Volume 10 Number 24 1982 Nucleic Acids Research

Nucleotide sequence at the site of junction between adenovirus type 12 DNA and repetitive hamster cell DNA in transformed cell line CLAC1

Silvia Stabel and Walter Doerfler

Institute of Genetics, University of Cologne, Cologne, FRG

Received 11 October 1982; Revised and Accepted 16 November 1982

ABSTRACT

The hamster cell line CLAC1 originated from a tumor induced by injecting human adenovirus type 12 (Ad12) into newborn hamsters. Each cell contained about 12 copies of viral DNA colinearly integrated at two or three different sites. We have cloned and sequenced a DNA fragment comprising the site of junction between the left terminus of Ad12 DNA and cellular DNA. The first 174 nucleotides of Ad12 DNA were deleted at the site of junction. Within 40 nucleotides, there were one tri-, two tetra-, one penta-, and one heptanucleotide which were identical in the 174 deleted viral nucleotides and the cellular sequence replacing them. In addition, there were patch-type homologies ranging from octa- to decanucleotides between viral and cellular sequences. There is no evidence for a model assuming adenovirus DNA to integrate at identical cellular sites. The cellular DNA sequence corresponding to the junction fragment was cloned also from BHK21(B3) hamster cells and sequenced. Up to the site of linkage with viral DNA, this middle repetitive cellular DNA sequence was almost identical with the equivalent sequence from CLAC1 hamster cells. Taken together with the results of previously published analyses (11, 12), the data suggest a model of viral (foreign) DNA integration by multiple short sequence homologies. Multiple sets of short patch homologies might be recognized as patterns in independent integration events. The model also accounts for the loss of terminal viral DNA sequences.

INTRODUCTION

We have previously analyzed in detail the patterns of integration of some 70 different adenovirus type 12 (Ad12)-transformed cell lines, Ad12-induced tumors or tumor lines and adenovirus type 2 (Ad2)-transformed cell lines of hamster, rat or mouse origin (for review, 1; 2 - 8). There was no indication of a specific site of integration as determined by restriction analyses and Southern blotting. In general, Ad12 DNA was integrated colinear and nearly intact into cellular DNA, while inserted Ad2

0305-1048/82/1024-8007$2.00/0

genomes had smaller or larger deletions. Usually, multiple copies of viral DNA persisted at a limited number of integration sites. There was evidence that viral DNA together with abutting cellular sequences was amplified (1 - 5; 9). Rearrangements or inversions of viral sequences were observed in some cases (5, 10).

The problem of the possible specificity of the insertion site was further investigated by determining the nucleotide sequence at the sites of junction between Ad12 DNA and hamster DNA in cell line CLAC3 (11), and between Ad2 DNA and hamster DNA in cell line HE5 (12). In both sequences multiple patch homologies were discovered between adjacent cellular and viral DNA sequences ranging from octa- to dodecanucleotides. Some of these homologies were remote from the site of junction. Similar homologies were described to occur at the site of junction between Ad5 and hamster cell DNAs in cell line BHK268-C31 (13). The sequences of these patch homologies were different in different cell lines investigated. The high frequency of occurrence of these patch homologies has prompted us to propose that they might have a function in the insertion of foreign (viral) DNA into the host chromosome (1, 11, 12). Thus, the integration event may be mediated by short homologies between viral and cellular DNAs. Similar conclusions have been derived at from work in the SV40 system (14 - 16). The host-virus junction between rat DNA and the right end of Ad2 DNA had also been sequenced (17). Another feature common to adenovirus DNA junction sites analyzed was the deletion of viral sequences at the site of junction, 45 and 5 nucleotides being deleted from Ad12 DNA (11) and from Ad2 DNA (12), respectively. In the Ad5-transformed rat cell line 5RK20, viral genomes were linked directly. At the site of this junction 107 and 62 nucleotides of the two viral termini were deleted (18). Thus, recombination of viral DNA with cellular or viral DNA seems to entail losses of nucleotides at the site of junction.

The computer-aided comparison of terminal adenoviral DNA sequences and adjacent cellular DNA sequences has revealed extensive patch homologies (11, 12). Patch homologies also exist between the right terminus of Ad2 DNA and many randomly selected DNA se-

quences of pro- and eukaryotic origins (12). Hence, it is conceivable that the abundance of patch homologies reflects the ability of adenovirus DNA and perhaps of any foreign DNA to integrate at a large number of different sites.

In the present communication we describe the cloning and sequencing of the site of junction between the left terminus of Ad12 DNA and hamster cell DNA from the Ad12-induced hamster tumor line CLAC1 (4). The nucleotide sequence comprising 529 base pairs (bp) of cellular and 128 bp of viral origin was determined. The first leftmost 174 bp of authentic Ad12 DNA were deleted. Patch homologies between viral and cellular DNA ranging from octa- to decanucleotides were observed. Moreover, the deleted viral sequence of 174 bp and the cellular sequence replacing it had one tri-, two tetra-, one penta-, and one heptanucleotide in common within a sequence of 40 nucleotide pairs. This finding suggests that distinct patterns of short sequence homologies between viral and cellular DNA may direct the insertion event. Sequence homologies between the cloned cellular DNA and DNA from untransformed primary hamster cells or from non-virus transformed BHK21(B3) cells were determined by Southern blotting. A 4 kilobase (kb) EcoRI fragment of cellular DNA occurring at high frequency in hamster DNA exhibited strong homology. This fragment was cloned from the DNA of B3 cells. From two independent clones containing this fragment, a 120 bp HaeIII fragment corresponding to the junction fragment (see above) was sequenced. Except for one or two nucleotides, depending on the clone sequenced, the cellular DNA sequence in B3 hamster cells was identical to the cellular DNA sequence at the site of junction and up to the joint with Ad12 DNA. A model for the mechanism of recombination between viral and cellular DNA will be presented.

MATERIALS AND METHODS

Cells and virus, cellular and viral DNA. Origin, mode of propagation and patterns of viral DNA integration of the Ad12-induced hamster tumor line CLAC1 were described earlier (4). Ad12 was propagated on KB cells growing in suspension cultures. The virus was purified and the DNA extracted as described elsewhere (19). The cloned EcoRI-C fragment of Ad12 DNA was a gift of A. van der

Eb, Leiden. Cellular DNA from CLAC1 cells was extracted as detailed earlier (2).

Agarose gel electrophoresis, blotting, and DNA-DNA hybridization procedures were performed according to standard protocols (2-8). The Southern (20) transfer method was employed. DNA was nick translated as outlined (21), and DNA-DNA hybridization was performed as described (22).

Molecular cloning procedures and vectors. The DNA of cell line CLAC1 was cleaved with the EcoRI restriction endonuclease, fragments (6.4 kb) corresponding to the left terminal junction site (c. f. ref. 4, Figs. 1 and 2) were selected by velocity sedimentation on a sucrose density gradient and were ligated with the "arms" of bacteriophage λgtWES·λB DNA (23) as described in detail earlier (11). A twofold excess of vector DNA was used in the ligation reaction (11), and confluent λ plaques were screened by the Benton-Davis procedure (24), using the [^{32}P]-labeled left terminal EcoRI-C fragment of Ad12 DNA as a probe. One of the clones obtained was designated λ 59. The Sau3A fragment (about 750 bp) comprising the junction site in this clone (c. f. Fig. 1) was subcloned into the BamHI site of plasmid pBR322 DNA, and the clone obtained was designated p9. Similarly, BHK21 (B3) DNA was cleaved with the EcoRI restriction endonuclease, and a 4 kb cellular DNA fragment was recloned in λgtWES·λB DNA as described above using clone p9 as hybridization probe which was [^{32}P]-labeled by nick translation. Routinely, positive λgtWES·λB plaques were plaque purified three to four times before the phage was propagated and DNA prepared for further analysis. A number of λ clones were obtained and characterized by Southern blotting. A 2 kb Sau3A fragment from clone λ 2 was subcloned into plasmid pBR322 DNA. This clone was designated p7.

Restriction maps of cloned DNA fragments were determined by routine procedures using many different restriction endonucleases, blotting and hybridization techniques to identify the junction fragment. Overlapping fragments and double cleavage with combinations of restriction endonucleases were also used.

Determinations of nucleotide sequences. The procedure of Maxam and Gilbert (25, 26) was applied. Both strands were sequenced as indicated (Fig. 1 c). DNA fragments were labeled at their 5'- or

3'-termini as described (25, 26) and subsequently cleaved with an appropriate restriction endonuclease to generate fragments with only one labeled terminus for sequence determination. Computer analyses of nucleotide sequences were carried out as described elsewhere (12).

RESULTS

Mapping of the cloned junction site between the left end of Ad12 DNA and hamster cell DNA. Restriction analyses of the patterns of Ad12 DNA integration in cell line CLAC1 had revealed one major (and a minor) off-size fragment containing the left end of Ad12 DNA (4). Four independent λgtWES·λB clones were isolated which contained the major left terminal off-size fragment of Ad12 DNA: λ 57, λ 59, λ 64, and λ 66. The clones λ 57, λ 64, and λ 66 contained additional hamster cellular DNA fragments of unknown origin. Clone λ 59 carried only the Ad12 DNA-hamster cell DNA junction fragment. By using a number of restriction endonucleases, the junction fragments of all 4 clones were mapped in detail. The fragments proved to be identical. A map of clone λ 59 is presented in Fig. 1 a. For nucleotide sequencing, a DNA fragment of manageable size and spanning the site of junction had to be produced. Clone λ 59 was, therefore, cleaved first with EcoRI to excise the insert and subsequently with a number of different restriction endonucleases to identify an appropriate off-size fragment. Ad12 virion DNA was cleaved with the same combination of enzymes. DNA fragments were then separated by gel electrophoresis, blotted, and Ad12-specific fragments were identified by hybridization to the cloned [^{32}P]-labeled EcoRI-C fragment of Ad12 DNA. The restriction endonuclease Sau3A generated an off-size fragment of about 750 bp which was subcloned by conventional techniques into the BamHI site of plasmid pBR322 to yield subclone p9 (Fig. 1 b). In this way, large quantities of the junction DNA fragment could be produced. A detailed restriction map of the Sau3A junction fragment was established using the restriction endonucleases AluI, DdeI, HaeIII, HhaI, MspI, PvuII, Sau96I, and TaqI (Fig. 1 b). The correlation of this map with the nucleotide sequence (Fig. 2 a) is perfect, except for the TaqI site (TCGA) starting at bp 11. This site might be methy-

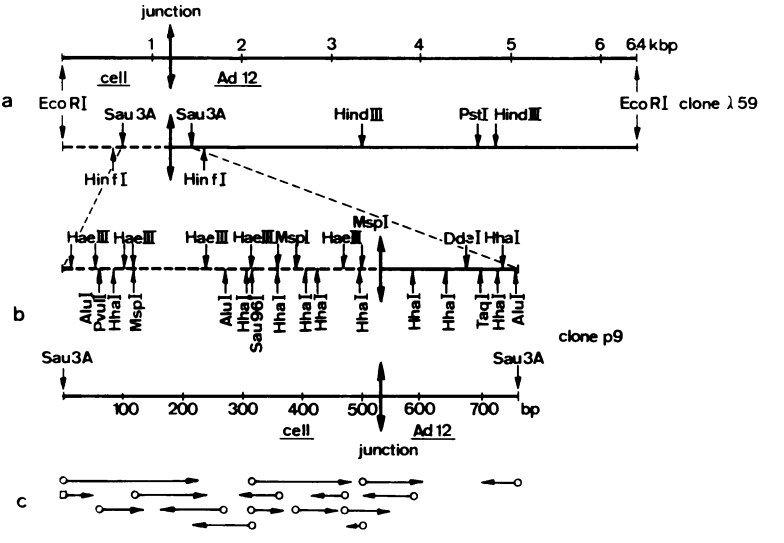

Fig. 1
Analysis of a cloned DNA fragment comprising the junction between the left end of Ad12 DNA and cellular DNA from cell line CLAC1.

The EcoRI off-size fragment containing the left terminus of Ad12 DNA and hamster cell DNA (c. f. Fig. 1 and 2 in reference 4) was cloned in λgtWES·λB DNA. Clone λ 59 contained only that fragment (a). The excised EcoRI junction fragment was subsequently cleaved with different restriction endonucleases in independent experiments. As explained in the text, Sau3A generated an off-size fragment of about 750 bp (b) which was subcloned in pBR322. This clone was designated p9. By using restriction endonucleases as indicated, the DNA in clone p9 was mapped in detail (b).
The scheme in part (c) of this figure describes which fragments were sequenced by the Maxam-Gilbert technique (25, 26). In brief: Most of the restriction endonuclease fragments were labeled at their 5'-termini by incubation with γ-[^{32}P]-ATP and polynucleotide kinase (o). Prior to the reaction the 5'-terminal phosphate had been removed by bacterial alkaline phosphatase. One fragment was labeled at its 3'-terminus (□) using the Klenow fragment of DNA polymerase I and the appropriate α-[^{32}P]-labeled deoxyribonucleoside triphosphate. The reaction was completed by adding the unlabeled deoxyribonucleoside triphosphate and continuing the incubation. The directions of the arrows designate which of the two strands were sequenced. The Ad12 sequence from bp 589 to bp 694 (b) was not determined.

lated in the clone, and TaqI is known to be blocked by N^6-mA. It had to be ascertained that in cloning and subcloning experiments the size of the junction fragment was not altered. The results of blotting and hybridization experiments demonstrate that

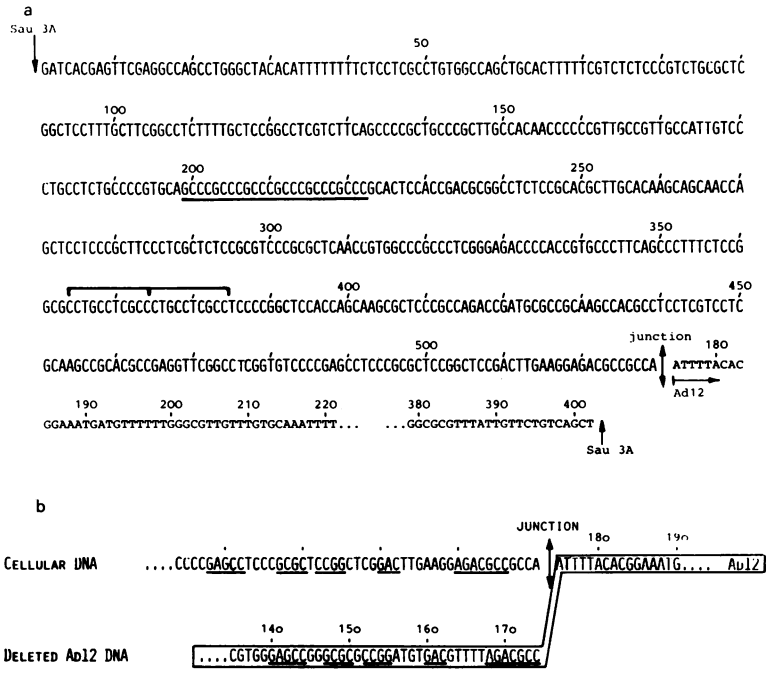

Fig. 2
The nucleotide sequence at the junction site between the left terminus of Ad12 DNA and hamster cell DNA.

a) A total of 529 nucleotides of cellular and of 128 of viral origin has been determined. As indicated in Fig. 1 c, about 100 bp of Ad12 DNA were not sequenced. The site of junction has been indicated by a vertical bidirectional arrow. From nucleotide 175 onward the viral DNA sequence is identical with authentic Ad12 DNA (27). Only part of the viral DNA sequence is therefore reproduced. Internal repeats in the cellular DNA have been underlined or designated by brackets.

b) Comparison between the cellular DNA sequence at the site of junction and the original viral sequence that has been deleted and been replaced by cellular DNA. One tri-, two tetra-, one penta-, and one heptanucleotide were identical in the replaced viral and the substituting cellular DNA sequences. Identical nucleotide strings have been underlined.

the EcoRI junction fragments in CLAC1 DNA and in all λ clones have identical sizes. The same holds true for the Sau3A junction fragments in λ 59 DNA and p9 DNA. Furthermore, the 757 bp Sau3A fragments (Fig. 1 b) from the λ 59 and p9 clones have identical sites for the DdeI, HaeIII, HhaI, and MspI restriction endonucleases (data not shown). It is concluded that cloning and subclo-

ning of junction fragments does not introduce major deletions or rearrangements. The same conclusion had been arrived at for the cloning of junction fragments from the Ad12-induced tumor line CLAC3 (11) and the Ad2-transformed hamster cell line HE5 (12). Nucleotide sequence at the site of junction between the left terminus of Ad12 DNA and hamster cell DNA in tumor line CLAC1. The Sau3A fragment from subclone p9 was used for nucleotide sequencing according to the Maxam-Gilbert method (25, 26). In general, DNA fragments were end-labeled either at the 5'- or the 3'-terminus by conventional techniques and subsequently cleaved by a suitable restriction endonuclease to generate two fragments of unequal size labeled at only one terminus. These fragments could then be separated by gel electrophoresis and directly sequenced. The sequencing strategy for the Sau3A fragment has been schematically indicated in Fig. 1 c. For some segments, both strands were sequenced as designated in Fig. 1 c. The total sequence of the cellular segment and part of the viral segment of the Sau3A fragment is presented in Fig. 2 a. The following features of this sequence are apparent.

1) The first 174 bp of the viral DNA sequence were deleted during or after integration. The Ad12 sequence starts with bp 175 and from there on represents the authentic Ad12 DNA sequence (27).
2) The sequence of the 100 cellular nucleotides immediately adjacent to the Ad12 DNA is strikingly rich in GC bp (72 %), the overall GC content of the 529 bp of cellular DNA is 69.9 %.
3) There is no apparent homology between immediately adjacent cellular and viral DNA sequences.
4) Patch homologies ranging from octa- to decanucleotides can be detected by computer analyses between the cellular DNA sequence in clone p9 and the left terminal 2320 nucleotides of Ad12 DNA; 24 octa-, 7 nona-, and 1 decanucleotide were detected. Shorter patch homologies exist in large numbers, but have not been recorded.
5) In the cellular sequence one internal undecanucleotide repeat (CCTGCCTCGCC) can be detected (position number 364 - 384; brackets in Fig. 2 a). These repeat units overlap. A 24 bp repeat of GCCC is apparent between positions 199 and 223 (sequence underlined).

6) The nucleotide combinations GCC, GCCC, or GCCCC occur 25-, 12-, and two-times in the cellular sequence, respectively. These repeats comprise 25 % of the entire cellular DNA sequence of 529 bp.
7) The trinucleotide GAC, the tetranucleotides GCGC and CCGG, the pentanucleotide GAGCC and the heptanucleotide AGACGCC in the cellular sequence close to the site of junction (underlined strings in Fig. 2 b) also occur in the deleted Ad12 sequence that has been displaced by cellular DNA.
8) The cellular DNA sequence presented in Fig. 2 a contains open reading frames in all three positions, one of them extends into the viral DNA sequence. It has not yet been tested whether these sequences are actually expressed.
9) A sequence comparison to cellular junction sequences adjacent to Ad12 and Ad2 DNA from cell lines CLAC3 (11) and HE5 (12), respectively, reveals sequence homologies from hexa- to octanucleotides but not more extensive sequence similarities (data not shown).
10) As will be shown below (Fig. 4), the nucleotide sequence in non-Ad12 transformed hamster B3 cells corresponding to the cellular sequence at the junction site in line CLAC1 is identical, except for one or two nucleotides depending on the clone analyzed, to the cellular insertion site up to the linkage point with Ad12 DNA. The DNA from normal primary hamster cells seems to contain the same sequence as determined by restriction analyses and blotting (data not shown).

Cloning of the cellular insertion sequence from BHK21 (B3) cells.

Since we have consistently found more or less extensive deletions at the termini of integrated Ad12 or Ad2 DNA (11, 12, Fig. 2 in this report), the possibility existed that the cellular sequences at the site of insertion of foreign DNA were also altered by the integration event. It was therefore necessary to clone these cellular DNA sequences from non-virus transformed hamster cells. Cell line B3, a subline of BHK21 cells, was chosen for these experiments. In order to investigate the location and the organization of the insertion DNA sequences in normal and Ad12-transformed hamster cells, cellular DNA from CLAC1 cells, from B3 cells, from primary hamster cells (not shown), and calf thymus

was cleaved with EcoRI, BamHI or BglII, the fragments were separated by agarose gel electrophoresis and blotted (20). The insertion sequences were visualized by hybridization to [^{32}P]-labeled clone p9 DNA followed by autoradiography. This probe would also hybridize to the left terminal Ad12 DNA fragments in DNA from transformed cells. The data shown in Fig. 3 demonstrate that the cellular sequence from the junction clone p9 hybridizes very intensely to an approximately 4 kb long EcoRI fragment, to a 4 kb BamHI fragment and to high molecular weight BglII fragments in hamster DNA. The homology to a 4 kb EcoRI fragment was also observed in DNA from cell lines T637, HA12/7, A2497-3, CLAC3, and from normal primary hamster cells (data not shown). Homologies to calf thymus or human KB cell DNA (not shown) are not observed. An additional band of higher molecular weight with weak homology can also be observed. In Ad12-transformed or tumor cell lines (CLAC1, CLAC3, T637, HA12/7, A2497-3) additional homologies due to the presence of Ad12 DNA sequences are also detectable, as expected. The 4 kb DNA fragment seems to occur a few hundred times in B3 DNA and perhaps more abundantly in Ad12-transformed cells. By using conventional cloning techniques and λgtWES·λB DNA as a vector, the 4 kb fragment of hamster cell DNA was cloned from B3 cells. Clones λ 2, λ 5, λ 6, λ 7, λ 9, λ 12, λ 13, and λ 14 were obtained in this way. By restriction analyses (data not shown) these clones were shown to be very similar but not identical to each other. The 4 kb fragment occurs in multiple internal repeats displaying staggered EcoRI and BamHI restriction sites. A possible arrangement of these sites and of BglII sites is shown in Fig. 4 a. This scheme indicates that EcoRI and BamHI sites alternate in a regular pattern in this repetitive sequence generating equal-sized EcoRI and BamHI fragments.

The 120 bp HaeIII fragment from clone p7 or λ 12 corresponding to the site of junction in cell line CLAC1 (Fig. 4 a) was excised, purified by polyacrylamide gel electrophoresis, labeled at the 5'-termini as described (25, 26) and subsequently cleaved with the Sau96I or HhaI restriction endonuclease. The fragments were then sequenced (Fig. 4 b). The results demonstrate that, with the exception of one or two nucleotides, depending on the clone sequenced (underlined in Fig. 4 b), the sequence is identical to

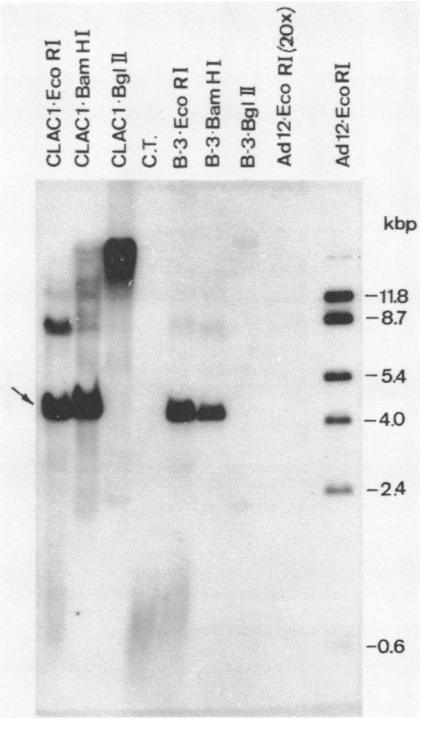

Fig. 3

Localization of the cellular site of Ad12 DNA insertion in hamster cell DNA.
Cellular DNA from CLAC1 cells, from B3 cells and calf thymus was cleaved with different restriction endonucleases as is apparent from the figure. CLAC1·EcoRI designates that 10 µg of CLAC1 DNA were cleaved with EcoRI. C. T. (calf thymus) DNA was cut with EcoRI. Ad12·EcoRI (20 x): 0.5 ng of Ad12 DNA (i. e. 20 genome equivalents, relative to 10 µg of hamster DNA), was cleaved with EcoRI. DNA fragments were separated and blotted as described in the text. The specific cellular DNA fragment carrying the DNA sequence corresponding to the cellular junction sequence was visualized by hybridization to clone p9 DNA labeled with [^{32}P] by nick translation. In the rightmost track Ad12 DNA (0.5 ng) was cut with EcoRI. After transfer, this strip was hybridized to α-[^{32}P]-labeled Ad12 DNA to indicate the EcoRI fragments of Ad12 DNA (in kilobase pairs = kbp) as marker. The 4 kb position is indicated by an arrow.

that linked to the left terminus of Ad12 DNA in cell line CLAC1. These findings lend further support to the interpretation that, during cloning and subcloning of the junction sequence or the

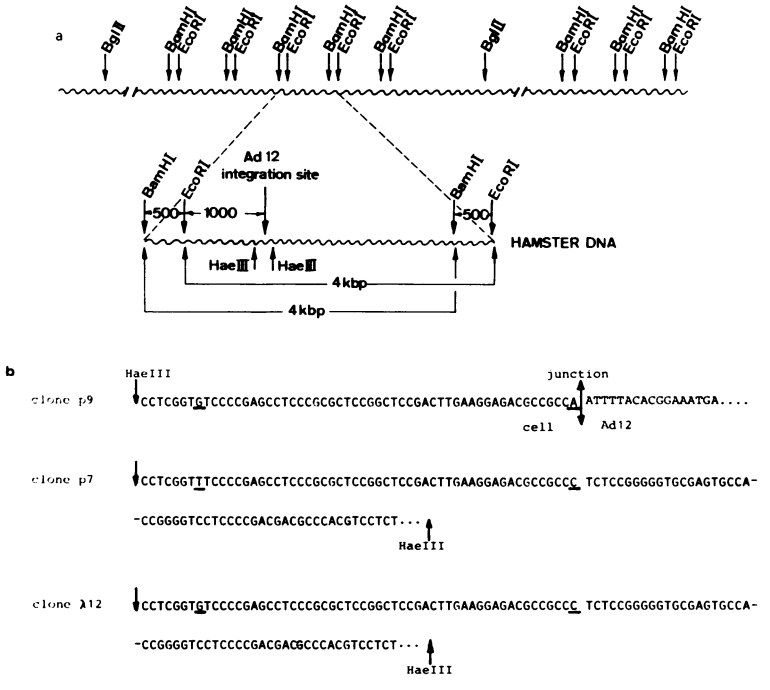

Fig. 4

Organization of the cloned hamster cell DNA fragment and nucleotide sequence of the 120 bp cellular HaeIII fragment corresponding to the site of junction.

a) As described in the text, the 4 kb hamster cell DNA fragment occurs in tandem repeats, and EcoRI and BamHI sites appear to be regularly spaced in this segment of repetitive cellular DNA. The positions of BglII sites are also indicated.

b) Nucleotide sequence of the 120 bp HaeIII fragment in subclone p7 and in clone λ 12. The nucleotide sequence of this DNA fragment from B3 cells was determined in clone p7 and in clone λ 12. For comparison the DNA sequence at the site of junction between cellular DNA and Ad12 DNA in CLAC1 cells is also shown (clone p9).

corresponding cellular DNA sequence, there were no deletions or rearrangements of nucleotide sequences. Of course, it cannot be decided yet whether cellular DNA sequences at the site of linkage had been deleted during integration.

DISCUSSION

One of the prime objectives of studying the sequence organization of integrated foreign (viral) DNA is to start elucidating the me-

chanism by which mammalian cells can insert foreign DNA sequences into their genomes. The nucleotide sequences at the site of junction between viral and cellular DNA from three different adenovirus-transformed cell lines (11, 12, this report) reveal patch homologies ranging from hexa- to dodecanucleotides closer to or more remote from the site of junction between viral and cellular DNA sequences. A computer-aided comparison between the right terminal sequence of Ad2 DNA and randomly selected DNA sequences from pro- or eukaryotic organisms has demonstrated that such patch homologies are rather ubiquitous. Since adenovirus DNA can integrate at many different sites (for review, reference 1), it is conceivable that short sequence homologies, possibly a set of clustered patch homologies (Fig. 5), could serve as guiding signals for the integration event. In cell line CLAC1, there are patchy sequence homologies comprising one tri-, two tetra-, one penta-, and one heptanucleotide between the cellular DNA sequence immediately adjacent to viral DNA and the 174 bp sequence of Ad12 DNA origin that hat been deleted (Fig. 2 b). Patch homologies do not involve the site of linkage in line CLAC1. Thus actual recombination seems to have occurred outside the regions of homology which may merely help to align the recombining molecules (c. f. Fig. 5, 6). The possibility that short sequence homologies may play an important role in viral DNA insertion has also been suggested by findings in the SV40 system (14, 15, 16), in other adenovirus-transformed cell-lines (13), in the Ti-plasmid of Agrobacterium tumefaciens integrated into plant DNA (28), and in polyoma (29) transformed cell lines. Further work will be required to determine in a definitive way what function, if any, such short sequence homologies exert. Another apparently general feature observed in integrated adenovirus DNA sequences is the deletion of terminal viral nucleotides at the sites of junction. In different cell lines 45 (11), 5 (12), 174 (this report, Fig. 2 a), 2 (17) or 62 and 107 nucleotides (18) have been deleted, in the latter instance at the site of junction between two tandemly linked, integrated Ad5 genomes.

In many Ad12-transformed and tumor cells the integrated viral genomes persist almost intact and colinear with virion DNA. The finding that short viral sequences at the termini frequently

comprising the origins of viral DNA replication are missing in the integrated state, may explain the observation that Ad12 genomes cannot be rescued from Ad12-transformed cells.

The nucleotide sequences in the flanking cellular DNA sequences in the Ad12-induced hamster tumor cell lines CLAC1 (this report, Fig. 3), CLAC3 (11), and in the Ad2-transformed hamster cell line HE5 (12) have been compared. Apart from apparently randomly distributed sequence strings of hexa- to octanucleotides, which these sequences had in common, more extensive sequence homologies could not be detected. Thus, there is no reason to assume that adenovirus DNA is permanently fixed in the same or similar hamster cell DNA sequences.

The 4 kb cellular DNA sequence corresponding to the site of Ad12 DNA insertion in cell line CLAC1 has been recloned from B3 hamster cells and represents DNA of the intermediate repetitive class. The cellular DNA sequence at the site of junction has multiple sequence homologies ranging from octa- to dodecanucleotides to the human and hamster Alu sequences (30) as revealed by computer analyses.

It cannot yet be decided whether, in the insertion event, cellular DNA sequences at the site of junction are also deleted, since in cell line CLAC1 the opposite junction site has not yet been cloned and sequenced. Work is in progress along these lines with cell line HE5 (R. Gahlmann and W. Doerfler, unpublished results).

The question of whether foreign (viral) DNA was inserted at specific sites or rather at random in the mammalian genome has attracted much interest. At the first level of analysis, sequence data (11, 12) and restriction analyses (1, 2 - 8) of adenovirus-transformed cell lines have not provided evidence for the notion of specific insertion sites. However, for a number of reasons we should still like to reserve judgement about the problem of specificity:

i) Insertion events could be guided by specific, hitherto unrecognized structures in DNA rather than by specific sequences.

ii) The insertion event in the 70 different adenovirus-transformed and tumor lines investigated in this laboratory (1, 2 - 8)

could possibly have been specific, the specificity having been obscured by amplifications and/or rearrangements occurring subsequent to insertion. There is, at present, no evidence in support of this notion, but it cannot be ruled out either.

The schemes in Fig. 5 and 6 present a tentative model summarizing some of the features of adenovirus DNA integration. The model essentially encompasses the following observations:

1) In many instances investigated, adenovirus DNA is linked to cellular DNA by the termini of viral DNA or by sequences close to the termini.
2) Integration leads to the deletion of terminal viral nucleotides which are not inserted, at least not at the site of insertion of the bulk of the viral genome. The terminal viral nucleotides might be deleted or integrated at a remote site.
3) Insertion is somehow directed by multiple patch type homologies. It is conceivable that patterns of patches are recognized, possibly different sets of patches in integration events at different cellular sites (Fig. 6). The combination of patch homologies might determine the site of recombination

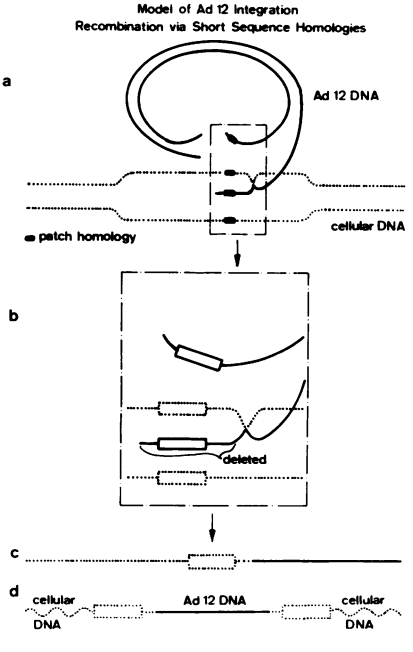

Fig. 5

A possible model of adenovirus DNA integration. In this tentative model, the terminus of one strand of adenovirus DNA invades double stranded cellular DNA. The site of invasion may by determined by a certain pattern of non-contiguous patch homologies (c. f. Fig. 6). Recombination can occur inside or outside (this scheme) these patches. In the process of recombination the terminal viral DNA sequence is deleted or displaced to a site remote from the site of insertion of the bulk of the viral DNA molecule. The model does not make predictions about the events at the opposite terminus of viral DNA, nor about the structure of the recombining adenovirus DNA molecule.

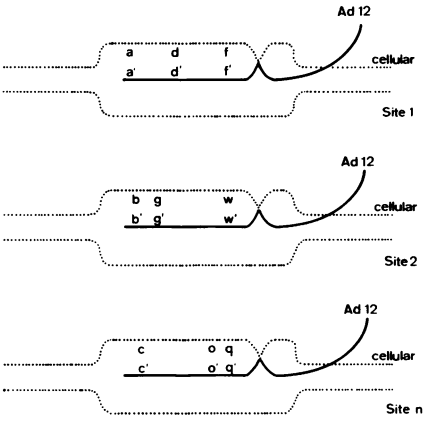

Fig. 6
Patterns of patch homologies determine the site of insertion. Letters a, b, c, ... designate short nucleotide strings, a', b', c'...complementary sequence strings. Depending on the site of recombination, different patterns of homologies in viral DNA match those in cellular DNA and thus determine the site of recombination.

with the host genome. The sequence data presented suggest that the actual site of recombination between cellular and viral DNA does not necessarily coincide with one of these patches.

4) The model presented in Figs. 5 and 6 does not preclude the possibility of a circular or concatemeric intermediate of adenovirus DNA in integration. The scheme attempts to describe the constellation at the moment of recombination. It will also be interesting to determine what function, if any, the 5' terminal protein of adenovirus (31) can exert in the insertion event.

ACKNOWLEDGEMENTS

We thank C. M. Radding, Yale University, for discussions on the model of adenovirus DNA integration.
We are indebted to Ute Winterhoff for help with some of the cloning experiments and to Rainer Leisten for performing the computer analyses of nucleotide sequences. We thank Gertrud Deutschländer for typing this manuscript.
This research was supported by the Deutsche Forschungsgemeinschaft through SFB 74-C1.

REFERENCES

1. Doerfler,W. (1982) Current Topics Microbiol. Immunol. 101, 127-194.
2. Sutter,D., Westphal,M. and Doerfler,W. (1978) Cell 14, 569-585.

3. Ibelgaufts,H., Doerfler,W., Scheidtmann,K.H. and Wechsler,W. (1980) J. Virol. 33, 423-437.
4. Stabel,S., Doerfler,W. and Friis,R.R. (1980) J. Virol. 36, 22-40.
5. Vardimon,L. and Doerfler,W. (1981) J. Mol. Biol. 147, 227-246.
6. Kuhlmann,I. Achten,S., Rudolph,R. and Doerfler,W. (1982) EMBO J. 1, 79-86.
7. Kuhlmann,I. and Doerfler,W. (1982) Virology 118, 169-180.
8. Starzinski-Powitz,A., Schulz,M., Esche,H., Mukai,N. and Doerfler,W. (1982) EMBO J. 1, 493-497.
9. Yasue,H. and Ishibashi,M. (1982) Virology 116, 99-115.
10. Eick,D. and Doerfler,W. (1982) J. Virol. 42, 317-321.
11. Deuring,R., Winterhoff,U., Tamanoi,F., Stabel,S. and Doerfler,W. (1981) Nature 293, 81-84.
12. Gahlmann,R., Leisten,R., Vardimon,L. and Doerfler,W. (1982) EMBO J. 1, 1101-1104.
13. Westin,G., Visser,L., Zabielski,J., van Mansfeld,A.D.M., Pettersson,U. and Rozijn,T.H. (1982) Gene 17, 263-270.
14. Gutai,M.W. and Nathans,D. (1978) J. Mol. Biol. 126, 275-288.
15. Stringer,J.R. (1981) J. Virol. 38, 671-679.
16. Stringer,J.R. (1982) Nature 296, 363-366.
17. Sambrook,J., Greene,R., Stringer,J., Mitchison,T., Hu,S.-L. and Botchan,M. (1980) Cold Spring Harbor Symp. Quant. Biol. 44, 569-584.
18. Visser,L., Reemst,A.C.M.B., van Mansfeld,A.D.M. and Rozijn, T.H. (1982) Nucl. Acids Res. 10, 2189-2198.
19. Doerfler,W., Lundholm,U., Hirsch-Kauffmann,M. (1972) J. Virol. 9, 297-308.
20. Southern,E.M. (1975) J. Mol. Biol. 98, 503-517.
21. Rigby,P.W.J., Dieckmann,M., Rhodes,C. and Berg,P. (1977) J. Mol. Biol. 113, 237-251.
22. Wahl,G.M., Stern,M. and Stark,G.R. (1979) Proc. Natl. Acad. Sci. USA 76, 3683-3687.
23. Tiemeier,D., Enquist,L. and Leder,P. (1976) Nature 263, 526-527.
24. Benton,W.D. and Davis,R. (1977) Science 196, 180-182.
25. Maxam,A.M. and Gilbert,W. (1977) Proc. Natl. Acad. Sci. USA 74, 560-564.
26. Maxam,A.M. and Gilbert,W. (1980) Methods Enzym. 65, 499-560.
27. Sugisaki,H., Sugimoto,K., Takanami,M., Shiroki,K., Saito,J., Shimojo,H., Sawada,Y., Uemizu,Y., Uesugi,S. and Fujinaga,K. (1980) Cell 20, 777-786.
28. Simpson,R.B., O'Hara,P.J., Kwok,W., Montoya,A.L., Lichtenstein,C., Gordon,M.P. and Nester,E.W. (1982) Cell 29, 1005-1014.
29. Hayday,A., Ruley,H.E. and Fried,M. (1982) J. Virol. 44, 67-77.
30. Schmid,C.W. and Jelinek,W.R. (1982) Science 216, 1065-1070.
31. Robinson,A.J., Younghusband,H.B. and Bellett,A.J.D. (1973) Virology 56, 54-69.