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Evidence for a Transcription-Control Region of Simian Virus 40 in the Adenovirus 2–Simian Virus 40 Hybrid, $Ad2^{+}ND_{1}$

(DNA virus/strand separation/transcription/late and early RNA/initiator-terminator)

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ABSTRACT **The complementary DNA strands of the nondefective adenovirus 2 (Ad2)–simian virus 40 (SV40) hybrid virus, $Ad2^{+}ND_{1}$, were separated by isopycnic banding in a CsCl density gradient in the presence of synthetic polyribonucleotides. Separated strands were used in DNA–RNA hybridization reactions with RNA from cells productively infected by Ad2 or SV40, and with complementary SV40 RNA transcribed asymmetrically *in vitro*. About five times as much Ad2 RNA hybridized to the light stand of $Ad2^{+}ND_{1}$ as to the heavy strand. Complementary RNA and early SV40 RNA (RNA synthesized before viral DNA replication) had significant homology only with the light strand. Only half as much of a preparation of RNA synthesized before and after viral DNA replication (early-plus-late SV40 RNA) hybridized to the light strand as to the heavy strand. These results indicate that templates for both late and early SV40 RNA are present in $Ad2^{+}ND_{1}$. Therefore, the small SV40 segment within this virus (10–18% of the SV40 genome) must contain a transcription-control region. $Ad2^{+}ND_{1}$ should thus be useful in the selective study of transcription as it occurs in cells infected by the oncogenic virus SV40 .**

Separation of complementary strands of denatured viral DNA by hybridization with synthetic polyribonucleotides and isopycnic banding in CsCl density gradients was first accomplished by Hradecna and Szybalski (1). This method was used by these workers and others to demonstrate that the strands of bacteriophage λ DNA are transcribed sequentially during the course of lytic infection in *Escherichia coli* (2–4). Landgraf-Leurs and Green (5) used the polyribonucleotide-banding technique to separate the complementary DNA strands of the human virus, adenovirus 2 (Ad2). By means of DNA–RNA hybridization of virus-specific RNA synthesized during the early and late phases of lytic infection, it was demonstrated that the two strands of Ad2 DNA are transcribed differentially during these phases (6). Early RNA (RNA synthesized before viral DNA synthesis has begun) hybridizes almost equally with both strands, while early-plus-late RNA (RNA synthesized before and after viral DNA synthesis has begun) hybridizes primarily with the light strand.

Studies of transcription similar to those performed with bacteriophage λ and Ad2 would be of interest in the case of the small oncogenic DNA virus, simian virus 40 (SV40). In the SV40 lytic cycle, as with Ad2, only a portion of the viral genome is transcribed before replication of viral DNA begins. The entire genome is transcribed after viral DNA replication has begun, so that the RNA synthesized late in infection consists of both early and late sequences (early-plus-late RNA) (7–9). Although the strands of SV40 cannot be separated with synthetic polyribonucleotides (10), Westphal (11) demonstrated that superhelical SV40 DNA (Form I) is transcribed asymmetrically (from one strand) by *E. coli* RNA polymerase *in vitro*. This finding has recently been exploited by Khoury *et al.* (12, 13) and by Sambrook *et al.* (J. Sambrook, P. A Sharp, and W. Keller, submitted for publication) who separated the strands of SV40 by forming hybrids between asymmetrically transcribed, complementary SV40 RNA (SV40 cRNA-I) and fragments of denatured SV40 DNA. SV40 cRNA-I hybridized with only one strand (the "−" strand) which was separated from the unhybridized strand (the "+" strand) by hydroxyapatite chromatography. Using separated strands, Khoury *et al.* have shown that about one-third of the − strand of SV40 DNA is transcribed before viral DNA synthesis begins, while none of the + strand is transcribed during the early phase of infection. On the other hand, the RNA transcribed in the late phase of infection is homologous with one-third of the − strand and two-thirds of the + strand. Thus, the early DNA template occupies about one-third of the − strand and the late DNA template occupies about two-thirds of the + strand.

The nondefective Ad2–SV40 hybrid virus $Ad2^{+}ND_{1}$ (14) contains about 10–18% of the SV40 genome covalently linked to Ad2 DNA, as determined by reconstruction–hybridization experiments (10%; refs. 15 and 16) and by electron microscopic examination of Ad2–$Ad2^{+}ND_{1}$ heteroduplexes (18%; T. Kelly and A. M. Lewis, Jr., manuscript in preparation). During lytic infection by $Ad2^{+}ND_{1}$, one SV40 antigen is induced (the early "U" antigen; ref. 17) and only early SV40 DNA sequences are transcribed (ref. 8; J. Sambrook, P. A. Sharp, and W. Keller, submitted for publication). If we assume that there exist regions in SV40 DNA for initiation and termination of RNA transcription, as in bacteriophage λ (18, 19), it seemed probable to us that either an SV40 initiator or terminator region was present within $Ad2^{+}ND_{1}$.

In this report, we demonstrate that heavy and light strands of $Ad2^{+}ND_{1}$ DNA, and hence the complementary strands of the SV40 moiety, can, as anticipated, be separated by isopycnic banding in the presence of synthetic polyribonucleotides. Moreover, it is shown that early SV40 RNA hybridizes only with the light $Ad2^{+}ND_{1}$ strand, whereas early-plus-late SV40 RNA hybridizes with both strands. From these data, we are led to the conclusion that an SV40 transcription-control region is contained in $Ad2^{+}ND_{1}$.

MATERIALS AND METHODS

Tissue Culture. BSC-1 and Vero lines of African green-monkey kidney cells were maintained on Eagle's minimal

Abbreviations: cRNA, complementary RNA; $Ad2^{+}ND_{1}$, a nondefective adenovirus 2–simian virus 40 hybrid; SV, simian virus; PFU, plaque-forming units.

essential medium supplemented with penicillin (250 units/ml) and streptomycin (250 μg/ml) (Medium) plus 10% fetal-bovine serum. Human-embryonic kidney cells were maintained in Medium plus 2% fetal-bovine serum. All monkey-kidney cell lines were shown to be free of mycoplasma by anaerobic culture on Hayflick's medium.

Viruses. SV40 strain 777 has been maintained by low-multiplicity infection of BSC-1 cells (8). Ad2 (strain Ad6) and Ad2$^+$ND$_1$ were maintained in primary human-embryonic kidney cells. The derivation, propagation, and biological characterization of the Ad2$^+$ND$_1$ virus have been discussed (8, 14–17). All virus pools were demonstrated to be free of adeno-associated virus, types 1–4, by complement fixation, and of mycoplasma by anaerobic culture on Hayflick's medium.

Radiolabeling of Viral DNA and Virus Purification. Pools of Ad2 and Ad2$^+$ND$_1$ to be used for strand separation, were grown in cultures of Vero cells, in 1-liter bottles, by inoculation of confluent monolayers with 20–40 plaque-forming units (PFU) per cell. [*2*-^{14}C]Thymidine (20–30 Ci/mol; New England Nuclear Corp.) was added to 20 ml of Medium–2% fetal-bovine serum, 3–4 hr after infection, at 0.04 μCi/ml. Cells were scraped into the medium 48–50 hr after infection, pelleted at 500 × *g*, and suspended in 0.01 M Tris·HCl–0.85% NaCl (pH 7.8). The cell suspension was treated for 30 min at 37° with 1% sodium deoxycholate and 0.1% trypsin (20), and then centrifuged into a cushion of CsCl (density = 1.40 g/cm^3). Virus was banded twice in CsCl (density = 1.34 g/cm^3), and the bands were collected and dialyzed against 0.01 M Tris·HCl for 24 hr.

Separation of Ad2 and Ad2$^+$ND$_1$ DNA Strands. Single-stranded DNA was prepared from purified virus by the method of Szybalski *et al.* (21), with the following modifications: virus was first treated with Pronase (100 μg/ml, B grade, nuclease-free; Calbiochem) for 30 min at 37°, and poly(uridylic, guanylic)acid [poly(U,G); Shwartz–Mann] was used at a poly(U,G):DNA ratio of 2–3:1. After isopycnic banding, 0.1-ml fractions were collected from the bottom of the gradient, and aliquots were counted in Aquasol (New England Nuclear) in a Packard Tricarb scintillation spectrometer. Fractions containing the heavy or light DNA strands were separately pooled, and the DNA in each pool was self-annealed in 6 M CsCl for 3–5 hr at 65° (2). Heavy and light DNA pools were then diluted 25-fold in 0.9 M NaCl–0.09 M Na$_3$-citrate (pH 6.9) and collected by gravity on nitrocellulose filters as described (22). Each 13-mm filter contained about 0.1–0.2 μg of DNA.

Preparation of In Vivo Early and Early-plus-Late SV40 RNA and Early-plus-Late Ad2 RNA. Tritiated, early SV40-specific RNA was prepared by infection of confluent cultures of Vero cells, in 1-liter bottles, with 40–60 PFU of SV40 per cell in the presence of 20 μg of arabinosyl cytidine per ml (8). [*5*-^{3}H]Uridine (more than 20 Ci/mmol; New England Nuclear Corp.) was added to 20 ml of Medium–2% fetal-bovine serum 6 hr after infection at a final concentration of 100 μCi/ml, and the cells were harvested 30 hr after infection. Glass cover slips seeded with harvested cells and stained by an indirect fluorescent-antibody procedure (23) demonstrated that 70% of the cells were Tumor antigen-positive while less than 0.01% of the cells were Virion antigen-positive.

Tritiated, early-plus-late SV40-specific RNA was prepared in Vero cells infected with 40–60 PFU of SV40 per cell, and labeled 3 hr after infection with [*5*-^{3}H]uridine, [*2,8*-^{3}H]-adenosine, and [*5*-^{3}H]cytidine (more than 20 Ci/mmol, 20–30 Ci/mmol, and 15–30 Ci/mmol, respectively; New England Nuclear Corp.), each at a final concentration of 20 μCi/ml. Infected cells were harvested 50–60 hr after infection.

Labeled early-plus-late Ad2-specific RNA was prepared similarly to early-plus-late SV40-specific RNA, except that infected Vero cells were harvested 24 hr after infection. Uninfected Vero RNA was prepared from cells that had been cultured for 48 hr in the presence of the same isotopes used prepare early-plus-late RNAs from SV40 and Ad2.

Extraction of Nucleic Acids from Infected and Uninfected Cells. DNA from *E. coli* was extracted by the method of Marmur (24). RNA from infected and uninfected Vero cells was extracted by a hot phenol–sodium dodecyl sulfate procedure (22) and stored at −30° in 0.3 M NaCl–0.03 M Na$_3$-citrate plus 0.05% sodium dodecyl sulfate. The concentrations of DNA and RNA solutions were determined by a modified diphenylamine reaction (25) and an orcinol reaction (26), respectively. Radioactivity in viral DNA or virus-specific RNA was determined by precipitation with trichloroacetic acid on nitrocellulose filters.

Synthesis of SV40 cRNA-I. [^{3}H]RNA complementary to superhelical SV40 DNA (Form I) was synthesized *in vitro* by a modification (27) of the method of Chamberlin and Berg (28), with *E. coli* DNA-dependent RNA polymerase (containing sigma factor). The specific activity of the SV40 cRNA-I was 1.1 × 10^7 cpm/μg, estimated on the basis of equimolar uptake of isotopic precursors. The SV40 DNA used as a template in this reaction had been extracted from virus purified by a modification (8) of the method of Burnett *et al.* (29). The superhelical form of the viral DNA was obtained from an isopycnic ethidium bromide–cesium chloride gradient (30), and all of the DNA so obtained sedimented at 53 S as a single peak in an alkaline sucrose gradient.

RNA–DNA Hybridization. The procedure for hybridization of [^{3}H]RNA with single-stranded DNA immobilized on nitrocellulose membrane filters was that of Gillespie and Spiegelman (31) with slight modifications (22). Hybridization reactions were performed for 18 hr at 60° with 13-mm filters in a volume of 0.25 ml of 0.3 M NaCl–0.03 M Na$_3$-citrate plus 0.05% sodium dodecyl sulfate. Each hybridization vial contained one filter with viral DNA and one filter with 0.1 μg of *E. coli* DNA.

All RNA–DNA hybrids were washed extensively with 0.3 M NaCl–0.03 M Na$_3$-citrate at 60°, treated with pancreatic ribonuclease (20 μg/ml; Type XII-A, Sigma Chemical Co.) and ribonuclease T$_1$ (10 units/ml; B grade, Calbiochem) for 1 hr at room temperature (24°), washed again, dried, and counted in Liquifluor (New England Nuclear Corp.) in a scintillation spectrometer. All samples were counted for sufficient time to achieve a counting accuracy of at least ±4% in all channels; ^{3}H and ^{14}C counts were corrected for spill-over, and ^{3}H cpm bound to *E. coli* DNA (blank) were subtracted from ^{3}H cpm bound to viral DNA.

RESULTS

Separation of Ad2$^+$ND$_1$ DNA Strands. The distribution of single-stranded Ad2$^+$ND$_1$ DNA-poly(uridylic, guanylic) acid complexes in a CsCl gradient at equilibrium is shown in Fig. 1*A*. The difference in density between the two DNA peaks

is 10–12 mg/ml. When fractions containing the two DNA peaks were separately pooled, self-annealed, and rebanded in the absence of poly(U,G), two peaks were observed, one with the density of native DNA and one with the density of denatured DNA. However, when the two peaks were combined, reannealed, and rebanded in the absence of poly(U,G), a single peak was observed at the density of native DNA (experiments not shown). These results indicate that the complementary strands of Ad2$^+$ND$_1$ DNA were separated.

When fractions containing the separated strands were pooled and rebanded in the presence of poly(U,G), they exhibited single peaks at equilibrium that again differed in density by 10–12 mg/ml (Fig. 1*B* and *C*). A higher ratio of poly(U,G) to DNA was used in the rebanding experiment than in the initial banding experiment; consequently, the densities observed for the separated strand–poly(U,G) complexes are greater than in the initial experiment. Examination of Fig. 1*B* and *C* suggests that the heavy-strand peak is skewed toward the light-strand peak, and vice versa. Therefore, fractions containing the heavy or light strands were separately pooled and self-annealed for 3–5 hr. The self-annealed strands were then collected on nitrocellulose membrane filters. This self-annealing procedure should permit any contaminating minority strands to form double-stranded duplexes with an equivalently small amount of majority strands, leaving the excess majority strands available for RNA–DNA hybridization. The small amount of double-stranded DNA that may be formed should not be available for hybridization with RNA, since the hybridization conditions favor DNA–DNA hybrids (1). However, some double-stranded DNA might be retained by the filters, presumably bound to nitrocellulose by single-stranded tails. The efficacy of the 3–5 hr self-annealing step will become apparent as the RNA–DNA hybridization results are discussed; it should be noted here that Ad2-specific RNA eluted from filters with heavy or light Ad2$^+$ND$_1$ DNA could be hybridized again only with the homologous strands.

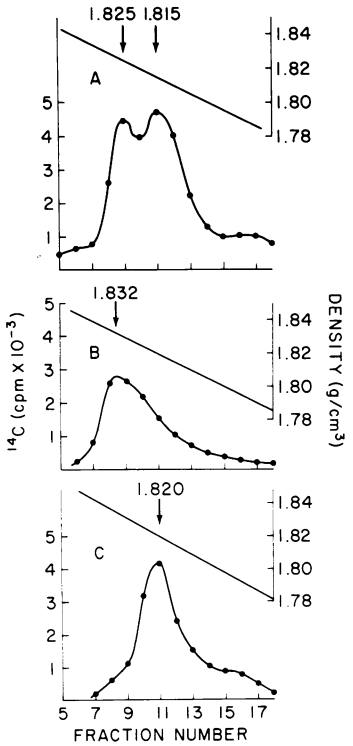

FIG. 1. Isopycnic banding of single-stranded Ad2$^+$ND$_1$ DNA in the presence of poly(U,G). (*A*) Initial strand separation. 20 μg of viral DNA was denatured in the presence of 50 μg of poly(U,G) as described in *Methods*. Separation was by centrifugation in Cs_2Cl (initial density 1.76 g/cm^3) in an SW39 rotor at 32,500 rpm for 65 hr at 8°. (*B*) Rebanding of the heavy strand (fractions 8 and 9 from the gradient shown in *A*). The gradient contained 7.4 μg of viral DNA and 50 μg of poly(U,G), and banding conditions were as stated for *A*. (*C*) Rebanding of the light strand (fractions 11–13 from the gradient shown in *A*). The gradient contained 7.2 μg of viral DNA and 50 μg of poly(U,G), with banding conditions as stated for *A*.

Hybridization of Heavy and Light Ad2$^+$ND$_1$ DNA Strands with Various RNAs. Nitrocellulose filters impregnated with either heavy or light Ad2$^+$ND$_1$ DNA strands were challenged with saturating amounts of early-plus-late RNA prepared from Vero cells infected with Ad2 or SV40, or with a saturating amount of SV40 cRNA-I. In addition, strands were challenged with nonsaturating amounts of early SV40 RNA. The results of these hybridization experiments are presented in Table 1. The data from a typical experiment are shown, as well as the observed homology distribution (the percentage of the total hybridizable RNA that binds to each strand), obtained by averaging the results from several experiments. Heavy and light strands of Ad2 DNA and Ad2$^+$ND$_1$ DNA were challenged with virus-specific RNA synthesized in Vero cells throughout the period of Ad2 infection. The homology distribution of early-plus-late Ad2 RNA between the heavy and light strands of Ad2 DNA is essentially the same as that between the heavy and light strands of Ad2$^+$ND$_1$ DNA (Table 1*A*), and is in good agreement with that reported elsewhere (6). When filters containing unseparated, denatured Ad2 DNA were challenged with early-plus-late Ad2 RNA, the counts bound (^{3}H/μg of DNA) were equal to the average of the counts bound to the heavy and light strands. [^{3}H]RNA prepared from uninfected Vero cells did not hybridize with either Ad2 or Ad2$^+$ND$_1$ DNA, indicating that our results were not perturbed by the presence of host-cell DNA sequences in the viral DNA strands.

The homology distribution of SV40 cRNA-I between heavy and light strands of Ad2$^+$ND$_1$ is shown in Table 1*B*. Only the light strand has significant homology with this RNA, and the very small amount of hybridization exhibited by the heavy strand most likely arises from a small amount of symmetrical transcription during *in vitro* synthesis of the cRNA (9). This result confirms that the two DNA strands are well separated and, in addition, serves to identify the SV40 moiety present in the light strand of Ad2$^+$ND$_1$ as a portion of the − strand of SV40 DNA (early RNA template) (13). This interpretation depends upon the assumption that the SV40 cRNA-I used in these experiments contained detectable sequences complementary to the − strand of SV40 DNA (or at least to all of that portion present in Ad2$^+$ND$_1$) and no sequences complementary to the + strand. That early SV40 information is contained only in the light strand is further supported by the results of an experiment in which the heavy and light strands of Ad2$^+$ND$_1$ were challenged with early SV40 RNA (Table 1*C*). As anticipated, early SV40 RNA is only homologous to the light strand of Ad2$^+$ND$_1$. The relatively low number of ^{3}H counts bound to DNA is explained by the low concentration of virus-specific RNA in cells treated with arabinosyl cy-

TABLE 1. *Distribution of homology on separated DNA strands of Ad2 and Ad2+ND$_1$ when challenged with various RNAs*

All experiments*			Typical single experiment				
DNA strand	Homology distribution %†	No. of experiments	μg DNA/Filter‡	RNA challenge, cpm × 10^{-6}	Net ^{3}H cpm/filter§	^{3}H/μg specific DNA, cpm × 10^{-3}¶	Homology distribution %†
			(*A*) Early + late Ad2 *in vivo* RNA‖				
Ad2-heavy	12.2 ± 2.9**	4	0.023	11.9	322	13.8	15.4
			0.021	11.9	338	16.1	
Ad2-light	87.8 ± 2.9	4	0.040	11.9	3630	90.4	84.6
			0.032	11.9	2343	73.4	
Ad2+ND$_1$-heavy	15.8 ± 3.5	2	0.106	18.4	1831	17.3	13.4
			0.102	18.4	1562	15.3	
Ad2+ND$_1$-light	84.2 ± 3.5	2	0.148	18.4	15,191	102.5	86.6
			0.183	18.4	19,962	109.2	
			(*B*) SV40 cRNA-I‖				
Ad2+ND$_1$-heavy	2.8 ± 0.6	3	0.129 (0.0030)	0.34	210	70.6	2.3
			0.139 (0.0032)	0.34	338	106	
Ad2+ND$_1$-light	97.2 ± 0.6	3	0.204 (0.0047)	0.34	18,191	3879	97.7
			0.199 (0.0046)	0.34	17,207	3757	
			(*C*) Early SV40 *in vivo* RNA‖				
Ad2+ND$_1$-heavy	0 0	2	0.093 (0.0021)	55.0	−10.1	—††	0
			0.097 (0.0022)	55.0	−8.8	—	
Ad2+ND$_1$-light	100 0	2	0.190 (0.0044)	55.0	41.8	—	100
			0.202 (0.0046)	55.0	62.5	—	
			(*D*) Early + late SV40 *in vivo* RNA‖				
Ad2+ND$_1$-heavy	63.8 ± 5.3	5	0.090 (0.0021)	24.4	112	53.1	62.4
			0.135 (0.0031)	24.4	175	57.2	
Ad2+ND$_1$-light	36.2 ± 5.3	5	0.205 (0.0047)	24.4	146	31.4	37.6
			0.160 (0.0037)	24.4	130	35.2	
Ad2-heavy	0 0	1	0.030	24.4	−5.4	—	0
			0.025	24.4	4.3	—	
Ad2-light	0 0	1	0.040	24.4	−1.2	—	0
			0.035	24.4	0	—	

* The left side of the table presents the results of all experiments with DNA strands from Ad2 and Ad2+ND$_1$ viruses. Each hybridization experiment was done with duplicate filters with viral and *E. coli* DNA as described in *Methods*. The right side of the table presents the results of a single typical experiment.

† The percentage of the total hybridizable RNA that binds to each strand.

‡ This column shows the total amount of DNA on each filter. The numbers in parentheses show the amount of SV40 DNA on the filter, assuming that 2.3% of the Ad2+ND$_1$ genome is SV40 (T. Kelly and A. M. Lewis, Jr., in preparation). Amounts of total DNA per filter were calculated from these specific activities: Ad2, 2420 cpm/μg; Ad2+ND$_1$, 2250 cpm/μg.

§ ^{3}H cpm have been corrected for spill-over of ^{14}C, and ^{3}H cpm bound to *E. coli* DNA (blank) have been subtracted from ^{3}H cpm bound to viral DNA. Average ^{3}H cpm on *E. coli* blanks: (*A*) Early-plus-late Ad2 RNA, 87,160; (*B*) SV40 cRNA-I, 200; (*C*) Early SV40 RNA, 55; (*D*) Early-plus-late SV40 RNA, 114.

¶ cpm of [^{3}H]RNA/μg of that DNA for which it is specific. The calculation is only presented for those RNAs used at saturation.

‖ Specific activities of the challenging RNAs are: Early-plus-late Ad2, 1.33×10^5 cpm/μg; SV40 cRNA-I, 1.1×10^7 cpm/μg; Early SV40, 2.72×10^5 cpm/μg; Early-plus-late SV40, 0.93×10^5 cpm/μg. The specific activities stated are for whole-cell RNA, of which the virus-specific moiety is a fractional component.

** Standard deviation.

†† —, not applicable.

tidine (8). However, the difference between ^{3}H cpm bound to heavy and light strands is statistically significant ($P < 0.01$), and SV40-specific RNA is not homologous to nonhybrid Ad2 DNA strands (Table 1*D*).

The distribution of homology between the heavy and light strands of Ad2+ND$_1$ DNA when challenged with early-plus-late RNA prepared from Vero cells infected with SV40 is presented in Table 1*D*. The data indicate that the heavy strand of Ad2+ND$_1$, whose SV40 component should be identical with a region of the + strand of SV40, is substantially more homologous with SV40 early-plus-late RNA than is the light strand. This difference may be exaggerated by a higher specific activity of pure late RNA with respect to early RNA, arising from differences in base composition and labeling conditions (7). However, it is probable that at least half of the SV40 moiety in Ad2+ND$_1$ contains genetic information that is expressed late in SV40 lytic infection.

DISCUSSION

The results of these experiments indicate that the SV40 moiety in Ad2+ND$_1$ contains a template for late RNA, as well as for early RNA, although the late DNA sequences are known not to be detectably expressed in cells infected with Ad2+ND$_1$ virus (8). The late SV40 homology is on the heavy strand of Ad2+ND$_1$, which is the strand that is homologous to a significant amount of early Ad2 RNA sequences (6). The early SV40 homology is on the light strand of Ad2+ND$_1$, which is the strand that is homologous to 85% of late Ad2 RNA (early-plus-late sequences) (6). Finally, the presence of both early and late information in the SV40 moiety of Ad2+ND$_1$ leads us to the conclusion that this piece of DNA contains an SV40 transcription-control region, which may include either an initiator(s) or a terminator(s).

Recent evidence from three laboratories strongly suggests that 30–40% of one SV40 strand is the template for early RNA, while 60–70% of the opposite strand is the template for late RNA (refs. 9, 12, and 13; J. Sambrook, P. A. Sharp, and W. Keller, submitted for publication). A recombination or other discontinuity within the SV40 DNA segment of Ad2+ND$_1$, yielding late sequences in the light strand, might exist. However, electron microscopic examination of heteroduplexes formed between Ad2+ND$_1$ DNA and SV40 DNA has ruled out this possibility (32). With these studies and the results of the present experiments in mind, the possible configuration of the SV40 segment within Ad2+ND$_1$ is presented in Fig. 2. Fig. 2*A* illustrates the early region of SV40 from which the fragment in Ad2+ND$_1$ originated. It is evident that, since the early region of SV40 is much larger (30–40% of the SV40 genome)

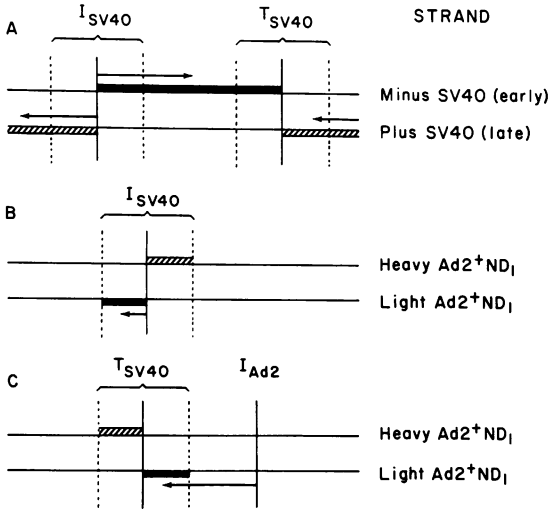

FIG. 2. Schematic representation of the early portion of SV40 DNA and insertion of a fragment of this DNA into Ad2 DNA to form the Ad2+ND1 molecule. (*A*) Early portion of the SV40 genome. The *heavy horizontal line* (—) represents the early RNA template. The *hatched horizontal line* (▨) represents the late RNA template. *Arrows* indicate the direction of transcription. *Solid vertical lines* indicate points of RNA synthesis initiation (I_{SV40}), or termination (T_{SV40}). *Broken vertical lines* demark the two possible fragments of the parental DNA that give rise to the SV40 moiety in Ad2+ND1. (*B*) Configuration of the Ad2+ND1 molecule if the initiator region (I_{SV40}) of SV40 DNA is inserted into Ad2 DNA. (*C*) Configuration of the Ad2+ND1 molecule if the terminator region (T_{SV40}) is inserted into Ad2 DNA. I_{AD2} represents an Ad2 initiator site.

than the fragment present in Ad2+ND1 (10–18% of the SV40 genome), it is unlikely that both the initiator and terminator of early SV40 RNA transcription are present in Ad2+ND1.

Fig. 2*B* shows the configuration of the Ad2+ND1 molecule if the SV40 initiator region is inserted into Ad2 DNA. The fragment is inverted in the illustration to maintain transcription polarity and to locate the early SV40 homology on the light strand of Ad2+ND1, where it was found in the present studies. In this configuration, the SV40 segment would contain its own initiator for early SV40 RNA synthesis. It might be presumed that the initiator of early SV40 RNA synthesis lies very close to the initiator of late RNA synthesis, but since Ad2+ND1 does not induce SV40 late RNA despite the presence of late DNA, other mechanisms must be considered. It is possible that an early SV40 protein, which is not made by Ad2+ND1, is required for late RNA synthesis, even though a template complete with late initiator is available.

Fig. 2*C* shows the configuration of the Ad2+ND1 molecule if the SV40 terminator region is inserted into Ad2 DNA. In this case, it must be assumed that SV40 expression is under the control of an Ad2 initiator. However, recent evidence indicates that Ad2+ND1 contains a strong initiator for *in vitro* SV40 cRNA synthesis (B. S. Zain, R. Dahr, S. M. Weissman, P. Lebowitz, and A. M. Lewis, Jr., submitted for publication), a finding that offers support to the model shown in Fig. 2*B*.

We believe that the experimental results reported here provide strong evidence that the small SV40 moiety of Ad2+ND1 contains a transcription-control region. Moreover, the failure of the template for late SV40 RNA to be expressed in Ad2+-ND1 virus lytic infection might be analogous to the failure of complete RNA transcription observed in most SV40-transformed cells (33). The nondefective hybrid virus Ad2+ND1 should thus provide a useful tool in the study of the structure and function of transcription regulator sites in the genome of the oncogenic virus SV40.

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