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Use of Nondefective Adenovirus-Simian Virus 40 Hybrids for Mapping the Simian Virus 40 Genome

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A series of viable recombinants between adenovirus 2 (Ad2) and simian virus 40 (SV40) (nondefective Ad2-SV40 hybrids) have been isolated. The members of this series (designated $Ad2^+ND_1$ through $Ad2^+ND_5$) differ from one another in the early SV40-specific antigens and the SV40-specific RNA species which they induce in infected cells. They also contain different amounts of SV40 DNA as shown by RNA-DNA hybridization techniques. We have examined the structure of the DNA molecules from these hybrids, using electron microscope heteroduplex mapping techniques. Each hybrid was found to contain a single segment of SV40 DNA of characteristic size covalently inserted at a unique location in the adenovirus 2 DNA molecule. The SV40 segments of the various hybrids formed an overlapping series with a common end point. When the results of the electron microscopic study were combined with data on antigen induction, it was found that a self-consistent map could be constructed which related specific regions of the SV40 genome to the induction of specific antigens. The order of these early SV40 antigen inducing regions in the SV40 DNA segments contained in the nondefective hybrids is: U antigen, tumor specific transplantation antigen, and T antigen with the U antigen region being nearest the common end point.

Adenovirus (Ad)-simian virus 40 (SV40) hybrids are virus particles which contain both SV40 and adenovirus genetic information in the same adenovirus capsid. The genomes of all Ad-SV40 hybrids which have been tested thus far have been shown to contain part or all of the SV40 DNA molecule covalently inserted into the adenovirus DNA molecule (1, 3, 4, 7, 10, 18). The first Ad-SV40 hybrids which were isolated were defective; i.e., they required coinfection with nonhybrid adenovirus for their propagation (2, 13, 15, 20). Recently, however, a series of five nondefective Ad2-SV40 hybrids have been isolated and plaque purified (11, 12). The members of this series (designated $Ad2^+ND_1$ through $Ad2^+ND_5$) are genetically stable, capable of independent replication without helper virus, and differ from one another in the early SV40-specific antigens which they induce in infected cells (Table 1). It seemed likely that the induction of different SV40 functions by the various nondefective Ad2-SV40 hybrids was a consequence of the incorporation into their genomes of different portions of the SV40 genome. This was confirmed by Henry et al. (6), who showed by RNA-DNA hybridization techniques that each nondefective Ad2-SV40 hybrid contained a different amount of SV40 DNA and by Levine et al. (9), who showed that each hybrid induced qualitatively different (but overlapping) species of SV40-specific RNA in infected cells (Table 1).

We studied the structure of the DNA molecules from the nondefective Ad2-SV40 hybrids, using electron microscope heteroduplex techniques. We found that each hybrid contains a single segment of SV40 DNA inserted at a unique location within the Ad2 DNA molecule. The sizes of the SV40 segments ranged from 7 to 43% of the SV40 genome. The SV40 segments of the various hybrids were mapped relative to one another, and the results were compared with the biological data on antigen induction. It was found that a self-consistent map could be constructed which related specific regions of the SV40 genome to the induction of specific antigens.

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TABLE 1. *Properties of the nondefective Ad2-SV40 hybrid viruses*

Virus	Host Range	SV40 antigen induction				Relative amount of SV40 DNA within hybrid genome as determined by hybridization experiments[a]	Extent of competition between hybrid virus RNA and early SV40-specific ^{3}H RNA induced by Ad2$^+$ND$_4$[a]
		T	U	TSTA	V		
Ad2$^+$ND$_1$	HEK[b]	–	+	–	–	0.29	30
	AGMK						
Ad2$^+$ND$_2$	HEK	–	+	+	–	0.75	72
	AGMK						
Ad2$^+$ND$_3$	HEK	–	–	–	–	0.06	8
Ad2$^+$ND$_4$	HEK	+	+	+	–	1.00	100
	AGMK						
Ad2$^+$ND$_5$	HEK	–	–	–	–	0.63	58

[a] Data from Levine et al. (9).
[b] HEK, human embryonic kidney cells.

MATERIALS AND METHODS

Viruses, virus purification, and viral DNA extraction. The isolation and properties of the five nondefective Ad2-SV40 hybrids have been described (Table 1; references 6, 9, 11, 12, 14, 17). Pools of these agents used for our experiments contained $10^{8.3}$ to $10^{8.6}$ PFU per ml. The virions in each of these pools represented the progeny of the third tissue culture passage after clonal isolation (11). To prepare hybrid virions for DNA extraction 32-oz bottle cultures of human embryonic kidney cells were infected with 10 to 40 PFU per cell of third passage virus. The virions were purified, and DNA was extracted as previously described (6). The pool (titer $10^{9.6}$ PFU/ml) of nonhybrid Ad2 (strain Ad2$^-$) used in these experiments represents the third human embryonic kidney passage of a plaque isolated at high dilution ($>10^{-6}$) from a titration of the Ad2^{++} pool 2 (13, 14) in human embryonic kidney cells. This strain of nonhybrid Ad2 failed to pass serially in African green monkey kidney (AGMK) cells and did not induce detectable SV40 antigens or SV40 RNA during lytic infection (A. M. Lewis, Jr. and A. S. Levine, unpublished observations). In addition, DNA extracted from Ad2$^-$ virions failed to react with SV40 cRNA. Ad2 cRNA, prepared by using Ad2$^-$ DNA as a template, failed to bind to SV40 DNA (A. S. Levine and A. M. Lewis, Jr., unpublished observations). Thus, the Ad2$^-$ strain appears to be a representative of the nonhybrid Ad2 component of the Ad2^{++} population (13, 14) rather than an unrecognized type of Ad2-SV40 hybrid which contains SV40 DNA but induces neither SV40 antigen nor SV40 RNA.

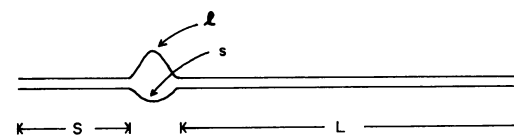
To prepare viral DNA, Ad2$^-$ virions were grown in Vero cells (a continuous line of AGMK cells which is permissive for Ad2, 12, 22) and purified by banding in cesium chloride as described for the purification of the nondefective hybrids.

Heteroduplex formation and electron microscopy. Heteroduplexes were prepared by the method of Davis, Simon, and Davidson (5). A mixture of two DNAs (each at a concentration of 5 μg/ml) was adjusted to 0.1 N NaOH and incubated at room temperature for 10 min. The resulting solution was neutralized with one-tenth volume of 1.8 M tris(hydroxymethyl)aminomethane (Tris)-hydrochloride, 0.2 M Tris-OH. Formamide (Matheson, Coleman, and Bell) was added to a final concentration of 48%, and the DNA was allowed to renature at room temperature for 1 h. The renatured product was dialyzed against 0.01 M Tris, and 0.001 M EDTA (pH 8.6). As a control, homoduplexes of Ad2 DNA and each of the nondefective Ad2-SV40 hybrids DNAs were prepared by this denaturation and renaturation procedure. Homoduplexes of Ad2, Ad2$^+$ND$_1$, Ad2$^+$ND$_2$, Ad2$^+$ND$_3$, and Ad2$^+$ND$_5$ did not contain

TABLE 2. *Heteroduplexes between Ad2 and the nondefective Ad2-SV40 hybrids*[a]

Heteroduplex	S/(S + L)[a]	s (SV40 units)[b]	l (SV40 units)[b]
Ad2/Ad2$^+$ND$_1$	0.148 (0.006)[c]	0.178 (0.019)	0.376 (0.027)
Ad2/Ad2$^+$ND$_2$	0.151 (0.007)	0.324 (0.040)	0.425 (0.045)
Ad2/Ad2$^+$ND$_3$	0.146 (0.008)	0.065 (0.013)	0.369 (0.029)
Ad2/Ad2$^+$ND$_4$	0.148 (0.007)	0.310 (0.038)	0.434 (0.043)
Ad2/Ad2$^+$ND$_5$	0.149 (0.008)	0.278 (0.045)	0.493 (0.042)

[a] Each Ad2/hybrid heteroduplex contained a single substitution loop. The following diagrammatic representation shows the contour lengths which were measured:



S and L are the lengths of the short and long duplex segments, respectively. s and l are the lengths of the short and long single-stranded segments, respectively.

[b] Single-stranded SV40 rings were used as internal length standards; the length of one complete SV40 genome = 1 SV40 unit.

[c] The numbers in parentheses equal one standard deviation. The numbers of heteroduplexes measured were 35 Ad/Ad2$^+$ND$_1$, 38 Ad2/Ad2$^+$ND$_2$, 37 Ad2/Ad2$^+$ND$_3$, 28 Ad2/Ad2$^+$ND$_4$, and 30 Ad2/Ad2$^+$ND$_5$.

single stranded loops. A fraction (about 40%) of the Ad2$^+$ND$_4$ homoduplexes contained a small deletion loop with a length equal to about 1% of the Ad2 genome. These molecules have been described by J. Morrow et al. (15a).

DNA samples were mounted for electron microscopy by the formamide method of Davis, Simon, and Davidson (5). Micrographs were taken on a Siemens Elmiskop IA graciously provided by Albert Kapikian. Contour lengths of traced molecules were measured with a Keuffel and Esser 620300 map measurer. Single-stranded circular SV40 DNA molecules mounted on the same grid as the sample served as an internal length standard.

RESULTS

Structure of Ad2/hybrid heteroduplexes. The genomes of the five nondefective Ad2-SV40 hybrids have been shown to contain a fraction of the SV40 genome covalently linked to the Ad2 DNA molecule (6). To determine the size and location of the SV40 DNA segment(s) we prepared heteroduplexes between the DNA of each of the hybrids and Ad2 DNA. The DNA of a given hybrid was mixed with an equal amount of Ad2 DNA and then denatured and renatured. Electron microscopy revealed that about half of the renatured molecules in each of the five preparations contained a small substitution loop. Examples are shown in Fig. 1. Length measurements were made on these Ad2/hybrid heteroduplexes by using single-stranded SV40 rings as internal length standards. The results (Table 2) showed: (i) that heteroduplexes prepared from a particular hybrid DNA formed a homogeneous population, (ii) that the lengths of the two unpaired strands of the substitution loop were different for heteroduplexes prepared from different hybrids, and (iii) that the location of the substitution loop relative to the ends of the molecule was roughly the same for heteroduplexes prepared from all five hybrids.

Models for the genomes of the nondefective Ad2-SV40 hybrids. Two possible models for the genome of each nondefective Ad2-SV40 hybrid can be constructed, depending on which of the two unpaired strands of the corresponding heteroduplex is assigned to Ad2 and which to the hybrid. The unpaired strand assigned to Ad2 represents a segment of Ad2 DNA deleted from the hybrid genome. The other unpaired strand represents a segment of DNA in the hybrid which is heterologous to Ad2 DNA and which, by inference, contains the inserted SV40 DNA. By using the data of Table 2, the two possible models for each hybrid genome were computed. The results of this computation are given in Table 3.

Determination of the correct model. To determine which of the two possible strand assignments was correct, a number of hybrid/hybrid heteroduplexes were prepared and examined in the electron microscope. Of particular interest were the heteroduplexes between Ad2$^+$ND$_4$ and each of the other hybrids, i.e., Ad2$^+$ND$_4$/Ad2$^+$ND$_1$, Ad2$^+$ND$_4$/Ad2$^+$ND$_2$, Ad2$^+$ND$_4$/Ad2$^+$ND$_3$, and Ad2$^+$ND$_4$/Ad2$^+$ND$_5$. Unlike the Ad2/hybrid heteroduplexes which contained substitution loops, each of these Ad2$^+$ND$_4$/hybrid heteroduplexes contained a single deletion loop (Fig. 2). As illustrated in Fig. 3 this observation has two important implications: (i) the SV40 segment of one of the two constituents of each Ad2$^+$ND$_4$/hybrid heteroduplex must be completely homologous to the SV40 segment of the other, and (ii) the two SV40 segments must have a common end point and this end point must be located at the same position within the Ad2 genome. These statements are probably accurate to within about 50 to 100 nucleotide pairs, the estimated resolution limit for the electron microscopic method (5). Two types of structures for the Ad2$^+$ND$_4$/hybrid heteroduplexes are possible. The common end point of the SV40 segments can be adjacent to the short Ad2 duplex segment (Fig. 3A) or adjacent to the longer Ad2 duplex segment (Fig. 3B). For type A structures S/(S + L) will be greater for the Ad2$^+$ND$_4$/hybrid heteroduplexes than for the corresponding Ad2/hybrid heteroduplexes due to the presence of homology between the SV40 segments of the two hybrids. The opposite will be the case for type B structures. We have shown in Table 2 that S/(S + L) is 0.151 or less for the Ad2/hybrid heteroduplexes. The data in Table 4 indicate that S/(S + L) is greater than this value for all four Ad2$^+$ND$_4$/hybrid heteroduplexes. It follows that these heteroduplexes are type A structures and that the SV40 segments of all of the nondefective Ad2-SV40 hybrids share a common end point which is adjacent to the short Ad2 duplex segment.

Given the foregoing interpretation for the structure of the various Ad2$^+$ND$_4$/hybrid heteroduplexes it becomes a relatively simple task to determine the correct model for each hybrid genome. The first step is to use the data in Table 3 to calculate 4 possible structures for each Ad2$^+$ND$_4$/hybrid heteroduplex. This procedure is illustrated in Fig. 4 for the Ad2$^+$ND$_4$/Ad2$^+$ND$_1$ heteroduplex. There are two possible models for the genome of Ad2$^+$ND$_4$ and also two possible models for the genome of Ad2$^+$ND$_1$. It follows that there are four ways of combining strands from these models to produce an Ad2$^+$ND$_4$/Ad2$^+$ND$_1$ heteroduplex. The dimen-

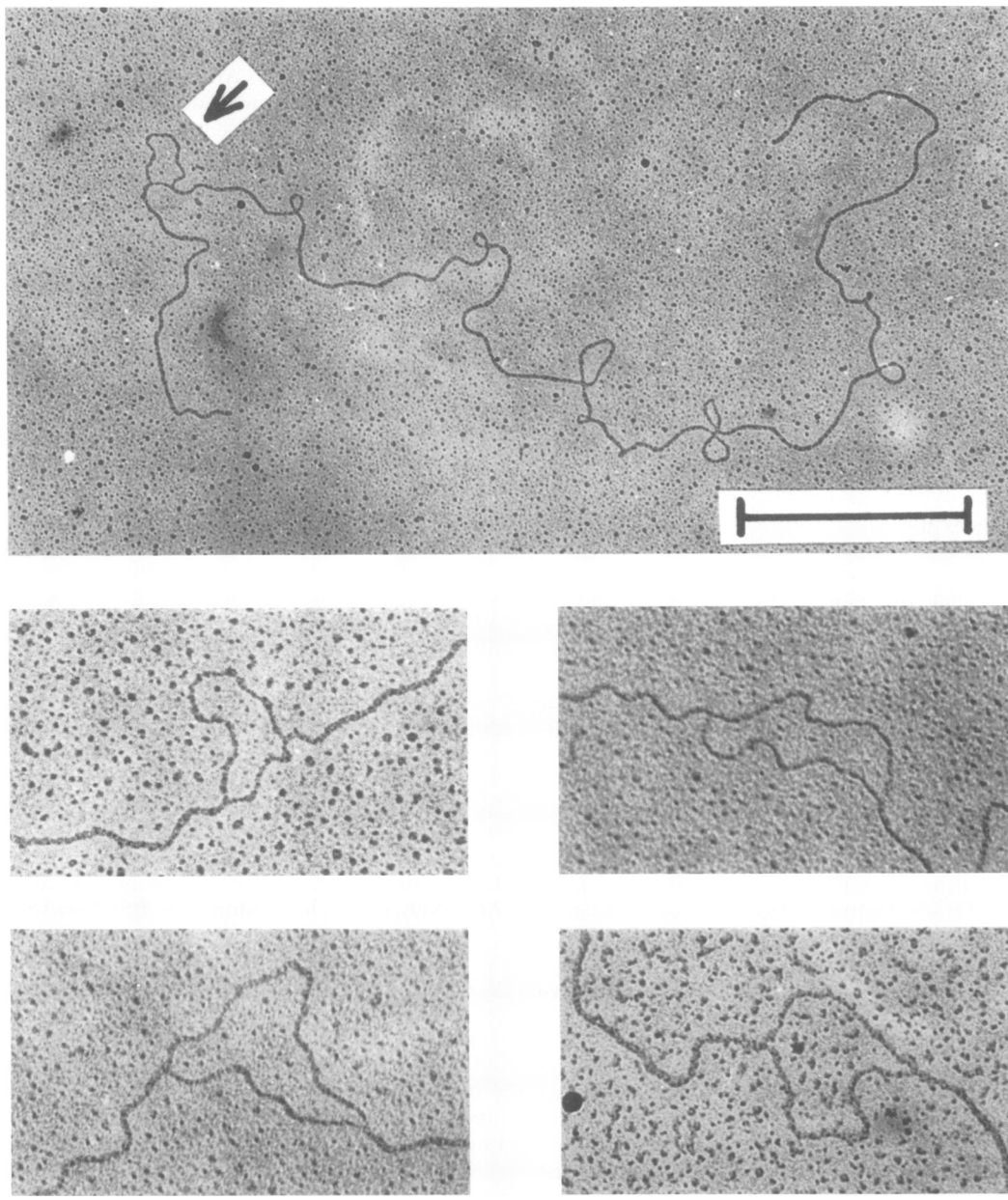
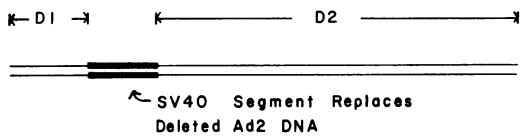


FIG. 1. *Ad2/hybrid heteroduplexes. Top: Ad2/Ad2$^+$ND$_3$ heteroduplex. Arrow indicates the position of the substitution loop. The length of the bar corresponds to 1 μm. Bottom (clockwise from top left): substitution loops of Ad2/Ad2$^+$ND$_1$, Ad2/Ad2$^+$ND$_2$, Ad2/Ad2$^+$ND$_4$, and Ad2/Ad2$^+$ND$_5$. These loops are located at approximately the same position as that of the Ad2/Ad2$^+$ND$_3$ heteroduplex (Table 2). Magnification is twice that of the micrograph at top.*

TABLE 3. *Models for the genomes of the nondefective Ad2-SV40 hybrids*[a]

Hybrid	Model	D1 (Ad units)[a]	D2 (Ad units)	SV40 (Ad units)[b]	Ad2 deletion (Ad units)
$Ad2^+ND_1$	1[c]	0.140	0.806	0.026 (0.18)	0.054
	2	0.144	0.830	0.054 (0.38)	0.026
$Ad2^+ND_2$	1[c]	0.142	0.797	0.047 (0.32)	0.061
	2	0.144	0.809	0.061 (0.43)	0.047
$Ad2^+ND_3$	1[c]	0.138	0.808	0.009 (0.07)	0.053
	2	0.145	0.846	0.053 (0.37)	0.009
$Ad2^+ND_4$	1	0.139	0.799	0.045 (0.31)	0.063
	2[c]	0.141	0.814	0.063 (0.43)	0.045
$Ad2^+ND_5$	1[c]	0.138	0.790	0.040 (0.28)	0.071
	2	0.143	0.817	0.071 (0.49)	0.040

[a] The following diagrammatic representation of an Ad2 SV40 hybrid shows the lengths which were computed for each model:



The dimensions of the two possible models for each hybrid were computed from the data of Table 2 in the following way: model 1: s = length of SV40 in the hybrid; l = length of Ad2 DNA deleted from the hybrid; D1 = (1 − l)[S/(S + L)]; D2 = (1 − l) [1 − (S/{S + L})], where l, s, S, and L are defined in the legend to Table 2. Model 2: l = length of SV40 in hybrid; s = length of Ad2 DNA deleted from the hybrid; D1 = (1 − s) [S/(S + L)]; D2 = (1 − s) [1 − (S/{S + L})]. In these formulae all lengths are expressed in Ad units. One Ad unit = length of one complete Ad2 genome. Conversion of the data in Table 2 to Ad units was made by using the relation: one Ad unit = 6.9 SV40 units. This relation was obtained from length measurements on a mixture of Ad2 and SV40 DNA molecules.

[b] Numbers in parentheses are the lengths of the SV40 segments expressed in SV40 units.

[c] This marks the correct model as determined by the methods described in the text.

sions of each of the four possible heteroduplexes can be calculated from the data in Table 3. Two of the calculated structures contain substitution loops and can, therefore, be eliminated (Fig. 4). The remaining two structures contain deletion loops, but they can be distinguished because the loops are located at different positions (S/(S + L) is 0.171 for structure b and 0.190 for structure c. As indicated in Fig. 4 and Table 4 the observed S/(S + L) for the $Ad2^+ND_4/Ad2^+ND_1$ heteroduplex was 0.167 indicating that structure b is the correct representation of the molecule. It immediately follows that model 2 is correct for the genome of $Ad2^+ND_4$ and model 1 is correct for the genome of $Ad2^+ND_1$, since these were the two models used to generate structure b (Table 3).

Table 5 shows a similar analysis for the remaining $Ad2^+ND_4$/hybrid heteroduplexes and two other hybrid/hybrid heteroduplexes. For each heteroduplex, the calculated dimensions of the four possible structures are given together with the dimensions actually observed. In most cases the observed dimensions are close to only one of the four possible structures. However, in two cases ($Ad2^+ND_4/Ad2^+ND_5$ and $Ad2^+ND_4/Ad2^+ND_2$) the observed dimensions are consistent with two of the calculated structures. In these cases, knowledge that model 2 is correct for the genome of $Ad2^+ND_4$ resolves the ambiguity. The data of Table 5 are mutually consistent and indicate that Model 1 is correct for $Ad2^+ND_1$, $Ad2^+ND_2$, $Ad2^+ND_3$, and $Ad2^+ND_5$ and that Model 2 is correct for $Ad2^+ND_4$.

Thus, $Ad2^+ND_4$ contains the largest SV40 genome segment. The SV40 segments of the other hybrids are completely contained within that of $Ad2^+ND_4$ and, as indicated above, share a common end point (Fig. 5). The common end point of the SV40 segments is fixed within the Ad2 genome at a point about 0.140 fractional Ad2 lengths from one end, so that the Ad2 deletions also share a common end point (Fig. 5).

DISCUSSION

It has been previously shown by RNA-DNA hybridization techniques that the genomes of the nondefective Ad2-SV40 hybrids contain different amounts of SV40 DNA and that this SV40 DNA is covalently linked to Ad2 DNA (6). In this paper we have shown that the genome of each nondefective hybrid contains a single continuous segment of heterologous DNA of characteristic size, and further, that these segments form an overlapping series with a common end point. Although it is not directly demonstrated here, we assume that these segments contain SV40 DNA. Direct evidence on this point is presented in the accompanying report which shows that the heterologous DNA in the $Ad2^+ND_4$ genome represents a single uninterrupted segment of the SV40 genome (15a). The same must be true of the other hybrid segments, since they all represent subparts of the $Ad2^+ND_4$ segment. The genomes of the nondefective hybrids also contain overlapping deletions of Ad2 DNA with a common end point (at 0.140 fractional Ad2 length units from one end of the molecule). The largest deletion is in $Ad2^+ND_5$, where the Ad2 DNA segment from 0.140 to 0.211 fractional Ad2 length units is absent. It is interesting that this deletion is ac-

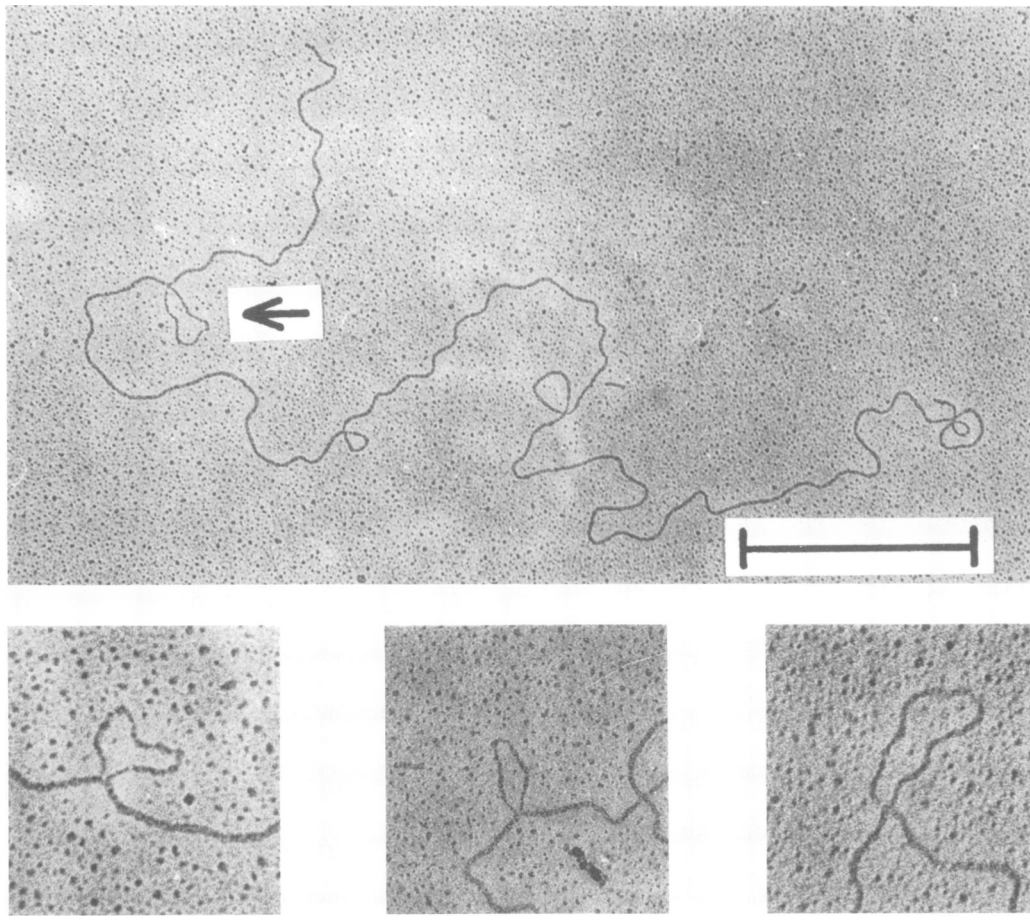


FIG. 2. *Ad2$^+$ND$_4$/hybrid heteroduplexes. Top: Ad2$^+$ND$_4$/Ad2$^+$ND$_3$ heteroduplex. Arrow indicates the position of the deletion loop. The length of the bar corresponds to 1 μm. Bottom (left to right): deletion loops of Ad2$^+$ND$_4$/Ad2$^+$ND$_1$, Ad2$^+$ND$_4$/Ad2$^+$ND$_2$, and Ad2$^+$ND$_4$/Ad2$^+$ND$_5$. The locations of these loops are given in Table 4. Magnification is twice that of the micrograph at the top.*

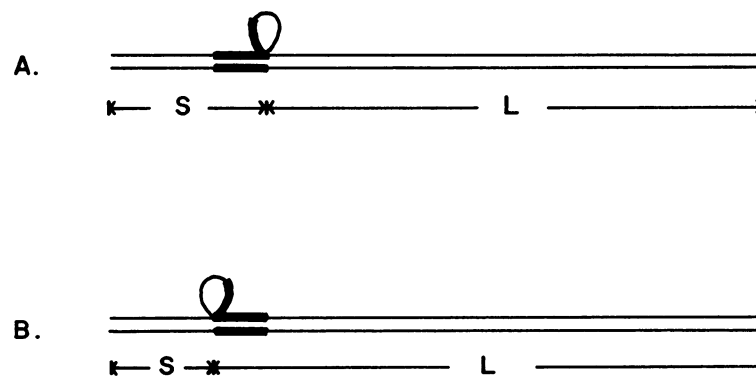


FIG. 3. *Two possible structures for the Ad2$^+$ND$_4$/hybrid heteroduplexes. Each of the Ad2$^+$ND$_4$/hybrid heteroduplexes contains a deletion loop (a single strand which originates and terminates at the same point on the heteroduplex). This requires: (i) that the SV40 segment of one of the two constituents of the heteroduplex be completely homologous to the SV40 segment of the other, and (ii) that the two SV40 segments have a common end point and that this end point be located at the same position in the Ad2 genome. As shown in the figure, two types of structures are possible; the common end point can be nearer the short Ad2 duplex segment (A) or nearer the long Ad2 duplex segment (B). Since S/(S + L) is greater for the Ad2$^+$ND$_4$/hybrid heteroduplexes than for the Ad2/hybrid heteroduplexes, the Ad2$^+$ND$_4$/hybrid heteroduplexes must be type A structures. Thin lines represent Ad2 DNA. Thick lines represent SV40 DNA.*

companied by no detectable decrease in infectivity (11).

In Fig. 5, the data on the relationship between the SV40 segments of the nondefective hybrids is combined with biological data on the induction of various SV40-specific antigens. It has been shown that $Ad2^+ND_1$ induces U antigen, $Ad2^+ND_2$ induces U antigen and SV40 tumor specific transplantation antigen (TSTA), and that $Ad2^+ND_4$ induces U, TSTA, and T antigens (Lewis and Rowe, manuscript in preparation; reference 11). This biological data correlates well with the physical data on the relationship of the SV40 segments to one another (Fig. 5). A self-consistent map can be constructed relating specific regions of the SV40 genome to the induction of specific antigens. From this map, the order of the early SV40 antigen inducing regions in the SV40 DNA segment contained in the $Ad2^+ND_4$ genome appears to be U, TSTA, and T proceeding from the short toward the long duplex segment in Ad2/$Ad2^+ND_4$ heteroduplex DNA molecules. Two other hybrids, $Ad2^+ND_3$ and $Ad2^+ND_5$, do not induce any known SV40-specific antigens. This is not surprising in the case of $Ad2^+ND_3$, as it contains only about 7% of the SV40 genome. However, $Ad2^+ND_5$ contains more SV40 DNA than $Ad2^+ND_1$ (in fact, the SV40 segment of $Ad2^+ND_5$ contains the entire SV40 segment of $Ad2^+ND_1$), and yet it does not induce detectable SV40 U antigen. The reason for this is not understood, but is apparently not due to a failure of transcription since it has been shown that the SV40-specific RNA species induced in cells infected with $Ad2^+ND_5$ contain all of the SV40-specific RNA species induced in cells infected with $Ad2^+ND_1$ (9). It is interesting that $Ad2^+ND_5$ contains the largest deletion of Ad2 DNA of any of the hybrids. It is possible that the deleted segment includes information required for the translation of the SV40 segment in $Ad2^+ND_5$.

TABLE 4. *Location of the deletion loops in the $Ad2^+ND_4$/hybrid heteroduplexes*

Heteroduplex	S/(S + L)[a]
$Ad2^+ND_4/Ad2^+ND_1$	0.167
$Ad2^+ND_4/Ad2^+ND_2$	0.188
$Ad2^+ND_4/Ad2^+ND_3$	0.156
$Ad2^+ND_4/Ad2^+ND_5$	0.182

[a] S/(S + L) is defined in the legend to Table 2.

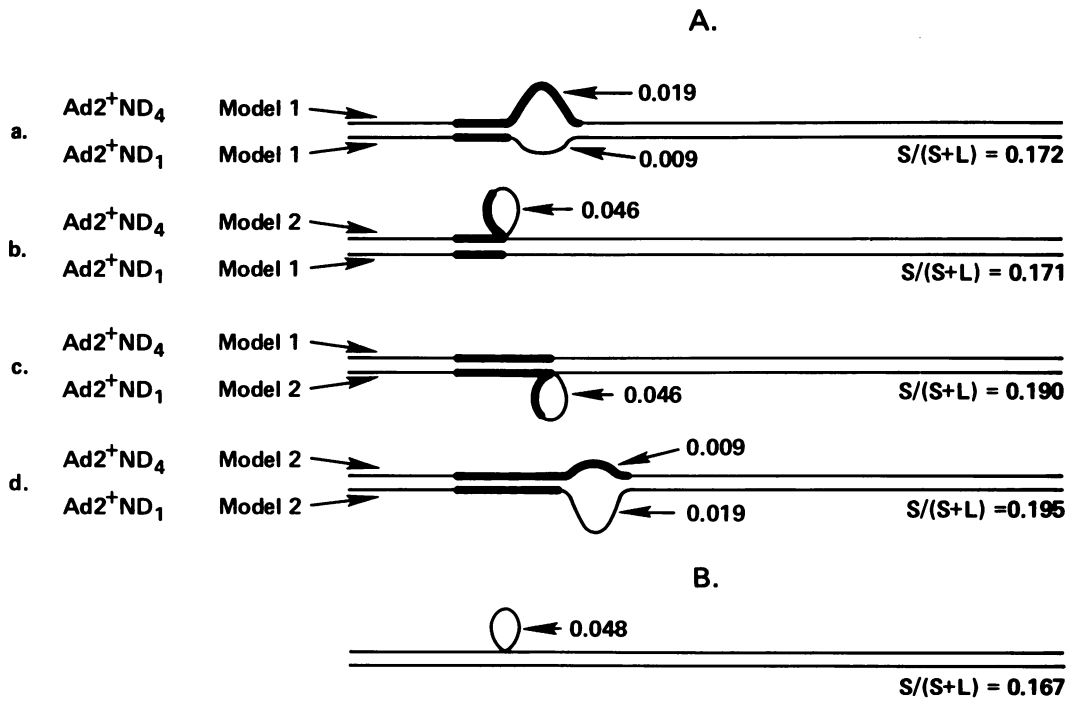


FIG. 4. *A, Possible structures for the $Ad2^+ND_4/Ad2^+ND_1$ heteroduplex. B, Observed structure of the $Ad2^+ND_4/Ad2^+ND_1$ heteroduplex. Thin lines represent Ad2 DNA. Thick lines represent SV40 DNA. Drawings are not to scale.*

TABLE 5. *Hybrid/hybird heteroduplexes,[a] calculated dimensions vs. dimensions observed*

Heteroduplex	Models	S/(S + L)	Loop[b] type	Loop length (Ad units)
$Ad2^+ND_4$/ $Ad2^+ND_1$	1/1	0.172	subs	0.019/0.009
	2/1[c]	0.171	del	0.046
	1/2	0.190	del	0.046
	2/2	0.195	subs	0.009/0.019
	observed	0.167	del	0.048
$Ad2^+ND_4$/ $Ad2^+ND_3$	1/1	0.156	subs	0.036/0.010
	2/1[c]	0.155	del	0.062
	1/2	0.190	del	0.062
	2/2	0.194	subs	0.010/0.036
	observed	0.156	del	0.066
$Ad2^+ND_4$/ $Ad2^+ND_5$	1/1	0.184	del	0.013
	2/1[c]	0.185	del	0.049
	1/2	0.189	del	0.049
	2/2	0.201	del	0.013
	observed	0.182	del	0.052
$Ad2^+ND_4$/ $Ad2^+ND_2$	1/1	0.189	del	0.004
	2/1[c]	0.191	del	0.032
	1/2	0.190	del	0.032
	2/2	0.201	del	0.004
	observed	0.188	del	0.033
$Ad2^+ND_2$/ $Ad2^+ND_5$	1/1[c]	0.186	del	0.017
	1/2	0.192	del	0.045
	2/1	0.187	del	0.045
	2/2	0.202	del	0.017
	observed	0.180	del	0.019
$Ad2^+ND_1$/ $Ad2^+ND_5$	1/1[c]	0.173	subs	0.017/0.014
	1/2	0.172	del	0.059
	2/1	0.187	del	0.059
	2/2	0.195	subs	0.014/0.017
	observed	0.171	subs	0.021/0.019

[a] The dimensions of four possible structures for each heteroduplex were calculated from the data of Table 3. The second column shows which models in Table 3 were used in this computation. For example in the case of the $Ad2^+ND_4/Ad2^+ND_1$ heteroduplex 2/1 means that model 2 of $Ad2^+ND_4$ was used to obtain the dimensions of the $Ad2^+ND_4$ strand, and model 1 of $Ad2^+ND_1$ was used to obtain the dimensions of the $Ad2^+ND_1$ strand.

[b] Abbreviations: subs = substitution loop; del = deletion loop.

[c] This marks the calculated structure whose dimensions are closest to those actually observed for each hybrid/hybrid heteroduplex.

It should be pointed out that there is no evidence at present that the SV40-specific antigens are products of SV40 structural gene(s), or, if they are products of SV40 structural gene(s), whether they represent different proteins or different antigenic determinants on the same protein. Thus, one must be cautious in interpreting a map such as that presented in Fig. 5. Although in a formal sense we can associate different regions of the SV40 genome with induction of different antigens, the detailed nature of this association is not known.

The finding that the SV40 segments in the nondefective Ad2-SV40 hybrids form an overlapping series correlates well with the data of Levine et al. (9), who have examined the relationship between the SV40 RNA species induced by the nondefective hybrids after infection of Vero cells. These workers showed by hybridization competition experiments that the RNA species induced by the various hybrids are overlapping; i.e., the RNA species induced by $Ad2^+ND_4$ (which contains the largest SV40 segment) contain all of the RNA species induced by $Ad2^+ND_2$ (which contains the next largest SV40 segment). Similarly, the RNA species induced by $Ad2^+ND_2$ contain all those induced by $Ad2^+ND_5$, etc. These investigations have also shown that the SV40 specific RNA induced by $Ad2^+ND_4$ is essentially identical to "early" SV40 RNA. Since we have shown here that the SV40 DNA segment of $Ad2^+ND_4$ represents about 40% of the SV40 genome, this sets an upper limit on the fraction of the SV40 genome which is transcribed into early RNA. Previous estimates for the size of the early region of the SV40 genome (based mostly on hybridization saturation experiments) have been in the range of 35 to 40% (8, 16, 20). The fact that these estimates are very close to the size of the SV40 segment in $Ad2^+ND_4$, suggests that the regions of the SV40 genome which are transcribed early are contiguous to one another and not scattered throughout the genome. It should be noted in this context that the antigens induced by $Ad2^+ND_4$ (U, TSTA, T) are all early SV40 antigens.

The nondefective Ad2-SV40 hybrids are recombinants between Ad2 and SV40. Analysis of their structure reveals that a minimum of two crossovers between Ad2 and SV40 must have occurred to produce each of them. (See reference 7 for a possible mechanism). It is interesting that one of the two crossovers appears to have been the same for all five of the hybrids studied here. The reason for this is obscured by the fact that the nondefective hybrids were all derived from the same virus pool (11) and, therefore, do not represent independent isolates. Thus, it is not possible to say at present whether this particular crossover occurs with a higher probability than other crossovers between Ad2 and SV40. An equally plausible explanation is that the nondefective hybrids were derived from some common ancestor in which this crossover had already occurred.

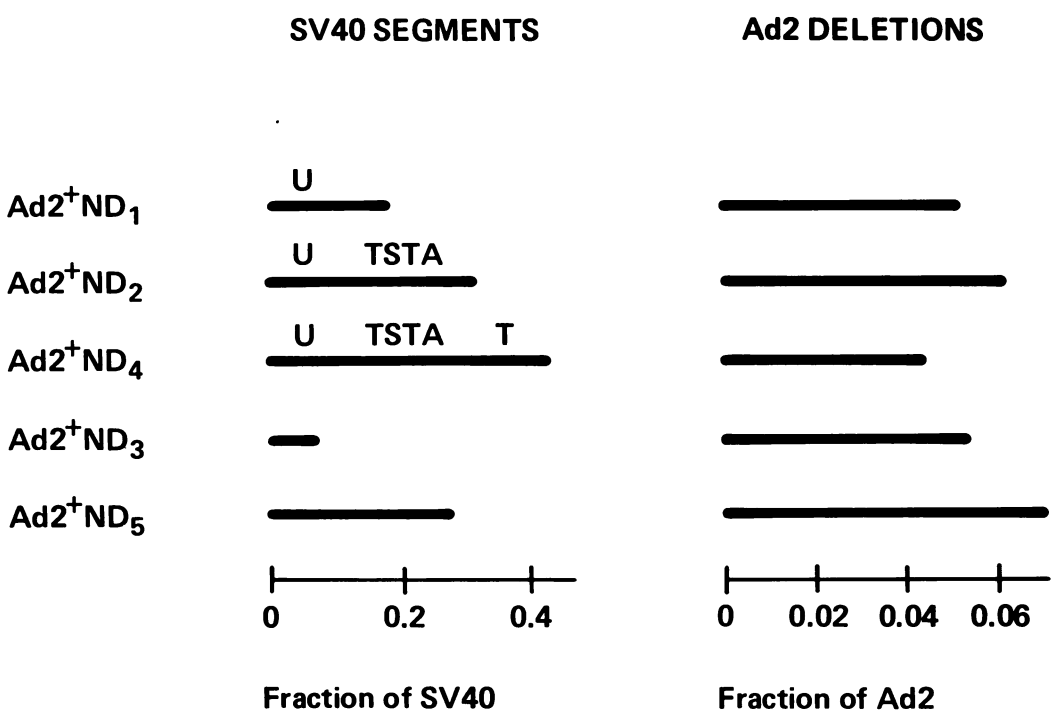


FIG. 5. *A, SV40 segments of the nondefective Ad2-SV40 hybrids. The SV40 segments form an overlapping series. The common end point is located at 0.140 fractional Ad2 lengths from one end of each hybrid. The SV40 segments extend toward the long end of the molecule. The letters above each hybrid segment represent the SV40-specific antigens induced by that hybrid. Abbreviations: U, SV40 U antigen; T, SV40 T antigen; TSTA, SV40 tumor specific transplantation antigen. B, Ad2 deletions of the nondefective Ad2-SV40 hybrids. The Ad2 deletions also overlap one another. The common end point is also located at 0.140 fractional Ad2 lengths from one end of each hybrid, e.g., the deletion of Ad2+ND$_1$ covers the region from 0.140 to 0.194 fractional Ad2 lengths from one end of the molecule.*

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