Simian Virus 40 DNA Segment of the Adenovirus 7-Simian Virus 40 Hybrid, E46⁺, and Its Transcription During Permissive Infection of Monkey Kidney Cells

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Nucleic acid hybridization methodology has been used to investigate the span of the simian virus 40 (SV40) DNA segment in the adenovirus 7-SV40 hybrid, E46⁺, and the extent of its transcription in lytically infected monkey kidney cells. The SV40 segment of E46⁺ comprises approximately 62% of the SV40 genome; it originates in the proximal region of *Hin-G* (the *G* fragment derived by cleavage of intact SV40 DNA with *Haemophilus influenzae* restriction endonuclease), extends sequentially through approximately 80% of this fragment, all of fragments *Hin-B*, *-I*, *-H*, and *-A*, and terminates approximately 70% of the distance through *Hin-C*. During E46⁺ lytic infection of permissive cells, the vast majority of stable cytoplasmic SV40-specific RNA is transcribed from the minus (E) strand of the fragments *Hin-A*, *-H*, *-I*, and *-B*, comprising the early template region. Transcripts of the minus strand of the *Hin-G* and *-C* fragments are detected in much lower concentrations, especially in the total lytic cellular RNA, whereas RNA complementary to the plus (L) strand is not detected. The transcriptional pattern of the SV40 segment within E46⁺ is thus very similar to that in a number of transformed cell lines and in some respects to the transcriptional pattern in a series of nondefective adenovirus 2-SV40 hybrid viruses. These results suggest a common transcriptional mechanism for integrated SV40 DNA.

Although it has been known for several years that the viral genome is covalently integrated into the host cell genome in mammalian cells transformed by simian virus 40 (SV40) (33), only recently has the extent of transcription of viral DNA in transformed cells been delineated (13, 25, 31). Uniformly transcribed in all transformed lines tested is a segment of viral DNA either identical or similar to that region transcribed before DNA replication in cells lytically infected with SV40. This segment of DNA, termed the early gene template, is transcribed in a counter-clockwise direction, as the SV40 map is conventionally drawn (15, 32), and comprises close to 50% of the minus strand, extending from a point in the distal *Hin-A* fragment of SV40 through the *Hin-H* and *-I* fragments to a point within the proximal region of *Hin-B* (15, 32) or in the distal portion of *Hin-G* (5). In certain transformed lines, other areas of the minus strand, designated anti-late regions, are also transcribed; although the concentration of RNA so derived may be variable (25; G. Khoury, M. Martin, T. N. H. Lee, and D.

Nathans, submitted for publication) in a large number of lines, it is present at much lower concentrations than RNA derived from the early gene region (Khoury et al., submitted for publication). The plus strand of SV40 DNA is rarely, if ever, transcribed into stable RNA species in transformed cell lines (13, 25; Khoury et al., submitted for publication). The presence of relatively large amounts of early RNA and limited amounts of anti-late RNA in many transformed cells suggests that in these lines initiation and termination of transcription occurs within the SV40 segment or that post-transcriptional processing occurs at an internal point(s) in the initial SV40 RNA transcript. The finding of SV40 RNA sequences immediately adjacent to 3' poly(A) termini in RNA isolated from transformed cell lines supports this conclusion (P. Ghosh, S. Sotman, and P. Lebowitz, manuscript in preparation).

The adenovirus 7 (Ad 7)-SV40 hybrid, E46⁺, provides another example of SV40 DNA integrated within a linear host DNA molecule. In this study we have examined the span of the

SV40 segment contained in E46⁺ and the extent of its transcription in infected monkey kidney cells. Our results indicate that the SV40 DNA sequences contained in E46⁺ include all of the early template and a significant portion of the late template, a total of about 62% of the entire SV40 genome. Furthermore, analysis of transcription of the SV40 segment in E46⁺ has indicated that the entire early gene region as well as portions of the anti-late template are transcribed, a pattern of transcription similar to that observed in a large number of transformed cells (Khoury et al., submitted for publication).

MATERIALS AND METHODS

Growth of SV40 and E46⁺ viruses, DNA, and RNA isolation. SV40 growth and DNA purification have been described previously (12, 16). Briefly, confluent BSC-1 monolayers were infected with strain 777 SV40 at a multiplicity of 0.5 to 2 PFU/cell. Culture medium contained Eagle minimal essential medium supplemented with 2 mM glutamine; penicillin, 250 U/ml; streptomycin, 250 µg/ml; and 2% heat-inactivated agammaglobulinemic calf serum. For propagation of radiolabeled virus, [³²P]H₂PO₄ was added to phosphate-free culture medium in a final concentration of 25 µCi/ml. Cells were disrupted according to the procedure of Hirt (9) when 90 to 100% exhibited cytopathic lesions. The Hirt supernatant was extracted twice with redistilled phenol and viral DNA precipitated with 2 volumes of ethanol. SV40 DNA_i was purified by equilibrium centrifugation in a gradient of CsCl₂ (ρ = 1.57)-ethidium bromide (200 µg/ml) (27). After removal of dye by extraction with isoamyl alcohol, DNA_i was exhaustively dialyzed against 0.1× SSC (SSC: standard saline citrate; 0.15 M sodium chloride, 0.015 M sodium citrate) and precipitated with 2 volumes of ethanol. [³²P]DNA had specific activities of 4 × 10⁶ to 5 × 10⁶ counts/min per µg.

E46⁺ was grown on human embryonic kidney or primary African green monkey kidney cells (AGMK), as described elsewhere (2). Its DNA, purified by slight modification (28) of the procedure of Green and Pina (7), was generously provided by S. Baum. RNA from E46⁺-infected cells was isolated from AGMK cells 48 h after infection. Total cellular RNA was prepared by methods previously described (26). Cytoplasmic RNA was prepared in a similar fashion after separation of nuclear and cytoplasmic fractions with the nonionic detergent, NP-40. *Escherichia coli* DNA was prepared by the procedure of Marmur (21).

SV40 DNA fragments. Restriction endonuclease from *Haemophilus influenzae* was prepared by slight modification (P. Lebowitz, W. Siegel, and J. Sklar, J. Mol. Biol., in press) of the method of Smith and Wilcox (34). SV40 [³²P]DNA_i was digested with this enzyme and the resultant 11 fragments (designated *Hin*-A through *K*) were separated on 4% polyacrylamide gels as previously described (P. Lebowitz et al., J. Mol. Biol., in press). Fragments were recovered from acrylamide by elution in 0.1× SSC, followed by precipitation with 2 volumes of ethanol.

Separation of SV40 DNA strands. Separation of the plus and minus strands of SV40 DNA *Hin* fragments A through K with SV40 complementary RNA has been previously described (15).

Preparation of SV40 RNA complementary to DNA fragments. Transcription of SV40 DNA_i in the presence of unlabeled ribonucleoside triphosphates and DNA fragments in the presence of [α-³²P]ATP (New England Nuclear Corp., 95 Ci/mmol) with *E. coli* RNA polymerase followed established methodology (16). After the transcription incubation, template DNA was degraded by incubation with 25 µg of pancreatic DNase per ml (Worthington, electrophoretically purified) for 10 min at 37 C. Incubations were terminated by addition of sodium dodecyl sulfate to 0.25% and extraction with an equal volume of redistilled phenol. RNA transcripts were then passed through Sephadex G-100 columns in 0.01 M Tris-hydrochloride, pH 7.9, 0.01 M MgCl₂, and 1.0 mM EDTA to remove unincorporated substrates, after which they were recovered by precipitation in the presence of 200 µg of carrier *E. coli* tRNA, 2% potassium acetate, and 66% ethanol.

Nucleic acid hybridizations. RNA-DNA hybridizations of two types were performed. For determination of the span of the SV40 genome present in the DNA of E46⁺, radiolabeled *Hin* fragment transcripts were tested for binding to E46⁺ DNA by modification (16) of the method of Gillespie and Spiegelman (6). In these experiments, comparisons were made between fragment transcript binding to approximately equimolar quantities of SV40 and E46⁺ DNA (0.03 and 2.0 µg, respectively) fixed to nitrocellulose filters. The determination of these quantities was based on the comparative molecular weights of the viral DNAs (SV40 = 3 × 10⁶, and E46⁺ = 20.5 × 10⁶ daltons), and the fact that owing to its defective nature, E46⁺ cannot be propagated in the absence of the parent adenovirus 7 (Ad 7) (30) and extracted DNA contains both Ad 7 and E46⁺ DNA in a ratio of about 9:1 (1). Approximately equimolar quantities of fragment transcripts were also introduced into the hybridization reaction. These calculations were derived from the percentage of SV40 DNA spanned by each fragment, as estimated from the yield of each fragment after endonuclease cleavage of uniformly labeled SV40 DNA_i: A, 22.5%; B, 15%; C, 10.5%; D, 10%; E, 8.5%; F, 7.5%; G, 7.0%; H, 5.5%; I, 5.0%; J, 4.5%; and K, 4% (4).

For determination of the segments of the SV40 genome transcribed into stable cellular RNAs in vivo, small quantities of the separated strands of the 11 SV40 DNA fragments (1 to 5 ng) were incubated with increasing concentrations of total cellular (0.2 to 3.2 mg/ml) or cytoplasmic (1.0 to 1.6 mg/ml) E46⁺ lytic RNA in 0.1 to 0.2 ml of 1.0 M NaCl, 0.04 M PB, and .01 M Tris-hydrochloride, pH 7.5. After 20 h of incubation at 68 C, the percentage of hybrid molecules was determined by hydroxyapatite chromatography (12, 15).

RESULTS

The SV40 DNA segment of E46⁺. In previously reported experiments (16), the segments

of SV40 DNA included in a series of five nondefective adenovirus 2 (Ad2)-SV40 hybrids was determined by quantitating the binding of transcripts of the 11 fragments derived by cleavage of SV40 DNA with *H. influenzae* restriction endonuclease (*Hin* fragments) to equimolar quantities of SV40 and hybrid virus DNA. The presence of a complete fragment in the SV40 DNA segment of a hybrid was indicated by equivalent degrees of binding of its radiolabeled transcript with equimolar quantities of hybrid and SV40 DNA. The proportion of a *Hin* fragment represented only in part in a hybrid genome could be approximated from binding of the transcript by the hybrid DNA relative to binding by an equimolar quantity of SV40 DNA. Since the size of each fragment and its position on the SV40 genome has been established (Fig. 1), it was possible to estimate the length of the SV40 DNA segment in each hybrid virus. These results were in agreement with size determinations made by electron microscopic methods (10, 22).

Identical methodology has been utilized to determine the span of the SV40 segment of the defective Ad 7-SV40 hybrid, E46⁺. Table 1 reveals binding of all 11 *Hin* fragment transcripts to SV40 DNA, but binding of only the *Hin*-G, B, I, H, A, and C transcripts to E46⁺ DNA. The transcripts of *Hin*-B, I, H, and A bind almost as well to E46⁺ DNA (84 to 90%) as to an approximately equimolar quantity of SV40 DNA. Since these four fragments are in se-

quence on the *Hin* cleavage map of SV40 and since at least one strand of each of the *Hin* fragments is transcribed in vitro with *E. coli* RNA polymerase in its entirety or nearly its entirety (Lebowitz, unpublished data), this data suggests that each of the fragments is represented in its entirety in the SV40 segment of E46⁺. Equivalent degrees of binding to E46⁺ and SV40 DNAs were not reached, probably because slightly less than an equimolar quantity

TABLE 1. Hybridization of transcripts of *Hin* fragments of SV40 DNA to E46⁺ and SV40 DNA^a

DNA fragment transcript	Input (counts/min)	Counts/min annealed to DNA ^b			Counts/min bound to E46 ⁺ /counts/min bound to SV40
		<i>E. coli</i>	SV40	E46 ⁺	
A	52,000	43	5,760	5,090	0.88
B	34,500	35	3,800	3,195	0.84
C	24,000	32	2,520	1,510	0.60
D	23,000	26	2,505	37	0.01
E	19,500	24	2,015	32	0.02
F	17,200	36	1,540	19	0.01
G	16,000	25	1,530	1,120	0.71
H	13,000	24	1,355	1,220	0.90
I	11,500	22	1,205	1,025	0.85
J	10,000	14	1,165	14	0.01
K	9,000	17	980	15	0.02

^a Transcriptions and hybridizations were performed as described.

^b Average counts/min from two determinations.

FIG. 1. A comparison of the SV40 segment of E46⁺ with those of the nondefective adenovirus-SV40 hybrid viruses (8, 10, 16, 18, 22). The location of the SV40 segments relative to the *Eco* R₁ (4) and *Hin* cleavage sites (16) of SV40 DNA, as well as the antigen-inducing capacities of certain of these hybrids (18, 19, 29) are indicated. While the position of the E46⁺ SV40 insertion in Ad 7 is shown, the orientation of this segment with respect to the Ad 7 DNA has not yet been determined. (*, A deletion in the E46⁺ segment of SV40 DNA. See text for discussion.)

of E46⁺ DNA was utilized in the hybridization reaction. The variability in the relative yields of E46⁺ and the parent Ad 7 in the lytic infection make this explanation most plausible.

The hybridization of transcripts from fragments *Hin-G* and *C* to E46⁺ DNA was only 73 and 60%, respectively, of the binding of these transcripts to SV40 DNA. Normalizing these values to equimolar quantities of the two viral DNAs, it may be concluded that approximately 80% of *Hin-G* and 70% of *Hin-C* are represented in the SV40 segment of E46⁺. Thus, the SV40 segment of E46⁺ originates within *Hin-G*, extends through all of *Hin-B*, *I*, *H*, and *A*, and terminates about two-thirds of the distance through *Hin-C* (Fig. 1).

Transcription of the SV40 segment of E46⁺. The transcriptional pattern of SV40 sequences in E46⁺ during lytic infection of AGMK cells was first approached in liquid phase hybridization experiments between fragments of the separated strands of ³²P-labeled SV40 DNA and unlabeled total cellular or cytoplasmic RNA. These hybridizations indicated essentially no binding of either RNA to the plus (late) strand (1 to 2%) (Table 2). On the other hand, there was extensive binding of E46⁺ total cellular RNA (43%) and cytoplasmic RNA (38%) to fragments of the minus (early) strand.

To topographically localize these transcripts, similar hybridization experiments were carried out between both whole cell and cytoplasmic RNAs from E46⁺ infected cells and the minus strand of the 11 SV40 *Hin* fragments. Figure 2 reveals the extent of binding of the minus strand of six of the ³²P *Hin* fragments (*Hin-A*, *B*, *C*, *G*, *H*, and *I*) by increasing amounts of total cellular RNA. For all six, it appears that maximal binding of the minus strand has been reached or nearly reached under our hybridization conditions. The hybridization data for all 11 fragments under conditions of maximal binding are summarized in Table 2. It is apparent that there is extensive hybridization between the minus strand of *Hin* fragments *A*, *H*, *I*, and *B* and E46⁺ RNA. With total cell RNA, there is nearly complete retention of *Hin-H*, *A*, and *B* on hydroxyapatite (80, 84, and 90%, respectively); on the other hand, retention of *Hin-I* is somewhat lower (43%). A similar, though somewhat less disparate, result was also obtained when cytoplasmic rather than total cell RNA was used in the molecular hybridizations; 85 to 90% of input *Hin-A*, *B*, and *H* and 55% of *Hin-I* were bound in duplex molecules by cytoplasmic RNA. Recently, we have found that *Hin-I* is usually contaminated (as much as 50%) with *Hin-J*, a late fragment which runs just behind

TABLE 2. Hybridization of E46⁺ lytic RNA to the separated strands of SV40 DNA^a

	DNA in hybrid molecules % ^a	
	Total cell RNA	Cytoplasmic RNA
A. Fragments of SV40 DNA		
Minus strand	43	38
Plus strand	2	1
B. Minus DNA strands of SV40 <i>Hin</i> fragments		
<i>A</i>	84	95
<i>B</i>	90	95
<i>C</i>	15	10
<i>D</i>	<1	<1
<i>E</i>	<1	<1
<i>F</i>	1	<1
<i>G</i>	22	10
<i>H</i>	80	95
<i>I</i>	43	55
<i>J</i>	<1	2
<i>K</i>	<1	1

^a In preliminary experiments (A), E46⁺ RNA was shown to bind almost exclusively to the minus strand of SV40 DNA. Subsequently (B), total cellular and cytoplasmic E46⁺ RNAs were hybridized with the minus strands of SV40 *Hin* fragments *A* to *K*, under conditions described.

^b DNA in hybrid molecules assayed by hydroxyapatite chromatography.

Hin-I in the preparative gels (unpublished data). This almost certainly accounts for our inability to saturate the minus strand of *Hin-I* with either lytic (15) or E46⁺ RNA.

Compared to the extensive binding of the minus strand of these four fragments to E46⁺ RNA, only 10% of the minus strands of *Hin* fragments *C* and *G* formed duplex molecules with cytoplasmic RNA and only 15 and 22%, respectively, with whole cell RNA. Even at the highest concentrations of whole cell RNA, the remaining *Hin* fragments failed to form duplex molecules.

These findings suggest that transcripts of *Hin* fragments *A*, *H*, *I*, and *B*, which comprise the early gene template of SV40 (15, 32), are present in abundance in AGMK cells infected with E46⁺, whereas transcripts from the minus strands of *Hin* fragments *C* and *G*, representing the anti-late template, are either scarce or span a small portion of these fragments. We have recently shown that fragments the size of *Hin-C* and *G* (as opposed to *Hin-A*) are broken only slightly during strand separation (Khouri et al., submitted for publication), and so it appears

FIG. 2. Extent of hybridization of radiolabeled *Hin* fragments with increasing quantities of *E46*⁺ total cellular RNA. Increasing concentrations of total cellular RNA from *E46*⁺-infected primary AGMK cells were incubated in reaction mixtures with 1 to 5 ng of the ³²P-labeled minus DNA strands of *Hin* fragments A, B, C, G, H, and I under conditions detailed in Materials and Methods. The percent [³²P]DNA in duplex molecules was determined by hydroxyapatite chromatography. The inability to saturate *Hin*-I is possibly related to its contamination with *Hin*-J (see text).

more likely that the anti-late transcripts are present in lower concentrations than the early transcripts.

Since hydroxyapatite chromatography does not distinguish between partial and complete duplex molecules, the extent of transcription of *Hin* fragments A and B was assayed by the single-strand nuclease, S1, as previously described (13, 15). Almost complete protection of *Hin*-A (87%) and B (88%) from S1 nuclease with *E46*⁺ total cell RNA was obtained, suggesting transcription of most, if not all, of the sequences of these fragments. Thus, the abundant transcript in *E46*⁺-infected cells appears to span most, if not all, of *Hin*-A and B and probably all of *Hin*-H and I, the total extent of the early gene template, as most recently delineated by both nucleic acid hybridization (Khouri et al., unpublished data) and sequencing (Dhar et al., unpublished data) methods. Transcription from *Hin*-C and G was not assayed by S1 nuclease

since the highest concentrations of RNA used were insufficient to saturate these fragments when assayed by hydroxyapatite chromatography.

DISCUSSION

The present experiments were conducted to determine the quantity of SV40 genetic information integrated within the Ad 7 genome of the hybrid, *E46*⁺, and the extent of expression of this information during infection of permissive mammalian cells. Our findings on the extent of the SV40 genome included within the *E46*⁺ genome are summarized in Fig. 1. The SV40 segment within *E46*⁺ originates in the proximal region of *Hin*-G. It includes about 80% of this fragment, all of *Hin*-B, I, H, and A, and terminates approximately 70% of the distance into *Hin*-C. By summation of the lengths of the component fragments, it may be deduced that the SV40 segment of *E46*⁺ comprises about 62% of the SV40 genome. This estimate of the size of the SV40 segment in *E46*⁺ is intermediate between the value of 45 to 50% obtained by Baum and Fox (1) and S. Palmar and J. A. Rose (personal communication) by DNA-DNA hybridizations and the value of 75% obtained by Kelly and Rose (11) from electron microscope heteroduplex mapping. The value of 62% derived from hybridization experiments with the in vitro transcripts would, of course, reflect only unique SV40 sequences. Thus, one possible explanation for the higher values seen in heteroduplex studies would be a redundancy of part of the SV40 segment. This possibility is presently under investigation.

It has previously been demonstrated that the SV40 DNA segments in five nondefective hybrids all originate at a common site (10) within *Hin*-G (16), approximately 10% of the distance from the proximal terminus of this fragment and 0.11 map units from the unique *E. coli* R₁ restriction endonuclease cleavage site of SV40 DNA (10, 16, 22) (Fig. 1). Our data suggest that the representation of *Hin*-G in *E46*⁺ in approximately 10% less than that in the nondefective hybrids. However, recent nucleic acid sequencing studies have revealed that the SV40 segment of *E46*⁺ originates at precisely the same location as the SV40 segments of the five nondefective hybrids. Furthermore, a deletion of 30 nucleotides, representing 8.5% of *Hin*-G exists in the *Hin*-G fragment of *E46*⁺, at the *Hin*-G-B junction (S. Zain, P. Lebowitz, S. Baum, and S. M. Weissman, manuscript in preparation). The common point of origin of the SV40 segments in *E46*⁺ and the five nondefective hybrids is of great interest in that it

suggests a preferred site on the SV40 genome susceptible to specific endonuclease cleavage and recombinational integration into another viral genome. It is also of interest that the terminus of the SV40 segment of E46⁺ within *Hin*-C corresponds closely to a proposed cleavage site for integration of SV40 DNA in several transformed cell lines (Khouri et al., submitted for publication) and to a terminus in a triplicated SV40 DNA segment resulting from passage of virus at high multiplicities (G. Khouri, G. C. Fareed, K. Berry, M. A. Martin, T. N. H. Lee, and D. Nathans, *J. Mol. Biol.*, in press).

Prior studies have revealed that the minus strand of SV40 DNA serves as the template for viral RNAs transcribed before DNA replication during lytic infection (12, 20, 31). Early nucleic acid hybridization studies utilizing total cellular RNA indicated that this "early" template extends from a site in the distal *Hin*-A region to a site within the *Hin*-B fragment (15, 32). More recent nucleic acid hybridization (Khouri et al., unpublished data) and sequencing studies (Dhar et al., unpublished data), utilizing only cytoplasmic RNA, have shown that the early region extends from a point very close to the *Hin* A-C junction to a point very close to the *Hin* G-B junction, a span covering almost 50% of the SV40 genome. Transcription of this region proceeds from right to left as the SV40 map has been drawn (15, 32) (Fig. 1). Like the nondefective Ad 2-SV40 hybrid virus, Ad2⁺ND₄, E46⁺ contains the entire early gene region of SV40 DNA and expresses the three early SV40 antigens—U, tumor-specific transplantation, and T (29; S. Baum, personal communication)—during lytic infection. Although E46⁺ contains a considerably greater expanse of DNA coding for late SV40 functions than Ad2⁺ND₄, it is still incapable of synthesizing the viral capsid antigen (29).

Despite the fact that the SV40 segment of E46⁺ includes 62% of the SV40 genome, the nucleic acid hybridization data of Table 2 clearly indicate differential expression of this information in stable cytoplasmic as well as total cellular RNA species in monkey kidney cells infected with E46⁺. Specifically, the pattern of transcription as determined by hybridization with the separated strands of *Hin* fragments revealed that the most abundant species of RNA was similar if not identical to early lytic RNA, in agreement with earlier results derived from filter competition hybridization experiments (23). This conclusion is based on the findings that both RNAs hybridized most extensively with the minus strand of *Hin* frag-

ments A, H, I, and B, and that A and B, the terminal fragments in this contiguous segment, were almost completely protected from S1 nuclease digestion by total cellular RNA. Detectable at much lower levels were RNA molecules homologous to the minus strands of *Hin* fragments C and G, the so-called "anti-late" template. The absence of any significant reaction between E46⁺ RNA and *Hin* fragments D, E, F, J, and K supports the DNA mapping studies which showed that these fragments were not included in the SV40 segment of E46⁺.

As compared to total cellular RNA, there appears to be a relatively greater concentration of SV40-specific sequences in the cytoplasmic preparations of RNA, since under the same experimental conditions, lower RNA concentrations resulted in greater saturation of the early *Hin* fragments. Furthermore, there may be a greater relative concentration of early transcripts (from the minus strands of *Hin* fragments A, H, I, and B) as opposed to anti-late transcripts (from *Hin* fragments C and G) in the cytoplasmic RNA.

The pattern of transcription of the SV40 segment of E46⁺ is by present methods indistinguishable from that observed in several SV40-transformed cell lines (Khouri et al., submitted for publication), and very similar to that described for the five nondefective adenovirus-SV40 hybrid viruses, Ad2⁺ND₁₋₅ (14). In all cases, transcription is predominantly, if not exclusively, from the minus (early) strand. Furthermore, in cells infected with E46⁺ (Table 2) or the nondefective hybrids (Khouri, unpublished data), or transformed by SV40 (Khouri et al., submitted for publication), there appears to be an abundant species of RNA derived from the early SV40 gene region and a less abundant component(s) from the anti-late region. Of interest, however, preliminary experiments suggest that the amount of anti-late transcript relative to early template transcript in cells infected with the nondefective hybrids is greater than that in E46⁺-infected cells. Whether the deletion of 30 nucleotides in the distal *Hin*-G fragment of E46⁺ may be implicated in this disparity is not presently known.

In considering mechanisms responsible for the observed pattern of transcription of the integrated SV40 DNA in E46⁺, transformed cells, and the nondefective hybrids, one might assume that integration of SV40 DNA leads to preferential transcription of the minus (early) strand. Whether this specificity is related to a specific promoter site or the specificity of the active polymerase is only speculation. Furthermore, it is not known whether transcription of

FIG. 3. Models for the transcription of SV40 DNA sequences in E46⁺. (A) Recognition of an early SV40 promoter, P_E, and terminator, T_E. (B) Tandem transcription of adenoviral and SV40 sequences with subsequent degradation of the "anti-late" SV40 RNA. See text for discussion of these models. (—) Ad 7 DNA, (---) SV40 DNA, (~~~~) abundant RNA transcript, (.....) scarce RNA transcript.

the early region of the SV40 genome in lytic infection requires integration of the viral DNA.

An explanation for the abundance of RNA sequences from the early region of the minus strand and a scarcity of RNA sequences from the anti-late region in E46⁺-infected cells presents the same dilemma as does the transcriptional pattern of SV40 DNA in transformed cell lines. It is possible that the putative promoter and terminator for the early SV40 genes are recognized in most rounds of transcription; the anti-late RNA would then arise from a less frequent transcription which might "read through" from the proximal adenoviral genes (Fig. 3). Alternatively, this extensive "read through" transcription of the minus SV40 strand (in tandem with adenoviral transcription) might predominate. The abundance of the early SV40 RNA could then arise by a selective post-transcriptional cleavage mechanism. In either case, one might expect to find some RNA molecules containing both adenoviral and SV40 specific sequences, similar to those detected during the lytic cycle of Ad2⁺ND₄ (24). There is little data at present, however, to permit a choice between these alternatives.

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ADDENDUM IN PROOF

After submission of this paper, we became aware of the results of T. J. Kelly (J. Virol. 15:1267-1272) indicating a duplication of a portion of *Hin-A* and *-C* genetic information in the SV40 segment of E46⁺

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