JOURNAL OF VIROLOGY, Mar. 1981, p. 887-892
0022-538X/81/030887-06$02.00/0

Vol. 37, No. 3

Integration of Adenovirus Type 2 DNA at a Limited Number of Cellular Sites in Productively Infected Cells

RAINER NEUMANN AND WALTER DOERFLER*

Institute of Genetics, University of Cologne, Cologne, Germany

Several experimental findings supported the notion that adenovirus type 2 DNA is covalently linked to cellular DNA in productively infected human cells. Although the significance of this linkage for the process of viral replication is unknown, the recombination of viral DNA with host DNA may simply reflect the efficacy of the recombination systems in mammalian cells. In this report, evidence is presented for the existence of selective sites of recombination between viral and host DNAs. These sites are presumably located in repetitive sequences of cellular DNA. All parts of the viral genome appear to be represented in the recombinant form.

The problem of recombination between virus and host chromosomes has attracted much interest. In particular, the possible significance of virus DNA integration in the process of malignant transformation by viruses has been studied extensively (for a review, see reference 6). Adenovirus DNA has been shown to integrate in abortively infected cells (4, 5), in productively infected cells (2, 16, 23), and in transformed cells (10-13, 20).

In human cells permissively infected by adenovirus type 2 (Ad2), a large number of viral DNA molecules recombine with the host DNA (8, 23). The host-virus DNA hybrids have been shown to exhibit buoyant densities in alkaline CsCl density gradients intermediate between those of host and viral DNAs (2). Furthermore, the host-virus DNA hybrids have been found to anneal sequentially first to viral DNA and then to host DNA and vice versa in DNA-DNA filter hybridization experiments (17). The existence of these hybrids can also be documented by the network technique (23). Viral DNA sequences can be excised from the high-molecular-weight DNA of Ad2-infected cells by restriction endonucleases (1, 16). Several lines of evidence indicate that viral DNA replication is not required for host-virus DNA recombination to occur (9, 18, 23), whereas cellular replication seems to be essential (9).

The role that Ad2 DNA integration may play in viral replication in productively infected cells is not understood. The generation of the host-virus DNA hybrids may simply reflect the efficiency of the recombination system of the host which apparently enables the cell to incorporate practically any foreign DNA into its genome (25). The acquisition of heterologous DNA sequences may either be catastrophic for the cell or bestow upon it entirely novel properties. Malignant transformation as a consequence of viral gene insertion constitutes a readily recognized and extensively investigated new phenotype. More complex alterations of the cellular phenotype which are due to the insertion of foreign DNA and which could supply the cell with evolutionary advantages may be much harder to assess and have hitherto not been investigated. Irrespective of the nature of the incorporated heterologous DNA sequences, the sites of insertion into the host genome could determine the consequences of such recombination events for the cell. Investigations on host-virus DNA recombination may help elucidate important aspects of these fundamental mechanisms in eucaryotic cells.

In this communication, evidence will be presented that, in permissively infected human cells, Ad2 DNA sequences become linked to cellular DNA at selective sites, presumably in redundant cellular DNA sequences. Although a considerable number of viral DNA molecules are involved in recombination with host DNA, only a limited number of sites of host-virus DNA linkage have been observed.

MATERIALS AND METHODS

Cells and virus. The origin of the human KB cells and human embryonic kidney (HEK) cells and of Ad2 and the methods of propagating and purifying Ad2 have been described previously (2, 4, 8, 16).

Purification of Ad2 DNA. Viral DNA which was used as a probe in blotting experiments (see below) was prepared as follows. Ad2 was plaque purified twice in HeLa cells and then propagated to high titers on KB cells. Ad2 was centrifuged to equilibrium three times in CsCl density gradients (0.02 M Tris-hydrochloride, pH 8.1) as described previously (5). The virus was subsequently dialyzed into 0.02 M Tris-hydrochlo-

ride (pH 7.2)–0.001 M Mg^{2+} for 4 h and incubated with DNase I (Worthington Diagnostics; 0.2 μg/ml) at room temperature for 10 min. EDTA was then added to a final concentration of 0.002 M. The treatment with DNase I was introduced to remove possible trace contaminations of the virion with cellular DNA. The virus was subsequently rebanded once in a CsCl-sucrose gradient (22), and the viral DNA was extracted as described previously (2).

Purification of the *Bam*HI fragments of Ad2 DNA. Ad2 DNA was cleaved with restriction endonuclease *Bam*HI, according to published protocols, and the DNA fragments were preparatively separated by electrophoresis in 0.5% agarose gels in TEB buffer (0.1 M Tris-hydrochloride, 0.077 M H_3BO_3 [pH 8.3], 0.0025 M EDTA). Each viral DNA fragment was excised from the gels and reelectrophoresed twice to obtain highly purified DNA fragments. Details of this procedure have been outlined elsewhere (7, 20).

Cloned DNA fragments. *Hin*dIII fragments C, D, E, and I of Ad2 DNA were cloned in pBR322. The clones were kindly provided by S.-L. Hu and D. Solnick of the Cold Spring Harbor Laboratory. Plasmid DNA was isolated and purified by published procedures (7). The locations on the Ad2 map of the *Hin*dIII fragments used as probes are indicated in Fig. 3.

Elution of DNA fragments from gel slices. Specific DNA fragments were excised from horizontal gel slices under UV light control and with appropriate marker DNA fragments in parallel tracks for orientation. Published elution methods were employed (21). In brief, a small ditch was cut into the gel immediately in front of the band to be eluted. This ditch was filled with hydroxyapatite suspended in TEB buffer, and electrophoresis was continued for several hours until all of the DNA to be eluted was bound to hydroxyapatite (UV light control). The DNA was subsequently eluted from the hydroxyapatite with 1 M sodium phosphate (pH 7.25) and further purified by filtration over a Sephadex G-50 column in 0.2 M NaCl–0.01 M Tris-hydrochloride (pH 7.5)–0.001 M EDTA. The DNA was finally concentrated by ethanol precipitation and suspended in 0.01 M Tris-hydrochloride (pH 7.5)–0.001 M EDTA.

Extraction, fractionation, and purification of high-molecular-weight nuclear DNA from Ad2-infected human cells. KB cells or HEK cells were infected with CsCl-purified Ad2 at a multiplicity of infection of 100 PFU per cell. At various times postinfection, nuclei were isolated as described elsewhere (1). The total intranuclear DNA was then extracted, and the high-molecular-weight DNA was selected by zone velocity sedimentation on neutral sucrose density gradients as described earlier (1). The high-molecular-weight nuclear DNA was subsequently chromatographed on benzoylated-naphthoylated DEAE-cellulose columns (1) to eliminate all single-stranded DNA molecules which might resist cleavage by restriction endonucleases. The DNA was further purified by treatment with pancreatic RNase at low and high salt concentrations, by incubation (60 min) with proteinase K (250 μg/ml), and by equilibrium sedimentation in neutral CsCl density gradients. Details of these procedures have been reported previously (20). DNA from uninfected cells was prepared by the same procedure.

Analysis of high-molecular-weight nuclear DNA by Southern blotting and hybridization with intact Ad2 DNA or Ad2 DNA fragments. The high-molecular-weight DNA from uninfected or from Ad2-infected cells was subsequently cleaved with restriction endonuclease *Eco*RI or *Bam*HI by routine protocols. The DNA fragments generated were separated by electrophoresis in horizontal 0.5% agarose slab gels in TEB buffer and then transferred by the Southern blotting technique (19) to nitrocellulose filters as described earlier (7, 20). Ad2-specific DNA sequences were detected on the blots by hybridization of the DNA fixed on the nitrocellulose filters to intact Ad2 DNA, to *Bam*HI fragments, or to cloned *Hin*dIII fragments of Ad2 DNA which had previously been ^{32}P labeled by the nick translation procedure (15). After extensive washing, the hybridized filters were autoradiographed on XR-5 film (Kodak). Details of these techniques have been described elsewhere (7, 20).

RESULTS AND DISCUSSION

Recombination between viral and host DNAs in human cells productively infected with Ad2. The initial events of host-virus DNA recombination obviously cannot be studied in adenovirus-transformed cells, as these cells represent highly selected products of these interactions. Therefore, we investigated the recombination between virus and host DNAs in human cells productively infected with Ad2. Several lines of evidence have suggested that a rather large number of Ad2 DNA copies become covalently linked to cellular DNA at as early as 2 to 4 h postinfection, i.e., at a time when the synthesis of unit-length viral DNA has not yet started (1, 8, 16, 17). We analyzed the high-molecular-weight form of viral DNA isolated from Ad2-infected human cells at various times early after infection for the sites of viral DNA integration. The DNA was selected for size by zone velocity sedimentation and for double-stranded molecules by chromatography on benzoylated-naphthoylated DEAE-cellulose columns as described previously (1). The DNA was subsequently cleaved with restriction endonuclease *Bam*HI or *Eco*RI and analyzed by Southern blotting and hybridization, using intact Ad2 DNA which was ^{32}P labeled by nick translation. Figure 1a shows that, at least as early as 4 h postinfection, there were two to three Ad2-specific bands migrating in 0.5% agarose gels in molecular weight positions higher than that of *Bam*HI fragment A (14.1 kilobase pairs) of Ad2 DNA. The high-molecular-weight fragments were apparent as late as 10 h postinfection (Fig. 1a) and probably later (data not shown). Ad2-specific bands comigrating, but not necessarily identical, with the known *Bam*HI fragments were also apparent. In cleaving the DNA from Ad2-infected cells, *Bam*HI concentrations about fivefold higher than required to digest com-

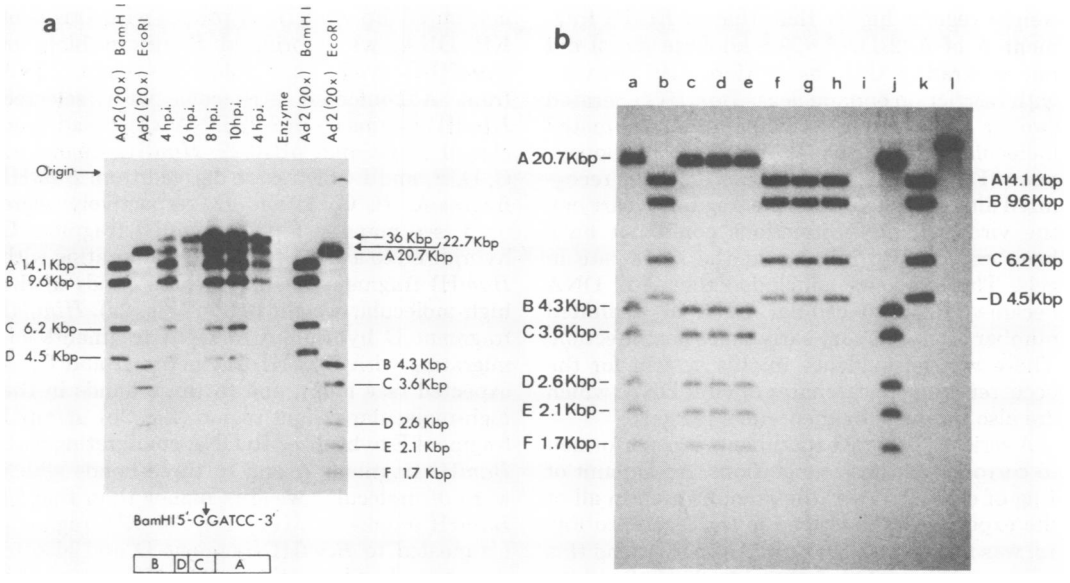

FIG. 1. *Integration of Ad2 DNA in productively infected human KB cells. (a) The high-molecular-weight DNA from Ad2-infected KB cells was isolated at time periods postinfection as indicated and selected as described in the text. The DNA was then cleaved with restriction endonuclease BamHI (5 U/μg of DNA). The fragments (1 μg per slot) were separated by electrophoresis in horizontal 0.5% agarose (SeaKem) slab gels and blotted to nitrocellulose filters. As a molecular weight marker, Ad2 virion DNA was cleaved with restriction endonuclease BamHI or EcoRI. Intact, ^{32}P-labeled Ad2 DNA was used as a hybridization probe. The BamHI map of Ad2 DNA is also indicated. Marker fragment sizes are given in kilobase pairs (Kbp). Ad2(20×) BamHI, 20 genome equivalents per cell (0.05 ng) and 1 μg of cell DNA, cleaved with restriction endonuclease BamHI. Ad2(20×) EcoRI, 20 genome equivalents per cell (0.05 ng) and 1 μg of cell DNA, cleaved with restriction endonuclease EcoRI. Genome equivalents were calculated on the basis of 1 μg of cellular DNA per slot as described previously (20). DNA from Ad2-infected cells was isolated at 4, 6, 8, and 10 h postinfection. Restriction endonuclease BamHI was used as a control in the slot designated Enzyme. Fragment sizes in molecular weight ranges outside the linear scale on the gel (>BamHI fragment A) were estimated by an extrapolation procedure (E. M. Southern, personal communication). (b) Control and reconstitution experiments. An amount of 1 μg of DNA from uninfected cells was mixed with 0.05 ng of Ad2 DNA (corresponding to 20 genome equivalents per cell) and cleaved with 40 (a), 20 (c and j), 10 (d), or 5 (e) U of restriction endonuclease EcoRI or 20 (b), 10 (f), 5 (g and k), or 1 (h) U of restriction endonuclease BamHI. An amount of 1 μg of DNA from mock-infected KB cells was cleaved with 20 U of restriction endonuclease EcoRI (i). In addition, 1 μg of cellular DNA was cleaved with 20 U of restriction endonuclease EcoRI, the enzyme was inactivated, and 0.025 ng of uncleaved Ad2 DNA was added (l). All DNA preparations were electrophoresed, transferred to a nitrocellulose sheet by the Southern blotting procedure, and analyzed by DNA-DNA hybridization using ^{32}P-labeled Ad2 DNA as described previously (20, 22).*

pletely an equivalent amount of Ad2 DNA were used (Fig. 1a and b). Thus, it is unlikely that the Ad2-specific high-molecular-weight DNA bands were due to incompletely cut Ad2 DNA. Moreover, the size of the largest of these bands equaled or even exceeded that of unit-length Ad2 DNA (Fig. 1a). Hence, the high-molecular-weight bands of Ad2-specific DNA could have been derived from concatenates of Ad2 DNA. In a similar experiment, the high-molecular-weight form of Ad2 DNA was also cleaved with restriction endonuclease *Eco*RI. As reported previously (1), there were three to four Ad2-specific bands intermediate in size between those of *Eco*RI fragments A and B which could also be detected by the transfer technique (data not shown). In addition, Ad2-specific sequences were also found to comigrate with the known *Eco*RI marker DNA fragments. There was no virus-specific DNA with a molecular weight higher than that of *Eco*RI fragment A of Ad2 DNA. These data provided further evidence against the possibility of concatenates of Ad2 DNA. The absence of Ad2-specific fragments with molecular weights higher than that of *Eco*RI fragment A indicated either that the site of junction to cellular DNA is near the terminus of Ad2 DNA or that fragments and not the intact Ad2 DNA are linked to cellular DNA. The finding that Ad2-specific DNA was absent from molecular

weight regions higher than that of *Eco*RI fragment A of Ad2 DNA (20.8 kilobase pairs) did not contradict the observation that cleavage with restriction endonuclease *Bam*HI generated two or three intense bands in the estimated molecular weight ranges of 23 to 36 kilobase pairs (Fig. 1a). Clearly, the two enzymes recognized different sites; thus, the fragments carrying the virus-cell DNA junctions could not have been expected to migrate at the same rate in gels. Therefore, we concluded that Ad2 DNA became linked to cellular DNA at a limited number of sites at very early times postinfection. There was no evidence in this system for the occurrence of concatenates of viral DNA, which are also unlikely for theoretical reasons.

A series of control experiments was performed to corroborate these conclusions. An amount of 1 μg of cellular DNA, the amount used in all of the experiments described in the legend to Fig. 1a, was mixed with 0.5 ng of Ad2 DNA, and this mixture was incubated with 1 to 20 U of restriction endonuclease *Bam*HI (Fig. 1b, tracks b, f, g, h, and k) or 5 to 40 U of restriction endonuclease *Eco*RI (Fig. 1b, tracks a, c, d, e, i, and j). Figure 1b shows that 1 U of the *Bam*HI and 5 U of the *Eco*RI enzymes were sufficient to cleave 1 μg of DNA to completion. In the experiments described in the legend to Fig. 1a, 5 U of *Bam*HI enzyme per μg of DNA was used, i.e., an amount five times higher than the minimal amount shown to cut 1 μg of DNA to completion. Similarly, the excess of restriction endonuclease *Eco*RI was fourfold. In further control experiments, 1 μg of KB DNA was cleaved with 20 U of restriction endonuclease *Eco*RI (Fig. 1b, track i) or with 5 U of restriction endonuclease *Bam*HI (data not shown). There was no hybridization detectable between Ad2 DNA and the DNA from uninfected KB cells (Fig. 1b, track i). Lastly, uncleaved Ad2 DNA was mixed with *Eco*RI-cut cellular DNA, and the mixture was electrophoresed. Upon transfer and hybridization to ^{32}P-labeled Ad2 DNA, only one Ad2-specific band was detectable on the blot (Fig. 1b, track l). We concluded that the Ad2-specific off-size bands detected in the experiment described in the legend to Fig. 1a could not have been due to incomplete digestion of DNA with restriction endonucleases or to adventitious homologies between viral and cellular DNAs.

In this study, we also used as hybridization probes each of the highly purified *Bam*HI fragments of Ad2 DNA which were ^{32}P labeled by nick translation. The results of these analyses (data not shown) indicated that all segments of the Ad2 genome were represented in the high-molecular-weight bands (>14 kilobase pairs). To rule out artifacts of a trivial nature, e.g., contamination of the viral DNA probes with traces of KB DNA, we hybridized Southern blots of *Bam*HI-cleaved high-molecular-weight DNA from Ad2-infected KB cells with selected *Hin*dIII fragments of Ad2 DNA which had been cloned in plasmid pBR322. *Hin*dIII fragments C, D, E, and I which were derived from *Bam*HI fragments B, C, A, and D, respectively, were used (see maps in Fig. 2). *Hin*dIII fragment C hybridized to a DNA fragment comigrating with *Bam*HI fragment B and to three bands in the high-molecular-weight region (Fig. 2a). *Hin*dIII fragment D hybridized to DNA fragments comigrating with *Bam*HI fragments D and C, as expected (see map), and to three bands in the high-molecular-weight region (Fig. 2b). *Hin*dIII fragment E hybridized to DNA comigrating with *Bam*HI fragment A and to three bands which were of molecular weights higher than that of *Bam*HI fragment A (Fig. 2c); *Hin*dIII fragment I annealed to *Bam*HI fragment D and also to the three bands in the high-molecular-weight region (Fig. 2d). These results were not compatible with the argument of incomplete digestion of Ad2 DNA and ruled out the possibility that hybridization of Ad2 DNA to high-molecular-weight DNA was caused by weak homologies between viral and KB cellular DNAs. The data further documented the notion that the entire Ad2 DNA molecule was represented in the virus-host recombinants. Figure 2a through d showed that the amounts of restriction endonuclease *Bam*HI used cleaved 1 μg of DNA to completion.

The data presented did not argue per se for covalent linkage of viral to cellular DNA. The results shown in Fig. 1 and 2 would also be compatible with a model assuming modifications or rearrangements in the Ad2 DNA molecule. Modifications of Ad2 DNA in the sense of DNA methylation have not been found in productively infected cells (24). Moreover, previously published results of sequential hybridization experiments with high-molecular-weight DNA from Ad2-infected cells clearly supported the notion of covalently linked viral and host DNAs (17).

Further evidence against the possibility of incomplete digestion of intracellular Ad2 DNA was provided by the results of the following experiment. The high-molecular-weight DNA from Ad2-infected KB cells was cleaved with restriction endonuclease *Bam*HI, and the DNA from the region of higher molecular weight than the *Bam*HI fragment A (>BamHI-A) was eluted from the gel as described previously (21). This DNA was subsequently cleaved with restriction endonuclease *Hin*dIII. Figure 3 showed that the cleavage pattern generated differed clearly from that of Ad2 DNA cut with the same enzyme. On the other hand, when Ad2 DNA used as a con-

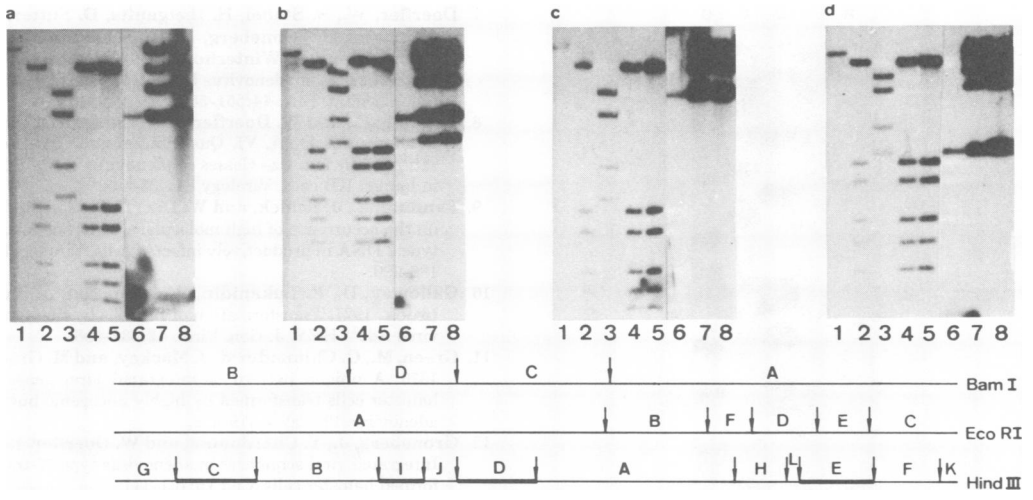

FIG. 2. *Each BamHI fragment is represented in the >BamHI fragment A DNA. The high-molecular-weight DNA from Ad2-infected KB cells was isolated and purified as described in the text. This DNA was then cleaved with restriction endonuclease BamHI, and the DNA fragments were separated by electrophoresis on 0.5% agarose gels and transferred to nitrocellulose filters as described in the legend to Fig. 1. The filters were subsequently cut into two parts. The DNA on the left parts comprising slots 1 to 5 was hybridized with ^{32}P-labeled Ad2 DNA; the DNA on the right parts (comprising slots 6 to 8) was hybridized with ^{32}P-labeled, cloned HindIII fragment C (a), D (b), E (c), or I (d) of Ad2 DNA. The following DNA samples were analyzed in the slots arranged from left to right: (1) 0.025 ng (10 genome equivalents per cell) of uncleaved Ad2 DNA; (2) 0.025 ng of EcoRI-cut (15 U/μg) Ad2 DNA; (3) 0.025 ng of BamHI-cut (5 U/μg) Ad2 DNA; (4) as in (2); (5) 0.025 ng of EcoRI-cut (20 U/μg) Ad2 DNA (in slots 1 to 6, each DNA preparation contained 1 μg of DNA from uninfected cells as a carrier); (6) 0.05 ng (20 genome equivalents per cell) of BamHI-cut (5 U/μg) Ad2 DNA; (7) High-molecular-weight DNA (1 μg) from Ad2-infected cells isolated at 3 h postinfection and cleaved with restriction endonuclease BamHI (5 U/μg); (8) as in (7), except the DNA was isolated at 4 h postinfection. The BamHI, EcoRI, and HindIII maps of Ad2 DNA were also shown; the HindIII fragments of Ad2 DNA which were cloned and used as probes are designated* ■.

trol was electrophoresed and eluted from the gel before cleavage with restriction endonuclease *Hin*dIII, its cleavage pattern was identical to that of Ad2 DNA that had not been electrophoresed in and eluted from an agarose gel (data not shown).

These data confirmed that the high-molecular-weight DNA fragments exhibiting homology to Ad2 DNA (Fig. 1 and 2) were not due to incomplete digestion or putative inherent homologies between viral and cellular DNAs. The high-molecular-weight DNA molecules constituted recombination products between viral and cellular DNAs. When viral DNA probes were used which were extracted from DNase-treated Ad2 virions as described above, homologies between Ad2 DNA and the DNA from uninfected KB cells could not be detected with the techniques employed in this study. Apparent weak homologies between Ad2 DNA and KB cell DNA could, however, be detected when we used viral DNA isolated from virions which had not been treated previously with DNase I. Therefore, we attribute these apparent homologies to contaminations of Ad2 DNA with trace amounts of host DNA.

Since previous results have indicated that there are a large number of copies of Ad2 DNA per cell in the high-molecular-weight form (8), the present data imply that viral DNA is linked to cellular DNA at selective sites, presumably in repetitive DNA. Alternatively, virus-host sequences that become linked may become amplified very rapidly. This amplification must occur at very early times postinfection. Further work is in progress to characterize the structure of the virus-host hybrid DNA molecules and the sites of virus-host DNA linkage.

Recombinants between Ad 12 DNA and host DNA with a highly regular, symmetric structure have been discovered recently in our laboratory (3). It is conceivable that these recombinants, which carry the origin of adenovirus DNA replication and the site required for packaging of the viral DNA (14), represent just one member in a family of recombinants between adenovirus and host DNA which we have been analyzing in this study.

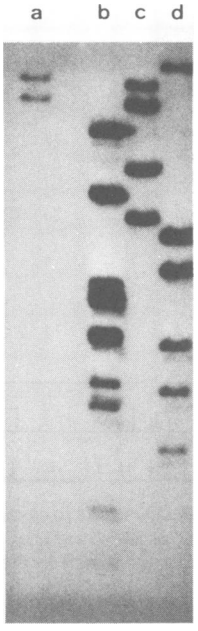

FIG. 3. *Cleavage of >BamHI fragment A DNA, which was eluted from a gel, with restriction endonuclease HindIII. The high-molecular-weight DNA was isolated from Ad2-infected KB cells at 4 h post-infection, and >BamHI fragment A DNA was prepared and eluted from the gel with hydroxyapatite method (21) as described in the text. This DNA was subsequently cleaved with restriction endonuclease HindIII (a). In control experiments, 0.05 ng (20 genome equivalents per cell) of Ad2 DNA was cleaved with restriction endonuclease HindIII (5 U/μg) (b), BamHI (5 U/μg) (c), or EcoRI (15 U/μg) (d).*

ACKNOWLEDGMENTS

This research was supported by the Deutsche Forschungsgemeinschaft through SFB 74.

We thank Birgit Kierspel for typing this manuscript.

LITERATURE CITED

1. **Baczko, K., R. Neumann, and W. Doerfler.** 1978. Intracellular forms of adenovirus DNA. VII. Excision of viral sequences from cellular DNA in adenovirus type 2-infected KB cells. Virology **85**:557–567.
2. **Burger, H., and W. Doerfler.** 1974. Intracellular forms of adenovirus DNA. III. Integration of the DNA of adenovirus type 2 into host DNA in productively infected cells. J. Virol. **13**:975–992.
3. **Deuring, R., G. Klotz, and W. Doerfler.** 1980. An unusual symmetric (SYREC) recombinant between adenovirus type 12 DNA and human cell DNA. Hoppe Seyler's Z. Physiol. Chem. **361**:1276–1277.
4. **Doerfler, W.** 1968. The fate of the DNA of adenovirus type 12 in baby hamster kidney cells. Proc. Natl. Acad. Sci. U.S.A. **60**:636–643.
5. **Doerfler, W.** 1970. Integration of the deoxyribonucleic acid of adenovirus type 12 into the deoxyribonucleic acid of baby hamster kidney cells. J. Virol. **6**:652–666.
6. **Doerfler, W.** 1977. Animal virus-host genome interactions, p. 279–399. *In* R. R. Wagner and H. Fraenkel-Conrat (ed.), Comprehensive virology, vol. 10. Plenum Publishing Corp., New York.
7. **Doerfler, W., S. Stabel, H. Ibelgaufts, D. Sutter, R. Neumann, J. Groneberg, K. H. Scheidtmann, R. Deuring, and U. Winterhoff.** 1980. Selectivity in integration sites of adenovirus DNA. Cold Spring Harbor Symp. Quant. Biol. **44**:551–564.
8. **Fanning, E., and W. Doerfler.** 1977. Intracellular forms of adenovirus DNA. VI. Quantitation and characterization of the four size-classes of adenovirus type 2 DNA in human KB cells. Virology **81**:433–448.
9. **Fanning, E., J. Schick, and W. Doerfler.** 1978. Studies on the occurrence of high molecular weight adenovirus type 2 DNA in productively infected cells. Virology **88**: 186–190.
10. **Galloway, D., E. Lukanidin, W. Topp, and J. Sambrook.** 1977. Transformation of rat cells by the hybrid virus $Ad2^{2+}$ HEY. J. Gen. Virol. **42**:339–356.
11. **Green, M., G. Chinnadurai, J. Mackey, and M. Green.** 1976. A unique pattern of integrated viral genes in hamster cells transformed by highly oncogenic human adenovirus 12. Cell **7**:419–428.
12. **Groneberg, J., Y. Chardonnet, and W. Doerfler.** 1977. Integrated viral sequences in adenovirus type 12-transformed hamster cells. Cell **10**:101–111.
13. **Groneberg, J., and W. Doerfler.** 1976. Integrated viral genomes in adenovirus type 12-transformed hamster cells. Hoppe Seyler's Z. Physiol. Chem. **357**:317.
14. **Hammarskjöld, M. L., and G. Winberg.** 1980. Encapsidation of adenovirus 16 DNA is directed by a small DNA sequence at the left end of the genome. Cell **20**: 787–795.
15. **Rigby, P. J. W., M. Dieckmann, C. Rhodes, and P. Berg.** 1977. Labeling deoxyribonucleic acid to high specific activity in vitro by nick translation with DNA polymerase I. J. Mol. Biol. **113**:237–251.
16. **Schick, J., K. Baczko, E. Fanning, J. Groneberg, H. Burger, and W. Doerfler.** 1976. Intracellular forms of adenovirus DNA. IV. Integrated form of adenovirus DNA appears early in productive infection. Proc. Natl. Acad. Sci. U.S.A. **73**:1043–1047.
17. **Schick, J., and W. Doerfler.** 1978. Integration of adenovirus type 2 DNA in productively infected cells: results of sequential hybridization experiments. J. Gen. Virol. **39**:365–370.
18. **Schick, J., and W. Doerfler.** 1979. High molecular weight virus DNA in KB cells infected with ts mutants of adenovirus type 2 under permissive and non-permissive conditions. J. Gen. Virol. **43**:222–227.
19. **Southern, E. M.** 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. **98**:503–517.
20. **Sutter, D., M. Westphal, and W. Doerfler.** 1978. Patterns of integration of viral DNA sequences in the genomes of adenovirus type 12-transformed hamster cells. Cell **14**:569–585.
21. **Tabak, H. F., and R. A. Flavell.** 1978. A method for the recovery of DNA from agarose gels. Nucleic Acids. Res. **5**:2321–2332.
22. **Tjia, S., E. Fanning, J. Schick, and W. Doerfler.** 1977. Incomplete particles of adenovirus type 2. III. Viral and cellular DNA sequences in incomplete particles. Virology **76**:365–379.
23. **Tyndall, C., H. B. Younghusband, and A. J. D. Bellett.** 1978. Some adenovirus DNA is associated with the DNA of permissive cells during productive or restricted growth. J. Virol. **25**:1–10.
24. **Vardimon, L., R. Neumann, I. Kuhlmann, D. Sutter, and W. Doerfler.** 1980. DNA methylation and viral gene expression in adenovirus-transformed and -infected cells. Nucleic Acids Res. **8**:2461–2473.
25. **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.