The Isolation of Defective Variants of Simian Virus 40 whose Genomes Contain Sequences Derived from Adenovirus 2 DNA

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SUMMARY

A new set of hybrid viruses has been isolated whose closed circular genomes 5 to 6 kbp in size, contain DNA sequences derived in part from adenovirus 2 and in part from SV40. The structure of these genomes is complex, but in the simplest case, analyses by restriction endonuclease digestion and hybridization indicate that the adenovirus 2 DNA is present as a continuous block, of maximum size 2.8 kbp. Different hybrids contain sequences derived from different segments of the adenovirus 2 genome.

INTRODUCTION

The idea that segments of foreign DNA can be propagated in mammalian cells as part of the SV40 genome was first raised by Lavi & Winocour (1972). They showed that serial passage of the virus in permissive simian cells leads to the appearance of genomes that consist of both host and virus DNA sequences. In the simplest case, part of the SV40 genome is replaced by monkey DNA of either the repetitive or unique classes (Rosenblatt et al. 1973; Frenkel, Lavi & Winocour, 1974; Segal et al. 1976) to create a defective genome that can multiply only in the presence of a helper virus. Subsequently Fareed and his colleagues (Ganem et al. 1976a, b; Nussbaum et al. 1976) used in vitro ligation to join a selected piece of bacteriophage λ DNA to a defined segment of the SV40 genome. The resulting chimeras were introduced into monkey cells together with wild-type SV40 DNA, which provided all the gene products required to replicate and pack the hybrid DNA molecules into virus particles. In principle, it seems possible to use this system and variants of it to amplify any desired segment of DNA that, together with its vector, is small enough to be packed into an SV40 virion.

In this paper the isolation of hybrid viruses is described, whose defective genomes consist partly of SV40 DNA and partly of the unrelated virus adenovirus 2. Unlike Fareed and his co-workers I have not used in vitro ligation but have relied upon in vivo cellular recombination enzymes during the construction of these hybrids; by contrast to Lavi & Winocour (1972), however, I have been able to select for hybrid molecules of a certain kind.

The isolation scheme is shown in Fig. 1. Ad2tD1 is a defective adenovirus 2-SV40 hybrid that can be propagated only in the presence of a helper adenovirus; under these conditions it grows well and forms a surprisingly high proportion (40%) of the virus yield (Lukanidin et al. 1977). Its genome contains an insertion of SV40 DNA about 3.2 kilobases (kbp) in length, in place of the 3.5 kbp segment of adenovirus 2 DNA which maps between 0.64 and 0.74 fractional genome lengths and includes the gene for the 72K DNA-binding protein. The right hand end of the inserted SV40 sequences begins at position 71 on the conventional
Selection of SV40-Ad2 hybrids

\[ \text{Ad}^+\text{D1 DNA} \]

Cleaved with restriction enzymes

Plaque formation on CV1 cells at 41 °C using SV40 tsa 30 DNA as helper

Virus from individual plaques passaged serially 3 times in CV1 cells at 40.5 °C

Closed circular DNA isolated (CsCl-ethidium bromide), and analysed by electrophoresis through 1 % agarose gels.

DNA transferred from agarose gels to nitrocellulose filters.

hybridized with \(^{32}\)P-labelled Ad2 DNA

Fig. 1. Isolation scheme for SV40-adenovirus 2 hybrids. For details see text.

map of that virus' genome. Stretching away to the left are the regions that code successively for the origin of replication, the A gene, the terminator at position 17 and, finally, part of the gene for the major coat protein, VP1: the integrated SV40 sequences ended at position 11 (see Fig. 2). During lytic infection by Ad2+D1 the integrated SV40 DNA sequences are transcribed and translated into a protein which can be precipitated by SV40-specific anti-T serum (Lukanidin et al. 1977) and whose mobility through SDS-polyacrylamide gels is indistinguishable from that of authentic T antigen – the product of the early SV40 A gene (Prives et al. 1975; Smith et al. 1975). On the basis of this evidence, it seemed entirely possible that AD2+D1 might complement ts mutants of the SV40 A gene. Because multiplication of SV40 is efficiently suppressed in cells infected by adenovirus 2 (Rabson et al. 1964), the simplest and most direct test for complementation is co-infection of cells with DNA afragments, using the techniques developed by Mertz & Berg (1974). Accordingly the DNA of Ad2+D1 was cleaved with one of a variety of restriction endonucleases and introduced into monkey cells at non-permissive temperature together with intact DNA of SV40 tsa 30 (Tegtmeyer, 1972). Amongst the resulting progeny were viruses whose closed circular genomes contained adenovirus 2 DNA sequences.

METHODS

Cell cultures. CV1 cells, a subline of African green monkey kidney cells (Jensen et al. 1964) were cultured in plastic dishes in Dulbecco's modification of Eagle's medium (Dulbecco & Freeman, 1959) with 10 % foetal bovine serum. Human KB cells (Eagle, 1955) used for production of high titre adenovirus stocks, were grown in suspension cultures in Eagle's F13 medium (F13; Gibco, Grand Island, New York), supplemented with 5 % horse serum.

Viruses. Ad2+D1 was propagated in cultures of CV1 cells: adenovirus type 2 in suspension cultures of HeLa cells. The virus particles were purified by a modification of the method of Green & Piña (1963) as described by Lonberg-Holm & Philipson (1969).
SV40 adenovirus 2 hybrid viruses

SV40 tsa 30, obtained from Dr Peter Tegtmeyer, was propagated at 32 °C in CV1 cells which had been infected at a multiplicity of 0.05 plaque-forming units (p.f.u.) per cell.

Plaque titrations with SV40 virions or DNA were performed at 41 °C or 40.5 °C in monolayers of CV1 cells as described by Mertz & Berg (1974).

Virus DNA. The DNAs of Ad2+D1 and adenovirus 2 were isolated from purified virus particles as described by Pettersson & Sambrook (1973). SV40 tsa 30 DNA was extracted by the method of Hirt (1967) from CV1 cells infected at a multiplicity of 1 p.f.u./cell, after 72 h incubation at 32.5 °C. Covalently closed virus DNA was purified by centrifugation to equilibrium in a solution containing CsCl and ethidium bromide (Bauer & Vinograd, 1968). After the band of component 1 DNA was collected, the ethidium bromide was removed by extraction with isopropanol and the CsCl was diluted by sequential dialysis against 0.01 M-tris-HCl, pH 7.8, 0.001 M-EDTA.

Restriction enzymes. Endonuclease Eco RI was prepared from Escherichia coli strain RY 13 (Yoshimori, 1971) as described by Gallimore, Sharp & Sambrook (1974). Hind III was isolated from Haemophilus influenzae serotype d (Smith & Wilcox, 1970) as described by Lai & Nathans (1974a). Endonuclease Hpa I was purified from Haemophilus parainfluenzae by the method of Sharp, Sugden & Sambrook (1973). Sma I was prepared from Serratia marcescens (C. Mulder, personal communication) as described by Böttner, Veres-Molnár & Green (1976). Endonuclease Hha I was prepared from Haemophilus haemolyticus as described by Roberts et al. (1976). Bgl I was isolated from Bacillus globigii according to an unpublished procedure of G. A. Wilson and F. E. Young.

Reaction mixtures for cleavage of virus DNAs by restriction endonucleases contained 1.0 µg DNA in a volume of 50 µl. For Eco RI the digestion buffer consisted of 0.1 M-tris-HCl, pH 7.7, 0.01 M-MgCl₂; for Sma I, 0.03 M-tris-HCl, pH 9.0, 0.01 M-MgCl₂, 0.01 M-KCl; for all other enzymes, 0.01 M-tris-HCl, pH 7.7, 0.01 M-MgCl₂. Digestions were carried out at 37 °C for periods of 30 min to 2 h depending on the enzyme used. The reactions were then stopped by addition of EDTA to a final concentration of 0.05 M.

Gel electrophoresis. Agarose gels (1 %) were prepared in tris-acetate buffer (0.04 M-tris-HCl, pH 7.8, 0.005 M-sodium acetate, 0.001 M-EDTA). Samples were applied in 50 µl of tris-acetate buffer containing sucrose (8 %, w/v) and electrophoresis was carried out for 20 h at 1.5 V/cm (Sugden et al. 1975). DNA bands were stained with ethidium bromide and photographed as originally described by Sharp et al. (1973).

Hybridization. Bands of DNA in agarose gels were denatured in situ and transferred to nitrocellulose filters as described by Southern (1975). Introduction of α-32P-nucleotides into DNA by 'nick-translation' (Kelly et al. 1970) was developed by P. W. J. Rigby, D. Rhodes, M. Dieckmann and P. Berg as a method to generate hybridization probes. In this work the conditions established by Maniatis et al. (1976) were used. Hybridization and autoradiography was carried out exactly as described (Botchan, Topp & Sambrook, 1976).

RESULTS

Complementation between fragments of Ad2+D1 and SV40 tsa 30

Ad2+D1 DNA was cleaved with various restricting endonucleases and the resulting fragments were purified by extraction with phenol, concentrated by precipitation with ethanol, mixed with SV40 tsa 30 DNA and used to infect monolayers of CV1 cells. Table 1 shows that the number of plaques obtained after 12 days incubation at 41 °C varies with the dose of Ad2+D1 fragments applied to the cells and with the particular restriction enzyme employed. No plaques were obtained when SV40 tsa 30 DNA was used alone or in con-
Table 1. Complementation between fragments of Ad2⁺DI DNA and SV4o tsa 30 DNA*

<table>
<thead>
<tr>
<th>Restriction endonuclease</th>
<th>Amount of fragments of Ad2⁺DI DNA (µg)</th>
<th>Amount of SV4o tsa 30 DNA (µg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>0,1</td>
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<tr>
<td></td>
<td>50</td>
<td>0,5</td>
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<tr>
<td>Sma I</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>0,1</td>
<td>1 × 10³</td>
</tr>
<tr>
<td></td>
<td>0,5</td>
<td>8 × 10³</td>
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<tr>
<td>Bgl I</td>
<td>0</td>
<td>0</td>
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<tr>
<td></td>
<td>0,1</td>
<td>6 × 10³</td>
</tr>
<tr>
<td></td>
<td>0,5</td>
<td>6 × 10³</td>
</tr>
<tr>
<td>Hha I</td>
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<td>0</td>
</tr>
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<td></td>
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</tr>
<tr>
<td></td>
<td>0,5</td>
<td>12 × 10²</td>
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<td>0,1</td>
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<td></td>
<td>0,5</td>
<td>8 × 10³</td>
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<tr>
<td>Hind III</td>
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<td>10 × 10³</td>
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<tr>
<td></td>
<td>50</td>
<td>10 × 10³</td>
</tr>
</tbody>
</table>

* Fifty µg aliquots of Ad2⁺DI DNA were digested with each of several restriction endonuclease. At the end of the reaction the NaCl concentration was adjusted to 0.1 M and SDS and EDTA were added to final concentrations of 0.5% and 0.1 M, respectively. The DNA fragments were purified by phenol extraction, concentrated by ethanol precipitation and dissolved in a small volume of tris-buffered saline. Confluent monolayers of CV1 cells were infected with mixtures of fragments and SV4o tsa 30 DNA in the presence of DNA as described by Mertz & Berg (1974). After 12 days incubation at 41°C the cells were stained with neutral red. The Table shows the average number of plaques present on day 14. The experiment was performed in triplicate.

Junction with either intact Ad2⁺DI DNA or fragments of it derived by digestion with endonucleases Hpa I and Hind III – enzymes which cleave within the sequences of SV4o gene A that are integrated into the Ad2⁺DI genome (Lukanidin et al. 1977). By contrast, co-infection of cells with SV4o tsa 30 DNA and fragments obtained by digestion of AD2⁺DI DNA by endonucleases Sma I, Bgl I, Hha I and Eco RI resulted in the appearance of plaques with morphology typical of those caused by SV4o. Neither Sma I, Hha I nor Eco RI cleave within the SV4o sequences present in the Ad2⁺DI genome (see Fig. 2): Bgl I cleaves the integrated SV4o sequences once, at position 67 (S. Zain, personal communication) close to the origin of DNA synthesis but not within the A gene. Approximately equal numbers of plaques were obtained in the presence of 0.1 µg and 0.5 µg of SV4o tsa 30 DNA – a result that was expected because preliminary experiments had shown 0.01 µg
Isolation of SV40-adenovirus 2 hybrids

Twenty-four plaques were picked from the complementation tests between SV40 tsα 30 DNA and each set of fragments obtained by digestion of Ad2+D1 DNA with endonucleases Hha I, Bgl I, Eco RI and Sma I. In all 96 isolates were obtained and used to infect small (1 cm diam.) monolayers of CV1 cells growing in plastic trays (Linbro Ltd, New Haven). After six days incubation at 40.5 °C, when they were showing severe cytopathic effect, the cells, together with their supernatant medium were frozen at −20 °C, thawed and removed from the plates by aspiration. The resulting fluid was used to infect 5 monolayers of CV1 cells, growing in 100 mm Petri dishes. After three days incubation at 40.5 °C the virus DNA was extracted by the method of Hirt (1967) and purified by centrifugation to equilibrium in CsCl-ethidium bromide gradients as described in Methods. About 1 µg of each preparation of closed circular DNA was applied to a 1.0 % agarose gel (20 × 20 cm) and subjected to electrophoresis for 20 h at 3 V. The DNA was then denatured in situ, transferred to nitrocellulose sheets (Southern, 1975) and hybridized to 32P-labelled adenovirus 2 DNA. The presence of adenovirus 2 DNA sequences in the various isolates was determined by autoradiography – a technique which reconstruction experiments have shown to be capable of detecting as little as 10−13 µg of DNA (Botchan et al. 1976).

The results of an experiment in which sixteen isolates were assayed simultaneously is shown in Fig. 3. Most of the isolates showed little or no homology to adenovirus DNA; one of them hybridized strongly and the positions of the two radioactive bands correspond
Fig. 3. Screening for SV40-adenovirus 2 hybrid genomes. About 0.1 μg of closed circular DNA isolated from cells infected with sixteen putative SV40-adenovirus 2 hybrids were applied to an agarose gel and subjected to electrophoresis as described in the text. The bands of DNA were then denatured and transferred to a sheet of nitrocellulose (Southern, 1975) and hybridized (Botchan et al. 1976) to about 10^6 cts/min of 32P-labelled adenovirus 2 DNA (sp. act. 10^8 cts/min/μg). After extensive washing, the nitrocellulose sheet was exposed to X-ray films for 120 h. Slot 17 contains a small quantity of 32P-labelled component II SV40 DNA that served as a marker. Slot 6 shows two bands of hybridization to adenovirus 2 that correspond in mobility to components I and II of SV40 DNA. The DNA applied to the slot comes from an isolate called Bgl 6.

to the location in the gel of the closed and relaxed circular forms of SV40 DNA. In all, three viruses (Hha 7, Sma 2 and Bgl 6) were found whose DNAs hybridized well to adenovirus 2 DNA: stocks of these were prepared by two further serial passages in CV1 cells. Four other isolates showed a lesser but still significant amount of homology to adenovirus 2 DNA: however, no further work was carried out with them.

Analysis of the DNAs of Bgl 6, Hha 7 and Sma 2

Closed circular DNA was prepared from each of the three isolates which hybridize well to adenovirus 2 DNA, digested with restriction endonucleases and examined by gel electrophoresis and hybridization. The results obtained are shown in Fig. 4 and 5. The immediate impression is one of heterogeneity and it is at once obvious that the preparations of DNA must contain several different species. However, the practised eye can discern amongst the complex pattern of fragments, familiar bands that are typical of digests of SV40 DNA. In all probability these are derived, at least in part, from the SV40 tsa 30 helper that is present in the stocks. New fragments of DNA, in abnormal molar quantities, are found in digests of both Bgl 6 and Sma 2 DNAs (Fig. 4): it is clear that the genomes present in these two virus stocks are not identical.

When the fragments of DNA were transferred from the gel to a sheet of nitrocellulose and hybridized to 32P-labelled adenovirus 2 DNA, it was found that not all fragments of DNA made visible by ethidium bromide staining contain adenovirus sequences. For example only one fragment (approx. 3 kB in size), obtained by cleavage of Sma 2 DNA with restriction endonuclease Hind III, hybridized to adenovirus 2 DNA. A similar result was found when endonuclease Hpa I was used except that the mol. wt. of the single DNA band which contained adenovirus sequences was approx. 2.8 kB in length. However when Sma 2 DNA...
SV40-adenovirus 2 hybrid viruses

Fig. 4. Analysis of the DNAs of Sma 2 and Bgl 6. About 1 µg of the DNAs of Sma 2 and Bgl 6 were digested with various restriction endonucleases and the resulting fragments were separated by electrophoresis through an agarose gel. The bands of DNA were stained with ethidium bromide and photographed (Sharp et al. 1973) before they were denatured and transferred to a nitrocellulose sheet (Southern, 1975) and hybridized (Botchan et al. 1976) to 5 x 10^6 ct/min of 32P-labelled adenovirus 2 DNA (sp. act. 10^8 ct/min/µg). After extensive washing the nitrocellulose sheet was exposed to X-ray film for 96 h. Slot 1 contains DNA cleaved with Bgl I, slot 2 DNA cleaved with Eco RI, slot 3 DNA cleaved with Hha I, slot 4 DNA cleaved with Hpa I and slot 5 DNA cleaved with Hind III.

was digested with Eco RI or Hha I — restriction endonucleases that cleave SV40 DNA at one and two sites respectively — more than one fragment was obtained that contained adenovirus sequences. The pattern of hybridization of fragments of Sma 2 DNA is different from that observed in the DNA of Bgl 6. In the latter case, no enzyme was found to yield a single fragment that contained all the adenovirus DNA sequences: and it was often difficult to identify the bands of DNA that hybridized to adenovirus DNA, so faintly did they stain with ethidium bromide. Apparently, in Bgl 6, adenovirus 2 sequences are contained within a species of DNA that is present in very low concentration. The same conclusion holds true for Hha 7 DNA, whose pattern of hybridization to adenovirus 2 DNA is shown in Fig. 5. With endonuclease Eco RI, adenovirus sequences are found to occur in one major and two subsidiary, smaller DNA fragments: treatment of Hha 7 DNA with endonuclease Hae III produces several very small species of DNA whose faint hybridization with adenovirus 2 DNA can be seen only after prolonged exposure of the autoradiogram (Fig. 5b). Most interesting, however, is the pattern of hybridization found amongst the fragments obtained by digestion of Hha 7 DNA with endonuclease Hpa I. Five evenly-spaced bands are seen (Fig. 5) whose ability to hybridize to adenovirus 2 DNA decreases with decreasing mol. wt. This result must mean that Hha 7 contains a heterogeneous population of DNA molecules carrying adenovirus 2 DNA sequences, which differ in length from each other by about 0.2 kb.

It is obvious from all this that the isolates called Hha 7, Bgl 6 and Sma 2 do not contain a single substituted virus DNA species. They were therefore re-purified by plaque isolation.
Fig. 5. Distribution of adenovirus 2 sequences in DNA fragments of Hha 7. About 0.3 μg of Hha 7 DNA was digested with restriction endonucleases, subjected to agarose gel electrophoresis, transferred to nitrocellulose filters and hybridized to 32P-labelled adenovirus 2 DNA. The autoradiogram was exposed for 4 days (a) or 10 days (b).

from monolayers of CV1 cells infected and maintained at 40.5 °C for 12 days. All three isolates formed plaques with two-hit kinetics (data not shown). Several plaques of each isolate were picked and the production of working stocks and the process of isolation of closed circular DNAs were repeated. The results of analysing seven of the resulting isolates are shown in Fig. 6. Obvious heterogeneity is still detected by ethidium bromide staining (left), although there seems to have been significant simplification of the species of DNA in the preparations. Each isolate contains at least two sorts of DNA which can be either slightly smaller (Fig. 6B; slots 1, 3, 4 and 5) or larger (slot 2) than standard-sized SV40 DNA. Some DNAs are resistant to cleavage by endonuclease Eco RI (Fig. 6; slots, 4, 5 6 and 7), while others seem to be almost completely sensitive to the enzyme (Fig. 6A; slot 2): one isolate (slot 1) consists primarily of a species of DNA that contains two Eco RI cleavage sites.

The DNAs of all the subisolates contain both closed superhelical and relaxed circular molecules, both of which hybridize with adenovirus 2 DNA (Fig. 6 right). There is wide
SV40-adenovirus 2 hybrid viruses

Fig. 6. Analysis of the DNAs of subisolates of Hha 7, Sma 2 and Bgl 6. About 1 µg of each isolate was applied to an agarose gel. After electrophoresis the bands of DNA were stained with ethidium bromide and photographed. The A photographs show the bands obtained after cleavage of the DNAs with Eco RI; the B photographs show the untreated DNAs. Slots 1, 3, 4 and 5 contain subisolates of Sma 2, slot 2 a subisolate of Hha 7 and slots 6 and 7 subisolates of Bgl 6 and 7. The DNAs shown in the left hand photographs were transferred to a nitrocellulose sheet (Southern, 1975) and hybridized (Botchan et al. 1976) to 2 x 10^6 ct/min ^32P-labelled adenovirus 2 DNA. Autoradiographic exposure was for 4 days.

variation in the amount of adenovirus sequences detected, the greatest quantities being found in the DNAs of subisolates 1, 3, 4 and 5, all of which are derived from Sma 2: in every case, one major and several minor species of DNA are present which hybridize with adenovirus 2 DNA. Subisolate number 2 is derived from Hha 7; those numbered 6 and 7 from Bgl 6. Again, heterogeneity is evident, and it seems that re-purification of the original isolates has improved the situation, but not eliminated the problem of variation within the hybrid virus stocks.

The origin of the adenovirus 2 DNA sequences in subisolates of Hha 7, Sma 2 and Bgl 6

To determine what part of the adenovirus 2 genome is present in each of the substituted genomes, hybridizations were carried out between unlabelled fragments of adenovirus 2 DNA, transferred directly from analytical agarose slab gels to nitrocellulose sheets, and the DNAs of subisolates 6, 4 and 2, which had been radiolabelled in vitro with ^32P. The results are shown in Fig. 7, 8 and 9. Knowing the map locations of the adenovirus 2 DNA fragments, it is a simple matter to work out the origin of the sequences that are present in the substituted genomes. Fig. 7 and 8 show that Sma 2 and Bgl 6 contain indistinguishable sets of adenovirus sequences, which originate between positions 60 and 64 on the map of the adenovirus genome – a result that might have been predicted from the structure of the original starting material – Ad^2+D1 DNA (see Fig. 2). However, when the same experiment was carried out with DNA of Hha 7 (subisolate 2), a very surprising result was obtained (Fig. 9): instead of those that flank the SV40 insertion in Ad^2+D1, Hha 7 (subisolate 2) carried sequences from a very distant part of the adenovirus genome which maps at the right hand end between map positions 94 and 98.
Fig. 7. The origin of adenovirus 2 DNA sequences in Sma 2. About 1 μg of adenovirus 2 DNA was treated with various restriction endonucleases. The resulting fragments were separated by gel electrophoresis, stained with ethidium bromide (Sharp et al. 1973) and photographed, and then transferred to a nitrocellulose (Southern, 1975) sheet for hybridization to Sma 2 DNA ³²P-labelled in vitro (Botchan et al. 1976). Slot 1 contains uncut adenovirus 2 DNA. The other slots contain fragments cleaved by the enzymes listed in the lower part of the figure. Fragments that hybridize strongly to Sma 2 DNA are marked on the maps with thick lines, and those that hybridize weakly, with thinner lines.
Fig. 8. The origin of adenovirus 2 DNA sequences in Bgl 6. For experimental details, see the legend to Fig. 7.
Fig. 9. The origin of adenovirus 2 DNA sequences in Hha I. For experimental details, see the legend to Fig. 7.

**DISCUSSION**

To summarize the data: defective viruses have been isolated whose closed circular genomes, ranging in size from 5 to 6 kB, consist of covalently-joined SV40 and adenovirus 2 DNA sequences. Each defective virus stock contains a population of hybrid genomes that are different from those of the other stocks and heterogeneous in their own right. The detailed arrangement of the substituted genomes is not known, but in the simplest case
SV4o-adenovirus 2 hybrid viruses

Analyses by restriction endonuclease digestion and hybridization indicate that the adenovirus 2 DNA sequences are contiguous and have a maximum size of 2.8 kB. Different hybrids contain sequences from different parts of the adenovirus 2 genome.

It was not surprising to find heterogeneity in the original isolates of Hha 7, Sma 2 and Bgl 6. With the techniques currently available, exposure of cultured cells to a mixture of DNA fragments will almost ensure that any infected cell will receive many kinds of molecules—probably in the form of a large clump. It seems entirely possible that this situation may promote recombination and result in the formation of several sorts of hybrid molecules. It is more disconcerting, however, to find the variegation persisting through subsequent plaque purification. The causes of this heterogeneity are not understood and it is not known whether the SV4o-adenovirus 2 hybrids are any more susceptible to amendment in vivo than are other sorts of substituted SV4o genomes. However, it should be pointed out that Ganem et al. (1976b), using gel electrophoresis, were able to detect within the DNA sequences of SV4o-λ hybrids, heterogeneity that appeared during passage of the substituted genomes in monkey cells. The hybridization techniques used here are considerably more sensitive and are capable of detecting rearranged molecules that are present at extremely low concentrations. It is obvious that the same techniques should be applied to other substituted SV4o genomes, both naturally-occurring and artificially generated.

How the SV4o-adenovirus 2 hybrids were formed is not understood. In two cases (Bgl 6 and Sma 2) the cells were infected with intact SV4o tsA 30 DNA and an assorted mixture of fragments amongst which was a piece of DNA that contained the SV4o A gene and flanking adenovirus 2 DNA sequences. Presumably this linear fragment was converted to a covalently-closed molecule by a process of cell-mediated ring closure similar to that discussed by Lai & Nathans (1974b) and Mertz et al. (1974). This hypothesis is supported by the observation that the DNA of Sma 2 contains no site of cleavage for the enzyme originally used to excise the SV4o segment from Adz D1 (data not shown): it may therefore correspond to the extended deletion mutants described by Lai & Nathans (1974b). The DNA of Bgl 6, however, contains at least one site cleaved by endonuclease Bgl I, and may be equivalent to the ‘excisional deletion’ mutants of SV4o (Lai & Nathans, 1974b). The SV4o sequences presumably provide to the helper ts virus, a supply of the SV4o A gene product: in turn, the helper virus donates capsid proteins which are used to pack the hybrid genome into virus particles.

The formation of Hha 7 is more mysterious. However, the following series of events seems plausible. Endonuclease Hha 1, by contrast to Bgl I and Sma 1 cleaves adenovirus 2 DNA into very small pieces. In fact it is known (J. Sambrook, unpublished data) that the distance from the SV4o insertion in Adz D1 to the nearest Hha 1 cleavage sites in both directions is less than 100 base-pairs. Even if this DNA could circularize without destroying the SV4o A gene, the resulting molecule would be too short to be packed efficiently into SV4o virions (Ganem et al. 1976a). The longer genome of Hha 7 must have been formed by recombination between the SV4o-containing fragment of Adz D1 and a piece of DNA that, although derived from a distant part of the adenovirus 2 genome, happened to be conveniently adjacent in the infected cell. This result once again indicates the bizarre processes of recombination that occur in cells infected by SV4o: it seems to be commonplace that DNAs which share little or no base sequence homology, are genetically remote from one another, show little or no organizational similarities, and on all these grounds could be expected not to interact, nevertheless manage to recombine to form hybrids. The substituted genomes described in this paper can therefore be added to a list of products of promiscuous recombination that already includes integration of virus DNA in transformed cells, generation
of adenovirus-SV40 hybrid viruses, and rearrangements and substitution within the SV40 genome itself.

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SV40-adenovirus 2 hybrid viruses


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