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E1a Regions of the Human Adenoviruses and of the Highly Oncogenic Simian Adenovirus 7 Are Closely Related

DAVID KIMELMAN, JACQUELINE S. MILLER, DAVID PORTER, AND BRYAN E. ROBERTS*

Department of Biological Chemistry, Harvard Medical School, Boston, Massachusetts 02115

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Simian adenovirus 7 (SA7) is a highly oncogenic virus, capable of causing tumors in hamsters upon the direct injection of viral DNA. We determined the transcriptional organization of the transforming region and compared it with that of the human adenoviruses. This analysis demonstrated that there are two independently promoted transcription units similar to the E1a and E1b regions of the human adenoviruses. The nucleotide sequence of the SA7 E1a region demonstrated considerable homology with the human adenoviruses, both in the sequences that regulate E1a expression and in the encoded polypeptides. The amino acid homology was reflected in the ability of SA7 to complement the growth of human adenoviruses mutant in the E1a region. Furthermore, we found two regions of amino acid homology unique to SA7 and the highly oncogenic human adenovirus 12.

Adenoviruses, a family of small DNA viruses with genomes of approximately 35 kilobase pairs, have evolved to infect a broad range of animal species from birds to humans. In humans, at least 30 adenovirus serotypes have been isolated and divided into subgroups which differ in their abilities to induce tumors upon injection into newborn rodents (21). Extensive studies of the human adenoviruses demonstrate that the leftmost 10% of the linear genome representing approximately 3,500 base pairs both regulates the expression of early adenovirus genes and induces the transformation of cells in culture (3, 9, 16, 18, 19, 22, 23, 31, 44–47). This region has been extensively characterized in adenovirus 5 (Ad5), a member of a nononcogenic subgroup. Molecular and genetic analyses demonstrate (i) two separately promoted transcription units designated E1a and E1b (4, 5, 11); (ii) the synthesis of five mature transcripts, three from E1a and two from E1b, differing in each case by the size of the intervening sequence removed (5, 11, 33); (iii) that the polypeptide encoded by the largest E1a RNA is the sole viral function responsible for the accumulation of RNAs from other adenovirus early genes (31, 41); and (iv) that a function(s) encoded by both E1a and E1b is necessary for transformation of cells in culture (55, 56). The analogous regions of serotypes from the oncogenic subgroups also demonstrate two complex transcription units, utilizing selective removal of intervening sequences to generate transcripts expressing overlapping polypeptides (13, 34, 42, 61). The comparison of the nucleic acid sequences of representatives from the different subgroups has been used to locate those invariant sequences potentially important in the transcriptional regulation of E1a and E1b and also to define highly conserved domains within the encoded polypeptides (57, 59).

We have examined the transforming region (17, 29, 37, 38) of a highly oncogenic simian adenovirus, simian adenovirus 7 (SA7), isolated in 1963 from an African monkey (27), to determine whether (i) the organization of this region resembles that of the human adenoviruses, (ii) the human and monkey adenoviruses have similar invariant regulatory sequences and conserved domains within the encoded polypeptides, and (iii) common features occur between the highly oncogenic human and simian adenoviruses that might define a domain in an encoded polypeptide involved in tumor induction.

We report here that the transforming region of SA7, like that of human adenoviruses, contains two distinct transcription units located at the leftmost end of the genome. The organization of the transcripts originating from these regions was determined and compared with the RNA transcripts derived from the E1a and E1b sequences of human adenoviruses. This comparison illustrated that, although the overall organization is similar, differences occur in the location of the intervening sequences removed and in the pattern of the transcripts produced late in infection. Nucleotide sequence analysis of the regions which in Ad5 are essential for E1a transcription and those that encode the E1a polypeptides demonstrates that the human and simian adenoviruses have a very similar organization of highly conserved domains separated by divergent sequences. In addition, we show that SA7 can activate the transcription of Ad5 early regions, demonstrating that the E1a transcriptional regulatory function is conserved between human and simian adenoviruses. This comparison between SA7 and different human adenoviruses demonstrates a conservation of structure and function of the E1a region, indicating that these highly conserved sequences are essential to the activity of the polypeptide. Moreover, this is the first comparison of the E1a regions of two oncogenic viruses, SA7 and Ad12, and it defines two regions of conservation not found in nononcogenic or weakly oncogenic viruses.

MATERIALS AND METHODS

Cells and viruses. Ad5 wild type was grown and titrated on HeLa cells in Dulbecco modified medium supplemented with 10% fetal calf serum. The Ad5 deletion mutant *dl*312 (23) and host range mutant Hr-1 (19) were grown and titrated on 293 cells in Dulbecco modified medium supplemented with 10% fetal calf serum. SA7 was a gift from Bruce Casto and Tom Benjamin (Harvard Medical School) and was grown and titrated on either HeLa or Vero cells in Dulbecco modified medium supplemented with 10% calf serum. All SA7 infections were at 2 to 5 PFU per cell, and all Ad5, Hr-1, and *dl*312 infections were at 5 to 10 PFU per cell.

* Corresponding author.

Isolation of viral DNA. A 150-mm dish of infected Vero cells at 16 to 20 h postinfection was rinsed with phosphate-buffered saline; then 8 ml of 10 mM Tris-hydrochloride (pH 7.0)–10 mM EDTA–0.6% sodium dodecyl sulfate (SDS)–1 mg of pronase per ml was added, and the plates were incubated for 2 h at 37°C. Two milliliters of 5 M NaCl was added, and the solution was poured into centrifuge tubes and incubated at 0°C for 6 h. The samples were spun at 35,000 rpm in an SW41 rotor (Beckman Instruments, Inc.) for 30 min at 4°C. The supernatant was extracted with phenol-chloroform and collected by precipitation with ethanol.

Cloning of viral DNA. To remove the terminal protein, viral DNA was treated with S1 nuclease by the addition of 400 U of S1 nuclease (Boehringer Mannheim Corp.) to 30 μl of 10% formamide–280 mM NaCl–50 mM sodium acetate (pH 4.6)–4.5 mM $ZnSO_4$ and incubated at 37°C for 15 to 30 min. The DNA was extracted with phenol-chloroform and collected by ethanol precipitation. *Bam*HI linkers (New England Biolabs) were ligated onto the DNA, using T4 DNA ligase. The DNA was digested with *Bam*HI and ligated with *Bam*HI-digested pBR322. Clones containing the *Bam*HI B fragment of SA7 (0 to 27 map units) were isolated and characterized. From this plasmid, the following subclones were created: *Sma*IG (0 to 5.1 map units), *Bgl*II-D (1.8 to 10.8 map units), and *Sma*I+ (5.5 to 10.8 map units).

RNA purification. Infected cells were lysed with ice-cold 4 M guanidine thiocyanate–25 mM sodium citrate (pH 7.0)–0.5% Sarkosyl–0.1 mM β-mercaptoethanol. The cell lysate was then passed through a 25-gauge syringe needle five times to shear the DNA and layered onto a 3-ml cushion of 5.7 M CsCl–0.1 M EDTA treated with diethylpyrocarbonate. The lysate was centrifuged for 18 h at 20°C in an SW50.1 rotor at 35,000 rpm. The RNA pellet was washed with 70% ethanol, suspended in water, and precipitated with ethanol after the addition of 0.1 volume of 2 M sodium acetate.

Hybridization selection. Hybridization selection of specific RNA was carried out with diazobenzoxymethyl-cellulose paper following the procedure of Alwine et al. (1). A 10-μg amount of viral DNA was cut with either *Bam*HI or *Sma*I and then electrophoretically separated on a 1% agarose gel. The DNA was transferred to diazobenzoxymethyl-cellulose paper overnight at 4°C in 1 M sodium acetate, pH 4.0. The filters were washed with water, 0.4 N NaOH, and water and then treated at 65°C for 1 h in 99% deionized formamide–10 mM Tris-hydrochloride (pH 7.4) (elution buffer).

Hybridization was carried out with 30 μg of total RNA in 100 μl of 50% deionized formamide–0.1% SDS–4× SET (20× SET is 3.0 M NaCl, 0.4 M Tris-hydrochloride, pH 7.4, 20 mM EDTA) at 37°C for 3 h. The filters were then washed extensively with 50% formamide–0.1% SDS–0.2× SET at 37°C to remove nonhybridized RNA. Specifically bound RNA was eluted in elution buffer at 65°C for 5 min, ethanol precipitated, and then used to program the synthesis of [^{35}S]methionine-labeled polypeptides in the rabbit reticulocyte system treated with micrococcal nuclease (32). The translation products were analyzed by electrophoresis on an SDS-polyacrylamide gel containing 10% acrylamide and 0.1% bisacrylamide and examined by fluorography (7).

RNA filter hybridization. RNA was fractionated on 1.5% horizontal agarose gels in 20 mM morpholinepropanesulfonic acid (pH 7.0)–50 mM sodium acetate–1 mM EDTA containing 6% formaldehyde as a denaturant (43). Before loading on the gel, the RNA was denatured in 50% formamide–6% formaldehyde–20 mM morpholinepropanesulfonic acid–50 mM sodium acetate–1 mM EDTA for 15 min at 60°C. After electrophoresis, gels were soaked in 0.05 M NaOH for 45 min, neutralized with 0.1 M Tris-hydrochloride (pH 7.5) for 1 h with one change, and then equilibrated with 20× SSC (1× SSC is 0.15 M NaCl, 0.015 M sodium citrate). The RNA was transferred to nitrocellulose overnight in 20× SSC. The filter was rinsed with 3× SSC, dried for 1 h, and then baked in a vacuum oven for 2 h at 80°C. The filters were prehybridized in 50% formamide–5× SSC–5× Denhardt solution (50× Denhardt solution is 1% bovine serum albumin, 1% Ficoll, 1% polyvinylpyrrolidone)–50 mM sodium phosphate–200 μg of denatured herring sperm DNA per ml (53). Hybridization was in 50% formamide–5× SSC–1× Denhardt solution–20 mM sodium phosphate (pH 7.0)–100 μg of denatured herring sperm DNA per ml plus denatured DNA ^{32}P-labeled by nick translation (53). The filters were washed in 2× SSC–0.1% SDS at 55°C for 1 h with one change and then in 0.3× SSC–0.1% SDS at 55°C for 1 h with one change and exposed to film.

Uniform labeling of plasmid DNA. Ampicillin-resistant plasmids containing a fragment of the SA7 genome were grown overnight in Luria broth with 50 μg of ampicillin per ml. A 0.3-ml portion of this culture was inoculated into 30 ml of 10 mM Tris-hydrochloride (pH 7.0)–10 mM NaCl–100 mM KCl–20 mM NH_4Cl–0.2 mM KH_2PO_4–0.2 mM Na_2SO_4–0.1 mM $MgCl_2$–0.4% glucose–1 mg of vitamin B1 per ml–0.3% Casamino Acids, with 50 μg of ampicillin per ml. At an optical density at 600 nm of 0.4, chloramphenicol was added to 15 μg/ml. A 2-mCi amount of $^{32}P_i$ (New England Nuclear) was added and the cultures were grown overnight. Plasmid DNA was isolated and digested with the appropriate restriction enzymes, and the viral fragments were purified from agarose gels.

S1 nuclease analysis. S1 nuclease analysis was performed according to the method of Berk and Sharp (4), using 10 ng of uniformly labeled viral DNA fragments and 25, 75, or 150 μg of total cellular RNA, as indicated. For each experiment, the S1 nuclease reaction was performed with the DNA in the presence or absence of RNA. DNA and RNA were suspended in 20 μl of 80% deionized formamide–40 mM PIPES [piperazine-*N*,*N*′-bis(2-ethanesulfonic acid)], pH 6.4–1 mM EDTA–400 mM NaCl and heated to 68°C for 10 min to denature the DNA. They were then incubated at 60°C for 3 h to allow hybridization to take place.

The reactions were treated with S1 nuclease by adding 200 μl of ice-cold S1 buffer (280 mM NaCl, 50 mM sodium acetate, pH 4.6, 4.5 mM $ZnSO_4$, 25 μg of herring sperm DNA per ml, 5% glycerol) with S1 nuclease (Boehringer Mannheim Corp.) at 500 U/ml. The reaction mixtures were quick chilled on ice and then incubated at 45°C for 30 min. The S1 reactions were terminated by ethanol precipitation.

The products were resolved by electrophoresis in 1.4% alkaline agarose gels in 50 mM NaOH–2 mM EDTA. The gel was dried and exposed to film.

DNA sequencing. All nucleotide sequences were determined by the partial chemical degradation method as described by Maxam and Gilbert (28) with slight modifications. For the guanine-plus-adenosine reaction 10 μl of DNA was added to 25 μl of 88% formic acid, and incubation was at room temperature for 4 min. Then 200 μl of hydrazine stop buffer was added, and the procedure for the hydrazine reactions was followed.

RESULTS

Organization of genes encoding polypeptides expressed after DNA replication. In the type C human adenoviruses the transforming region is located at the leftmost end of the genome and the majority of the capsid polypeptides are

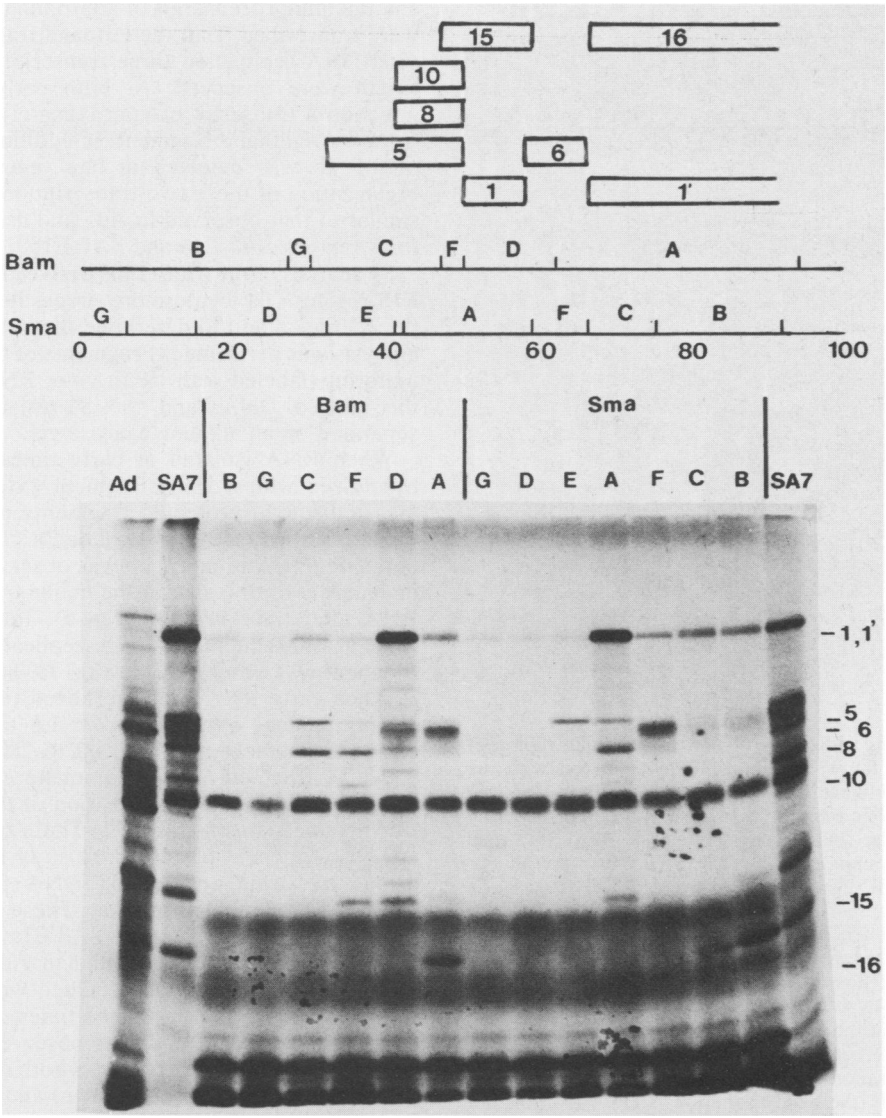

FIG. 1. Determination of the location of late SA7 polypeptides by hybridization selection and cell-free translation. Filters were made from DNA fragments created by *Bam*HI or *Sma*I digestion of viral DNA. RNA isolated from infected Vero cells at 27 h postinfection was selected on these filters, translated in a rabbit reticulocyte cell-free translation system, electrophoretically separated on a 10% SDS-polyacrylamide gel, and examined by fluorography. In the outside lanes are the cell-free translation products of total RNA from Ad5-infected HeLa cells and SA7-infected Vero cells. The bar diagrams indicate the location of eight SA7 polypeptides deduced from the hybridization selection results. The closely migrating polypeptides 1 and 1′ are separable on a longer gel (data not shown).

encoded in the righthand end (18, 25, 26, 30). Previous studies of SA7 have located the transforming region to the terminal restriction fragment *Bam*HI B (Fig. 1) (17, 38); however, the position of these sequences relative to the genes encoding the capsid polypeptides was unknown. To determine whether the genomic organization of SA7 was analogous to that of the human adenoviruses, the location of the sequences encoding the capsid proteins was determined by hybrid selection of mRNA to the *Bam*HI or *Sma*I restriction fragments followed by cell-free translation. The ^{35}S-labeled translation products were resolved on an SDS-polyacrylamide gel, and those sequences encoding certain polypeptides were located along the viral genome (Fig. 1). These data demonstrate that at least eight polypeptides are located in the righthand 70% of the genome. Based on molecular weight and relative abundance, peptide 1 appears to correspond to the hexon of Ad5 and peptide 1′ corresponds to the 100K polypeptide (25, 26, 30). On the basis of its ability to bind to single-stranded DNA (D. Kimelman and L. Cohen, unpublished data), peptide 6 most likely correlates to the 72K DNA-binding protein of Ad5 (30). The location of genes encoding these three polypeptides on the SA7 genome is similiar to the genomic location of the corresponding genes of Ad5. The overall organization of the genes expressed late in infection establishes that the genomic arrangement of SA7 is similar to that of Ad5 and that the transformation region contained within the *Bam*HI B fragment (Fig. 1) is located at the leftmost end of the genome.

RNA filter hybridization of the SA7 transforming region. The transforming region of Ad5 is located in the leftmost 10% of the genome and is comprised of two independent

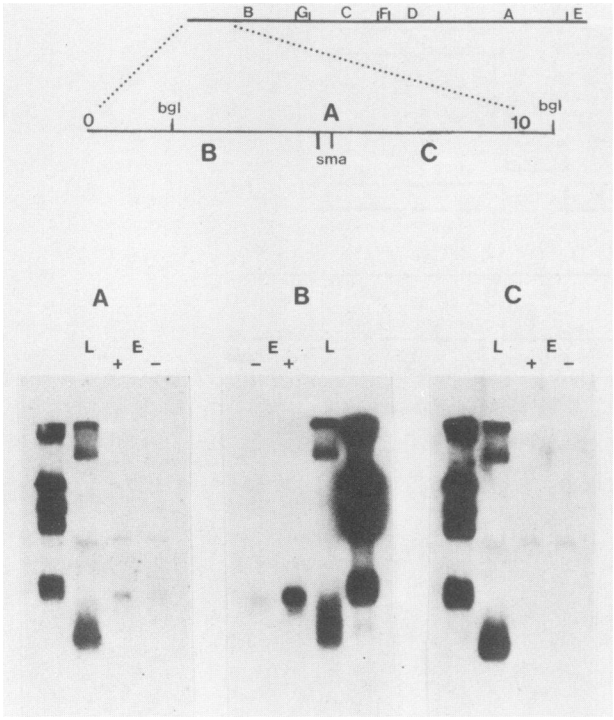

FIG. 2. RNA filter hybridization of the transforming region of SA7. The left 10% of the viral genome (shown at top with the *Bam*HI cleavage sites indicated as in Fig. 1) was subdivided into three regions, using either *Bgl*II or *Sma*I total digestion or partial digestion to produce the following fragments: A (0 to 10.8 map units), B (0 to 5.1 map units), and C (5.5 to 10.8 map units). For hybridization analysis, RNA was isolated from SA7-infected HeLa cells at 8 h postinfection (early times [E]), with (+) or without (−) cycloheximide added at 6 h postinfection, or at 27 h postinfection (late times [L]). The RNA was electrophoretically separated on a denaturing formaldehyde-agarose gel, transferred to nitrocellulose, and hybridized with DNA fragments labeled with ^{32}P by nick translation. The bands at the top of the late lanes are SA7 genomic DNA isolated in the RNA preparation. The unlabeled lane in each panel is a fixed concentration of SA7 DNA with the following sizes: 3.5, 2.4, 1.9, and 0.8 kb (the band at the top is DNA that has not entered the gel). Estimates of RNA sizes are only approximate, as RNA and DNA have different mobilities in this gel system. (B) was exposed to film for a longer period than either (A) or (C) to enhance the detection of the minor E1a early transcript.

transcription units, E1a and E1b (5, 11, 18). To determine whether an analogous organization exists in the transforming region of SA7, RNA from infected cells was analyzed by hybridization to fragments from the leftmost 10% of the SA7 genome. Total cellular RNA was isolated before DNA replication at 8 h after infection of HeLa cells with SA7; where indicated, cycloheximide was added at 6 h postinfection to boost the levels of viral RNA. Similarly, total cellular RNA was isolated after viral DNA replication at 27 h postinfection. The RNA was electrophoretically separated on formaldehyde-agarose denaturing gels, transferred to nitrocellulose filters, and then hybridized with ^{32}P-labeled fragments from the SA7 genome. A fragment which covers the left 10% of the genome hybridized to numerous transcripts (Fig. 2). These transcripts can be divided into two nonoverlapping groups, those which hybridized exclusively to a fragment extending from 1.8 to 5.1 map units and those which hybridized exclusively to a fragment extending from 5.5 to 10.8 map units. At early times of infection, one major and one minor transcript of approximately 1.0 kilobase (kb) were transcribed from the lefthand fragment, whereas after viral DNA replication three transcripts of 0.5 to 0.9 kb in length were observed. At both early and late times of infection a transcript of approximately 2.0 kb was derived from the righthand fragment; in addition, a major transcript of 0.5 kb was detected at late times. The location and organization of these two transcription units appeared very similar to that observed in Ad5 and are henceforth referred to as regions SA7 E1a and SA7 E1b.

S1 analysis of the transcripts derived from the SA7 E1a and E1b regions. To locate more precisely the transcripts from the E1 region and to determine their structure, S1 nuclease analysis was performed. Fragments of the SA7 genome were uniformly labeled with ^{32}P in vivo, RNA was hybridized to the labeled DNA, and the S1-resistant fragments were separated on an alkaline agarose gel.

When RNA isolated at early times after infection was hybridized with a DNA fragment extending from 0 to 5.1 map units, two protected fragments of 600 and 300 bases were observed (see Fig. 3a, lane 2). On nondenaturing gels only one fragment of 1.0 kb was observed (data not shown), in accord with the size of the major transcript observed by RNA filter hybridization (Fig. 2). In contrast, with RNA isolated subsequent to DNA replication only a 300-base fragment was observed (see Fig. 3a, lane 3).

When early RNA was hybridized to a uniformly labeled DNA sequence extending from 1.8 to 5.1 map units, the 600-base fragment was reduced to 280 bases, whereas the 300-base fragment remained unaltered (Fig. 3a, lane 1). These results predict the position of the 600- and 300-base fragments as shown in Fig. 3. The size and position of the 600-base fragment protected by early RNA have been confirmed by using end-labeled DNA fragments in the S1 analysis (our unpublished data). These results indicated only one early transcript from the SA7 E1a region, in contrast to the two early transcripts found in Ad5 (5, 33). However, when the S1 analysis was repeated with increased concentrations of RNA and prolonged times of autoradiography, a minor 400-base fragment was observed (Fig. 3b, lane +). The 600- and 400-base fragments both derive from the 5′ end of the transcript (data not shown) and, in conjunction with the 300-base 3′ fragment, comprise two transcripts comparable to that found in Ad5. In addition, when the cycloheximide pretreatment was omitted during the viral infection, the ratio of the two transcripts was closer (Fig. 3b, lane −).

When the uniformly labeled fragment extending from 1.8 to 5.1 map units was hybridized to late RNA, the 300-base fragment was completely protected, though 600- and 400-base fragments were absent, indicating that at least these transcripts are not present in detectable levels late in the infection (Fig. 3a, lane 4). The location of the 5′ end of the late messages that are joined to the 300-base 3′ fragment has yet to be determined. However, by analogy with late E1a RNAs encoded by Ad2 and Ad7, they could arise from splice sites very close to the start of the messages (12, 51, 60) and therefore would not be resolved on an alkaline agarose gel.

S1 nuclease analysis of the E1b region used four uniformly labeled DNA fragments: 1.8 to 10.8 , 5.5 to 10.8, 6.3 to 10.8, and 5.5 to 9.2 map units. The S1 nuclease-resistant fragments produced when the early RNA was hybridized to the four fragments are shown in Fig. 3a, lanes 5 to 8. These data are consistent with a single contiguous transcript derived from E1b extending from 5.1 to 9.5 map units. Information from RNA filter hybridization indicated that this transcript

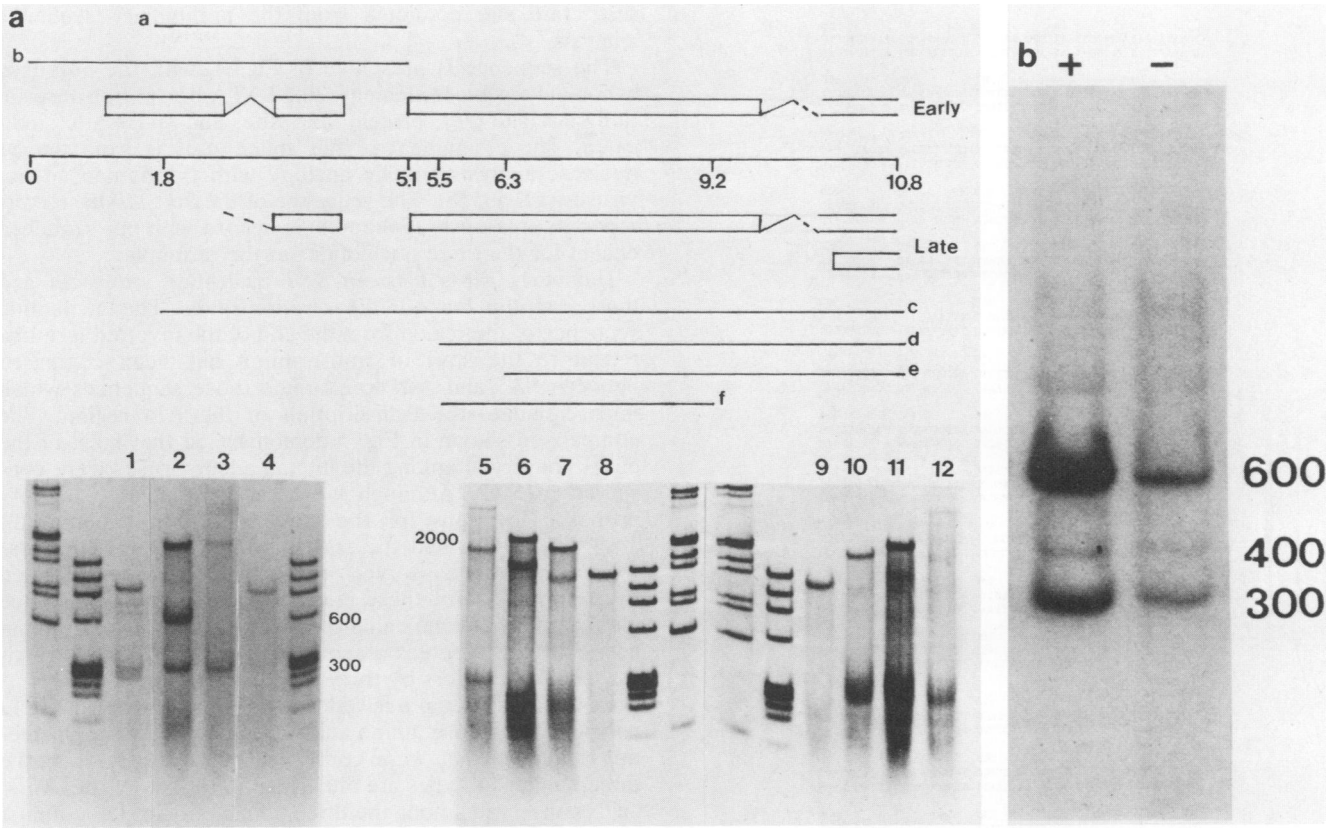

FIG. 3. S1 nuclease analysis of the transforming region of SA7. RNA from SA7-infected cells was isolated at 8 h (early times) or at 27 h (late times) postinfection. (a) A 75-μg amount of early RNA or 25 μg of late RNA was hybridized to uniformly labeled DNA fragments from the SA7 genome (a to f) and then treated with S1 nuclease. The resulting products were separated on a 1.4% alkaline agarose gel and exposed to film for 1 day. The markers (unlabeled lanes) are λ DNA digested with *Eco*RI and *Hin*dIII for the higher molecular weights and φX DNA digested with *Hae*III for the lower molecular weights. In all lanes from the nuclease reaction, the upper band is reannealed labeled DNA, which is not digested by the S1 nuclease. (Lane 1) Early RNA, fragment a; (lane 2) early RNA, fragment b; (lane 3) late RNA, fragment b; (lane 4) late RNA, fragment a; (lane 5) early RNA, fragment c; (lane 6) early RNA, fragment d; (lane 7) early RNA, fragment e; (lane 8) early RNA, fragment f; (lane 9) late RNA, fragment f; (lane 10) late RNA, fragment e; (lane 11) late RNA, frgment d; (lane 12) late RNA, fragment c. The bar diagrams are the predicted transcripts at both early and late times deduced from the results of the S1 nuclease analysis and the RNA filter hybridization (Fig. 2). The location of the acceptor site for the E1b message is not known, nor is the precise end of the E1b transcripts. (b) Analysis shown in (a), lane 2, was performed but with 150 μg of early RNA prepared from cells that were (+) or were not (−) pretreated with cycloheximide. The resulting gel was exposed to film for 3 days. The molecular weights are indicated.

extends beyond 10.8 map units (our unpublished data), suggesting a small 3′-proximal intervening sequence analogous to that found in the E1b transcript of Ad5.

When the same analysis was repeated with the four uniformly labeled fragments hybridized to late RNA, identical results were observed, except that an additional protected fragment was produced that was located at the 3′ end of the E1b region (Fig. 3a, lanes 9 to 12). This RNA corresponds to the message for protein IX observed in Ad5 in terms of map location, size, abundance, and appearance after DNA replication (35).

The data obtained by S1 nuclease analysis and RNA filter hybridization demonstrated that the location and transcript organization of the SA7 E1a and E1b transcription regions are similar to those observed in Ad5. Two E1a transcripts are produced throughout an Ad5 infection, with an additional transcript produced after viral DNA replication. In contrast, one major E1a transcript is produced at early times during an SA7 infection and is not detectable at late times. In an Ad5 infection a spliced transcript of 1,000 bases produced from the E1b region after viral DNA replication (11) was not observed in the SA7 E1b region.

Nucleotide sequence of the SA7 E1a region. To determine the nucleotide sequence of the E1a region, a series of overlapping deletions were constructed. Beginning at the unique *Bgl*II restriction site in the middle of the E1a region (Fig. 2), endonuclease Bal 31 was used to progressively digest the DNA in both directions. At various intervals corresponding to the digestion of 100 bases in each direction, samples of the reaction mix were removed and the reaction was terminated. The resulting fragments were then resealed in the presence of *Xho*I linkers in the presence of T4 DNA ligase. This generated a series of clones with a unique *Xho*I site 100 bases apart. As the Bal 31 digestion began from the middle of the E1a region, the deletions were generated simultaneously in both the 5′ and 3′ directions, so that each clone could be used to gather sequence information in both halves of the region. In addition, the *Xho*I site could be labeled on either strand with polynucleotide kinase or DNA polymerase, such that both strands could be sequenced from

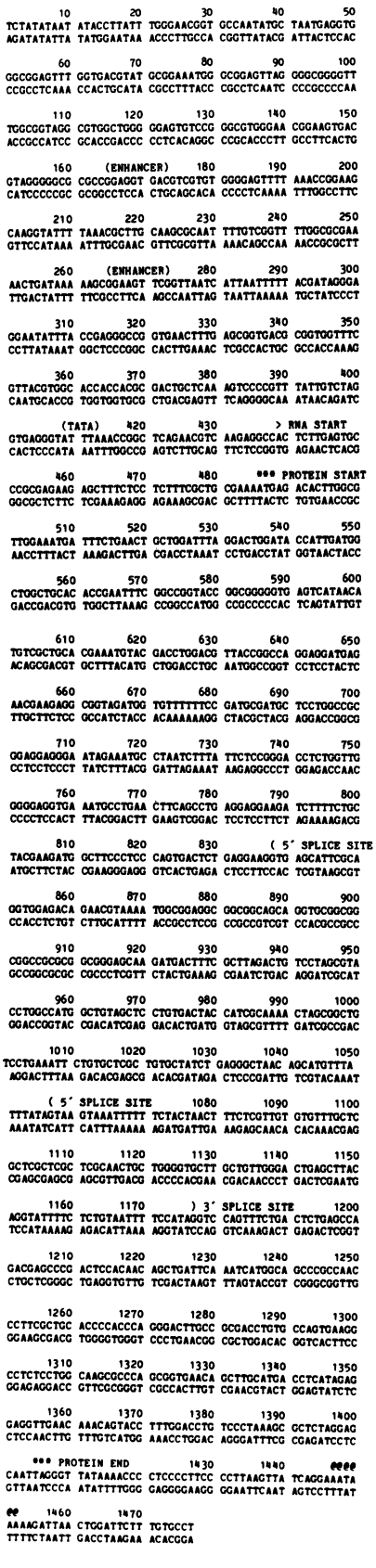

FIG. 4. Nucleotide sequence of the E1a region of SA7. A plasmid containing the SA7 E1a region (0 to 5.1 map units) was opened at the unique *Bgl*II site (at 1.8 map units), and the enzyme Bal 31 was used to processively digest the DNA in both directions. At various intervals corresponding to the digestion of 100 base pairs in each direction, the plasmids were religated in the presence of *Xho*I linkers. This series of deletions was used in the nucleotide sequence analysis. Each plasmid was labeled with ^{32}P, using either polynucleotide kinase to label one strand or DNA polymerase large fragment to label the other strand, and DNA fragments from the 5′ or 3′ end of the region were isolated after secondary cleavage of the DNA with *Bam*HI (which excises the cloned fragment). The nucleotide sequence of the labeled DNA was determined by the partial chemical degredation method of Maxam and Gilbert (28). For the entire region, the nucleotide sequence of both strands was determined. The proposed core enhancer elements, the TATA box, the start and end of transcription, the beginning and end of the coding sequence, and the splice donor and acceptor sites are indicated.

the same site. In some instances the DNA was labeled at a restriction site predicted from the preliminary sequence analysis.

The sequence is presented in Fig. 4, with the proposed canonical enhancer element (20), TATA box (39), transcription start site (59), protein start site, and splice sites indicated. The assignment of the splice sites is from the S1 nuclease analysis and by analogy with the human adenoviruses (12, 50, 58). The sequence of the first 120 bases was reported previously (54) and is in accord with our sequence except for the three nucleotides at the terminus.

Homology exists between SA7 nucleotide sequences and those essential for Ad5 E1a transcription. The nucleotide sequence of the region from the end of the inverted terminal repeat to the start of transcription has been compared between SA7 and Ad5 to examine those sequences which might regulate the transcription of the E1a region. The comparison shown in Fig. 5 demonstrated that most of the bases conserved among the human adenoviruses were conserved in SA7. Although we have been able to identify proposed locations for the core enhancer element (20), TATA box (39), and the start of transcription (59), there were many more conserved sequences than can be explained by the presence of these elements. We found a sequence identical to the distal enhancer element found in the human adenoviruses, whereas a sequence analogous to the proximal enhancer differs by three nucleotides (Fig. 5).

Comparison of the nucleotide sequences encoding the E1a polypeptides. Those amino acids predicted from the nucleotide sequence that were conserved between SA7 and the three human subtypes are illustrated in Fig. 6. Of the amino acids conserved among the three human viruses (59), almost all were conserved in SA7. The regions between the conserved amino acids were highly variable among the human adenoviruses and contained numerous insertions and deletions. Comparison of the SA7 sequence demonstrated the same pattern of variability with no homology to any particular human subtype. However, a sequence of seven of eight amino acids in the middle of the polypeptide and one of six of seven amino acids in the carboxy terminus are identical for SA7 and the oncogenic Ad12 (Fig. 6).

SA7 complements E1a mutants of Ad5. During the lytic infection by adenovirus, transcription of five early regions is regulated by the E1a gene product (3, 9, 19, 22, 41, 45). The transcriptional activating function of this protein has been studied, utilizing a wide variety of mutants in the E1a region such as Hr-1, a point mutant that alters only the larger E1a

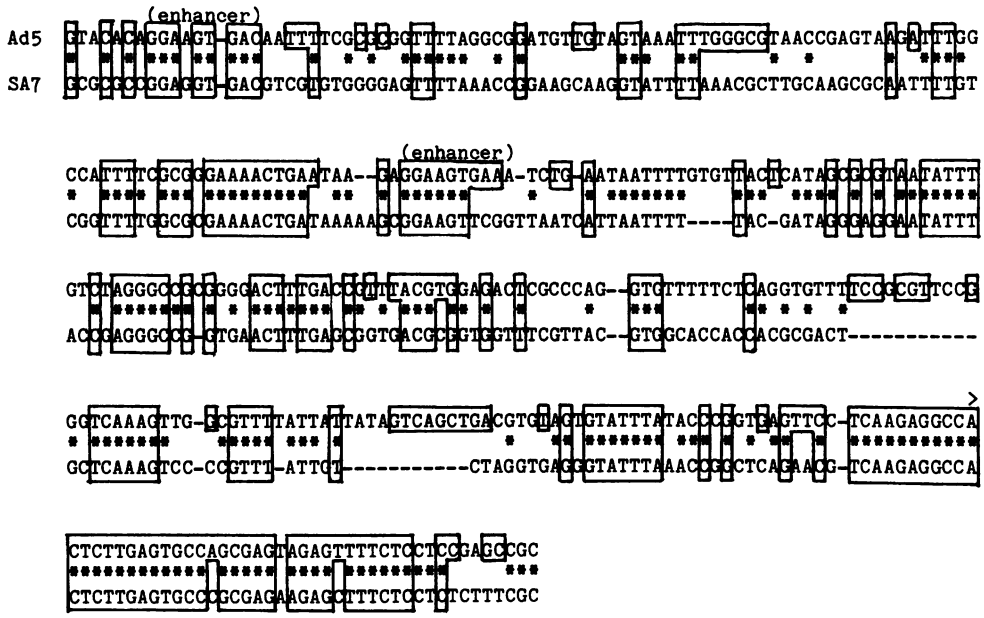

FIG. 5. Comparison of the nucleotide sequences of Ad5 and SA7 from the end of the inverted terminal repeat to the start of transcription. The nucleotide sequences of Ad5 and SA7 have been aligned to produce the maxium homology. Boxes have been drawn around the Ad5 sequences which are conserved among all human adenoviruses (59) and are drawn to include the SA7 sequences which are also homologous. The asterisks indicate nucleotides conserved between Ad5 and SA7. The locations of the proposed core enhancer elements are indicated as well as the proposed start of transcription (denoted by >).

gene product (19), and *dl*312 which is deleted for almost all of the E1a region (23). When cells are infected with these mutants, no expression of the other early regions occurs, and hence no viral replication or late gene expression is detected. If, however, the E1a function is supplied to these viruses, either by coinfection with another virus or by infection of cells that constitutively produce a functional E1a product, the mutant viruses will follow a productive cycle of infection.

To determine whether SA7 can directly activate transcription of the adenovirus early regions, we assayed the RNA transcription from early regions E1b, E2, E3, and E4 during coinfection of the E1a mutant viruses with SA7. After infection of HeLa cells by Hr-1 or *dl*312, alone or in the presence of SA7, RNA was isolated at 8 h (early times) or 20 h (late times) postinfection. The RNA was electrophoretically separated on formaldehyde-agarose denaturing gels, transferred onto nitrocellulose filters, and then hybridized with ^{32}P-labeled DNA fragments from the four early regions.

Infection of cells by the mutant viruses alone produced no viral RNA from regions regulated by E1a at either early or late times postinfection (Fig. 7, Hr and 312). However, when the mutants were coinfected with SA7, viral RNA was detected at both times (Fig. 7, lanes Hr + SA7 and 312 + SA7). This directly demonstrates the ability of SA7 to provide an E1a-type function, which can activate transcription of the Ad5 early regions.

DISCUSSION

A region of the viral genome capable of transforming cells in vitro is common to the human and simian adenoviruses (8, 9, 16, 17, 23, 29, 37, 38, 44–47). Three human viruses, Ad5, Ad7, and Ad12, representing the nononcogenic, weakly oncogenic, and strongly oncogenic subtypes, have been carefully compared. The nucleotide sequences of these three viruses have diverged to a large extent; however, pronounced conservation of the transforming region has been observed (57, 59). The transforming region is comprised of two separately promoted transcription units designated E1a and E1b, both of which are necessary for complete transformation (55, 56). Each region expresses one set of transcripts upon infection and additional transcripts after viral DNA replication, though only the early transcripts are expressed in transformed cells (15). The largest E1a transcript encodes a polypeptide required for the activation of the other early regions (31, 41). The other two E1a transcripts are dispensable for growth in cell culture, though their conservation in more than one subtype suggests their importance in the viral life cycle in the whole animal (5, 11, 13, 42, 61).

SA7 is the best studied of the simian adenoviruses and has been shown to be very efficient in the transformation of cells in culture (10; D. Kimelman and T. Benjamin, unpublished data) and in the promotion of tumor formation in hamsters upon the direct injection of virus or subgenomic fragments of DNA containing the transforming region (8, 29, 37, 38). DNA hybridization studies have shown that SA7 sequences share approximately 10 to 15% homology with those of the human adenoviruses (36). Using DNA filter hybridization (our unpublished data), we have located two regions of detectable homology between SA7 and the human viruses: one is located between 15 and 30 map units, which in Ad5 includes the major late promoter (2), and those sequences encoding the terminal protein and the DNA polymerase (48, 49); the other is located between 50 and 60 map units, which includes the sequences encoding the hexon protein in both Ad5 and SA7 (25, 26, 30).

The DNA hybridization results demonstrate that the transforming region, which we have located in the leftmost 10% of the SA7 genome, shows no hybridization to the equivalent sequence from Ad5 (our unpublished data). However, analysis of RNAs indicated that the structure and location of the two transcription units in this region were directly analogous to those found in Ad5, though there were three significant differences: (i) the smaller SA7 E1a early transcript is

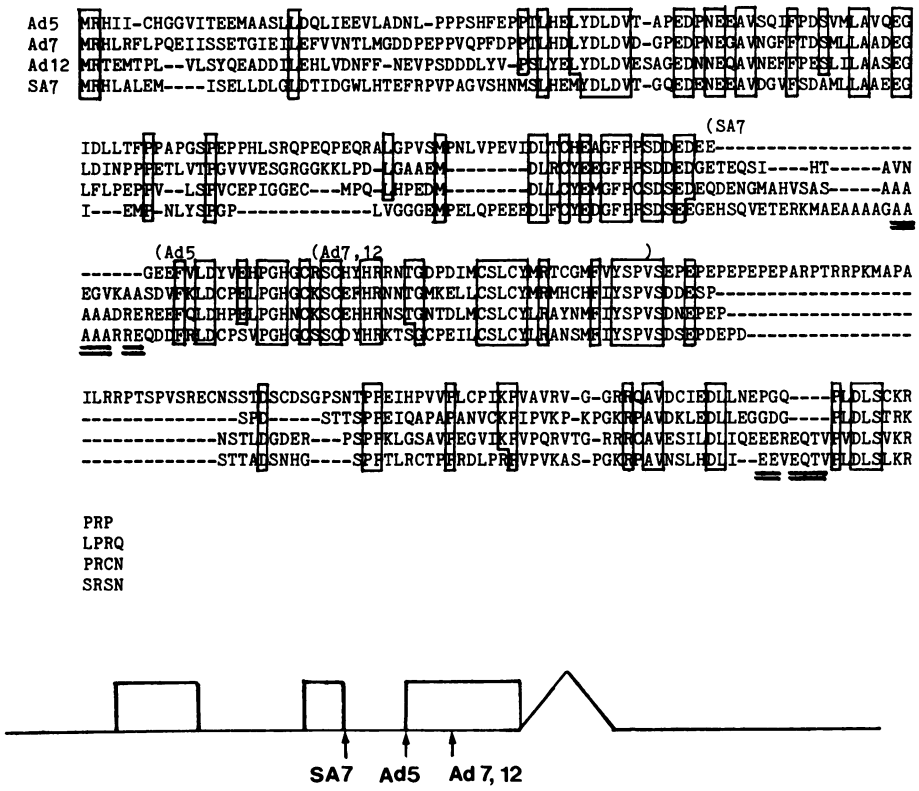

FIG. 6. Comparison of the amino acid sequences of the E1a region of the human adenoviruses and SA7. The amino acid sequences of the E1a polypeptides of the human adenoviruses Ad5, Ad7, and Ad12 (59) have been aligned with the corresponding sequences of SA7 to produce maximum homology. Boxes are drawn to indicate amino acids which are conserved in all viruses and those which are conserved only among the three human adenoviruses. The underscored double lines indicate two regions which are conserved between SA7 and the oncogenic human adenovirus Ad12. Parentheses indicate donor (opening parenthesis) and acceptor (closing parenthesis) splice sites for the smaller early E1a polypeptide. Included at the bottom is a diagram showing the three principal regions of conservation found among the four viruses. The splice for the 13S message is shown, along with the positions of the 12S donor sites for each of the viruses.

shorter than the corresponding transcript from the human adenoviruses; (ii) the two early SA7 E1a RNAs are not detectable late in infection; and (iii) the spliced E1b transcript produced late in infection by the human adenoviruses is not detectable at late times of an SA7 infection.

The limited hybridization that occurs between Ad5 and SA7 viral DNAs reflects the divergence at the nucleic acid level between the human and simian adenoviruses. However, nucleotide sequence analysis of the SA7 E1a region revealed that the sequences which may regulate the transcription of this region as well as those that encode the E1a proteins are highly homologous to those found in the human viruses. In fact, among the three human adenovirus subtypes, 80 amino acids are invariant (59); of these, only 8 are variant in SA7. These conserved amino acids define three major domains which are separated by highly variable sequences often containing insertions or deletions. The conservation of these three domains in SA7 strongly suggests that these regions are important in the functions of the E1a polypeptides. In the human adenoviruses, the larger E1a polypeptide contains all three of these domains, whereas the smaller polypeptide contains only two of these domains due to the removal of a larger intervening sequence from the primary transcript. In SA7 the donor splice site for the generation of the smaller transcript is positioned closer to the 5′ end of the message than in the human adenoviruses, though still retaining both domains in the smaller protein. Although recent evidence has shown that this transcript is dispensable for the growth of Ad5 in tissue culture (31), the existence of this transcript in the simian virus with a novel splice site suggests that the encoded protein may have an important role in the viral life cycle.

The high degree of conservation observed between the E1a proteins of the human and simian adenoviruses suggests that the SA7 E1a protein should complement that encoded by the human viruses. In fact, SA7 is able to activate the early regions of Ad5 viruses deleted for or containing mutations in the E1a region. These results now define a limit of 72 amino acids essential for the regulatory activity of the E1a polypeptide and provide a strong basis for mutational analysis in both SA7 and Ad5. As the conserved amino acids may represent important residues, mutations can be targeted to the highly conserved regions of the E1a polypeptide to define the function of each domain.

In addition to its role in regulation, the E1a region of the human adenoviruses is essential for the transformation of cells in culture and for tumor formation in animals by the highly oncogenic adenovirus subtype. Of all the adenoviruses, SA7 alone has been shown to be capable of causing tumors in hamsters by direct injection of viral DNA (8, 29). More recently, Ponomareva et al. have demonstrated that this oncogenic activity is contained within a DNA fragment from the terminal 20% of the genome (37, 38). Based on the close similarity between SA7 and the human adenoviruses, we believe that the transforming region of SA7 is comprised of the E1a and E1b transcription units located in the leftmost

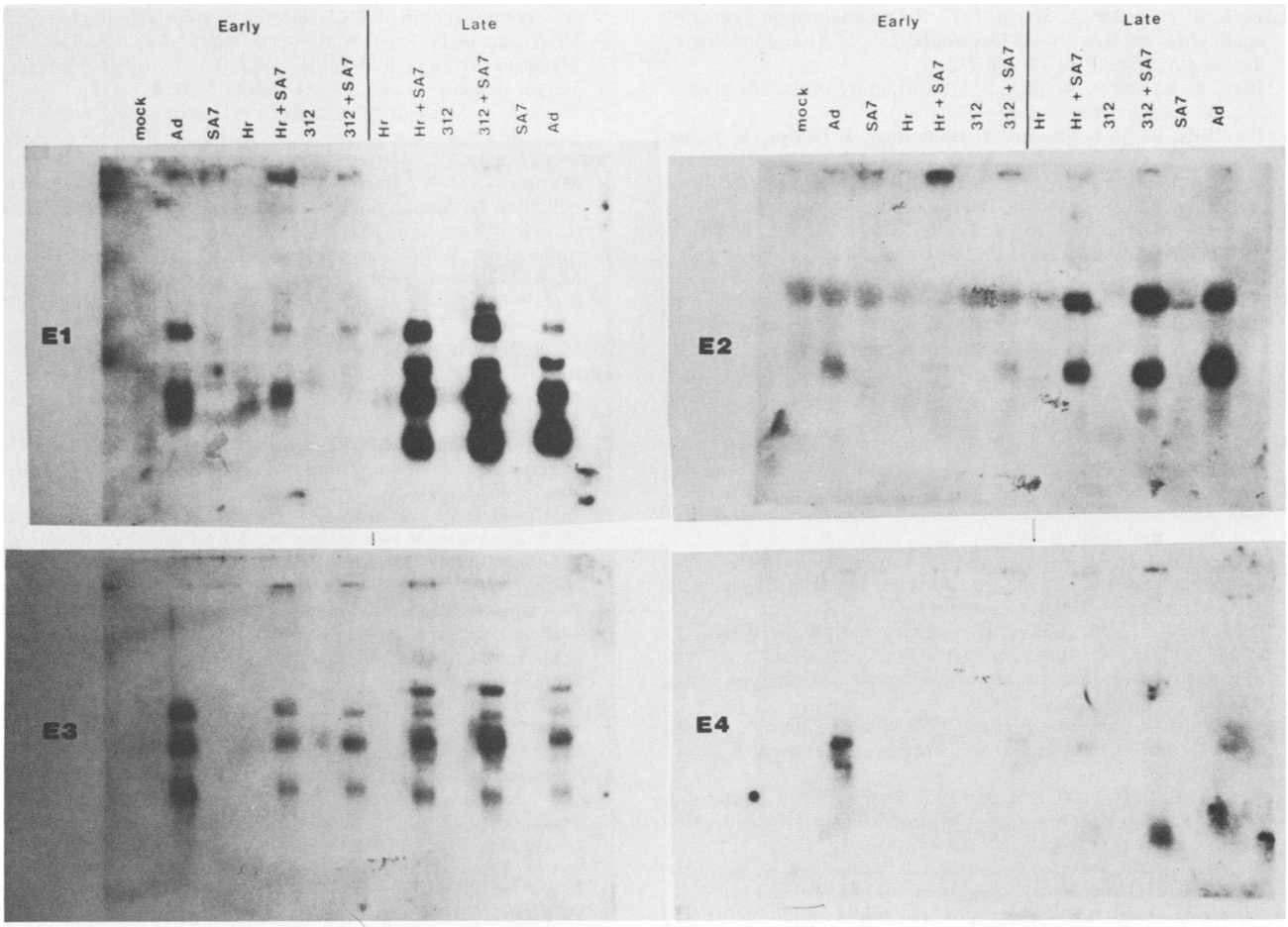

FIG. 7. SA7 activation of transcription of the early regions in Ad5 E1a mutant viruses. HeLa cells were infected with Hr-1 or *dl*312, alone or together with SA7, or with either Ad5 or SA7, and RNA was isolated at 8 h (early times) or 20 h (late times) postinfection. The RNA was separated on formaldehyde-agarose denaturing gels, transferred to nitrocellulose, and hybridized with fragments from each of the four Ad5 early regions, E1, E2, E3, and E4, labeled with ^{32}P by nick translation. The filters were washed and exposed to film.

10% of the genome. The definition of the transcriptional boundaries of these regions now permits the exchange of DNA fragments of E1a and E1b from SA7 and the human adenoviruses to determine which region is responsible for tumor formation. In the human adenoviruses, replacement of the E1a region of the nononcogenic Ad5 with the corresponding region from the oncogenic Ad12 confers the oncogenic phenotype, demonstrating that the oncogenic potential is located in the E1a region (6). Other than the highly conserved amino acid sequences present in both human and simian E1a regions, the only amino acid homology between SA7 and a human adenovirus is in two regions shared by SA7 and the oncogenic adenovirus Ad12. It remains to be determined whether these homologous sequences are involved in the tumorigenicity of both of these viruses. Whereas neither of these regions is very large, it has been shown that only a one-amino acid change in the c-*ras* gene is necessary to confer the transformed phenotype (40, 52). Site-directed mutagenesis can be utilized to determine whether either of these two homologous regions is involved in tumorigenicity.

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ADDENDUM

Dekker et al. (submitted for publication) have completed the E1a and E1b sequences of SA7P, an antigenic variant of SA7. Whereas this virus is of an independent origin, the E1a regions of the two viruses are remarkably conserved.

LITERATURE CITED

1. **Alwine, J. C., D. J. Kemp, B. A. Parker, J. Reiser, J. Renant, G. R. Stark, and G. M. Wehl.** 1980. Detection of specific RNAs on specific fragments of DNA by fractionation in gels and transfer to diazobenzyloxymethyl paper. Methods Enzymol. **68**:220–242.
2. **Akusjarvi, G., and U. Pettersson.** 1979. Sequence analysis of adenovirus DNA. IV. The genomic sequences encoding the common tripartite leader of late adenovirus messenger RNA. J. Mol. Biol. **134**:143–158.
3. **Berk, A. J., F. Lee, T. Harrison, J. Williams, and P. A. Sharp.** 1979. Pre-early adenovirus 5 gene product regulates synthesis of early viral messenger RNAs. Cell **17**:935–944.

4. **Berk, A. J., and P. A. Sharp.** 1977. Sizing and mapping of early adenovirus mRNAs by gel electrophoresis of S1 endonuclease-digested hybrids. Cell **12**:721–732.
5. **Berk, A. J., and P. A. Sharp.** 1978. Structure of the adenovirus 2 early mRNAs. Cell **14**:695–711.
6. **Bernards, R., P. I. Schrier, A. Houweling, J. L. Bos, A. J. van der Eb, M. Zijlstra, and C. J. M. Melief.** 1983. Tumorigenicity of cells transformed by adenovirus type 12 by evasion of T-cell immunity. Nature (London) **305**:776–779.
7. **Bonner, W. M., and R. A. Laskey.** 1974. A film detection method for tritium-labeled proteins and nucleic acids in polyacrylamide gels. Eur. J. Biochem. **46**:83–88.
8. **Burnett, J. P., N. Mayne, and L. Helton.** 1975. Retention of tumor-inducing capability by adenovirus DNA after cleavage by restriction endonucleases. Nature (London) **254**:158–159.
9. **Carlock, L. R., and N. C. Jones.** 1981. Transformation-defective mutant of adenovirus type 5 containing a single altered E1a mRNA species. J. Virol. **40**:657–664.
10. **Casto, B. C.** 1968. Adenovirus transformation of hamster embryo cells. J. Virol. **2**:376–383.
11. **Chow, L. T., T. R. Broker, and J. B. Lewis.** 1979. Complex splicing patterns of RNAs from the early regions of adenovirus-2. J. Mol. Biol. **134**:265–303.
12. **Dijkema, R., B. M. Dekker, and H. Van Ormondt.** 1980a. The nucleotide sequence of the transforming Bgl II-H fragment of adenovirus type 7 DNA. Gene **9**:141–156.
13. **Dijkema, R., B. M. Dekker, H. Van Ormondt, A. de Waard, J. Maat, and H. W. Boger.** 1980b. Gene organization of the transforming region of weakly oncogenic adenovirus type 7: the E1a region. Gene **12**:287–299.
14. **Farber, M. S., and S. G. Baum.** 1978. Transcription of adenovirus RNA in permissive and nonpermissive infections. J. Virol. **27**:136–148.
15. **Flint, S. J., P. H. Gallimore, and P. A. Sharp.** 1975. Comparison of viral RNA sequences in adenovirus 2-transformed and lytically infected cells. J. Mol. Biol. **96**:47–68.
16. **Fujinaga, K., Y. Sawada, Y. Uemizu, T. Yamashito, K. Shimojo, H. Shiroki, H. Sugisaki, K. Sugimoto, and M. Takanami.** 1979. Nucleotide sequences, integration and transcription of the adenovirus type 12 transforming genes. Cold Spring Harbor Symp. Quant. Biol. **44**:519–532.
17. **Gibadulin, R. A., R. Dejkema, T. S. Denisova, and H. Van Ormondt.** 1981. Simian adenovirus genome. II. Identification of specific fragments of viral DNA possessing transforming activity. Mol. Biol. (USSR) **16**:315–321.
18. **Graham, F. G., P. J. Abrahams, C. Mulder, H. L. Heijneker, S. O. Warner, F. A. J. de Vries, W. Fiers, and A. J. Van der Eb.** 1975. Studies on in-vitro transformation by DNA and DNA fragments of human adenoviruses and SV40. Cold Spring Harbor Symp. Quant. Biol. **39**:637–650.
19. **Harrison, T., F. Graham, and J. Williams.** 1977. Host range mutants of adenovirus type 5 defective for growth in Hela cells. Virology **77**:319–329.
20. **Hearing, P., and T. Shenk.** 1983. The adenovirus type 5 E1a transcriptional control region contains a duplicated enhancer element. Cell **33**:695–703.
21. **Huebner, R. J.** 1967. Adenovirus-directed tumor and T-antigens. Perspect. Virol. **5**:147–166.
22. **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.
23. **Jones, N., and T. Shenk.** 1979. Isolation of Ad5 host range deletion mutants defective for transformation of rat embryo cells. Cell **17**:683–689.
24. **Kitchingman, G. R., and H. Westphal.** 1980. The structure of adenovirus 2 early nuclear and cytoplasmic RNAs. J. Mol. Biol. **137**:23–48.
25. **Lewis, J. B., C. W. Anderson, and J. F. Atkins.** 1977. Further mapping of late adenovirus genes by cell-free translation of RNA selected by hybridization to specific DNA fragments. Cell **12**:37–44.
26. **Lewis, J. B., J. F. Atkins, L. W. Anderson, P. R. Baum, and R. F. Gesteland.** 1975. Mapping of late adenovirus genes by cell-free translation of RNA selected by hybridization to specific DNA fragments. Proc. Natl. Acad. Sci. U.S.A. **72**:1344–1348.
27. **Malherbe, H., and R. Harwin.** 1963. The cytopathic effects of Vervet monkey viruses. S. Afr. Med. J. **37**:407–411.
28. **Maxam, A. M., and W. Gilbert.** 1980. Sequencing end-labeled DNA with base-specific chemical cleavages. Methods Enzymol. **65**:499–580.
29. **Mayne, N., J. P. Burnett, and L. K. Butler.** 1971. Tumour induction by simian adenovirus SA7 DNA fragments. Nature (London) New Biol. **232**:182–183.
30. **Miller, J. S., R. P. Ricciardi, B. E. Roberts, B. M. Patterson, and M. B. Matthews.** 1980. Arrangement of messenger RNAs and protein coding sequences in the major late transcription unit of adenovirus 2. J. Mol. Biol. **142**:455–488.
31. **Montell, C., E. F. Fisher, M. H. 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.
32. **Pelham, H. R. B., and R. S. Jackson.** 1976. Efficient mRNA dependent translation system from reticulocyte lysates. Eur. J. Biochem. **67**:247–256.
33. **Perricaudet, M., G. Akusjarvi, A. Virtanen, and U. Pettersson.** 1979. Structure of two spliced mRNAs from the transforming region of human subgroup C adenoviruses. Nature (London) **281**:694–696.
34. **Perricaudet, M., J.-M. Moullec, P. Tiollais, and U. Pettersson.** 1980. Structure of two adenovirus type 12 transforming polypeptides and their evolutionary implications. Nature (London) **288**:174–176.
35. **Pettersson, U., and M. B. Matthews.** 1977. The gene and messenger RNA for adenovirus polypeptide IX. Cell **12**:741–750.
36. **Pina, M., and M. Green.** 1968. Base composition of the DNA of oncogenic simian adenovirus SA7 and homology with the human adenoviruses DNAs. Virology **36**:321–323.
37. **Ponomoreva, T. I., N. N. Chaplygina, B. S. Naroditsky, N. A. Grodnitskaya, R. S. Dreizin, and T. I. Tikchonenko.** 1976. Transforming activity and oncogenicity of simian adenovirus SA7 DNA. Byull. Eksp. Biol. Med. **N12**:698–700.
38. **Ponomoreva, T. I., N. A. Grodnitskaya, E. Z. Goldberg, N. M. Chaplygina, B. S. Naroditsky, and T. I. Tikchonenko.** 1979. Biological activity of the intact and cleaved DNA of the simian adenovirus 7. Nucleic Acids. Res. **6**:3119–3131.
39. **Proudfoot, N. J.** 1979. Eukaryotic promoters. Nature (London) **279**:376.
40. **Reddy, E. P., R. K. Reynolds, E. Santos, and M. Barbacid.** 1982. A point mutation is responsible for the acquisition of transforming properties by the T24 human bladder carcinoma oncogene. Nature (London) **300**:149–152.
41. **Riccardi, R. P., R. L. Jones, C. L. Cepko, P. A. Sharp, and B. E. Roberts.** 1981. Expression of early adenovirus genes requires a viral encoded acidic polypeptide. Proc. Natl. Acad. Sci. U.S.A. **78**:6121–6125.
42. **Sawada, Y., and K. Fujinaga.** 1980. Mapping of adenovirus 12 mRNAs transcribed from the transforming region. J. Virol. **36**:639–651.
43. **Seed, B.** 1982. Nucleic acid attachment to supports, p. 91–103. *In* J. K. Setlow and A. Hollaender (ed.), Genetic engineering, vol. 4. Plenum Press, New York.
44. **Sekikowa, K., K. Shiroki, H. Shimojo, S. Ojima, and K. Fujinaga.** 1978. Transformation of a rat cell line by an adenovirus 7 DNA fragment. Virology **88**:1–7.
45. **Shenk, T., N. Jones, W. Colby, and D. Fowlkes.** 1979. Functional analysis of adenovirus-5 host-range deletion mutants defective for transformation of rat embryo cells. Cold Spring Harbor Symp. Quant. Biol. **44**:367–375.
46. **Shiroki, K., H. Handa, H. Shimojo, S. Yano, S. Ojima, and K. Fujinaga.** 1977. Establishment and characterization of rat cell lines transformed by restriction endonuclease fragments of adenovirus 12 DNA. Virology **82**:462–471.
47. **Solsnick, D., and M. A. Andersson.** 1982. Transformation-deficient adenovirus mutant defective in expression of region 1a but not region 1b. J. Virol. **42**:106–113.
48. **Stillman, B. W., J. B. Lewis, L. T. Chow, M. B. Matthews, and**

J. E. Smart. 1981. Identification of the gene and mRNA for the adenovirus terminal protein precursor. Cell **23**:497–508.
49. **Stillman, B. W., F. Tamanoi, and M. B. Matthews.** 1982. Purification of an adenovirus-coded DNA polymerase that is required for initiation of DNA replication. Cell **31**:613–623.
50. **Sugisaki, H., K. Sugimoto, M. Takanami, K. Shiroki, I. Saito, H. Shimojo, Y. Sawada, Y. Uemizu, S. Uesugi, and K. Fuginaga.** 1980. Structure and gene organization in the transforming Hind III-G fragment of Ad12. Cell **20**:777–786.
51. **Svensson, C., U. Pettersson, and G. Akusjanvi.** 1983. Splicing of adenovirus 2 early region 1a mRNAs is non-sequential. J. Mol. Biol. **165**:475–495.
52. **Tabin, C. J., S. M. Bradley, C. I. Bargmann, R. A. Weinberg, A. G. Papageorge, E. M. Scolnick, R. Dhar, D. R. Lowry, and E. H. Chang.** 1982. Mechanism of activation of a human oncogene. Nature (London) **300**:143–149.
53. **Thomas, P. S.** 1980. Hybridization of denatured RNA and small DNA fragments transferred to nitrocellulose. Proc. Natl. Acad. Sci. U.S.A. **77**:5201–5205.
54. **Tolun, A., P. Alestrom, and U. Pettersson.** 1979. Sequence of inverted terminal repetitions from different adenoviruses: demonstration of conserved sequences and homology between SA7 termini and SV40 DNA. Cell **17**:705–713.
55. **Van der Elsen, P., S. de Pater, A. Houweling, J. van der Veer, and A. van der Eb.** 1982. The relationship between region E1a and E1b of human adenoviruses in cell transformation. Gene **18**:175–185.
56. **Van der Elsen, P., A. Houweling, and A. van der Eb.** 1983. Expression of region E1b of human adenoviruses in the absence of region E1a is not sufficient for complete transformation. Virology **128**:377–390.
57. **Van Ormondt, H., and B. Hesper.** 1983. Comparison of the nucleotide sequences of early region E1b DNA of human adenovirus types 12, 7 and 5 (Subgroups A, B and C). Gene **21**:217–226.
58. **Van Ormondt, H., J. Maat, A. De Waard, and A. J. van der Eb.** 1978. The nucleotide sequence of the transforming HpaI-E fragment of Ad5 DNA. Gene **4**:309–328.
59. **Van Ormondt, H., J. Maat, and R. Dijkema.** 1980. Comparison of nucleotide sequences of the early E1a regions for subgroups A, B, and C of human adenoviruses. Gene **12**:63–76.
60. **Virtanen, A., and U. Pettersson.** 1983. The molecular structure of the 9S mRNA from early region 1a of adenovirus serotype 2. J. Mol. Biol. **165**:496–499.
61. **Yoshida, K., K. Sekikawa, and K. Fujinaga.** 1979. Mappings of adenovirus type 7 cytoplasmic RNA species synthesized in lytically infected cells and synthesized in transformed cells. J. Virol. **32**:339–344.