Volume 10 Number 24 1982 Nucleic Acids Research

Sequence of the junction in adenovirus 2-SV40 hybrids: examples of illegitimate recombination

Leona E. Ling, M. Michele Manos and Yakov Gluzman

Cold Spring Harbor Laboratory, P.O. Box 100, Cold Spring Harbor, NY 11724, USA

Received 9 September 1982; Revised and Accepted 9 November 1982

ABSTRACT

The nucleotide sequences of six Ad2-SV40 junctions from three Ad2-SV40 hybrid viruses (Ad2^{++}HEY, Ad2^{++}LEY and Ad2^{+}D1) were determined. Comparison of parental adenovirus 2 and SV40 DNA sequences with the sequence at the Ad2-SV40 junctions revealed that 5 out of 6 junctions are abrupt transitions from Ad2 to SV40 DNA, and in one case (Ad2^{++}LEY, right junction) there is an additional nucleotide at the junction, which cannot be ascribed to either DNA. Ad2^{++}HEY and Ad2^{+}D1 right junctions are identical and Ad2^{++}LEY and Ad2^{+}ND4 left junctions are identical, a result that strongly suggests these Ad2-SV40 hybrids arose by recombination between the linear Ad2 DNA and circular SV40 DNA, followed by recombination between Ad2 DNA and SV40 DNA present in the Ad2-SV40 hybrid DNA. The unambiguous transition of Ad2 DNA into SV40 DNA at the junction sites is an example of recombination events which have apparently occurred without any homology at the recombination site.

INTRODUCTION

Recombination in eukaryotic cells has been studied extensively in viral systems. Viral recombination, rather than recombination between cellular sequences, has been used because recombination between viral mutants is readily assayed and the recombinants are easily analyzed. Recombination takes place at high frequency between regions of homology. For example, when adenovirus type 2 and type 5 recombine, the sites of exchange are found in sequences common to both types (1). However, recombination events also occur in the absence of extensive homology. Adenovirus and SV40 are nonhomologous in terms of DNA sequences, yet they recombine to form adenovirus-SV40 hybrids (for review see 2). The sequence of the two junctions of hybrids, Ad2^{+}ND1 (3) and Ad2^{+}ND4 (4), showed that adenovirus and SV40 sequences were joined at regions bearing no obvious homology. Surrounding the junctions, adenovirus and SV40 sequences contained only scattered stretches of homologous sequence, none greater than four basepairs. The sequence of an Ad5-SV40 junction of AD5^{++}D1 hybrid showed that there is a homology of five out of six nucleotides at

0305-1048/82/1024-8099$2.00/0

the junction (5). Thus, the recombination responsible for forming the Ad-SV40 hybrids appears to be either independent of homology, or involving very short stretches of homology.

Nonhomologous recombination is also important in forming SV40 variant genomes. During serial passage of SV40 on permissive monkey cells, variant subpopulations are produced that carry deletions, rearrangements, and cellular sequences (6,7,8). In viral-cell junctions of the variants, the exact extent of homology is difficult to assess since the parental cellular sequences are not known. Sequence analysis of the viral-viral junctions in these variants reveals 0 to 4 basepairs homologous at the junctions between the parental sequences. The comparison of parental to recombinant sequences in these studies suggests that viral recombination in eukaryotes can occur in the absence of obvious homology. Another example of nonhomologous recombination is the integration of exogenous viral DNA sequences into the cellular genome which occur infrequently and appear to happen at random sites. In the SV40 transformed cell lines 14B (9), SVRE9 and SVRE17 (10) the viral-cell junctions are abrupt transitions from cell to virus DNA, when the sequence of junctions were compared with SV40 DNA sequence. However, as in the case of the viral-cell junction of SV40 variants, it is difficlt to assess the extent of homology for 14B and SVRE17 junctions because the parental cell sequences are not known. In the case of the SVRE9 junction the cloned cellular DNA sequence from the unoccupied site of integration was compared with the corresponding cell-viral junction. It was found that SV40 and rat DNAs are linked at the point where two genomes shared 5 base pairs of homologous DNA sequence (11). Short stretches of homology also were found at the crossover junctions of excised SV40 DNA from the genome of 14B cells (12). These findings indicate that short stretches of homology may have a role in the integration of the SV40 genome into cellular DNA, or during the recombination of linear SV40 molecules into circular replicating DNA.

To further characterize nonhomologous recombination in eukaryotes, we have sequenced the junctions of three defective Ad2-SV40 hybrids: $Ad2^{++}HEY$, $Ad2^{+}D1$, and $Ad2^{++}LEY$. Because the DNA sequence of the parental viruses is established, an unambiguous comparison can be made with the Ad2-SV40 hybrid DNAs. Defective, rather than nondefective, hybrids were chosen for this study because they were presumably formed without the selective pressure of maintaining a viable genome. Thus, at least some of the hybrid genomes described here are likely to be products of a primary recombination between

Ad2 and SV40 genomes.

MATERIALS AND METHODS

Preparation of viral DNAs.

$Ad2^{++}$HEY and $Ad2^{++}$LEY hybrid viruses were obtained from Dr. A.M. Lewis (13). $Ad2^{+}$D1 hybrid virus was a gift of Dr. J. Sambrook (14). Viral DNAs were prepared from CV-1 cells infected with hybrid viruses 30-36 hours post-infection using the Hirt extraction procedure (15). The extraction was modified by adding 1mg/ml of pronase into the Hirt buffer and incubating for 2 hours at 37°C before adding NaCl. Viral DNA was digested with restriction enzymes, and the junction fragment was either cloned directly or cloned after purification from agarose gels.

Cloning of the adenovirus-SV40 junctions

The complete insert of SV40 DNA and the flanking Ad2 DNA from $Ad2^{++}$HEY was cloned into the modified λgtWESλB (10). Total $AD2^{++}$HEY DNA was digested with SstI enzymes, ligated to Sst-terminal λ-arms, packaged *in vitro* and screened for SV40 DNA by the method of Benton and Davis (16). The DNA (λHEY-II) was digested with EcoRI, and left (pBHEY-L) and right (pBHEY-R) Ad2-SV40 junctions were recloned into pBR322 at the EcoRI site.

$Ad2^{+}$D1 DNA was digested with EcoRI and subjected to electrophoresis on a 1.4% agarose gel. The 4.4kb fragment containing the SV40 insert and adjacent Ad2 DNA was recovered by electroelution, and cloned into the EcoRI site of pBR322.

The complete insert of SV40 DNA and adjacent Ad2 DNA from $Ad2^{++}$LEY was cloned into pMK16#1 (17). $Ad2^{++}$LEY DNA was digested with BglII, ligated to the BamHI digested pMK16#1, and kanamycin resistant bacterial colonies were screened for the presence of SV40 sequence by in situ hybridization to ^{32}P-labeled SV40 probe. Recombinant plasmid (pML12) was isolated which contained the complete SV40 insert and the adjacent Ad2 DNA. The right junction of the $Ad2^{++}$LEY was recloned by cutting pML12 with EcoRI, recircularizing by ligation, and transforming E.coli.

DNA sequence determination of the Ad2-SV40 junctions.

DNA fragments, depicted in Figure 2, were labeled at their 5' termini using kinase [P-L Biochemicals] and [γ-^{32}P] ATP, or 3' termini using the Klenow fragment of E.coli DNA polymeraseI (BRL) and appropriate [α-^{32}P] XTPs. Labeled fragments were sequenced by the procedure of Maxam and Gilbert (18). The nucleotide numbers for SV40 sequence are given using the SV system (2). The nucleotide numbers for adenovirus 2 DNA are taken from

different sources (10-24). The map units for adenovirus 2 DNA were calculated using 360 nucleotides as 1%, and counting nucleotides from the left end ($Ad2^{++}$HEY left junction), from the right end ($Ad2^{+}$D1, right, $Ad2^{++}$LEY both junctions), or from the BamHI site at 59.5% ($Ad2^{+}$D1 left junction).

RESULTS

Cloning the $Ad2^{++}$HEY Junctions

Stocks of $Ad2^{++}$HEY consist of three stable classes of hybrids and adenovirus 2 helper (13). 90% of the population is the adenovirus 2 helper whereas the hybrid classes, HEY I, II, and III, comprise 10% or less of the population (19). Out of these three, HEY II is the most abundant. The hybrid classes differ only in the amounts of SV40 DNA they contain. HEY I contains 40% of an SV40 genome (map units 0.26 to 0.66). This SV40 segment is flanked by Ad2 sequence from 0 to 0.30 on the left and 0.74 to 1.00 on the right. HEY II and III are identical to HEY I except that HEY II contains one complete SV40 genome and HEY III contains two SV40 genomes in addition to the 40% between 0.26 and 0.66. The additional SV40 sequences are integrated in tandem in adenovirus 2 DNA (Figure 1).

To isolate a junction carrying fragment from the bulk of the adenovirus 2 DNA sequence, $Ad2^{++}$HEY DNA was digested with Sst I. This enzyme leaves SV40 sequences intact, but cleaves adenovirus 2 more than 15 times. The mixture of Sst I fragments of $Ad2^{++}$HEY DNA was cloned into λgtWES λB adapted with Sst I-Eco RI linkers (10). One clone, λHEY-II, (Figure 2) was isolated and determined to contain the desired 9kb Sst I insert. To facilitate unambiguous sequencing of the hybrid, redundant SV40 sequences were separated by cloning the right and left junction fragments separately into pBR322. Eco RI cleaves the vector at the two Sst I-Eco RI linkers liberating the λ arms, and produces two junction-containing fragments. The 4.6kb left junction fragment extends from the left Sst I-Eco RI linker to 0 in the SV40 insert, and the 4.3kb right junction fragment extends from this EcoRI site in SV40 to the Eco RI site in the flanking Ad2 DNA near the right junction. The resultant plasmids pBHEY-L and pBHEY-R (Figure 2) were used in sequence analyses.

Nucleotide Sequences around Ad2++HEY Junctions

The strategy used in sequencing the HEY junctions in pBHEY-L and pBHEY-R are illustrated in figure 2 and nucleotide composition is shown in figures 3 and 4. The most striking aspect of the HEY junctions was the

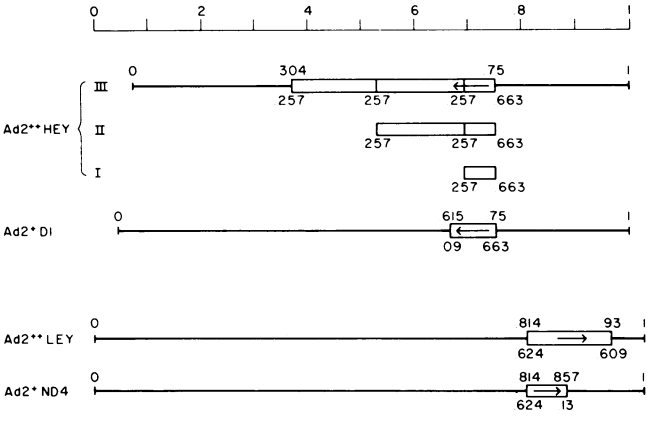

Figure 1. The structure of adenovirus-SV40 hybrids.

The structures of hybrid viruses are depicted according to: Kelly et al. (13) for Ad2^{++}HEY and Ad2^{++}LEY, Hassel et al. (14) for Ad2^{+}D1, Kelly and Lewis (37), Morrow et al. (38), Zain and Roberts (3) and Westphal (4) for Ad2^{+}ND4. The map units for adenovirus and SV40 DNAs are modified according to the data presented in this paper. To calculate adenovirus map units, 360 nucleotides were used as 1% equivalent of Ad2 genome. Numbers, shown above drawings, represent map units of Ad2^{++} genome; and below drawings, represent map units of SV40 genome. Ad2^{++}HEY and Ad2^{+}D1 are aligned at their right end because they possess common right Ad-SV40 junction; Ad2^{++}LEY and Ad2^{+}ND4 are aligned at their left end because they possess common left Ad-SV40 junction.
▭-SV40 DNA, —— Ad2 DNA. ⟶ - direction of SV40 early transcription.

abruptness with which the sequence changed from Ad2 to SV40 DNA. The junctions were unambiguous. The transition from Ad2 DNA sequence to SV40 occured at the nucleotide positions 10940 of Ad2 (20) and 3130 of SV40 DNA respectively (Figure 3). The right junction changes from SV40 DNA at the nucleotide position 15 to the Ad2 DNA at the nucleotide position 1283 (21). Some short runs of homology between the Ad2 and SV40 sequences were present on both sides of the joints, but these were no longer than 2 to 3 nucleotides. Some of the homologous sequences coincide with the AT-rich regions near the junctions. Previous studies have shown that AT rich sequences are often found near junctions that show no extensive homology in the parental sequences. Within 15 bp either side of the left junction, 22 nucleotides were AT bps. Fourteen were on the Ad2 side (93%) and 8 on the SV40 side (53%). The right junction was also AT rich. The AT pairs were distributed asymmetrically; the SV40 side was comprised of an AT bp stretch of 16 nucleotides. In contrast, the Ad2 side had only four AT basepairs within 15 basepairs of the junction (27%). The average AT content of Ad2

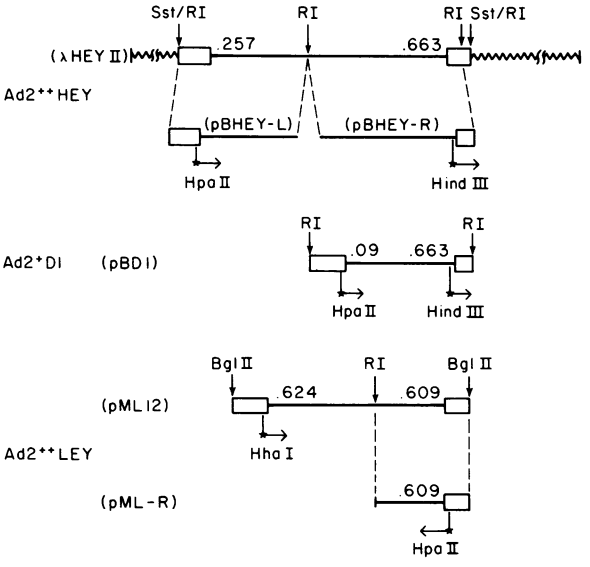

Figure 2. The structures of cloned Ad2-SV40 junctions, and sequencing strategies.

$Ad2^{++}$HEY fragment, produced by SstI digest cloned into the λ phage (λ-HEYII) Symbol Sst/RI denotes that near SstI site of Ad2 there is an EcoRI site of DNA. Recloning of $Ad2^{++}$HEY insert into two recombinant plasmids containing the left junction (pBHEY-L) and right junction (pBHEY-R). $Ad2^{+}$D1 fragment, produced by EcoRI digest cloned into the pBR322 (pBD1) $Ad2^{++}$LEY fragment produced by BglII digest cloned into pMK16 #1 (pML12). Right junction of $Ad2^{++}$LEY recloned into pMK16 #1 (pML-R).

DNAs were cut at the indicated sites and 5' or 3' termini were labeled using T4 kinase or Klenow fragment of E.coli DNA polymerase I.

and SV40 DNAs are 40-45% and 59% respectively. Each junction then has one side with a much higher than average AT content.

Cloning the Junctions of $Ad2^{+}$D1 Hybrids

In 1978, Hassell et al. (14) isolated a new defective virus from a stock of $Ad2^{++}$HEY. $Ad2^{+}$D1 contained less SV40 DNA than HEY and consisted of only one class of hybrid DNA and the Ad2 helper. Restriction mapping data demonstrates that 3 kb of SV40 from 0.09 to 0.66 is integrated between 61.5% and 74.5% of wild type Ad2. The sequences between 61.5 and 74.5% of Ad2 are missing (Figure 1).

Since there are no EcoRI sites in SV40 DNA between 0.09 and 0.66 map units, digestion of $Ad2^{+}$D1 with EcoRI produces a 4.4kb fragment containing both junctions. This fragment was purified and cloned into pBR322 yielding the plasmid pBD1 (Figure 2).

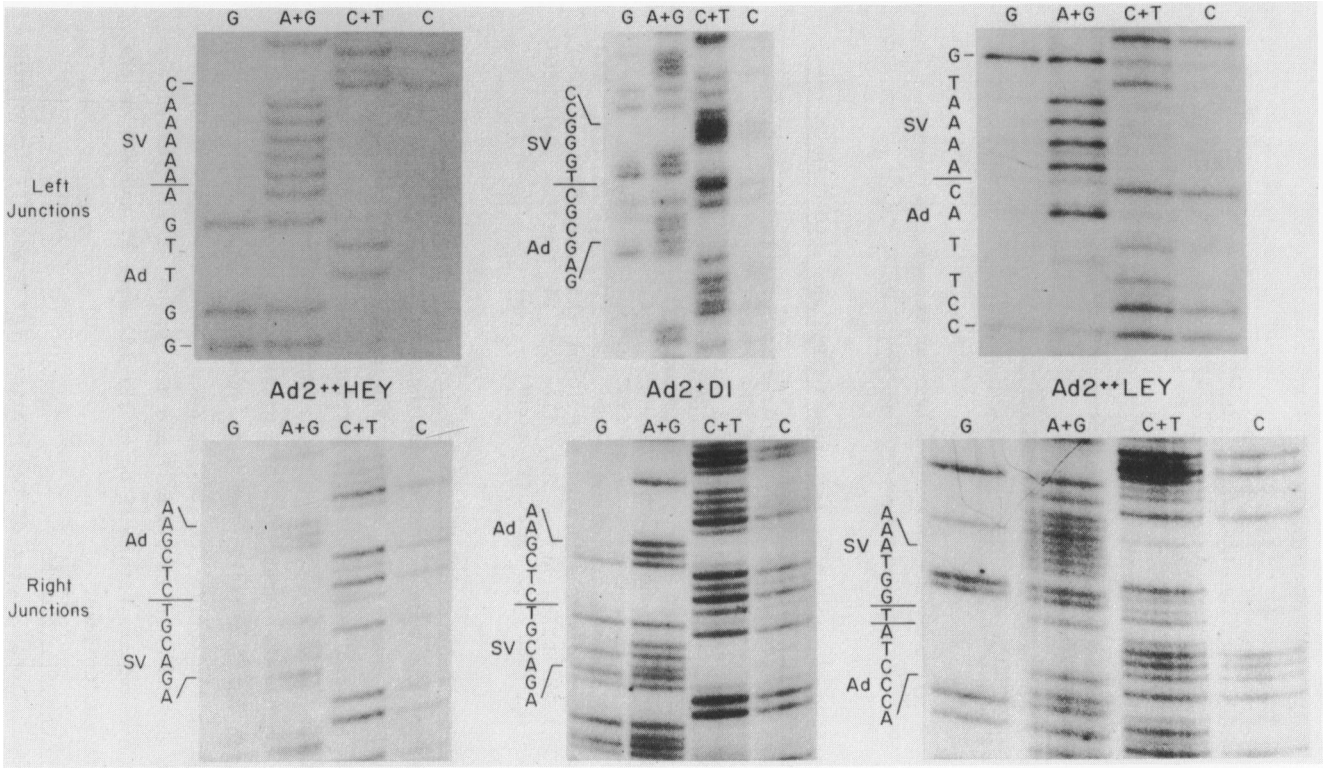

Figure 3. The nucleotide sequence of Ad2-SV40 junctions.
Six nucleotides of adenovirus and SV40 DNAs are shown at the junction sites of $Ad2^{++}$HEY, $Ad2^{+}$D1 and $Ad2^{++}$LEY hybrids. The line between two nucleotides shows the junction between two DNAs. In the case of $Ad2^{++}$LEY right junction two lines depicts an additional nucleotide T occurring between Ad2 and SV40 DNA.

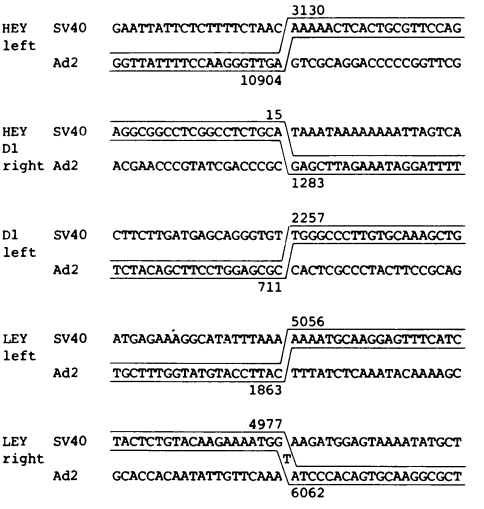

Figure 4. Comparison of nucleotide sequences of Ad2 and SV40 DNAs at the junctions of Ad2-SV40 hybrids.

All sequences are presented in 5' 3' direction, and they are oriented to correspond to left to right direction of the adenovirus chromosome. For this reason sequences of the Ad2^{++}HEY and Ad2^{+}D1 right junction shows complimentary strand to that on figure 3, and sequences of Ad2^{++}LEY right junction on figure 3 is read from top to bottom. The junction sequences are included between two horizontal lines. Numbers above or below sequences indicate the last nucleotide of SV40 or Ad2 DNA present at the junction. All SV40 numbers are given in SV numbering system (2). The adenovirus numbers are given according to: 10904 (Ad2^{++}HEY left) - (20) 1283 (Ad2^{++}HEY and Ad2^{+}D1 right) - (21) 711 (Ad2^{+}D1 left) - (22) 1863 (Ad2^{++}LEY left) - (23) and 6062 (Ad2^{++}LEY right) - (24)

Nucleotide Sequences at the Junctions of Ad2^{+}D1

Figure 2 shows the scheme for sequencing the D1 junctions in pBD1. Both junctions of D1 change from Ad2 to SV40 sequences without apparent homology between Ad2 and SV40 at the transition (Figures 3,4). The right junction of Ad2^{+}D1 is identical to the right junction of HEY, which supports the hypothesis that Ad2^{+}D1 originated from Ad2^{++}HEY by recombination with Ad2 DNA (14). The left junction occurs at nucleotide 2272 in SV40 and at nucleotide 711 (22) in Ad2. The left junction, unlike the right joint or those in HEY, was found not to be AT rich on either side. 30% of the basepairs within 15 bp on the Ad2 were AT and 40% on the SV40 side were AT nucleotides.

Cloning the Junctions of Ad2^{++}LEY

Ad2^{++}LEY populations consist of two hybrids, LEY I and II, containing 0.015 and 1.015 copies of SV40, respectively (Figure 1; only LeyII is

shown). The SV40 sequences are in the opposite orientation of those in $Ad2^{++}$HEY and $Ad2^{+}$D1. LEY II contains SV40 sequences in tandem repetition, and has adenovirus/SV40 junctions that are identical to those in LEY I.

Digestion of LEY DNA with Bgl II produces a 7kb fragment containing the complete SV40 sequences and flanking adenovirus sequences. This fragment was cloned into the BamHI site of pMK16#1, yielding the plasmid pML12 (Figure 2). pML12 contains both the left and right adenovirus/SV40 junctions. To facilitate unambiguous sequencing, a plasmid containing only the right junction was constructed. This was derived from pML12 after digestion with Eco RI which removed the left end of the hybrid sequence. Recircularization of the remaining plasmid sequences yielded pML-R, which contains the right Ad2-SV40 junction (Figure 2).

Sequence of the $Ad2^{++}$LEY Junctions

The sequence of the left junction was determined from pML12, and that of the right junction was obtained from pML-R. The strategies are diagrammed in figure 2. The left junction sequence revealed an unambiguous transition from adenovirus to SV40 DNA sequences. The joint occurs at nucleotide 1863 in Ad2 (23), and nucleotide 5056 in SV40. Interestingly, this is identical to the left junction of the non-defective hybrid Ad12+ND4 (4; Lee and Zain, personal communication), in agreement with the EM data obtained by Kelly et al (13). The right joint sequence shows an abrupt change from SV40 to Ad2 sequence, at nucleotide 4977 in SV40 and 6062 in Ad2 (24). In contrast to the other junctions, this joint contains one nucleotide which can be accounted for neither by SV40 or Ad2 adjacent sequences. Both the right and left junctions lie in AT rich regions of SV40 and Ad2. The parental SV40 sequences extending 15 bp to either side of the left junction site are 73% AT basepairs and the corresponding parental AD2 sequences are 70% AT. In the right junction, the parental SV40 Ad2 sequences are 70% and 63% AT, respectively.

DISCUSSION

Adenovirus-SV40 hybrids provide a model system to study nonhomologous recombination in eukaryotes. Since all of SV40 DNA and the junction sites in adenovirus 2 DNA have been sequenced, the origins of sequences in the hybrid recombinants could be identified. By comparing the parental adenovirus and SV40 DNA to the junction sequences of $Ad2^{++}$HEY, $Ad2^{+}$D1, and $Ad2^{++}$LEY, the location of the junctions were established unambiguously. The exact position of all the junctions could be defined because the

transition from adenovirus DNA to SV40 at these joints were abrupt. The unambiguous transition from SV40 to adenovirus 2 sequences in each of the junctions indicates that the two parent DNAs shared no common sequences at this site. In the surrounding sequences, only homologies of 3 bp or less were seen. Thus the recombination responsible for forming these joints appears to have occurred without homologous basepairing. Formally, this recombination looks like the joining of two free ends of adenovirus and SV40 DNAs by a sequence independent ligase. The ability of eukaryotic cells to promote such ligation was demonstrated by circularization of linear SV40 DNA during infection of monkey cells. Subramanian (25) used pairs of different restriction endonucleases to linearize SV40 prior to transfection so that the overhanging ends left by the restriction enzymes did not match. Nevertheless, these molecules were circularized in vivo by blunt-end ligation of the two nonhomologous ends. When permissive cells were infected with linear SV40 DNA from which the cohesive termini have been removed by S1 nuclease, cyclization of the linear DNA molecules occured intracellularly by a joining at or near the ends of the molecule (26). Two of the deletion mutants were produced by blunt-end ligation, three other mutants had one to four nucleotide common core sequences at the recombinant joint, and the last three had four to eight extra nucleotides present at the recombination joint (26). The ligation of SV40 and Ad2 sequences in $Ad2^{++}$HEY and $Ad2^{+}$D1 may represent the action of the nonspecific ligase similar to that which was involved in the circularizations of SV40 DNA. The extra nucleotide at the right junction of $Ad2^{++}$LEY could have a similar origin as extra nucleotides in SV40 mutants. Hybrid formation, however, occurs at a much lower frequency than the self-ligation of linear SV40, perhaps because hybrid formation requires intermolecular ligation or because free ends are generated very infrequently in vivo. An alternative mechanism could involve the integration of SV40 DNA into adenovirus DNA by illegitimate recombination. Recently, in vitro recombination mediated by DNA gyrase of Escherichia coli was described where circular pBR322 DNA was integrated into λ DNA (27). The analysis of recombinant junctions revealed that: 1) integration of pBR322 into DNA occurred randomly and within the nonhomologous DNA sequence; 2) seven out of nine recombinants suffered deletions on one of the genomes; and 3) the end point of deletions coincided with one end of the pBR322 insertion. Similar observations have been made in studies of adenovirus-SV40 recombinants. Furthermore, it was recently reported that

eukaryotic topoisomeraseI cleaves SV40 DNA at many sites, and these sites are specific for both human and calf enzymes (28). One can speculate that recombination between adenovirus and SV40 DNAs could be mediated by eukaryotic topoisomerases.

A constraint on hybrid formation is that the generated structures must be viable. Even if recombination does occur often between Ad2 and SV40, it is possible that only a very few of the recombinants have a viable genetic structure. The Ad2-SV40 hybrids evolved during high multiplicity passage of adenovirus 2 in simian cells (29). Since both genomes are able to replicate in these cells, the recombination might have occurred between circular, bidirectionally replicating SV40 and linear Ad2 DNAs. Although the primary recombination event must have been intermolecular, the junctions in the present structure may have been formed during a secondary intramolecular recombination. We have tried to minimize this possibility by studying defective hybrids rather than nondefective hybrids. A secondary intramolecular recombination event is unlikely in the case of $Ad2^{+}D1$ because the structure can be explained most simply by a single intermolecular recombination event. Since $Ad2^{+}D1$ was isolated from a stock of $Ad2^{++}HEY$ and its right junction was near that of $Ad2^{++}HEY$ by restriction mapping, Hassell et al. (14) suggested that $Ad2^{+}D1$ was produced by a recombination event between Ad2 and HEY II or III. Our data showing that the right junctions of $Ad2^{+}D1$ and $Ad2^{++}HEY$ are identical strongly supports this idea. It is very likely that $Ad2^{+}D1$ was formed by intermolecular recombination between linear adenovirus 2 and $Ad2^{++}HEY$ DNAs. The simplest recombination event would have been between the left end 0.615 of Ad2 DNA and 0.09 of the SV40 insert in HEY II or III. The evolution of $Ad2^{+}D1$ demonstrates that intermolecular recombination between linear DNA molecules can occur without homologous basepairing at the recombination junction. A similar situation exists in the case of $Ad2^{++}LEY$ and $Ad2^{+}ND4$ viruses. Because the left joint of $Ad2^{++}LEY$ (Figure 4) and $Ad2^{+}ND4$ (4; Lee and Zain, pers. comm.) are identical, the evolution of ND4 could be explained by recombination between $Ad2^{++}LEYII$ and Ad2 DNAs. Therefore the right junction of $Ad2^{+}ND4$ probably represents another example of nonhomologous recombination between two linear DNAs. Sequence analysis of the 6 revertants of the $Ad2^{+}ND1$ mutant 71, revealed that new Ad2-SV40 junctions produced by illegitimate recombination between two 71 mutant molecules also are unambiguous transitions from Ad2 DNA to SV40 (Tooze, 1982, p. 605, and p. 1060 (2), J. Sambrook, personal communication).

$Ad2^{+}D1$ is interesting in terms of transcription because it lacks the bonafide SV40 promoter for T antigen. Although $Ad2^{+}D1$ produces normal size T antigen, (14) which complements the replication of SV40 tsA mutant at the nonpermissive temperature (Gluzman, unpublished), it lacks the SV40 promoter region necessary for early transcription including the Hogness box which determines the 5' start and the upstream sequences necessary for transcription (30,31,32). The fact that $Ad2^{+}D1$ is viable and expresses T antigen implies that it is using an adenovirus promoter. The only adenoviral promoter in this area and in the correct orientation is the E2 early promoter. The junction between SV40 and Ad2 DNAs is located 176 nucleotides downstream from the cap site for mRNA of 72k protein (33). Two observations support the idea that T antigen is expressed using the E2 promoter. First, Hassell et al. (14) noted that D1 produced SV40 mRNA early in infection. Secondly, the plasmid pBD1, which codes for T antigen, was used to transform rat cells (Frisque and Gluzman, unpublished). Although the efficiency of transformation was 50-100 fold lower than that of wild-type DNA, the established lines produced SV40 T antigen of normal size. When these rat transformed cells were infected with Ad2, the level of T antigen expressed 24 hours later increased 5 fold. It is known that the E2 promoter requires the Ad2 E1A gene product for efficient transcription (34,35,36). Presumably, the infecting Ad2 provides the 1A gene. The basal level of T antigen produced before Ad2 infection would then be due to a weak activity of E2 promoter. Ad2 infection does not normally increase T antigen expression; in rat cells transformed by wild-type SV40 the expression of T antigen decreases 2 fold 24 hours after infection with adenovirus 2 (Frisque and Gluzman, unpublished).

Since other junctions formed without extensive homology often have a high AT content (7,8,10), we also compared the AT content of the $Ad2^{++}HEY$, $Ad2^{+}D1$, and $Ad2^{++}LEY$ junctions to the overall composition of SV40 and Adenovirus 2 DNA. In all but the left junction of $Ad2^{+}D1$, the percentage of AT bp was higher than average on at least one side of the junction. The significance of the AT-rich regions flanking all but one of the junctions is not known. This feature was also observed in the junctions of SV40 variants (7,8) and in the junctions of nondefective Ad2-SV40 recombinants (3,4, Zain, pers. comm.). AT rich sequences are more easily melted to produce single stranded regions. Perhaps the single strands are prone to breakage, or these regions may also be recognized by endonucleases or breaking and closing enzymes.

The analysis of the recombinant junctions in different systems reveals a great variety of final structures, which suggest that multiple pathways are available for recombination. The sequence analyses described here provide several examples of recombination without homologous basepairing in eukaryotic cells. These events are infrequent compared to homologous recombination between infecting viruses, but they may be important in processes such as a viral integration during transformation, SV40 variant genome formation, integration of transformed DNA into the cell genome, and recombination between other nonhomologous viruses.

ACKNOWLEDGEMENTS

We thank Drs. J. Stringer, S. Hughes and J. Sambrook for their critical reading of this manuscript. This work was supported by NCI grant CA 13106.

REFERENCES

1. Boursnell, M.E.G. and Mautner, V. (1981) Virology 112, 198-209.
2. Tooze, J. (1982) Cold Spring Harbor Lab., Cold Spring Harbor, N.Y.
3. Zain, B.S. and Roberts, R.J. (1978) J. Mol. Biol. 120, 13-31.
4. Westphal, H. (1981) J. Virol. 40, 526-532.
5. Gluzman, Y. and VanDoren, K. (1983) J. Virol. 45, in press.
6. Gutai, M.W. and Nathans, D. (1978) J. Mol. Biol. 126, 259-274.
7. Gutai, M.W. and Nathans, D. (1978). J. Mol. Biol. 126, 275-288.
8. Gutai, M.W. (1981) Virology 109, 344-352.
9. Botchan, M., Stringer, J., Mitchison, T. and Sambrook, J. (2980) Cell 20, 143-152.
10. Stringer, J.R. (1981) J. Virol. 38, 671-679.
11. Stringer, J.R. (1982) Nature 296, 363-366.
12. Bullock, P. and Botchan (1982) Gene Amplification, ed. R.T. Schimke, pp. 215-224. Cold Spring Harbor Laboratory, N.Y.
13. Kelly, T.J. Jr., Lewis, A.M. Jr., Levine, A.S. and Siegal, S. (1974) J. Mol. Biol. 89, 113-126.
14. Hassel, J.A., Lukanidin, E., Fey, G. and Sambrook, J. (1978) J. Mol. Biol. 120, 209-247.
15. Hirt, B. (1967) J. Mol. Biol. 26, 365-369.
16. Benton, W.D. and Davis, R. (1977) Science 196, 180-182.
17. Gluzman, Y., Frisque, R.D. and Sambrook, J. (1980a) Cold Spring Harbor Symp. Quant. Biol. 44, 293-300.
18. Maxam, A.M., and Gilbert, W. (1977) Proc. Natl. Acad. Sci. 74, 560-564.
19. van Roy, F. and Fiers, W. (1978) J. Mol. Biol. 126, 691-720.
20. Gingeras, T.R., Sciaky, D., Gelinas, R.E., Bing-Dong, J., Yen, C.E., Kelly, M.M., Bullock, P.A., Parson, B.L., O'Neill, E., and Roberts, J.R. (1982) J. Biol. Chem., in press.
21. Galibert, F., Herisse, J. and Courtois, G. (1979) Gene 6, 1-22.
22. Akusjarvi, G., Zabielski, J., Perricaudet, M. and Pettersson, U. (1981) Nucl. Acid Res. 9, 1-17.
23. Herisse, J., Courtois, G. and Galibert, F. (1980) Nucl. Acid Res. 8, 2173-2192.
24. Herisse, J., Rigolet, M., Dupont de Dinechin, S. and Galibert, F. (1981) Nucl. Acid Res. 9, 4023-4042.

25. Subramnian, K.N. (1979) Proc. Natl. Acad. Sci. USA 76, 2556-2560.
26. Gutai, M.W. (1981) Virology 109, 353-365.
27. Ikeda, H., Aoki, K. and Naito, A. (1982). Proc. Natl. Acad. Sci. USA 79, 3724-3728.
28. Edwards, K.A., Halligan, B.D., Davis, J.L., Nivera, N.L. and Liu, L.F. (1982) Nucl. Acid Res. 10, 2565-2576.
29. Hartley, J.W., Hubner, R.J. and Rowe, W.P. (1956) Proc. Soc. Exp. Biol. Med. 92, 667.
30. Benoist, C. and Chambon, P. (1981) Nature 290, 304-315.
31. Ghosh, P.K., Lebowitz, P., Frisque, R.J., and Gluzman, Y. (1981) Proc. Natl. Acad. Sci. USA 78, 100-104.
32. Gruss, P., Dhar, R. and Khoury, G. (1981) Prlc. Natl. Acad. Sci. USA 78, 943-947.
33. Baker, C.C., Herisse, J., Courtois, G., Galibert, F. and Ziff, E. (1979) Cell 18, 569-580.
34. Berk, A.J., Lee, F., Harrison, T., Williams, J., and Sharp, P.A. (1979) Cell 17, 935-944.
35. Jones, N. and Shenk, J. (1979) Proc. Natl. Acad. Sci. USA 76, 3665-3669.
36. Rossini, M., Jonak, G.J. and Baserga, R. (1981) J. Virol. 38, 982-986.
37. Kelly, T.J., Jr. and Lewis, A.M., Jr. (1973) J. Virol. 12, 643-652.
38. Morrow, J.F., Berg, P., Kelly, T.J., Jr., and Lewis, A.M., Jr. (1973) J. Virol. 12, 653-658.