late adenovirus functions.

MATERIALS AND METHODS

Cells and virus. HeLa spinner cells were grown and infected as described previously (3, 23). Multiplicities of infection were 10 PFU per cell in all experiments. COS cells (10; from Mia Horowitz) were grown on 10-

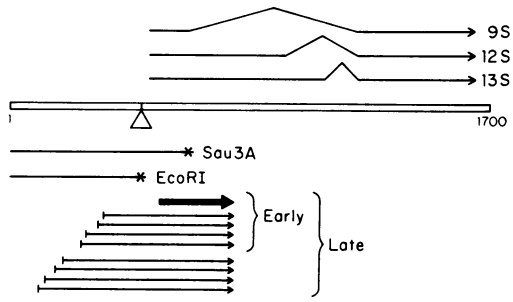

FIG. 1. E1A mRNA structure and organization. The open bar in the middle corresponds to the left terminus of the Ad2 genome marked off from nucleotides 1 to 1,700. The triangle denotes the synthetic *Eco*RI site and common endpoints of E1A deletion mutants discussed in the text. The structure of the three major E1A mRNAs is shown above the open bar. Each mRNA differs by the amount of sequence removed by RNA splicing (indicated by the carets). The 12S and 13S species are the predominant mRNAs during the early phase of infection (3) and the 9S mRNA predominates late in infection (29). Below the center bar are the structures of the 5′ end-labeled probes from wild type (*Sau*3A) or *dl*1503 (*Eco*RI) used in this study. The thick arrow represents the 5′ end of the major E1A mRNAs with their 5′ termini at nucleotide 498. The 5′ ends of the minor species observed early or late in infection (23; this work) are diagrammed at the bottom of the figure.

cm plates in Dulbecco modified minimal essential medium plus 10% newborn calf serum and transfected as described below. Total cytoplasmic RNA was prepared from cells infected for the indicated times by the Nonidet P-40 lysis–phenol-chloroform extraction method (3, 26). RNA was stored at −20°C in 10 mM Tris (pH 7.4)–1 mM EDTA–0.1% sodium dodecyl sulfate.

Nuclease S1 analysis of E1A mRNA. Nuclease S1 mapping of the 5′ termini of wild-type or mutant RNA was as described previously (23, 36), with minor modifications. Briefly, the left terminal *Sau*3A fragment (1 to 630 base pairs in wild-type Ad2) of a plasmid homologous to wild type or a particular mutant was 5′ end labeled with [γ-^{32}P]ATP and T_4 polynucleotide kinase, and the E1A-coding single strand was purified by gel electrophoresis (17). The labeled probe was hybridized to total cytoplasmic RNA isolated from cells infected with the homologous virus at the indicated times after infection. Hybridization in 1 M NaCl–10 mM Tris (pH 7.4)–1 mM EDTA was at 68°C for 1 h in DNA excess. This was followed by a 60-min room temperature digestion of the RNA-DNA hybrid mixture with 15 to 50 Vogt units (35) of nuclease S1 (Miles Laboratories). The S1-resistant material was denatured and subjected to electrophoresis on 13-cm-long, 1.5-mm-thick gels composed of 5% polyacrylamide–0.8% bisacrylamide with 8.3 M urea in Tris-borate-EDTA (16). Electrophoresis was for 400 to 600 V-h, after which the gels were soaked in cold 5% trichloroacetic acid for 1.5 h to remove the urea, dried, and exposed for autoradiography.

Insertion of E1A into SV40 and transcriptional analysis in COS cells. The large *Eco*RI-*Kpn*I fragment from BE5, a plasmid containing the *Bgl*II-E fragment of Ad2 (1 to 3,329 base pairs inserted into the *Pst*I site of pBR322), was ligated to the large *Eco*RI-*Kpn*I fragment of simian virus 40 (SV40). BE5 was a gift from Nigel Stow. A plasmid clone was identified by restriction enzyme cleavage analysis, isolated, and propagated in *Escherichia coli* C600. Plasmid DNA was isolated from large cultures of bacteria by Brij 58-deoxycholate lysis–ethidium bromide-CsCl gradient equilibrium centrifugation (22) and used to transfect COS cells (10). The calcium phosphate procedure (33) as modified by Mellon et al. (18) was used with minor variations. Supercoiled plasmid was added to 10-cm plates of COS cells subcultured 1:3 from confluent plates 24 to 36 h before transfection. A 10-μg portion of plasmid DNA was used for each plate of COS cells, and a glycerol shock was performed 4 h after transfection. After an additional incubation of 40 to 44 h at 37°C, total cytoplasmic RNA was harvested and analyzed for E1A-specific mRNA as described above, except that the hybridization was performed with the double-stranded left terminal *Sau*3A fragment 5′ end labeled at nucleotide 630, with 100 μg of RNA in 50 μl of 80% formamide–0.4 M NaCl–0.04 M PIPES [piperazine-*N*,*N*′-bis(2-ethanesulfonic acid)] (pH 6.4)–1 mM EDTA at 44°C for 14 h.

RESULTS

Adenovirus E1A mRNA 5′ termini exhibit a shift during lytic infection. To accurately map the 5′ termini of adenovirus E1A mRNAs synthesized at various times throughout the course of infection, total cytoplasmic RNA was harvested from Ad2-infected HeLa cells at different times after infection and hybridized to a single-stranded DNA probe. This probe corresponds to the coding strand of E1A mRNA, 5′ end labeled with ^{32}P at nucleotide 630 in the viral genome (Fig. 1). The DNA-RNA hybrids were digested with nuclease S1, and the S1-resistant material was denatured, subjected to electrophoresis, and detected by autoradiography (Fig. 2). The major 5′ end of the E1A mRNAs does not change throughout the course of infection (1) and yields an S1-resistant fragment of approximately 130 nucleotides (Fig. 2). However, a set of minor 5′ termini mapping further to the left and producing longer S1-resistant fragments is also evident before DNA replication begins (4 and 6 h). The occurrence of these minor species during the early phase of infection has been reported earlier (23). After viral DNA replication begins (approximately 8 h postinfection), another set of 5′ termini which correspond to RNA transcripts beginning still further upstream is synthesized. These transcripts are most visible at 16 and 32 h after infection and map at approximately −300, −325, −360, and −375. In the S1 mapping analysis shown (Fig. 2), these late specific E1A RNAs yield S1-protected frag-

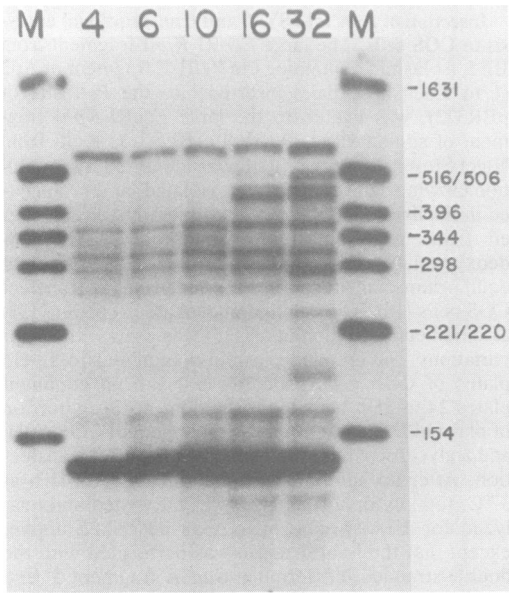

FIG. 2. Time course of appearance of E1A mRNA 5′ termini. Total cytoplasmic RNA extracted from HeLa cells after infection with Ad2 for the indicated times (hours) was analyzed for E1A mRNA as described in the text. The marker (M) is a *Hin*fI digest of pBR322 end-labeled with ^{32}P and denatured. The sizes indicated are in nucleotides. The largest band in all of the lanes corresponds to the undigested, full-length probe.

ments of approximate lengths 435, 460, 495, and 510. The 495- and 510-nucleotide S1-protected fragments are not well resolved on the gel shown. However, the 5′ ends of these late specific E1A RNAs were more accurately mapped with a probe which generated shorter S1-protected fragments (data not shown). This observation is in agreement with the results of Pettersson et al., who noted that more of the DNA sequence near the left end of the Ad2 genome is transcribed late in infection than before the onset of viral DNA replication (24). In addition, minor late specific S1-protected fragments of lengths 255, 190, and 110 were reproducibly observed, which result from E1A RNAs with 5′ ends mapping at approximately −125, −60, and +25. The 160-nucleotide S1-protected fragment seen in Fig. 2 was not reproducibly observed and may represent an artifact.

Viral mutants with deletions of the E1A T-A-T-A box also show the shift. The construction and analysis of Ad2/Ad5 mutants with small deletions engineered into the 5′ region of E1A were reported earlier (23). Mutants *dl*1501 through *dl*1504 all have deletions beginning at −44 with respect to the major E1A cap site at +1 and extend to the right to −39, −24, +21, and +62, respectively. The three largest deletion mutants lack the E1A T-A-T-A box region and cannot utilize the major wild-type start site (23). In addition, the steady-state level of E1A mRNA transcribed from these mutant templates is one-fifth to one-tenth the level observed during a wild-type infection. Nonetheless, they all grow with nearly wild-type kinetics in HeLa cells, where E1A functions are essential for growth.

The major E1A mRNA 5′ ends synthesized by these mutant viruses during the early phase of infection correspond to the minor wild-type species (23; Fig. 3A). When RNA is harvested

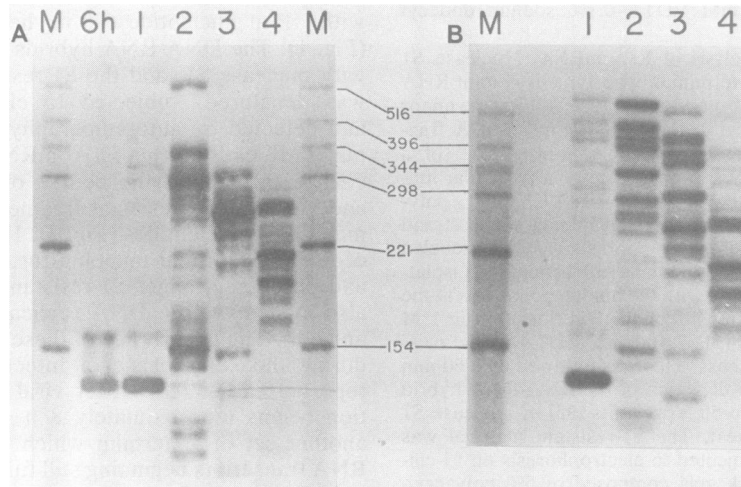

FIG. 3. Analysis of 5′ termini of E1A mRNA synthesized from deletion mutants. (A) Fragments protected from S1 digestion by RNA isolated 6 h postinfection; (B) pattern at 16 h postinfection. The marker (M) is the same as in Fig. 2. 6h denotes wild-type Ad2 RNA harvested at 6 h postinfection. Lanes 1, 2, 3, and 4 indicate RNA isolated from mutants *dl*1501 through *dl*1504, respectively. Again, the largest band in every track represents the full-length probe.

during the late phase of infection with each mutant virus and the E1A mRNA 5′ termini are analyzed and displayed as described above, it is apparent that the mutants synthesize late specific E1A mRNAs with the same 5′ termini observed during the late phase of a wild-type infection (Fig. 3B). Note that the S1-protected fragment sizes decrease as the size of the deletions in the mutants increases. These results confirm the late specific shift and indicate that synthesis of the late specific 5′ termini is not dependent on the sequence between −44 and +62.

Utilization of the late specific start sites is dependent on DNA replication. To determine whether synthesis of the late specific E1A mRNAs requires viral DNA replication, HeLa cells were infected with wild-type Ad2 in the presence or absence of the inhibitor of DNA synthesis, cytosine arabinoside (ara-C). The drug was added every 8 to 10 h, and each culture was harvested 32 h after infection. Total cytoplasmic RNA was extracted and analyzed as in Fig. 2 and 3. Figure 4 shows that ara-C very effectively prevents the synthesis of the late specific E1A mRNAs. Since blocking DNA replication also prevents the expression of late viral functions (30), it is not clear from this experiment whether the effect of ara-C was due to the inhibition of DNA replication directly or to the failure to express a late viral function.

Late specific mRNA start sites are observed in the absence of late adenovirus functions. The large *Eco*RI-*Kpn*I fragment of SV40 was cloned into a pBR322-derived plasmid which has a copy of E1A and part of the neighboring transcription unit E1B (Fig. 5A). This plasmid molecule contains the SV40 replication origin and therefore can replicate to high copy number after DNA transfection into COS cells (10, 20). Forty-four hours after transfection, when the plasmid was replicated to high copy number, E1A-specific mRNA was analyzed. Figure 5B shows that the E1A mRNAs synthesized from the plasmid replicating in COS cells have the same 5′ termini as E1A mRNAs synthesized after the initiation of viral DNA replication in adenovirus-infected HeLa cells (Fig. 2). Therefore, late viral functions are not required for synthesis of the late specific E1A mRNAs.

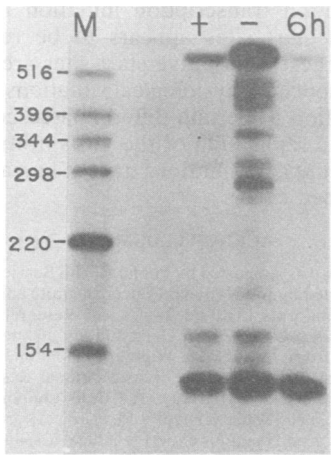

FIG. 4. E1A mRNA 5′ termini in the absence of DNA replication. See text for details. The marker (M) is a *Hin*fI digest of pBR322. (+) and (−) denote RNA harvested 32 h postinfection in the presence or absence, respectively, of ara-C. 6h represents wild-type E1A mRNA isolated at 6 h after infection.

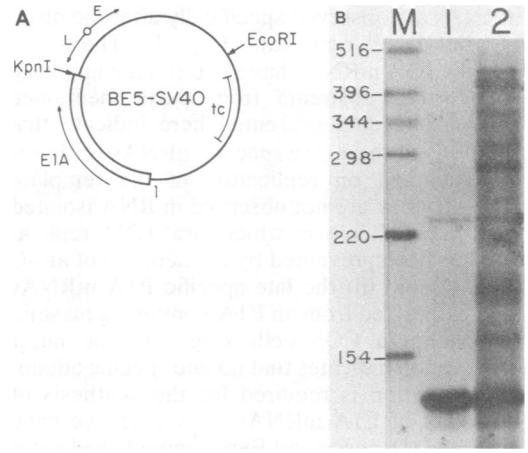

FIG. 5. Transcriptional analysis in COS cells. (A) Structure of E1A SV40 plasmid (BE5-SV40). Its construction is described in the text. The large SV40 *Eco*RI-*Kpn*I fragment is shown as a small line bracketed by *Eco*RI and *Kpn*I restriction sites. The origin of SV40 replication (o) and the direction of early (E) and late (L) SV40 transcription are shown. Ad2 DNA from nucleotide 1 at the left end of the genome to the *Kpn*I site at nucleotide 2,048 is represented by the curved open bar (bottom left). The direction of E1A transcription is shown. Plasmid pBR322 DNA is represented by the thin line connecting the SV40 DNA to the adenovirus sequence, and the tetracycline resistance gene (tc) is indicated. (B) The marker (M) is a *Hin*fI digest of pBR322. Lane 1, S1-protected fragments generated by RNA isolated from cells infected with wild-type Ad2 at 6 h after infection; lane 2, S1-protected fragments generated by RNA isolated 44 h after DNA transfection of COS cells with BE5-SV40 (shown in A).

DISCUSSION

The major 5′ terminus of the E1A mRNAs does not change from the early to late stage of infection in HeLa cells (1). In addition to these major E1A mRNAs, there is a minor set of E1A mRNAs which are synthesized throughout the course of infection and have 5′ ends that map upstream from the major cap site (23; this work). Here we show that another set of minor E1A

mRNAs are observed specifically after the onset of viral DNA replication (Fig. 1). These late specific E1A mRNAs have 5′ termini which map still further upstream from those mentioned above. The data presented here indicate that synthesis of these late specific mRNAs is directly dependent on replication of the template DNA: (i) they are not observed in RNA isolated from infected cells in which viral DNA replication has been prevented by the addition of ara-C (Fig. 4), and (ii) the late specific E1A mRNAs are synthesized from an E1A-containing plasmid replicating in COS cells (Fig. 5). The latter observation indicates that no late specific adenovirus function is required for the synthesis of this class of E1A mRNAs. However, we have observed (Osborne and Berk, unpublished data) that the late-phase-specific E1A splice which generates a 9S mRNA (29) occurs in transfected COS cells. Therefore, we cannot rule out that a protein translated from the 9S mRNA is involved in the shift. Since transcription initiation probably occurs at the mRNA cap site (4, 9, 12), these observations suggest that transcription initiation is altered on templates undergoing replication or on templates that have been recently replicated. Perhaps such replicating templates have a structure which makes the 5′ region of E1A accessible to RNA polymerase and other factors involved in transcription initiation. This could lead to the recognition of weak transcription initiation signals present in the DNA sequence, but masked in nonreplicating molecules. This suggestion is supported by the observation that initiation in this region occurs during in vitro transcription of naked DNA templates (5). Although the DNA sequence (34) shows the presence of adenine-thymine-rich regions approximately 30 base pairs upstream from the late specific starts at −300, −325, and −360, these sequences show little homology to the canonical T-A-T-A-A-A sequence. The sequence approximately 30 bases upstream from the −375 late specific start is in fact guanine-cytosine rich.

A similar observation has been made for the early mRNAs of SV40. The 5′ termini of early SV40 mRNAs are heterogeneous, with major initiation occurring approximately 30 nucleotides downstream from the early T-A-T-A homology (7, 11, 15). However, during the late phase of an SV40 infection the major 5′ termini shift 35 to 40 nucleotides upstream from the early major 5′ termini (7, 13). This shift to the utilization of upstream start sites is blocked during infection at the nonpermissive temperature with a temperature-sensitive (*ts*) mutant of SV40 large-T antigen (7). Also, the shift can be mimicked in vitro by addition of the T-antigen derivative, D2T protein (13). Therefore, it was postulated that T-antigen binding (possibly to site I, which is very close to the early transcription start sites [13, 31]) was involved in the shift. However, because the *ts*A mutation prevents DNA replication and since an SV40 mutant which lacks T-antigen binding site I nonetheless shows the shift in vivo (13), it is possible that the utilization of the upstream sites is in some manner directly coupled to DNA replication.

An important question is whether or not the minor E1A mRNAs encode distinct, functionally significant proteins. Translation of the identified E1A proteins begins with the first AUG triplet in the major E1A mRNAs at nucleotide 559 in the Ad2 sequence (28). In the mRNAs containing the early minor 5′ termini, this same AUG is the first AUG from the 5′ end. Thus, the early minor mRNAs probably translate the same E1A proteins as the major E1A mRNAs. On the other hand, whereas the viral DNA sequence (34) predicts that other AUG triplets occur near the 5′ ends of the late specific E1A mRNAs, all of these are closely followed by termination codons. It therefore seems unlikely that these minor late specific mRNA species encode functional proteins initiated at their first AUG codons.

The chief significance of the minor upstream starts documented in this work is their potential role in the regulation of region E1A expression. This potential is underscored by the observation that when the T-A-T-A box region for the major E1A start site is deleted, the virus compensates by turning up the level of transcription initiation from the upstream start sites (23). This allows production of sufficient essential E1A proteins to permit a full burst of infectious virus. Thus, in this situation transcription initiation from the upstream start sites appears to be regulated. Perhaps these alternative start sites are utilized in other specific physiological situations, such as the infection of certain differentiated cell types during the replication of the virus in its normal host, the upper respiratory tract of humans, or in transformed cells.

ACKNOWLEDGMENTS

This work was supported by Public Health Service grant CA 25235 awarded by the National Cancer Institute and by grants from the California Institute for Cancer Research and Academic Senate, UCLA. T.F.O. gratefully acknowledges a fellowship from the UCLA alumni foundation. T.F.O. is supported by Public Health Service National Research Service Award 2 T32 GM 07104. A.J.B. is supported by an American Cancer Society Faculty Research Award.

We thank Ann Healy for expert technical assistance, Debra Bomar for preparation of the manuscript, and Owen N. Witte for helpful comments and suggestions.

LITERATURE CITED

1. **Baker, C., and E. Ziff.** 1981. Promoters and heterogeneous 5′ termini of the messenger RNAs of adenovirus serotype 2. J. Mol. Biol. **149**:189–221.

2. **Berk, A., F. Lee, T. Harrison, J. Williams, and P. Sharp.** 1979. Pre-early adenovirus 5 gene product regulates synthesis of early viral messenger RNAs. Cell **17**:935–944.
3. **Berk, A., and P. Sharp.** 1978. Structure of the adenovirus 2 early mRNAs. Cell **14**:695–711.
4. **Contreras, R., and W. Fiers.** 1981. Initiation of transcription by RNA polymerase II in permeable, SV40 infected or noninfected, CV1 cells; evidence for multiple promoters of SV40 late transcription. Nucleic Acids Res. **9**:6305–6322.
5. **Fire, A., C. Baker, J. Manley, E. Ziff, and P. Sharp.** 1981. In vitro transcription of adenovirus. J. Virol. **70**:703–719.
6. **Gaynor, R. B., A. Tsukamoto, C. Montell, and A. J. Berk.** 1982. Enhanced expression of adenovirus transforming proteins. J. Virol. **44**:276–285.
7. **Ghosh, P., and P. Lebowitz.** 1981. Simian virus 40 early mRNAs contain multiple 5′ termini upstream and downstream from a Hogness-Goldberg sequence; a shift in 5′ termini during the lytic cycle is mediated by large T antigen. J. Virol. **40**:224–240.
8. **Ghosh, P., V. Reddy, P. Swinscoe, P. Lebowitz, and S. Weissman.** 1978. The 5′ terminal leader sequence of late 16S mRNA from cells infected with SV40. J. Biol. Chem. **253**:3643–3647.
9. **Gidoni, D., C. Kahana, D. Canaani, and Y. Groner.** 1981. Specific *in vitro* initiation of transcription of simian virus 40 early and late genes occurs at the various cap nucleotides including cytidine. Proc. Natl. Acad. Sci. U.S.A. **78**:2174–2178.
10. **Gluzman, Y.** 1981. SV40-transformed simian cells support the replication of early SV40 mutants. Cell **23**:175–182.
11. **Haegeman, G., and W. Fiers.** 1980. Characterization of the 5′-terminal cap structures of early simian virus 40 mRNA. J. Virol. **35**:955–961.
12. **Hagenbuchle, O., and U. Schibler.** 1981. Mouse β-globin and adenovirus-2 major late transcripts are initiated at the cap site *in vitro*. Proc. Natl. Acad. Sci. U.S.A. **78**:2283–2286.
13. **Hansen, U., D. Tenen, D. Livingston, and P. Sharp.** 1981. T antigen repression of SV40 early transcription from two promoters. Cell **27**:603–612.
14. **Jones, N., and T. Shenk.** 1979. An adenovirus type 5 early gene function regulates expression of other early viral genes. Proc. Natl. Acad. Sci. U.S.A. **76**:3665–3669.
15. **Kahana, C., D. Gidoni, D. Canaani, and Y. Groner.** 1981. Simian virus 40 early mRNAs in lytically infected and transformed cells contain six 5′-terminal caps. J. Virol. **37**:7–16.
16. **Maniatis, T., A. Jeffrey and H. van de Sande.** 1975. Chain length determination of small double and single-stranded DNA molecules by polyacrylamide gel electrophoresis. Biochemistry **14**:3787–3794.
17. **Maxam, A., and W. Gilbert.** 1980. Sequencing end-labeled DNA with base-specific chemical cleavages. Methods Enzymol. **65**:499–560.
18. **Mellon, P., V. Parker, Y. Gluzman, and T. Maniatis.** 1981. Identification of DNA sequence required for transcription of the human α-1-globin gene in a new SV40 host-vector system. Cell **27**:279–288.
19. **Montell, C., E. Fisher, M. Caruthers, and A. J. Berk.** 1982. Resolving the functions of overlapping viral genes by site-specific mutagenesis at a mRNA splice site. Nature (London) **295**:380–384.
20. **Myers, R., and R. Tjian.** 1980. Construction and analysis of simian virus 40 origins defective in tumor antigen binding and DNA replication. Proc. Natl. Acad. Sci. U.S.A. **77**:6491–6495.
21. **Nevins, J., H. Ginsberg, J. Blanchard, M. Wilson, and J. Darnell.** 1979. Regulation of the primary expression of the early adenovirus transcription units. J. Virol. **32**:727–733.
22. **Norgard, M., K. Emigholz, and J. Monahan.** 1979. Increased amplification of pBR322 plasmid deoxyribonucleic acid in *Escherichia coli* K-12 strains RR1 and X1776 grown in the presence of high concentrations of nucleoside. J. Bacteriol. **138**:270–272.
23. **Osborne, T., R. Gaynor, and A. Berk.** 1982. The T-A-T-A homology and the mRNA 5′ untranslated sequence are not required for expression of essential adenovirus E1A functions. Cell **29**:139–148.
24. **Pettersson, U., C. Tibbetts, and L. Philipson.** 1976. Hybridization maps of early and late messenger RNA sequences on the adenovirus type 2 genome. J. Mol. Biol. **101**:479–501.
25. **Ricciardi, R., R. Jones, C. Cepko, P. Sharp, and B. Roberts.** 1981. Expression of early adenovirus genes requires a viral encoded acidic polypeptide. Proc. Natl. Acad. Sci. U.S.A. **78**:6121–6125.
26. **Sharp, P., P. Gallimore, and J. Flint.** 1974. Mapping of adenovirus 2 RNA sequences in lytically infected cells and transformed cell lines. Cold Spring Harbor Symp. Quant. Biol. **39**:457–474.
27. **Shaw, A., and E. Ziff.** 1980. Transcripts from the adenovirus major late promoter yield a single early family of 3′ coterminal mRNAs and five late families. Cell **22**:905–916.
28. **Smart, J., J. Lewis, M. Mathews, M. Harter, and C. Anderson.** 1981. Adenovirus type 2 early proteins: assignment of the early region 1A proteins synthesized *in vivo* and *in vitro* to specific mRNAs. Virology **112**:702–713.
29. **Spector, D., M. McGrogan, and H. Raskas.** 1978. Regulation of the appearance of cytoplasmic RNAs from region 1 of the adenovirus-2 genome. J. Mol. Biol. **126**:395–414.
30. **Thomas, G., and M. Matthews.** 1980. DNA replication and the early to late switch in adenovirus infection. Cell **22**:523–533.
31. **Tjian, R.** 1978. The binding site on SV40 DNA for a T antigen related protein. Cell **13**:165–179.
32. **Tooze, J. (ed.).** 1980. DNA tumor viruses. *In* Molecular biology of tumor viruses, part 2, p. 383–575. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
33. **van der Eb, A., and F. Graham.** 1980. Assay of transforming activity of tumor virus DNA. Methods Enzymol. **65**:826–839.
34. **van Ormondt, H., J. Maat, A. de Waard, and A. van der Eb.** 1978. The nucleotide sequence of the transforming HpaI-E fragment of adenovirus type 5 DNA. Gene **4**:309–328.
35. **Vogt, V.** 1973. Purification and further properties of single-strand specific nuclease from Aspergillus oryzae. Eur. J. Biochem. **33**:192–200.
36. **Weaver, R., and C. Weissmann.** 1979. Mapping of RNA by a modification of the Berk-Sharp procedure: the 5′ termini of 15S β-globin mRNA precursor and mature 10S β-globin mRNA have identical map coordinates. Nucleic Acids Res. **7**:1175–1193.