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Genetics

An unusual symmetric recombinant between adenovirus type 12 DNA and human cell DNA

(symmetric DNA molecules/defective adenovirus DNA/recombination/hairpin molecules/end-labeling of adenovirus DNA)

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ABSTRACT **On purification of human adenovirus type 12 (Ad12) by equilibrium sedimentation in CsCl density gradients, two bands of particles, Ad12-3 and Ad12-3a, are observed. The particles from band Ad12-3a contain a recombinant of human host cell DNA and of Ad12 DNA. The human cell DNA sequences contain repetitive DNA recurring 200 to 500 times in cellular DNA. Ad12 DNA and the recombinant genomes exhibit the same or similar lengths. This finding suggests that a constant amount of DNA is packaged into complete Ad12 particles. On cleavage of KB cellular DNA with *Eco*RI, *Bam*HI, *Hin*fI, *Msp* I, *Mbo* I, *Pst* I, or *Bgl* II, the ^{32}P-labeled cellular DNA from Ad12-3a particles hybridizes on Southern blots to distinct bands of KB DNA. There is also less-specific background hybridization that is not observed in the control. The cellular DNA from Ad12-3a particles is not methylated, whereas the same cellular sequences in KB cell DNA appear to be extensively methylated. On denaturation and renaturation, the recombinant DNA molecules are converted to molecules half as long as Ad12 DNA, as determined by gel electrophoresis and electron microscopy. The recombinant DNA molecules were terminally labeled by exonuclease III treatment and subsequent refilling of the depleted segments with [^{32}P]dNTPs by using DNA polymerase I (Klenow fragment). When these molecules were cleaved with *Eco*RI, *Bam*HI, *Msp* I, or *Pst* I, only one terminal DNA fragment was found to be labeled. The results of partial digestion experiments using *Msp* I, *Hin*fI, or *Mbo* I are consistent with a model in which 700–1150 base pairs from the left terminus of Ad12 DNA are linked to host cell DNA containing repetitious sequences, and this structure is symmetrically duplicated as a large inverted repeat of the type ABCDD'C'B'A'. The Ad12 DNA sequences are flanking the entire molecule, which consists mainly of human KB cell DNA. The recombinants appear to be stable on serial passage of the virus preparation for many years, although variations in the sequence of the recombinants occur. These symmetric recombinant (SYREC) molecules suggest a way to use adenovirus DNA as a eukaryotic vector. Their occurrence provides further evidence for the generation of virus–host DNA recombinants and may help elucidate the role this interaction may have in adenovirus replication and oncogenesis.**

Purification of human adenovirus type 12 (Ad12) by CsCl equilibrium sedimentation gives two bands of Ad12 particles that have buoyant densities of 1.327 (Ad12-3) and 1.325 (Ad12-3a) g/cm^3 (1, 2). The two types of particles are morphologically identical. They contain DNA of the same size. The relative amounts of particles in bands Ad12-3 and Ad12-3a vary from experiment to experiment. The amount of Ad12-3a particles seems to increase with the number of viral passages. Particles comparable in buoyant density with the noninfectious Ad12-3a particles have not been observed with adenovirus type 2 (Ad2). The Ad12-3a particles are clearly different from incomplete adenovirus particles (1–8). Density variants containing deletions in Ad12 DNA have also been investigated (3, 8).

Recombinants between cellular and simian virus 40 DNA have been detected (9), and several have been analyzed in great detail (10–13). They contain cellular DNA sequences of both the unique and the repetitive types and are generated on multiple viral passages at high multiplicities.

In the present report, we describe recombinants between human Ad12 DNA and human KB cell DNA. These recombinants are produced on repeated high-multiplicity passage of Ad12 on human KB cells. The DNA is a symmetric molecule, a huge inverted repeat, in which 700–1150 base pairs from the left end of Ad12 DNA flank human cell DNA sequences, which contain repetitive DNA and make up the bulk of the molecule. On denaturation and rehybridizing, hairpin molecules are generated that are half the length of Ad12 DNA. The occurrence of Ad12–cell DNA recombinants provides further evidence for virus–host DNA recombination (14) in productively infected cells.

MATERIALS AND METHODS

Cells and Virus. The propagation and purification of Ad12 (Huie strain) in suspension or monolayer cultures of human KB cells were as described (15). Ad12 was passaged repeatedly at high multiplicities, and infected cells were harvested 48–72 hr after infection. In some experiments, Ad12 was purified by three cycles of CsCl equilibrium sedimentation and then treated with DNase I (0.2 μg/ml) and further purified as described (16). Bands Ad12-3 and Ad12-3a (1.2) were separated from each other by several cycles of CsCl equilibrium sedimentation, selecting after each centrifugation step for the heavy or light band (2).

Ad12 DNA. Ad12 DNA was extracted as described (17).

Restriction Enzyme Analysis of DNAs. The Ad12-3 and Ad12-3a DNAs, KB cell DNA, and pBR322 DNA (18) were analyzed by using restriction endonucleases *Eco*RI, *Bam*HI, *Hin*dIII, *Pst* I, *Mbo* I, *Hin*fI, *Msp* I, *Hpa*II, or *Bgl* II according to published protocols [New England BioLabs, Bethesda Research Laboratories (Rockville, MD), or Boehringer Mannheim]. Fragments were separated on horizontal 0.4–2.2% agarose slab gels as described (19) and transferred by the Southern blotting procedure to nitrocellulose filters (20). In some experiments, Ad12-3a DNA (see Fig 1*a*) was excised and eluted from agarose gels, nick translated (21), and used in DNA–DNA hybridization experiments. In control experiments, ^{32}P-labeled pBR322 DNA was used as hybridization probe. Details of the

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Abbreviations: Ad12, Ad12-3, and Ad12-3a, adenovirus type 12, type 12-3, and type 12-3a, respectively; bp, base pair(s); SYREC, symmetric recombinant.

hybridization (22) and autoradiographic techniques have been described (23, 24).

Terminal Labeling of Viral DNA (19, 25). Ad12 DNA and Ad12-3a DNA were ^{32}P-labeled at their 3′ termini by published methods (25). The DNA was digested to a limited extent with exonuclease III from *Escherichia coli* (26) at 13°C for 20 min. Subsequently, the depleted termini were refilled by using the Klenow fragment of DNA polymerase I (27) and the four [α-^{32}P]dNTPs. Terminally labeled DNA was partially digested (28) as described (19) using *Msp* I, *Hin*fI, or *Mbo* I.

Quantitation of Cellular Sequences from Ad12-3a DNA in Human Cell DNA. A published method (24) was used. KB cell DNA was cut with various restriction endonucleases, the fragments were separated by electrophoresis on 0.5% agarose gels and blotted onto nitrocellulose filters, and Ad12-3a-specific sequences were detected by hybridization to purified ^{32}P-labeled Ad12-3a DNA. The intensities of bands on the autoradiograms were compared with those of a reconstitution series by using known amounts of unlabeled Ad12-3a DNA cleaved with the same restriction endonucleases and subjected to electrophoresis on the same gel.

Denaturation and Renaturation of DNA; Electron Microscopy of DNA and of Virions. DNA ($\approx$10 μg/ml) was completely denatured in 0.05 M NaOH for 10 min at room temperature and renatured in 50% formamide/0.17 M Tris·HCl (pH 7.7) at room temperature for 1 hr. DNA samples were spread with cytochrome *c*, mounted on low-noise carbon supports, and positively stained with 0.1 mM uranyl acetate in 50% ethanol (29). A Siemens Elmiscope 102 was operated in dark field at a magnification of 10,000–40,000. Purified virus particles were negatively stained with aqueous uranyl acetate and visualized in bright field at a magnification of 50,000–100,000.

RESULTS

Two Buoyant Density Populations of Ad12 Virions. On repeated high-multiplicity passage of Ad12 on human KB cells, two populations of virions are generated that can be separated by repeated cycles of equilibrium centrifugation in CsCl density gradients (1, 2). By electron microscopy of negatively stained virions, the two types of particles are morphologically indistinguishable. The DNA molecules extracted from Ad12-3 or Ad12-3a particles have identical lengths, as shown by gel electrophoresis (Fig. 1*b*) and electron microscopy, and nearly the same buoyant densities in neutral CsCl density gradients (2). The basis for the small difference in density between the Ad12-3 and Ad12-3a particles is not understood.

The DNA was extracted from Ad12-3 and Ad12-3a particles and cleaved with *Eco*RI or *Bam*HI (Fig 1*a*). The fragments were analyzed by electrophoresis on horizontal 0.5% or 0.8% agarose slab gels. On cleavage with *Eco*RI, the known Ad12 DNA fragments and an aberrant band (pre-A) that is larger than the *Eco*RI A fragment are generated. Cleavage with *Bam*HI produces at least two additional bands, one larger (>A) and one smaller (<A) than the *Bam*HI A fragment. The nature of the minor bands (see Fig. 1*b*) has not been investigated.

Termini of the Ad12-3a DNA Molecule. The DNA extracted from a mixture of Ad12-3 and Ad12-3a particles was labeled at the termini (see Fig. 1*d*) as described (19, 25). The DNA was subsequently cleaved with *Eco*RI, *Bam*HI (Fig. 1*a*), or *Msp* I (Fig. 1*c*). Fragments were separated by gel electrophoresis, and the labeled terminal fragments were identified by autoradiography. The results shown in Fig. 1*a* demonstrate that, on cleavage with *Eco*RI, the two terminal *Eco*RI fragments of Ad12 DNA (A and C) and the pre-A DNA are labeled. On cleavage with *Bam*HI, the two terminal *Bam*HI fragments (A and E) of Ad12 DNA and the <A fragment are labeled. The *Eco*RI pre-A band and both *Bam*HI-generated bands, the one larger and the one smaller than the *Bam*HI fragment A, are presumably derived from Ad12-3a DNA. On cleavage of terminally labeled Ad12-3a DNA with *Msp* I, the terminal *Msp* I fragment of Ad12 DNA, which is 143 base pairs (bp) long (19), is exclusively labeled (Fig. 1*c*).

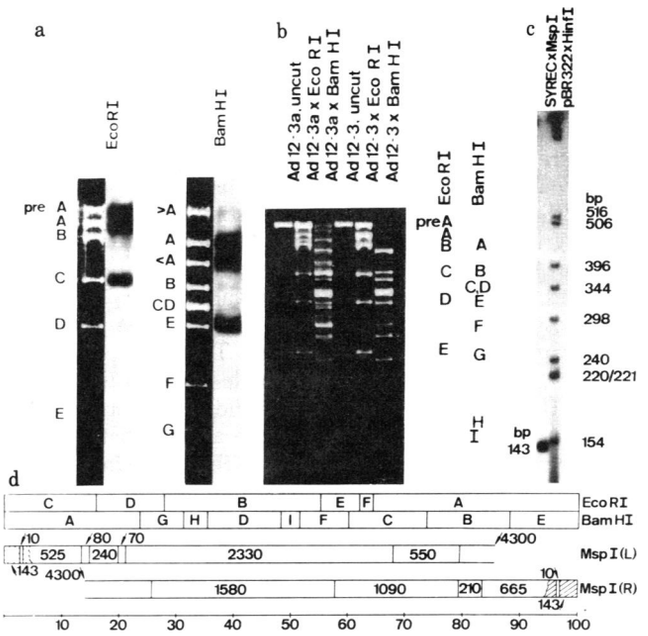

FIG. 1. Characterization of Ad12-3 and Ad12-3a DNAs. (*a*) DNA was extracted from a mixture of Ad12-3 and Ad12-3a particles and cleaved with *Eco*RI or *Bam*HI as indicated. Fragments were separated by electrophoresis on agarose slab gels and visualized by staining with ethidium bromide (0.5–1 μg/ml) and by photography using a UV light source. Terminal labeling: The termini of the same DNA molecules were ^{32}P-labeled as described (19, 25). Subsequently, the DNA was cleaved with *Eco*RI or *Bam*HI as indicated, and the fragments were separated by electrophoresis on horizontal 0.8% agarose slab gels. The locations of the ^{32}P-labeled terminal fragments were determined by autoradiography. (*b*) Virion particles Ad12-3 and Ad12-3a were separated and partly purified from each other by repeated cycles of equilibrium sedimentation in CsCl density gradients. DNA was extracted from either population, cleaved with *Eco*RI or *Bam*HI, and analyzed as described. Uncut Ad12-3 and Ad12-3a DNAs were also subjected to electrophoresis. (*c*) DNA from highly purified Ad12-3a particles was terminally labeled (19, 25) as described and cleaved with *Msp* I, and the fragments were separated by electrophoresis on a 5% polyacrylamide gel. The DNA of plasmid pBR322 was cut with *Hin*fI, and the fragments were terminally ^{32}P-labeled by polynucleotide kinase as described (19) and used as markers. The locations of labeled fragments were determined by autoradiography. (*d*) *Eco*RI and *Bam*HI maps of Ad12 DNA and *Msp* I maps of the right (R) and left (L) termini (19). The *Msp* I maps are drawn at a different scale as compared with the *Eco*RI and *Bam*HI maps. Numbers indicate bp. The scale represents the percent of genome length relative to the *Eco*RI and *Bam*HI maps.

In the experiment described in Fig. 1*b*, the Ad12-3 and Ad12-3a particles were partly purified from each other by repeated cycles of equilibrium centrifugation. Subsequently, each DNA preparation was cleaved with *Eco*RI or *Bam*HI, and the fragments were separated. The results show that Ad12-3 DNA gives the expected *Eco*RI or *Bam*HI cleavage patterns of Ad12 DNA, whereas cleavage of Ad12-3a DNA with *Eco*RI gives pre-A DNA and cleavage with *Bam*HI gives a series of new bands. Uncut Ad12 DNA has the same apparent size as pre-A (Ad12-3a) DNA. Each DNA preparation is still contaminated with DNA from the partner band.

Ad12-3a DNA Represents an Inverted Repeat of the Type ABCDD′C′B′A′. The possibility existed that Ad12-3a DNA

consisted at least partly of authentic Ad12 DNA sequences that might enable Ad12-3a DNA to hybridize with Ad12 DNA. A mixture of Ad12-3 and Ad12-3a DNAs was denatured and rehybridized. The renatured DNA was examined in the electron microscope and by gel electrophoresis. Heteroduplex molecules were not observed. The majority of the molecules represent double-stranded DNA. Contour length measurements showed that up to 60% of the double-stranded molecules on parallel grids of one spreading represent halves of unit-length Ad12 DNA (30). A total of 38 half-length and 25 unit-length molecules (Fig. 2*a*) were actually measured. Complex structures consisting of double- and single-stranded DNA in various arrangements have also been observed. These complex structures were far less abundant than double-stranded DNA molecules. The simplest explanation for the occurrence of half-length double-stranded molecules is to assume that Ad12-3a DNA represents a giant inverted repeat (Fig. 2*c*). On denaturation and renaturation, each strand would fold back on itself, giving rise to a hairpin-like molecule that is double-stranded and half the length of Ad12 DNA. The full-length double-stranded DNA molecules originate from denaturation and rehybridization of Ad12-3 or Ad12-3a DNA molecules with complementary strands of unit length. The partly single-stranded filaments may be due to hybridization of DNA fragments.

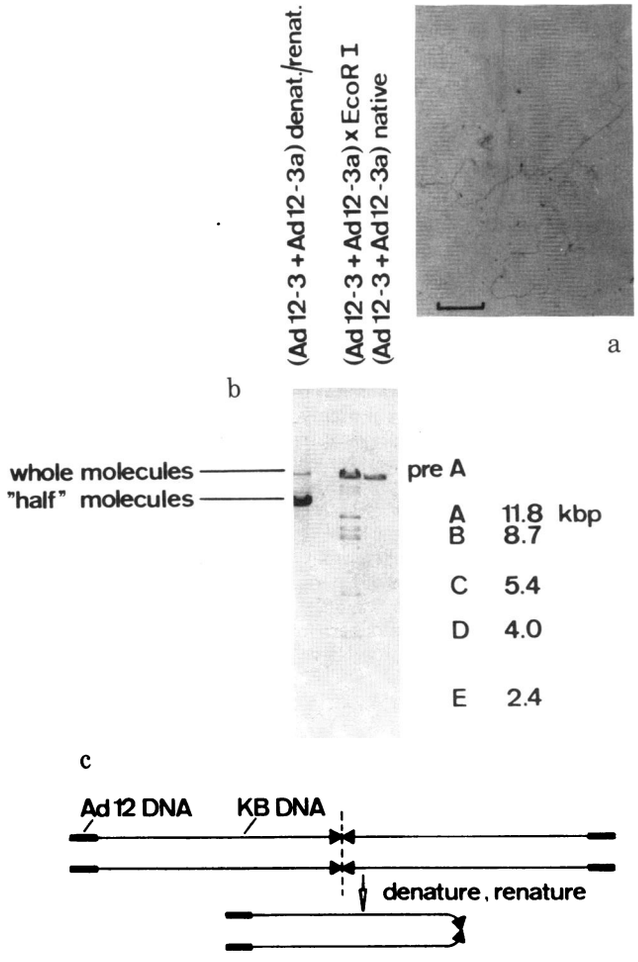

FIG. 2. Symmetry of the Ad12-3a molecule. DNA from a mixture of Ad12-3 and Ad12-3a, in which Ad12-3a was predominant, was denatured and rehybridized as described in *Materials and Methods*. The DNA was analyzed (*a*) by electron microscopy or (*b*) by electrophoresis on horizontal 0.5% agarose slab gels. Native DNA and a mixture of Ad12-3 and Ad12-3a DNA cleaved with *Eco*RI in different tracks were used as M_r markers. A scale of M_r in kilobase pairs (kbp) derived for the *Eco*RI fragments A–E is also indicated. (*c*) Model for the symmetric Ad12-3a molecules before and after denaturation and renaturation. The bar in *a* equals 1 μm.

The denatured and rehybridized molecules were also analyzed by electrophoresis (Fig. 2*b*). DNA preparations containing a high proportion of Ad12-3a DNA give predominantly half-length molecules, and these half-length molecules hybridize to a DNA preparation strongly enriched for Ad12-3a DNA. These findings are in agreement with those adduced by electron microscope analysis. It is also apparent that cleavage of a mixture of Ad12-3 and Ad12-3a DNAs produces the known pattern of Ad12 DNA fragments and pre-A DNA that comigrates on electrophoresis with uncut DNA. Minor aberrant bands have not been analyzed further. Native Ad12-3 and Ad12-3a DNAs migrate at the same rates and hence have the same lengths. Ad12-3a DNA is identical in size with pre-A DNA—i.e., Ad12-3a DNA does not have an *Eco*RI site. Cleavage of a mixture of Ad12-3 and Ad12-3a DNAs with *Eco*RI and subsequent separation of the Ad12-3 DNA fragments from the uncut Ad12-3a DNA molecules is a convenient way to purify Ad12-3a DNA.

Ad12 DNA Sequences in the Ad12-3a Variant. To elucidate further the structure and composition of the Ad12-3a molecules, the segments of authentic Ad12 DNA represented in the variant were determined. Ad12-3a DNA was cleaved with *Msp* I, and the fragments were separated by agarose gel electrophoresis and transferred to a nitrocellulose filter (20). The DNA was then hybridized to the ^{32}P-labeled terminal *Msp* I fragment of Ad12 DNA (143 bp) which had been prepared as described (24). This highly specific probe hybridizes exclusively to a 143-bp fragment (data not shown), indicating that Ad12-3a DNA contains the Ad12 DNA terminus and has at least 143 bp in common with authentic Ad12 DNA on either end. Moreover, Ad12 DNA hybridizes to the left-terminal *Eco*RI C fragment of authentic Ad12 DNA and not to other segments of Ad12 DNA. Finally, the left-terminal fragment of Ad12 DNA (see Fig. 1*d*) or Ad12-3a DNA was terminally labeled as described (19, 25) and detailed in the legend to Fig. 3. Subsequently, the DNA was subjected to partial digestion by *Msp* I, *Mbo* I, or *Hin*fI as described (28). By comparing the cleavage patterns of the variant DNA with those of Ad12 DNA, the extent of authentic viral DNA sequences in the variant has been deduced. The data based on partial digests with *Hin*fI show that Ad12 DNA has at least 700 and maximally 1150 bp from the left terminus in common with variant DNA (Fig. 3). Similar findings have been derived from partial digestion experiments with *Msp* I or *Mbo* I (data not shown). We conclude that the Ad12-3a recombinant carries 700–1150 bp derived from the left end of Ad12 DNA on either terminus. The data also suggest that the Ad12 DNA sequences in the variant have not been significantly rearranged relative to virion DNA.

Ad12-3a Variant DNA Represents a Recombinant Between Ad12 and KB Cell DNAs. The bulk of the DNA sequences in the variant DNA molecule are not of viral but of cellular origin. Human KB cell DNA or a mixture of Ad12-3 and Ad12-3a DNA was cleaved with *Eco*RI, *Bam*HI, *Bgl* II, *Hin*fI, *Msp* I, *Hpa* II, *Mbo* I, or *Pst* I, and the DNA fragments were separated by agarose gel electrophoresis, blotted, and hybridized to ^{32}P-labeled Ad12-3a DNA as a probe. Cleavage of KB cell DNA gives rise to distinct hybridization patterns with Ad12-3a DNA for each restriction endonuclease (Fig. 4). Some of the Ad12-3a specific bands are identical regardless of whether KB cell DNA or Ad12-3a DNA had been hybridized to the probe; other bands are unmatched. Ad12-3a DNA does not hybridize with salmon sperm DNA or DNA from bacteriophage λ (data not shown). Ad12-3a DNA contains human cell DNA sequences of the repetitive type; on cleavage with eight different restriction en-

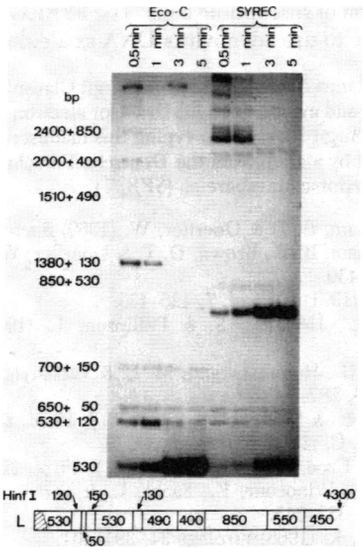

FIG. 3. Common sequences between Ad12 DNA and Ad12-3a variant DNA. A mixture of Ad12 DNA and Ad12-3a DNA was terminally labeled by controlled exonuclease III digestion and subsequent resynthesis using the Klenow fragment of DNA polymerase I and [^{32}P]dNTPs (19, 25). The DNA was then cleaved with *Eco*RI, and the *Eco*RI C fragment and Ad12-3a DNA, which lacks an *Eco*RI site, were isolated by electrophoresis on horizontal 0.5% agarose slab gels. Both DNA preparations were subjected to partial digestion (28) with *Hin*fI. An amount equivalent to ≈1500 cpm of each ^{32}P-labeled DNA preparation was mixed with 0.5 μg of salmon sperm DNA as carrier and incubated with 0.85 unit of *Hin*fI for 0.5, 1, 3, or 5 min as indicated. The reaction was stopped by adding EDTA and $NaDodSO_4$, and the preparation was heated to 70°C for 5 min. Reaction products were analyzed by electrophoresis on horizontal 1.8% agarose slab gels. Comparison of the reaction products derived from the *Eco*RI C fragment of authentic Ad12 DNA with those of the Ad12-3a variant indicates that fragments of 530 bp, 530 +120 = 650 bp, and 650 + 50 = 700 bp are common to both types of molecules (cf. the *Hin*fI map given in ref. 19). The next larger fragment obtained with Ad12-3a DNA, 1100–1150 bp long, is absent from the *Eco*RI C-fragment cleavage products. Hence, Ad12 DNA and recombinant DNA share a sequence that is 700–1150 bp long.

donucleases, distinct bands of homology between KB cell DNA and Ad12-3a DNA are observed. On extended exposure of the autoradiograms, enhanced background blackening of the film is apparent, which may indicate the presence of more heterogeneous populations of cellular DNA. The data also show that the cellular DNA sequences incorporated into the Ad12-3a molecules are not methylated at the 5′-C-C-G-G-3′ sequences, whereas the same sequences in KB cell DNA are completely methylated (*Msp* I and *Hpa* II tracks in Fig. 4; refs. 31–33). This conclusion is derived from the finding that *Msp* I cleaves cellular DNA sequences in KB cell DNA and in Ad12-3a DNA in the same way, whereas *Hpa* II cleaves the same cellular DNA sequences in Ad12-3a DNA but not in KB DNA.

We conclude that Ad12-3a DNA constitutes a symmetric recombinant (SYREC) between 700 and maximally 1150 bp from the left end of Ad12 DNA and KB cell DNA. The presumptive structure of this recombinant is depicted in Fig. 2. The cellular DNA has repetitive sequences in it and is not extensively methylated in the recombinant, whereas the same sequences in the host genome are methylated at the 5′-C-C-G-G-3′ sites.

The Cellular DNA Sequences Contain Repetitive DNA. The abundance in the KB genome of the cellular DNA sequences present in SYREC DNA was determined in a reconstitution experiment. Different amounts of SYREC DNA (0.13–1 ng) were subjected to electrophoresis in different tracks of the same agarose gel together with 1 μg of KB cell DNA that had been

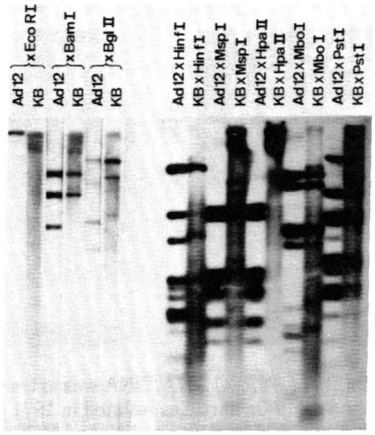

FIG. 4. Ad12-3a DNA contains KB cell DNA. KB cell DNA (5 μg) or 0.47 ng of Ad12-3 and Ad12-3a DNA (Ad12) mixed with 5 μg of salmon sperm DNA were cleaved with restriction endonucleases as indicated. Salmon sperm DNA was devoid of homology to SYREC DNA and hence can be used as carrier to balance effects of DNA concentration on rate of migration. The DNA fragments were separated by electrophoresis on horizontal 1.7% agarose slab gels and transferred to nitrocellulose filters (20). SYREC DNA had been purified by gel electrophoresis after cleaving a mixture of Ad12-3 and Ad12-3a DNAs with *Eco*RI. SYREC DNA was then ^{32}P-labeled by nick translation and used as probe in the hybridization experiments.

cleaved with *Eco*RI. Subsequently, the DNA was blotted to nitrocellulose filters and hybridized with ^{32}P-labeled purified SYREC DNA. The relative autoradiographic intensities were compared photometrically as described (24), and the amount of SYREC sequences in normal KB cell DNA was estimated. The SYREC DNA sequences are present 200 to 500 times per KB cell genome. This value takes into account the presence of heterogeneously sized complementary sequences in KB cell DNA.

Stability of the Symmetric Recombinants. Ad12 virions containing a mixture of Ad12-3 and Ad12-3a particles have been serially passaged in our laboratory at high multiplicities for about a decade. We have analyzed DNA preparations from particles grown in 1971, 1974, 1977, and 1980 for stability of the SYREC DNA structure. It is striking that the cleavage patterns (Fig. 5 *Left*) obtained with *Eco*RI and *Bam*HI are similar, in some details even identical, and that all SYREC DNA molecules from virus preparations grown since 1974 exhibit homologies to a recently prepared SYREC DNA probe (Fig. 5 *Right*). It is concluded that SYREC DNA, which is generated in an unknown way, is serially propagated and encapsidated into Ad12 virions. It should be pointed out that there is some variation with respect to aberrant bands in the cleavage patterns shown in Fig. 5. Some of these bands appear related in their sequences to that of SYREC DNA (Fig. 5 *Right*). Nevertheless, the SYREC population proper has remained stable over a long period of time.

DISCUSSION

On repeated passage of Ad12 at high multiplicities in human KB cells, variants of Ad12 are produced; one of these variants has been examined in detail. The variant DNA molecule is a highly symmetric structure that is flanked on either end by the left 700–1150 bp of Ad12 DNA. The bulk of the DNA consists of host cell DNA containing repetitious sequences. On denaturation and rehybridizing of the recombinant molecules, half-length hairpin structures are produced (see Fig. 2). The host DNA sequences in the recombinant are not methylated, whereas in KB cell DNA, all 5′-C-C-G-G-3′ sites in the same

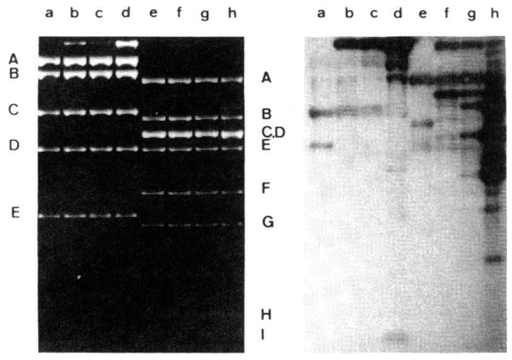

FIG. 5. Stability of SYREC DNA. DNA was extracted from a mixture of Ad12-3 and Ad12-3a particles isolated in 1971 (tracks a and e), 1974 (tracks b and f), 1977 (tracks c and g), and 1980 (tracks d and h). The DNA was then cut with *Eco*RI (tracks a–d) or *Bam*HI (tracks e–h). Cleavage products were separated by electrophoresis on 0.5% agarose gels. (*Left*) Fragments were visualized by ethidium bromide staining. The *Eco*RI and *Bam*HI fragments are also indicated. (*Right*) The DNA was transferred to nitrocellulose filters (20) and hybridized to ^{32}P-labeled purified SYREC DNA. It is apparent that not all bands visible in *Left* hybridize to the SYREC probe. On the other hand, due to the high sensitivity of the hybridization procedure, SYREC DNA-specific bands are apparent that are not detectable by ethidium bromide staining.

sequences are methylated. As virion DNA almost completely lacks 5-methylcytosine (31), it is likely that the cellular DNA in the recombinant has been synthesized simultaneously with viral DNA. In addition to the majority population of SYREC molecules analyzed here, minor species of variants are also produced, but these have not been examined.

For the DNA of Ad16, the left-terminal 390–400 nucleotides of the molecule have been found essential for packaging DNA into the viral capsid (34). SYREC DNA fulfils this sequence requirement for packaging; the variant DNA contains 700–1150 nucleotides from the left end of Ad12 DNA (see Fig. 3). This viral DNA sequence also encompasses an origin of DNA replication (35).

A recombinant between Ad12 and monkey cell DNA has been described (36). Its origin is unknown and may be very different from that of SYREC DNA. A symmetric recombinant consisting of ≈10% of the left end of Ad5 DNA and of multiple copies of simian virus 40 DNA has been discovered recently (37). Its basic structure is similar to that of SYREC DNA. Thus, the generation of such symmetric DNA molecules may be a general, more commonly occurring, event.

The presence of large amounts of KB cell DNA in a variant of Ad12 that is abundant in many Ad12 preparations raises the question to what extent KB DNA sequences can be incorporated into the genomes of Ad12-transformed hamster cells and of Ad12-induced hamster and rat tumor cells (19, 23, 24).

We can only speculate about the origin of the SYREC molecules. The SYREC DNA population may have been selected from a sizable pool of host-virus DNA recombinants occurring in productively infected cells. Such recombinants have been extensively characterized in our laboratory (14, 19, 38). Once encapsidated into Ad12-3a particles, they are able to transport human DNA sequences. SYREC DNA can be perpetuated over many years together with authentic Ad12 DNA. It is not yet known whether SYREC molecules will replicate autonomously on transfection of susceptible cells. The SYREC structures may suggest a way to use adenovirus DNA as a eukaryotic vector.

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