JOURNAL OF VIROLOGY, May 1981, p. 671-679
0022-538X/81/050671-09$02.00/0

Vol. 38, No. 2

Integrated Simian Virus 40 DNA: Nucleotide Sequences at Cell-Virus Recombinant Junctions

JAMES R. STRINGER

Cold Spring Harbor Laboratory, Cold Spring Harbor, New York 11724

Received 15 December 1980/Accepted 13 February 1981

DNA fragments containing the integrated viral DNA present in the simian virus 40 (SV40)-transformed rat cell lines SVRE9 and SVRE17 were cloned in procaryotic vectors, and the DNA sequences linking SV40 and cell DNA were determined. Comparison of the DNA sequences at the SV40-cell junctions in SVRE9 and SVRE17 cells with those of a previously characterized viral insertion from SV14B cells shows that no specific viral or cellular sequences occur at SV40-cell junctions and that the cellular DNA sequences adjacent to integrated SV40 DNA do not display the direct repeat structure characteristic of transposons and retrovirus proviruses.

Transformation of cells by simian virus 40 (SV40) is stabilized by integration of all or part of the viral genome into that of the cell. Previous studies (4, 5, 7, 14), in which the sizes of restriction endonuclease fragments bearing SV40 DNA were determined by Southern blot analysis of DNA extracted from a variety of rat and mouse transformed cell lines, have shown that integration of SV40 DNA is nonspecific with respect to the position on the SV40 genome at which viral DNA is linked to cellular DNA. These studies also suggested that SV40 DNA does not integrate at specific sites in the cellular genome. However, this conclusion is subject to two caveats. First, short stretches of specific cellular DNA sequence could border viral insertions and not be detected by restriction endonuclease analysis. Second, cellular DNA could be altered upon SV40 DNA integration. Thus, integration at the same site in the cellular genome could give rise to restriction fragments of various sizes if different alterations occurred in the surrounding cellular DNA in different cell lines. This possibility gains credence from recent experiments in which molecular cloning was used to isolate a DNA fragment which contains the viral insertion and flanking cellular sequences in cell line SV14B (3, 21). Analysis of the cloned DNA fragment revealed that in SV14B cells a rearrangement of cellular sequences had occurred at the site of SV40 insertion.

In the study reported here, I cloned the SV40 DNA insertions present in the genomes of two transformed rat cell lines, SVRE9 and SVRE17, and have analyzed these insertions by restriction endonuclease digestion and DNA sequencing. The results of these experiments, combined with those obtained with the cell line SV14B referred to above, show that integrated SV40 DNA can be linked to cellular DNA at numerous positions on the viral genome without sequence specificity. The cellular DNA sequences adjacent to integrated SV40 DNA are also nonspecific and do not display the repeat structures characteristic of transposons or retrovirus proviruses.

MATERIALS AND METHODS

Cell lines. The SVRE9 and SVRE17 cell lines were first isolated by Pollack et al. (19) after infection of primary cultures of Fisher rat embryo cells with virions of a thrice plaque-purified stock of SV40 strain 776. SV14B cells were obtained by infecting a continuous line of Fisher rat cells with SV40 DNA (4).

DNA cloning. The lambda phage λgtWESλB has been described by Leder et al. (15). The phage was modified to accept *Sst*I fragments as follows. λgtWESλB DNA was digested with restriction endonuclease *Eco*RI, and the large terminal fragments (λ-arms) were prepared by sucrose density centrifugation as described by Maniatis et al. (16). A short DNA fragment containing λ DNA from the *Sst*I site at 53.9 map units to the *Eco*RI site at 54.3 map units of the λ genome was isolated by polyacrylamide gel electrophoresis and used as a linker between the *Eco*RI termini of the λ-arms and DNA fragments produced by cleavage with *Sst*I. A mixture of WESB λ-arms, *Sst*I-RI linkers, and *Sst*I-digested Charon 4A was ligated, and the ligation products were packed in vitro as described by Blattner et al. (2) and Maniatis et al. (16). A resultant phage was plaque purified, propagated, and used to prepare λ-arms with *Sst*I termini by the method of Maniatis et al. (16). Cellular DNA was prepared as described previously (4). DNA fragments bearing SV40 sequences were cloned from transformed cell DNA by ligation of *Sst*I digests of total cell DNA to *Sst*I-terminal λ-arms, followed by packaging in vitro and screening for SV40 by the method of Benton and Davis (1).

Restriction endonuclease analysis. Restriction

endonucleases were purchased from either Bethesda Research Laboratories, Inc., Rockville, Md., or New England Biolabs, Inc., Beverly, Mass., and DNA digestions were done under the conditions specified by the enzyme supplier. Samples of digested DNA were electrophoresed on agarose gels and transferred to nitrocellulose by the method of Southern (24). SV40 DNA sequences were detected by hybridization to radioactive SV40 DNA followed by radioautography as described previously (4).

DNA sequencing. DNA sequences were determined by the method of either Maxam and Gilbert (18) or Sanger et al. (22). Both SV40-cell junctions in SVRE9 were analyzed by the Maxam and Gilbert method. The junction at nucleotide 2320 (see Fig. 3) was sequenced from the SV40 *Hae*III site at nucleotide 2242 (SV40 sequence numbers are by Buchman et al.; see reference 25). The SVRE9 junction at nucleotide 475 was sequenced from a cellular *Hin*dIII site within 15 base pairs of the joint. The SVRE17 junction at nucleotide 1502 in Fig. 3 was sequenced by the Maxam and Gilbert technique. DNA was labeled at a *Hin*fI site in the cell DNA. The other junction in SVRE17 was sequenced by the chain termination method of Sanger et al. (22). λSst-17 DNA was digested with exonuclease III and annealed to an SV40 DNA primer, consisting of SV40 sequences from nucleotide 418 to nucleotide 453. The primer was extended by the *Klenow* fragment of DNA polymerase I from *Escherichia coli* in the presence of different dideoxynucleotide triphosphates, and the products were separated by thin-gel electrophoresis.

RESULTS

Molecular cloning of the viral insertions present in SVRE9 and SVRE17 cells. Both SVRE9 and SVRE17 contain a single insertion of SV40 DNA. A Southern blot analysis of the DNA fragments produced by digestion of total DNA from these lines with the restriction endonuclease *Sst*I is shown in Fig. 1. In each case, a single DNA fragment hybridized to radioactive SV40 DNA. The *Sst*I DNA fragments which contain the viral insertions were cloned by ligating *Sst*I-digested samples of total cellular DNAs to a modified λgtWESλB phage vector which would accept *Sst*I fragments. Recombinant phage were screened for the presence of SV40 DNA sequences by hybridization with radioactive SV40 DNA. The cloned DNA fragments obtained from the recombinant phage comigrated on agarose gels with the SV40-containing *Sst*I fragments detected in DNA from SVRE9 and SVRE17 cells (Fig. 1).

Arrangement of the integrated SV40 DNA in SVRE9 cells and SVRE17 cells. Physical maps of the DNA fragments cloned from SVRE9 and SVRE17 cells were determined by restriction endonuclease digestion, followed by agarose gel electrophoresis, Southern blotting, and hybridization to radioactive SV40

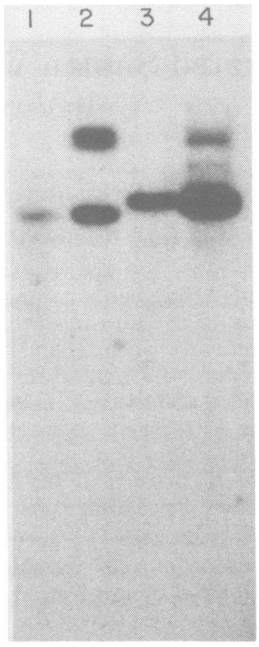

FIG. 1. *Detection of DNA fragments containing SV40 sequences in cell lines SVRE17 and SVRE9 and their identity with fragments cloned from these cells. Ten micrograms of total cellular DNA was digested with SstI. The fragments were separated by electrophoresis through 0.7% agarose, and the viral sequences were detected by blotting and hybridization as described by Southern (24). A 100-pg amount of recombinant λ-phage DNAs bearing the SV40 DNA sequences cloned from cell lines SVRE17 and SVRE9 were digested with SstI and run in parallel with cellular DNA samples. The cloned DNA fragments comigrated with the fragments in total cellular DNA which hybridize to SV40 DNA. The low mobility bands in the λ-phage reconstruction migrate with the λ vector arm fragments and are partial digestion products. Tracks: (1) SVRE17 DNA digested with SstI; (2) SstI digest of a recombinant phage bearing the SV40 DNA insertion from SVRE17 cells; (3) SVRE9 DNA digested with SstI; (4) SstI digest of a recombinant phage bearing the SV40 DNA insertion from SVRE9 cells.*

DNA. The results of some of these experiments are shown in Fig. 2, and structures of the viral insertions are diagrammed in Fig. 3. All numbering of SV40 sequences is by Buchman et al.; see reference 25.

The SV40 insertion in SVRE9 cells is a partial tandem repeat of viral sequences. This structure was deduced from the following observations. Digestion of the DNA from the λ-clone carrying SVRE9 sequences (λSst-9) with *Eco*RI produced three DNA fragments which hybridized to SV40 DNA (Fig. 2A). The largest of these

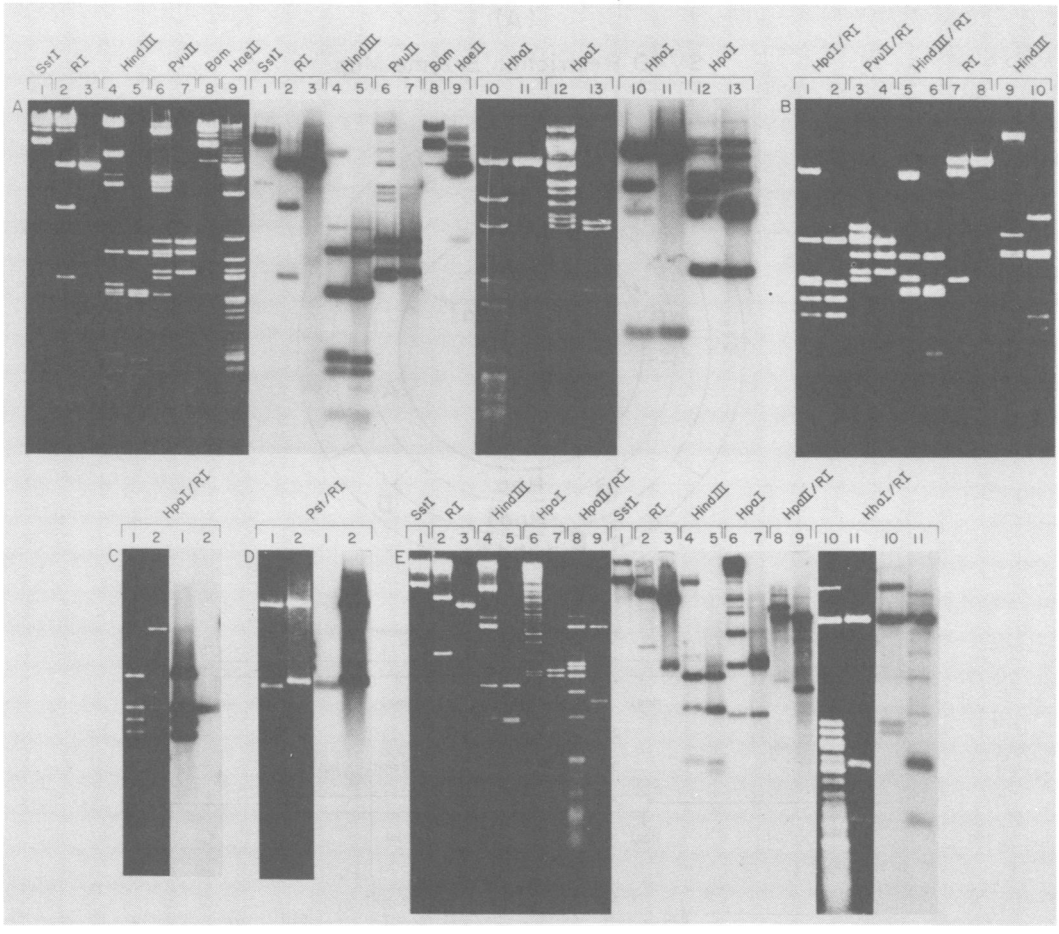

FIG. 2. *Electrophoretic patterns of DNA fragments produced by restriction endonuclease digestion of recombinant lambda phages λSst-9 and λSst-17. (A) DNA from λSst-9 and SV40 DNA were digested with the indicated enzymes, electrophoresed on 1.0% agarose, stained with ethidium bromide, transferred to nitrocellulose and hybridized to radioactive SV40 DNA to reveal the DNA fragments containing SV40 sequences. SV40 DNA markers are in tracks 3, 5, 7, 11, and 13. White on black gels are ethidium stained. Black on white gels are autoradiograms of Southern blots of the same gels. (B) DNA from a recombinant pBR322 plasmid containing the largest and smallest EcoRI fragments present in λSst-9 DNA (A; track 2). Tracks 1, 3, 5, 7, and 9 are digests of the recombinant plasmid DNA, and tracks 2, 4, 6, 8, and 10 are SV40 DNA fragments. (C) DNA from a recombinant pBR322 which contains the second largest EcoRI fragment in λSst-9 DNA. Track 1 is SV40 DNA; track 2 is recombinant plasmid DNA. (D) DNA from a recombinant pBR322 which contains the smallest EcoRI fragment in λSst-9 DNA. Track 1 is plasmid DNA, and track 2 is SV40 DNA. (E) DNA from λSst-17 and SV40 DNA were digested with the indicated enzymes and treated as described for (A). Tracks 1, 2, 4, 6, 8, and 10 are λSst-17 DNA, and tracks 3, 5, 7, 9, and 11 are SV40 DNA.*

fragments comigrated with form III SV40 DNA, suggesting that the *Eco*RI site in circular SV40 DNA was present twice in the linear array of SV40 DNA in λSst-9. The SV40 form III DNA marker in Fig. 2A, track 3 migrated a little faster than the largest SV40-containing band from λSst-9 DNA shown in track 2. This apparent size difference is not real, but is probably due to the large amount of λ-phage vector DNA present in the λSst-9 DNA sample. When a mixture of form III SV40 DNA and *Eco*RI-digested λSst-9 DNA was electrophoresed through an agarose gel, there was no difference in the mobilities of form III SV40 DNA and the largest SV40-containing *Eco*RI fragment in λSst-9 DNA (data not shown). Corroborating evidence for a tandem repeat arrangement was the presence (in λSst-9) of all the SV40 DNA fragments produced by *Hin*dIII, *Pvu*II, *Hpa*I, and *Hha*1 (Fig. 2A). Confirmation that the largest *Eco*RI fragment

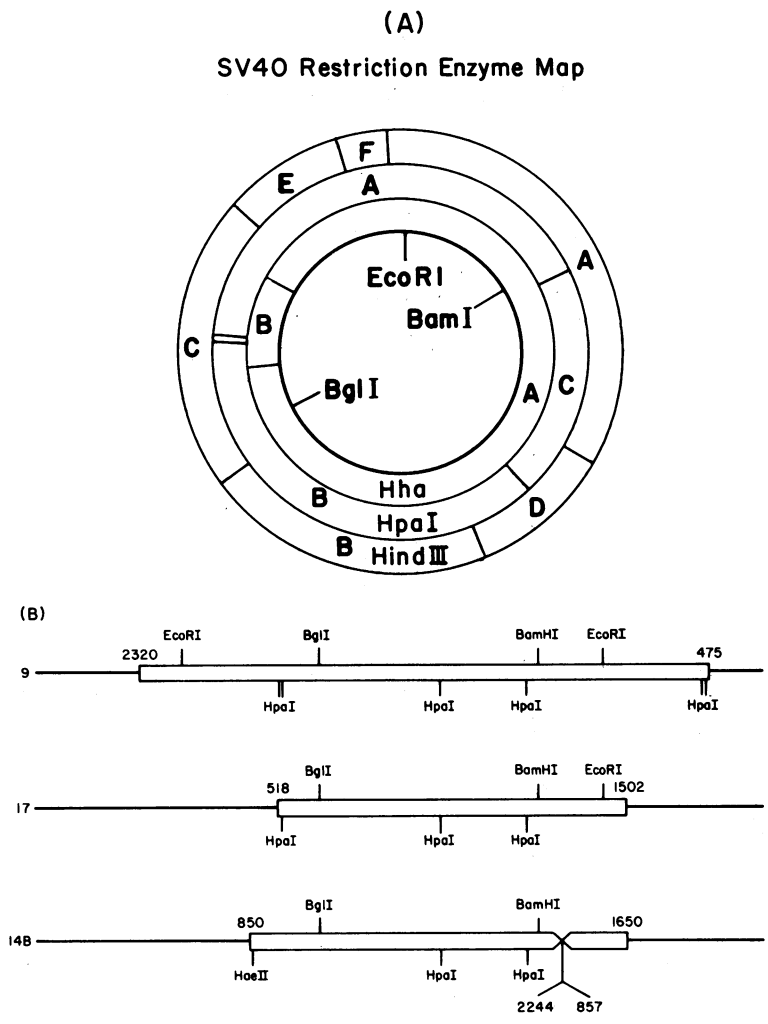

FIG. 3. *Physical maps of SV40 DNA and of the DNA fragments cloned from SVRE9, SVRE17, and SV14B cells. (A) restriction endonuclease map of SV40 DNA. (B) DNA fragments cloned from transformed cells. Open bars represent SV40 sequences and are drawn to scale. Lines represent flanking cellular sequences and are not drawn to scale. The numbers at the SV40-cell junctions indicate the nucleotide numbers in the SV40 DNA sequence at which the viral genomes join cellular DNA. The numbering is by Buchman et al. (see reference 25). The structure of the SV14B viral insertion is reproduced from Botchan et al. (3). The viral insertion in SV14B consists of two segments of viral DNA which are separated by 40 nucleotides of nonviral sequence. The position of this junction is indicated by the indentation in the open bar. The endpoints of viral sequences separated by the 40 nucleotides of nonviral DNA are indicated by the numbers below the indentation.*

from λSst-9 is identical to form III SV40 DNA was obtained by subcloning this fragment into the plasmid pBR322. Double digestions of this plasmid with *Eco*RI and either *Hpa*I, *Pvu*II, or *Hin*dIII showed the identity of the subcloned fragment with SV40 DNA linearized with *Eco*RI (Fig. 2B). The plasmid DNA used in the experiments shown in Fig. 2B contained both the largest and smallest *Eco*RI fragments from λSst-9 DNA. The smallest λSst-9 *Eco*RI fragment is visible in each of the tracks containing recombinant plasmid DNA. This fragment was not digested with either *Hpa*I, *Pvu*II, or *Hin*dIII, and its presence did not interfere with the interpretation of these experiments.

The extent to which the viral insertion extends beyond the two *Eco*RI sites in λSst-9 DNA was estimated by restriction endonuclease analysis of the two *Eco*RI fragments which migrated faster than form III SV40 DNA (see Fig. 2A,

track 2). Each of these two fragments was subcloned in pBR322 and analyzed as shown in Fig. 2C and D. Digestion of the larger subcloned *Eco*RI fragment with RI and *Hpa*I together generated a single fragment which hybridized to SV40 DNA and comigrated with the 1,263-base-pair DNA fragment, corresponding to viral DNA between the *Hpa*I site at nucleotide 502 and the *Eco*RI site at nucleotide 1765 (Fig. 2C). The absence of a second detectable SV40-containing DNA fragment of novel size indicated that viral sequences did not continue much past the *Hpa*I site at nucleotide 502. Therefore, one endpoint of the viral insertion in λSst-9 DNA is close to the *Hpa*I site at nucleotide 502. That the other endpoint of the viral insertion in λSst-9 extends beyond the *Pst* site at nucleotide 1971 was indicated by the fact that digestion of the small *Eco*RI subcloned fragment with *Eco*RI and *Pst* together produced two DNA fragments containing the SV40 sequence: one which comigrated with the SV40 marker DNA corresponding to the viral sequence from the *Eco*RI site to the *Pst* site at nucleotide 1971 and another larger fragment of novel size (Fig. 2D). The viral insertion must end before the *Bam*HI site at nucleotide 2516 because digestion of λSst-9 DNA with *Bam*HI did not produce a fragment the size of linear SV40 DNA (Fig. 2A, track 8).

The arrangement of the viral sequences in the DNA fragment cloned from SVRE17 cells (λSst-17) was deduced from the data shown in Fig. 2E. Digestion of λSst-17 DNA with *Eco*RI produced two SV40-containing DNA fragments of novel size, indicating that a single *Eco*RI site is present in the viral insertion. The largest stretch of SV40 DNA sequence present in λSst-17 DNA is the 3,790 base pairs between the *Hpa*II site at nucleotide 329 and the *Eco*RI site at nucleotide 1765. Digestions with *Hha*I, *Hpa*I, and *Hin*dIII localized the endpoints of the viral insertion as follows. The presence of the SV40 *Hin*dIII fragment A and absence of the *Hin*dIII fragments C, E, and F (Fig. 2E) indicated that viral sequences continue past the *Eco*RI site at least as far as the *Hin*dIII site at nucleotide 1691 but end before the next *Hin*dIII site at nucleotide 1476. The other end of the SV40 insertion was positioned between the *Hha*I site at nucleotide 816 and the *Hpa*I site at 482. As shown in Fig. 2E, λSst-17 does not contain the small *Hha*I fragment of SV40 but does contain the viral *Hpa*I fragment B.

DNA sequences linking SV40 and cellular genomes in SVRE9 and SVRE17 cells. The DNA sequences which link viral and cellular DNA were determined either by the method of Maxam and Gilbert (17) or by the chain termination technique of Sanger et al. (22). Each viral insertion ends at a different point on the SV40 map. In Fig. 3 the SV40 nucleotide numbers at which viral sequences are joined to cellular DNA are shown. The sequences at the four virus-cell junctions in SVRE9 and SVRE17 cells are shown in Fig. 4 together with the SV40-cell junctions in line SV14B, as previously described by Botchan et al. (3). In Fig. 4A is shown the sequence of one DNA strand of each insertion running left to right from cell sequence into viral sequence and back out to cell sequence in 5′ to 3′ polarity. The cellular DNA sequences which abut the SV40 insertions are all different, and they are not repeated in either direct or inverse orientation. Fig. 4A also shows that different SV40 sequences occur at the end of each insertion. This is more visible in Fig. 4B, in which all of the virus-cell junctions are displayed in alignment and in the same polarity. Four junction sequences for cell line SV14B are shown in Fig. 4B. The first two lines of SV14B sequence correspond to those at the ends of the complete viral insertion. The next two lines of SV14B sequence are those found interposed at the junction of the two tracts of SV40 DNA which comprise the complete viral insertion in these cells (see the legend to Fig. 3 and reference 3).

If the sequences linking viral and cellular DNA in these cell lines are the direct result of the primary integration event, then SV40 DNA does not integrate at a specific sequence in either the viral or cellular DNA. The question arises as to whether there are other, less specific sequence factors, such as unusual nucleotide base composition or repetitious sequence organization, which are common to the sequences of virus-cell recombinant joints. The compositions of the first 12 nucleotide bases on either side of recombinant junctions are shown in Fig. 4C. The average base composition of SV40 DNA is 59.3% adenine plus thymine (A+T) (11). A+T nucleotide residues occur more frequently than this in the SV40 sequences adjacent to recombinant joints in five of six cases. However, A+T richness is generally not a feature of the adjoining cellular sequences or the SV40 sequences which may have participated in the recombination event.

Two virus-cell junctions show strikingly unusual DNA sequence organization. The junction sequences shown in lines 1 and 4 of Fig. 4B are both composed of a simplified set of nucleotides. The SVRE17 junction shown in line 4 of Fig. 4B has 27 cytosines and thymidines in a row; the SVRE9 junction shown in line 1 of Fig. 4B has 22 consecutive cytosines and adenosines. Within these stretches of simplified base composition there are direct sequence repeats. In SVRE17,

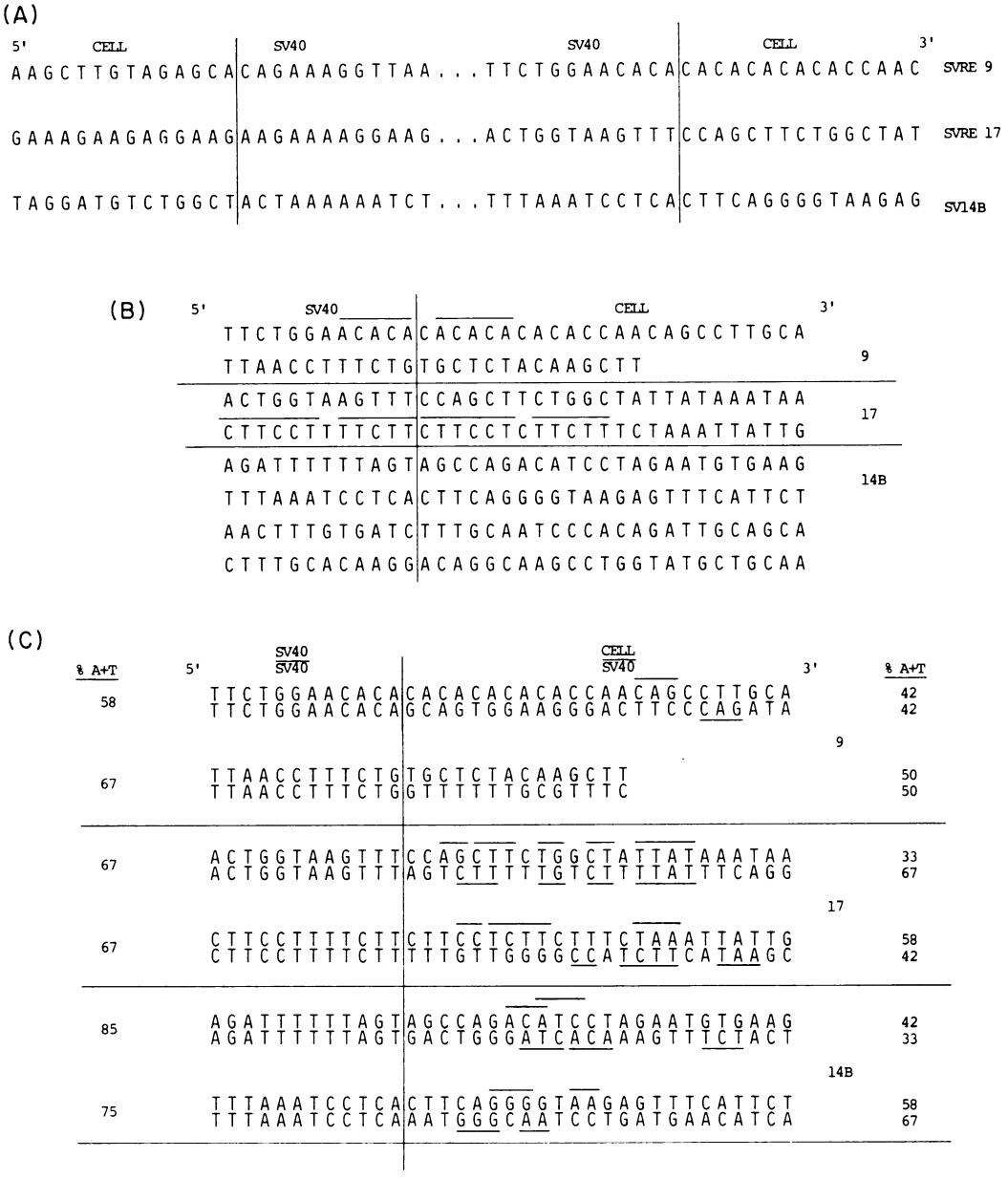

FIG. 4. *DNA sequences linking SV40 DNA to cellular DNA. (A) Sequences of one strand of DNA from each insertion running from cellular sequences into the viral insertion, through the viral sequences and back into cell DNA in 5' to 3' orientation. (B) Six SV40-cell junctions written in the same polarity. There are two junctions from SVRE9, two from SVRE17, and four from SV14B. The top two SV14B junctions are those which bound the complete viral insertion; the bottom two lines of SV14B sequence are those at the junction of the two segments of SV40 DNA which comprise the whole SV14B insertion (see Fig. 3 and reference 3). Sequences overscored with lines are directly repeated on either side of the SV40-cell junction. (C) Sequences at SV40-cell junctions compared with SV40 sequences which would have participated in the integration event. The top line of each doublet is the sequence of the SV40-cell junction; the bottom lines are the parental SV40 sequences. Possible remnants of parental SV40 sequences are overscored with lines and the putative donor sequences are underscored. The percentage of adenosines and thymidines in the parental SV40 sequences and the cell sequences adjacent to SV40 insertions are indicated in the margins. These numbers were calculated for the 12 proximal residues on either side of each junction.*

the terminal SV40 sequences, CTTCCT and TTCTT, are directly repeated in the adjacent cellular DNA. In SVRE9, the sequence ACACA is repeated on either side of the virus-cell junction. Whether or not these repeat structures are significant is unknown, but it is interesting that in immunoglobulin gene rearrangements, recombination occurs near blocks of repeated heptamers (20).

The question of the role of base pairing in SV40 integration is not directly addressed by the data presented here because the sequences of parental cellular DNAs which took part in recombination are still unknown. However, some clues may be found by comparing the cellular sequences which flank SV40 insertions with the SV40 sequences which could have taken part in partially mismatched base pairing at the site of integration. It is possible that during integration, cellular and viral DNA sequences sharing partial sequence homology form partially mismatched heteroduplexes. Mismatches in the heteroduplex region could be repaired randomly, sometimes conserving DNA sequences of SV40 origin and sometimes copying cellular sequences. This process would be expected to leave remnants of SV40 sequences interspersed with nonviral sequences in the DNA proximal to the cell-virus junction. Sequences which may be vestigial SV40 DNA are present in various amounts in the DNA adjacent to several virus-cell joints (Fig. 4C). However, the presence of such putative remnants of viral DNA sequence is not common to all virus-cell DNA junctions sequenced.

DISCUSSION

My analysis of DNA fragments cloned from cell lines SVRE9 and SVRE17, coupled with the work of Botchan et al. (3) on SV14B cells, shows that no specific viral or cellular sequences occur at the SV40-cell junctions of three different SV40 insertions. These findings extend to the level of DNA sequence the conclusion that SV40 integration is not site specific on the viral genome and provide the first unambiguous evidence that no single DNA sequence in the cellular genome serves as an obligatory integration site for SV40 DNA. The DNA sequences which occur at the recombinant junctions between SV40 and cell DNA show structural similarities in some cases, but there is no structural feature common to all of the junctions. There is some preference for A+T richness in the SV40 sequences at the termini of viral insertions, but adjacent cell sequences are not generally rich in A+T residues. Two SV40-cell junctions are striking in that DNA sequences are repeated on either side of the joint. However, these repeat structures are not present at every SV40-cell junction sequenced.

SV40-cell junctions are quite nondescript compared with the junctions of retrovirus proviruses (8, 23), copia elements in *Drosophila* species (9), and Ty*1* elements in yeast cells (10, 12). These elements all have specific sites in their DNA sequence at which they attach to cell DNA, whereas SV40 insertions show no such specificity. Retroviruses, copia elements, and Ty*1* elements can insert at multiple sites in cell DNA, but their insertion is accompanied by duplication of a short sequence which preexisted in the cell DNA at the integration site, generating a short repeat of host sequences flanking the DNA insertion. Integrated SV40 DNA is not flanked by direct repeats of cell DNA. Transposition and retrovirus integration do not perturb host DNA sequences beyond generation of the short duplications at the site of insertion (6). Integration of SV40 DNA can result in extensive changes in the host genome. Botchan et al. (3) have shown that a rearrangement of cell DNA occurs at the site of SV40 insertion in SV14B cells. Preliminary results indicate that the same is true in SVRE9 and SVRE17 cells (J. R. Stringer, manuscript in preparation).

Clearly, the mechanism of SV40 integration is quite different from those acting in transposition and in integration of retrovirus proviruses. It is probable that SV40 DNA integrates via the function of some generalized cellular recombination system which can rather indiscriminantly integrate heterologous DNAs. Cultured cells apparently possess a mechanism which can incorporate any foreign DNA introduced to the cell (26). Because integration is a general phenomena not unique to viral genomes, the function of SV40 gene products is probably not required for integration, although SV40 proteins may enhance the action of a cellular recombination system. It is of course possible that the nucleotide sequences of the cell-virus recombinant junctions analyzed in this work are the result of secondary rearrangements after integration and that SV40 integration may proceed via a transposition-like mechanism. For this to be true, SV40 insertions would have to (i) be very unstable after the original insertion event and then, after rearrangement, become very stable structures and (ii) still be different from movable genetic elements and retroviruses by virtue of this temporary structural instability.

Whatever the mechanism of SV40 integration, it is similar to that which mediates the formation of defective variants of SV40 which are substi-

tuted with cellular DNA (13). The recombination sites in these variants are similar to those found linking integrated SV40 DNA to the cellular genome in that (i) they are not sequence specific; (ii) they often occur in regions of the SV40 genome which are rich in adenosine and thymidine; and (iii) putative remnants of SV40 sequence occur in cellular sequences of some of the recombinants, suggesting that the DNA strands participating in the recombination event sometimes share patches of sequence homology.

These characteristics are suggestive of a mechanism of recombination which does not require perfect DNA sequence homologies between parental DNA strands. Of course to resolve fully whether or not DNA sequence homologies contribute to integrative recombination, it will be necessary to use the flanking cellular sequences in the DNA clones described here as probes to clone cellular DNA fragments containing the viral integration sites in untransformed rat cells. The sequences of both parental DNAs, viral and cellular, could then be directly compared with each other and with the DNA sequences at the virus-cell junctions. Two studies of other SV40-recombinants have been reported in which both parental DNA sequences were determined and compared with those of the recombinant. Zain and Roberts (27) analyzed an adenovirus-SV40 recombinant and found no sequence homology between the regions of adenovirus DNA and SV40 DNA involved in the recombination event. Gutai studied the nucleotide sequences at viral-viral recombinant joints in naturally arising defective variants of SV40 and found some instances of patchy homology between parental DNA sequences, but other recombinants showed little or no such homology. (M. W. Gutai, Virology, in press).

The studies cited above and others have shown that eucaryotic cells have the ability to recombine DNA illegitimately, i.e., without the mediation of extensive DNA sequence homology. It will be particularly interesting to determine whether DNA sequence homology is involved in integration in SVRE9 and SVRE17 cells. The directly repeated DNA sequences on either side of the virus-cell DNA joints in these two insertions (Fig. 4B) must have presented the cell with regions of homology which could have been used in the recombination reaction if DNA homology were important in the mechanism. If SV40 DNA did integrate in these cells via illegitimate recombination, it was not for lack of sequence homologies between proximal cellular and viral DNAs.

ACKNOWLEDGMENTS

I thank Laurel Garbarini and Jeanne Lanzarone for excellent technical assistance and Patti Pope, Mike Ockler, and Cindy Carpenter for preparation of this manuscript.

I was supported by fellowships from the American Cancer Society (PF-1565) and Public Health Service grant 1 F32 CA06460-01 from the National Institutes of Health.

LITERATURE CITED

1. **Benton, W. D., and R. Davis.** 1977. Screening λgt recombinant clones by hybridization to single plaques. Science **196**:180–182.
2. **Blattner, F. R., A. E. Blechl, K. Penniston-Thomson, H. E. Faber, J. E. Richards, J. L. Slightom, P. W. Tucker, and D. Smithies.** 1978. Cloning human fetal γ globin and mouse α-type globin DNA: preparation and screening. Science **202**:1279.
3. **Botchan, M., J. Stringer, T. Mitchison, and J. Sambrook.** 1980. Integration and excision of SV40 DNA from the chromosome of a transformed rat cell. Cell **20**: 143–152.
4. **Botchan, M., W. Topp, and J. Sambrook.** 1976. The arrangement of simian virus 40 sequences in the DNA of transformed cells. Cell **9**:269–287.
5. **Botchan, M. R., W. C. Topp, and J. Sambrook.** 1979. Studies on simian virus 40 excision from cellular chromosomes. Cold Spring Harbor Symp. Quant. Biol. **43**: 709–719.
6. **Calos, M. P., and J. H. Miller.** 1980. Transposable elements. Cell **20**:579–595.
7. **Chepelinsky, A. B., R. Seif, and R. G. Martin.** 1980. Integration of the simian virus 40 genome into cellular DNA in temperature-sensitive (N) and temperature-insensitive (A) transformants of 3T3 rat and Chinese hamster lung cells. J. Virol. **35**:184–193.
8. **Dhar, R., W. L. McClements, L. W. Enquist, and G. F. Van de Woude.** 1980. Nucleotide sequences of integrated Moloney sarcoma provirus long terminal repeats and their host and viral junctions. Proc. Natl. Acad. Sci. U.S.A. **77**:3937–3941.
9. **Dunsmuir, P., W. J. Brorein, M. A. Simon, and G. M. Rubin.** 1980. Insertion of the drosophila transposable element copia generates a 5 base pair duplication. Cell **21**:575–579.
10. **Farabaugh, P. J., and G. R. Fink.** 1980. Insertion of the eucaryotic transposable element Ty 1 creates a 5-base pair duplication. Nature (London) **286**:352–356.
11. **Fiers, W., R. Contreras, G. Haegeman, R. Rogiers, A. van de Voorde, J. Van Herreweghe, G. Volckaert, and M. Ysebaert.** 1978. Complete nucleotide sequence of SV40 DNA. Nature (London) **273**:113–120.
12. **Gafner, J., and P. Philipsen.** 1980. The yeast transposon Ty 1 generates duplications of target DNA on insertion. Nature (London) **286**:414–418.
13. **Gutai, M. W., and D. Nathans.** 1978. Evolutionary variants of simian virus 40: cellular DNA sequences and sequences at recombinant joints of substituted variants. J. Mol. Biol. **126**:275–288.
14. **Ketner, G., and T. Kelly, Jr.** 1976. Integrated simian virus 40 sequences in transformed cell DNA: Analysis using restriction endonucleases. Proc. Natl. Acad. Sci. U.S.A. **73**:1102–1106.
15. **Leder, P., D. Tiemier, and L. Enquist.** 1977. EK2 derivatives of bacteriophage lambda useful in the cloning of DNA from higher organisms: The λgtWES system. Science **196**:175–177.
16. **Maniatis, T., R. C. Hardison, E. Lacy, J. Lauer, C. O'Connell, D. Quon, G. K. Sim, and A. Efstratiadis.** 1978. The isolation of structural genes from libraries of eucaryotic DNA. Cell **15**:687–701.
17. **Maxam, A. M., and A. Gilbert.** 1977. A new method for sequencing DNA. Proc. Natl. Acad. Sci. U.S.A. **74**:560–564.
18. **Maxam, A. M., and A. Gilbert.** 1980. Sequencing end-

labeled DNA with base-specific chemical cleavages. Methods Enzymol. **65**:499–560.

19. **Pollack, R., R. Risser, S. Conlon, and R. Rifkin.** 1974. Plasminogen activator production accompanies loss of anchorage regulation in transformation of primary rat embryo cells by simian virus 40. Proc. Natl. Acad. Sci. U.S.A. **71**:4792–4796.
20. **Sakano, H., R. Maki, Y. Kurosawa, W. Roeder, and S. Tonegawa.** 1980. Two types of somatic recombination are necessary for the generation of complete immunoglobulin heavy-chain genes. Nature (London) **286**:676–683.
21. **Sambrook, J., R. Greene, J. Stringer, T. Mitchison, S. L. Hu, and M. R. Botchan.** 1980. Analysis of the sites of integration of viral DNA sequences in cells transformed by adenovirus 2 and SV40. Cold Spring Harbor Symp. Quant. Biol. **43**:569–584.
22. **Sanger, R., S. Nicklen, and A. R. Coulson.** 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. U.S.A. **74**:5463–5467.
23. **Shimotohno, K., S. Mizutani, and H. M. Temin.** 1980. Nucleotide sequences flanking retrovirus proviruses. Nature (London) **285**:550–554.
24. **Southern, E.** 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. **98**:503–517.
25. **Tooze, J.** 1980. The molecular biology of tumor viruses, 2nd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
26. **Wigler, M., R. Sweet, G. K. Sim, B. Wold, A. Pellicer, E. Lacy, T. Maniatis, S. Silverstein, and R. Axel.** 1979. Transformation of mammalian cells with genes from procaryotes and eucaryotes. Cell **16**:777–785.
27. **Zain, B. S., and R. Roberts.** 1978. Characterization and sequence analysis of a recombination site in hybrid virus Ad2$^+$ND1. J. Mol. Biol. **120**:13–32.