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Specific Fragmentation of DNA of Adenovirus Serotypes 3, 5, 7, and 12, and Adeno-Simian Virus 40 Hybrid Virus Ad2$^+$ND1 by Restriction Endonuclease R·*Eco*RI

CAREL MULDER, PHILLIP A. SHARP, HAJO DELIUS, AND ULF PETTERSSON[1]

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

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The products of complete digestion of duplex DNA of each of seven human adenoviruses with restriction endonuclease R·*Eco*RI ranged from two fragments for adenovirus 7 DNA (Ad7) to six fragments for Ad12 and Ad2 DNA. Viral serotypes from the same subgroups appeared to have related cleavage sites; Ad3 DNA and Ad7 (cl E46-LL) DNA were each cleaved into three fragments, and Ad7 (cl 19) DNA lacked one of the cleavage sites present in Ad3 and Ad7 (cl E46-LL) DNA. One of the cleavage sites in Ad2 DNA was deleted in the DNA of adeno-SV40 hybrid virus Ad2$^+$ND1, and three of the cleavage sites in Ad2 DNA were missing in Ad5 DNA. Thus, Ad2$^+$ND1 DNA was cleaved into five and Ad5 DNA into three fragments. Each fragment represented a unique segment of viral DNA since each fragment was obtained in equimolar amounts and since the sum of the molecular weights of the fragments equaled the molecular weight of the homologous intact adenovirus DNA.

Restriction endonucleases have been used to generate defined segments of DNA from the chromosomes of several DNA viruses (2, 4, 14, 18, 25). Such fragments have been used to determine the origin and direction of simian virus 40 (SV40) DNA replication (3, 5) and the location of "early" and "late" SV40 genes (11, 23), for the study of defective genomes (1, 21, 22), and will become a valuable tool for sequence analysis of DNA. We reported recently (18) that endonuclease R·*Eco*RI (endo R·*Eco*-RI) cleaves the DNA of adenovirus type 2 (Ad2) into six unique fragments. These fragments have been used to determine the amount of Ad2 viral DNA in a viral transformed cell line (24) and to map the in vitro transcription products of Ad2 DNA by *Escherichia coli* RNA polymerase (20). To extend this method of mapping of the viral genome to other serotypes of human adenovirus, we have now studied the cleavage by endo R·*Eco*RI of DNA from four additional serotypes and from the adeno-SV40 hybrid virus, Ad2$^+$ND1.

Endo R·*Eco*RI, a restriction enzyme from *E. coli* carrying the drug-resistance transfer factor RTF-RI (R. N. Yoshimori, Ph.D. thesis, Univ. of California, San Francisco Medical Center, 1971) cuts circular duplex SV40 DNA at one unique site (15, 16). This endonuclease cleaves duplex DNA at the hexanucleotide sequence $\frac{(5')GAATTC(3')}{(3')CTTAAG(5')}$ (9), and it acts by introducing single-strand scissions in the complementary strands between the guanine and adenine base, thereby generating short, identical cohesive ends (9, 13).

The adenoviruses comprise a widespread group of animal viruses which contain linear duplex DNA with a molecular weight of 21×10^6 to 24×10^6 (8, 30). Thirty-one human adenovirus serotypes can be distinguished by type-specific antigens in their capsids (for review, see 17). The human adenoviruses are classified into three subgroups according to their oncogenic potential for newborn hamsters; "highly-oncogenic", "weakly-oncogenic" and "non-oncogenic" serotypes. The non-oncogenic adenoviruses (e.g., Ad2 and Ad5) are able to transform cells in vitro (6) but they are not known to cause tumors in vivo after injection into newborn rodents. However, the highly oncogenic serotypes (i.e., Ad12) will produce tumors when injected into newborn hamsters (for review, see 29), whereas the weakly-oncogenic serotypes (i.e., Ad3 and Ad7) infrequently produce tumors when similarly injected. Viruses that belong to the same group have other biological properties in common and their genomes are closely related as determined by DNA-DNA hybridization (12) and heteroduplex mapping (7).

[1] Present address: The Wallenberg Laboratory, Department of Microbiology, Uppsala, Sweden.

MATERIALS AND METHODS

Virus stocks. Ad2 was originally obtained from J. Rose. The stock of adeno 2-SV40 hybrid virus, Ad2$^+$ND1, was a gift from A. M. Lewis, Jr. The Ad3 stock was a gift from L. Prage and Ad7, clones 19 and E46-LL, were obtained from J. Butel; adenovirus 7 (cl E46-LL) is the parental strain of Ad7 from which adeno 7-SV40 hybrid virus E46$^+$ or PARA was derived.

The techniques for labeling with [^{32}P]phosphate, purifying and extracting the DNA from adenoviruses grown in suspension cultures of KB cells have been described (19). Ad12 DNA (from strain Huie) was a gift from C. Tibbetts, Uppsala, Sweden.

Identification of serotypes. Virus stocks were identified by antibody neutralization assays. Monolayer plates of KB cells were infected with virus stocks which had been preincubated for 30 min at 37 C with neutralizing antisera, specific for different adenovirus serotypes. The antisera were kindly provided by L. Philipson, Uppsala, Sweden. The titers were determined by the fluorescent focus assay (28). Stocks of serotypes 2, 3, 5, and 7 showed more than a 1,000-fold reduction in titer in the presence of homologous antiserum, whereas heterologous antiserum neutralized the infectivity less than twofold.

Endonuclease R·EcoRI. The new nomenclature (26) for sequence-specific endonucleases has been followed replacing earlier descriptions of the enzyme (endonuclease R_I [16], endonuclease $R \cdot R_I$ [18]).

Endo R·*Eco*RI was isolated from *E. coli* RY13 as described (16). Incubation mixtures contained 10 mM $MgCl_2$, 90 mM Tris-hychochoride, pH 7.9, and 5 μliters of enzyme (0.5 μg of protein) per μg of DNA; the DNA concentration was between 20 and 200 μg/ml. After completion of the incubation, the enzyme reaction was stopped by addition of EDTA to a final concentration of 50 mM and was deproteinized with chloroform-isopropanol (24:1). The DNA was precipitated with 2.5 volumes of ethanol in a Spinco SW56 rotor at 35,000 rpm for 30 min at 0 C.

Gel electrophoresis. Samples were prepared for gel electrophoresis and the electrophoresis was performed essentially as described (18). Three types of gels were used: (i) 2.2% acrylamide, 0.11% *N'N'*-methylene *bis*-acrylamide-0.7% agarose (18); (ii) 1% acrylamide (Bio-Rad), 0.05% *N'N'*-methylene *bis*-acrylamide (Eastman-Kodak), and 0.7% agarose (Sigma); (iii) 1.4, 1, and 0.7% agarose (Sigma). Cylindrical gels were polymerized as described (18). Slab gels were poured (27) between glass plates (130 by 160 mm) separated by two plastic spacers (130 by 7 mm and 1-mm thick); one of the glass plates had a notch (132 by 20 mm) cut out from the top (27). A mixture of 2.2% acrylamide—0.11% *N'N'*-methylene *bis*-acrylamide—0.7% agarose at 50 C (18) was poured, and a comb with 24 teeth (3 by 15 by 1 mm each) was placed in the top before gelling, creating 24 loading slots for 10-μliter samples (27). Electrophoresis was performed in a device as described by Studier (27) by using a buffer, pH 7.8, containing 40 mM Tris-hydrochloride, 5 mM sodium acetate, and 1 mM EDTA at 4 V/cm for 20 h or as indicated in the text. Gels were stained with ethidium bromide either by including 0.5 μg of the dye per ml in the buffer during electrophoresis or by soaking the gel in this buffer containing 0.5 μg of the dye per ml after completion of electrophoresis (21, 25). The DNA bands were visualized under UV light (25). Radioactivity was measured as Čerenkov radiation or after dissolving gel slices in Aquasol (New England Nuclear Corp.).

Electron microscopy. DNA was mounted by the basic protein film technique for observations in an electron microscope, and length measurements were performed as described (16).

RESULTS

Digestion of adenovirus DNAs with endonuclease R·EcoRI. DNA from purified virus of five different adenovirus serotypes and the adeno-SV40 hybrid virus, Ad2$^+$ND1, was digested with endonuclease R·*Eco*RI for 30 min using an enzyme to substrate ratio which had been shown to digest Ad2 DNA to completion in less than 5 min (18). The digested DNAs were analyzed by gel electrophoresis and electron microscopy. Figure 1 is a picture of an ethidium bromide-stained slab gel electropherogram of seven different adenovirus DNAs after cleavage with endo R·*Eco*RI. We have included in two slots of the gels as size markers Ad2 DNA cleaved by endo R·*Eco*RI into six fragments whose molecular weights are 13.6×10^6, 2.7×10^6, 2.3×10^6, 1.7×10^6, 1.4×10^6, and 1.1×10^6 (18). A prolonged period of electrophoresis was required in order to demonstrate differences in mobility between some of the large fragments (e.g., Ad2$^+$ND1 DNA, slot c), and during this time small DNA fragments could have migrated off the gel. Therefore, one set of samples was loaded in slots a to g, and an electric field was applied to the gel. After electrophoresis for 19 h, an identical set of samples was loaded in slots h to n and electrophoresis was continued for 18 h. A comparison of the pattern in both sets of samples showed that Ad12 DNA was cleaved into six fragments by endo R·*Eco*RI, whereas Ad5, Ad2$^+$ND1, and Ad3 DNA were cut into three, five, and three fragments, respectively. DNA from the two different strains of Ad7 examined were cleaved into three (E46-LL) and two (cl 19) fragments. The smallest fragment produced by endo R·*Eco*RI cleavage of DNA from Ad12, Ad3, and Ad7 (E46-LL) had indeed migrated off the gel in the set of samples that were resolved by electrophoresis for 37 h (Fig. 1d, e, and g).

Because duplex DNA with a molecular weight greater than 6×10^6 comigrates under these conditions with all larger duplex DNA segments (18), it was possible that the slowest migrating bands shown in Fig. 1 consisted of heterogeneous populations of DNA segments. Therefore, it was necessary to determine independently the length of the fragments, produced by endo

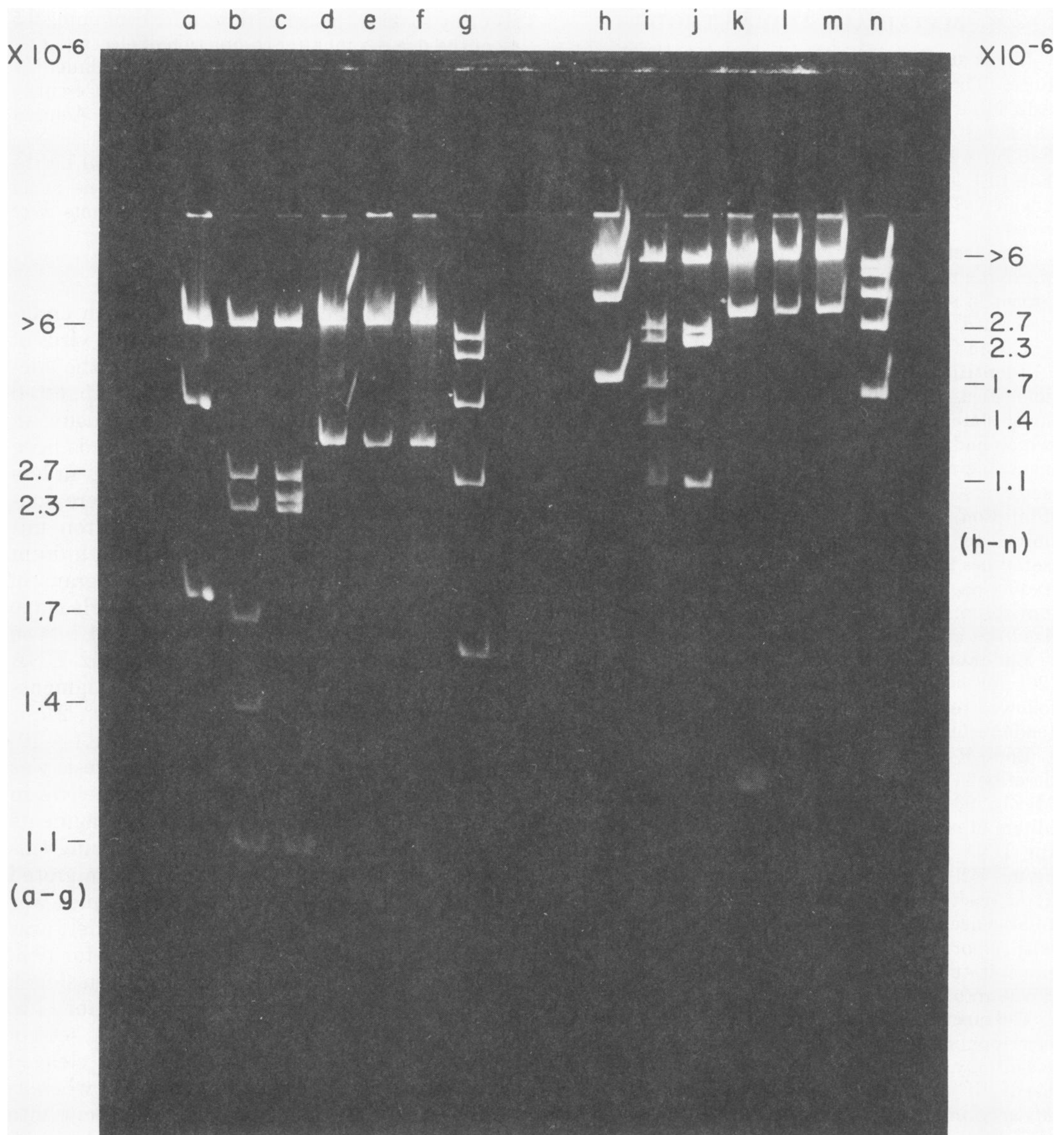

FIG. 1. *Slab gel electropherogram of endo R·EcoRI fragments of DNA from various adenovirus serotypes. DNA was incubated with endo R·EcoRI and deproteinized as described in Materials and Methods. These samples (1 μg of DNA in slots a to g, 2 μg in slots h to n) were loaded in slots (3 by 1 by 15 mm) of a 2.2% acrylamide—0.7% agarose slab gel as described in Materials and Methods. The gel was prerun in E buffer for 75 min at 100 V. Electrophoresis of samples a to g was performed for 19 h at 25 V. Then samples were loaded on slots h to n, and electrophoresis was continued for another 18 h at 20 V. After electrophoresis, the gel was stained for 1 h in E buffer containing 0.5 μg of ethidium bromide per ml. The DNA bands were visualized under UV light (25). (a) and (h), Ad5; (b) and (i), Ad2; (c) and (j), Ad2⁺ND1; (d) and (k), Ad3; (e) and (l), Ad7 cl E46-LL; (f) and (m), Ad7 cl 19; (g) and (n), Ad12.*

R·*Eco*RI cleavage of the DNA from different adenovirus serotypes by electron microscopy.

Electron microscopy. After incubation with endo R·*Eco*RI, the deproteinized samples were mounted for electron microscopy. Nicked circular DNA of bacteriophage PM2 was added to each sample as an internal molecular weight standard. Intact DNA of each viral serotype was separately spread in the presence of PM2 DNA and the length of both the intact DNA and its fragments was measured. Assuming that PM2 DNA has a molecular weight of 6.40×10^6 (18),

the molecular weight of each intact DNA and its fragments was calculated from their lengths relative to the length of PM2 DNA measured on the same grid. Histograms of the length measurements of fragments from six different adenovirus DNAs are shown in Fig. 2.

Cleavage products of Ad5 DNA consisted of three peaks corresponding to DNA molecular weights of 18.1×10^6, 3.72×10^6, and 1.70×10^6; the sum of these three fragment lengths is equal

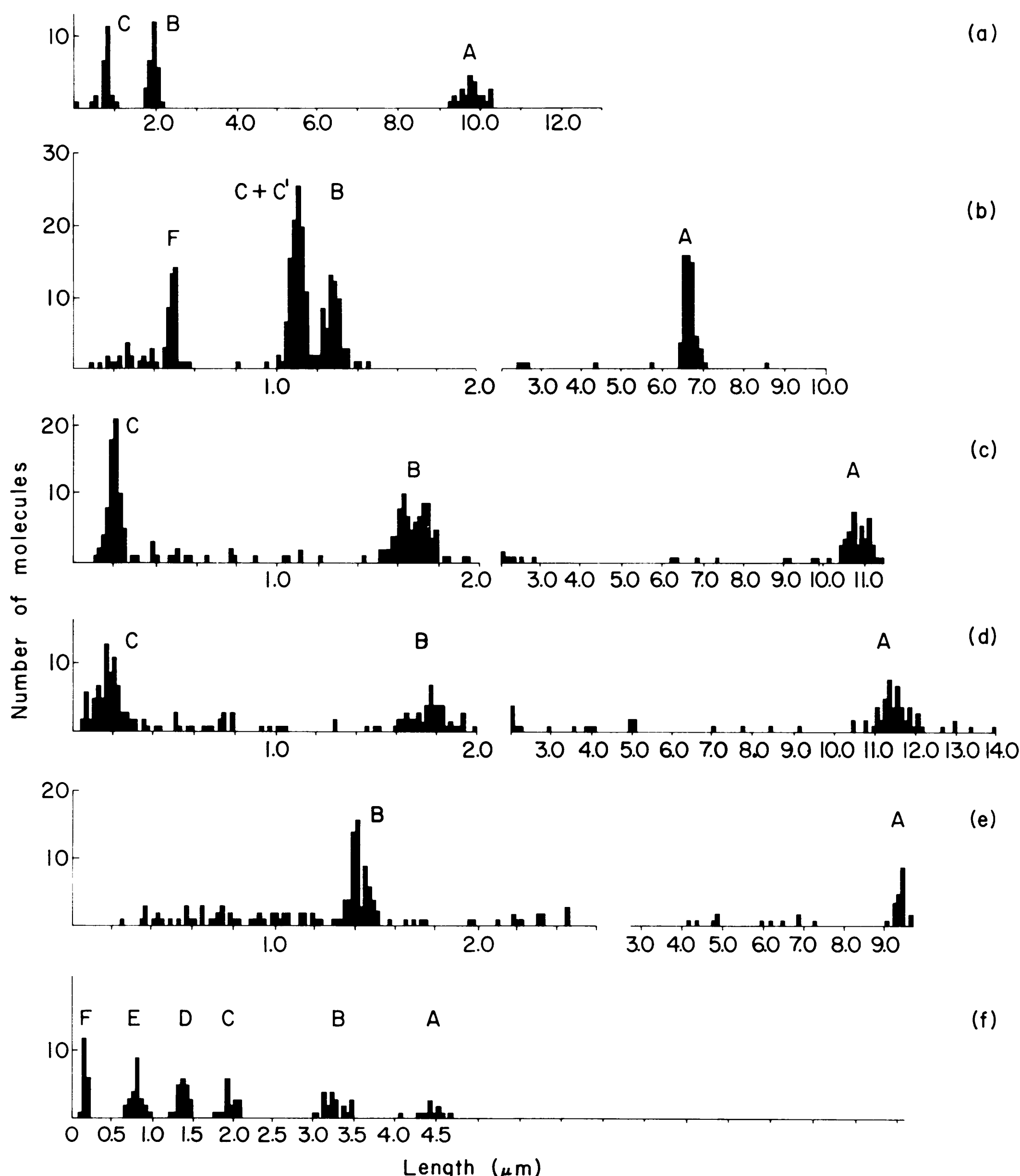

FIG. 2. *Histograms of length measurements of endo R·EcoRI fragments of DNA of various adenovirus serotypes. The DNA was incubated with endo R·EcoRI; the digestion was stopped by addition of EDTA to 50 mM. The mixture was spread for electron microscope length measurements as described in Materials and Methods. Small molecules were preferentially scored because large molecules had a higher probability of being entangled and, therefore, impossible to measure. In panels b to e, the histograms for fragments smaller than 2 or 2.5 μm are plotted on a fivefold expanded scale; panel f is plotted on a twofold expanded scale. (a), Ad5 DNA; (b), Ad2⁺ND1 DNA; (c), Ad3 DNA; (d), Ad7 (cl E46-LL) DNA; (e), Ad7 (cl 19) DNA; (f), Ad12 DNA.*

to the measured length of the Ad5 genome (see Table 1). The narrow distribution of DNA segment lengths around the mean for each of three peaks is that expected from the length measurements of a homogeneous population of DNA molecules. Therefore, the three different length segments of viral DNA resolved by electron microscopy of endo R·*Eco*RI digested Ad5 DNA correspond to the three bands of Ad5 DNA resolved by gel electrophoresis (Fig. 1).

The electron microscopic length measurements of endo R·*Eco*RI digested Ad2⁺ND1 DNA can be resolved into four peaks corresponding to molecular weights of 13.9×10^6, 2.66×10^6, 2.31×10^6, and 1.04×10^6. When endo R·*Eco*RI cleavage products of Ad2⁺ND1 were analyzed by gel electrophoresis, five bands were resolved (Fig. 1c). The third and fourth bands migrated in the gel at similar rates, suggesting that there were two DNA segments of approximately the same length in the digested Ad2⁺ND1 DNA. Assuming that there are two 2.31×10^6-dalton fragments per viral genome, the sum of the molecular weights of Ad2⁺ND1 fragments is equal to the molecular weight of the intact viral DNA (see Table 2).

The length histogram of endo R·*Eco*RI fragments of both Ad3 and Ad7 (E46-LL) DNA consisted of three peaks that corresponded to the three bands of digested Ad3 and Ad7 (E46-LL) DNA resolved by gel electrophoresis. The molecular weights calculated from the

TABLE 1. *Properties of endo R·EcoRI fragments from adenovirus type 5 DNA*

DNA fragment	Length (μm)	Mol wt[a] ($\times 10^{-6}$)	Fraction length (%)	Distribution[b] of ^{32}P (%)	Relative[c] mobility	Mol wt[c] ($\times 10^{-6}$)
A	9.889 ± 0.277	18.08 ± 0.51	76.94	76.43 ± 2.06	1.00	(>6)
B	2.037 ± 0.082	3.72 ± 0.15	15.83	16.12 ± 1.90	1.14 ± 0.02	3.8
C	0.932 ± 0.073	1.70 ± 0.13	7.23	7.45 ± 0.46	1.77 ± 0.07	2.0
Total	12.858	23.50				
Intact Ad5	12.755 ± 0.215	22.91 ± 0.39	(100)	(100)	1.0	

[a] Samples were mixed with nicked circular PM2 DNA before electron microscope measurements. The molecular weight of each DNA fragment was calculated from its length relative to the measured length of nicked circular PM2 DNA on the same grid. PM2 DNA is assumed to have a molecular weight of 6.40×10^6 (18) and was measured as 3.501 ± 0.125 μm in the sample with fragments and as 3.563 ± 0.055 μm in the sample with intact DNA.

[b] ^{32}P-labeled DNA was cleaved by endo R·*Eco*RI and separated by gel electrophoresis. The amount of radioactivity in each peak was determined as Čerenkov radiation as described in Materials and Methods.

[c] The mobility of each fragment was determined on 14-cm cylindrical gels which contained 0.7% agarose and 2.2% acrylamide after 20 h of electrophoresis at 50 V in buffer E (without ethidium bromide). The corresponding molecular weight is estimated from an empirical graph (18).

TABLE 2. *Properties of endo R·EcoRI fragments from DNA of adeno-SV40 hybrid virus Ad2⁺ND1*

DNA fragment	Length (μm)	Mol wt[a] ($\times 10^{-6}$)	Fraction length (%)	Distribution[b] of ^{32}P (%)	Relative[c] mobility	Mol wt[c] ($\times 10^{-6}$)
A	6.770 ± 0.135	13.90 ± 0.28	62.55	59.10 ± 4.31	1.0	>6
B	1.298 ± 0.034	2.66 ± 0.07	11.99	13.08 ± 3.52	1.50 ± 0.04	2.55
C′[d]	1.123 ± 0.027	2.31 ± 0.06	10.39	22.99 ± 2.79	1.58 ± 0.05	2.35
C[d]					1.64 ± 0.05	2.25
D	0.509 ± 0.020	1.04 ± 0.04	4.69	4.81 ± 0.58	2.90 ± 0.08	1.10
Total[e]	10.823	22.22				
Intact Ad2⁺ND1	11.139 ± 0.127	22.22 ± 0.25	(100)	(100)		

[a] Deproteinized samples were mixed with intact Ad2⁺ND1 DNA before electron microscope measurements. The molecular weight of each DNA fragment was calculated from its length relative to the measured length of intact Ad2⁺ND1 DNA (11.056 ± 0.138 μm) on the same grid. The molecular weight of intact Ad2⁺ND1 DNA was calculated from separate measurements of spreadings of a mixture of intact Ad2⁺ND1 DNA and nicked circular PM2 DNA. PM2 DNA is assumed to have a molecular weight of 6.40×10^6 (18) and was measured as 3.209 ± 0.073 μm in the sample with intact DNA.

[b] and [c] As in Table 1.

[d] A difference in molecular weight between C′ and C could not be distinguished on electron micrographs nor in the distribution of radioactivity and is given as one value.

[e] The observed values for C′ + C were doubled to obtain the total value assuming that the observed data represented values of two fragments of (almost) equal size.

mean lengths of the fragments of Ad3 DNA were 19.2×10^6, 2.98×10^6, and 0.38×10^6 (Table 3), and Ad7 (E46-LL) DNA were 19.47×10^6, 3.0×10^6, and 0.34×10^6 (Table 4, experiment 1). DNA extracted from another strain of Ad7 virus, clone 19, was cut by endo R·*Eco*RI into fragments whose lengths were resolved into two size classes. The molecular weights of the Ad7 (cl 19) fragments were 19.5×10^6 and 2.95×10^6 (Table 4, experiment 2). The sum of the molecular weights of the endo R·*Eco*RI digestion products of DNA from Ade, Ad7 (E46-LL), and Ad7 (cl 19) was equal to the molecular weights of the undigested viral DNA.

When the length of the endo R·*Eco*RI cleavage products of Ad12 DNA was measured, six peaks were obtained that corresponded to molecular weights of 7.57×10^6, 5.81×10^6, 3.47×10^6, 2.44×10^6, 1.52×10^6, and 0.42×10^6. The sum of these molecular weights is equal to the molecular weight of intact Ad12 DNA (see Table 5) suggesting that each of the six fragments is found once in each Ad12 genome.

Gel electrophoresis. For each of the adenovirus serotypes, the sum of the measured lengths of the endo R·*Eco*RI cleavage fragments as determined by electron microscopy was equal to that of the intact viral genome, suggesting that each viral genome contained one of each of the endo R·*Eco*RI cleavage fragments that were resolved by gel electrophoresis or electron microscopic length measurements. However, since the experimental error accumulated in the summing of the molecular weights of several fragments was approximately equal to the molecular weight of the smaller fragments, the electron microscopy data alone did not conclusively establish whether all fragments occurred at an equimolar frequency in the viral genome. This point can be resolved by showing that when the endo R·*Eco*RI digestion products of a uniformly labeled viral DNA are resolved by gel electro-

TABLE 3. *Properties of endo R·EcoRI fragments from adenovirus type 3 DNA*

DNA fragment	Length (μm)	Mol wt[a] ($\times 10^{-6}$)	Fraction length (%)	Distribution[b] of ^{32}P (%)	Relative[c] mobility	Mol wt[c] ($\times 10^{-6}$)
A	10.980 ± 0.263	19.21 ± 0.46	85.11	85.80 ± 1.54	1.0	>6
B	1.700 ± 0.088	2.98 ± 0.15	13.20	12.72 ± 1.46	1.36 ± 0.02	2.9
C	0.217 ± 0.029	0.38 ± 0.05	1.68	1.48 ± 0.11	5.86 ± 0.25	0.34
Total	12.897	22.57				
Intact Ad3	12.861 ± 0.261	22.50 ± 0.46	(100)	(100)		

[a] [b] and [c] As in Table 1; PM2 DNA measured in this experiment as 3.641 ± 0.071 μm in the sample with fragments and as 3.657 ± 0.101 μm in the sample with intact Ad3 DNA.

TABLE 4. *Properties of endo R·EcoRI fragments from DNA of two different clones of adenovirus type 7*

DNA fragment	Length (μm)	Mol wt[a] ($\times 10^{-6}$)	Fraction length (%)	Distribution[b] of ^{32}P (%)	Relative[c] mobility	Mol wt[c] ($\times 10^{-6}$)
Expt. 1[d]						
A	11.526 ± 0.380	19.47 ± 0.64	85.33	86.0	1.0	(>6)
B	1.781 ± 0.092	3.00 ± 0.15	13.18	12.6	1.35	2.9
C	0.201 ± 0.070	0.34 ± 0.12	1.49	1.4	5.98	0.33
Total	13.508	22.56	(100)			
Intact Ad7 (cl E46-LL) DNA	11.187 ± 0.090	22.81 ± 0.18	(100)	(100)		
Expt. 2						
A	9.483 ± 0.139	19.46 ± 0.28	86.84	86.70 ± 0.3	1.00	
B	1.437 ± 0.042	2.95 ± 0.08	13.16	13.30 ± 0.3	1.38 ± 0.04	2.9
Total	10.920	22.41	(100)	(100)		
Intact Ad7 (clone 19) DNA	11.328 ± 0.185	22.34 ± 0.37				

[a] [b] and [c] As in Table 1.

[d] Experiment 1 describes DNA of clone E46-LL; experiment 2 DNA of clone 19 of adenovirus type 7. PM2 DNA was measured in experiment 1 as 3.787 ± 0.201 μm in the sample with fragments and as 3.139 ± 0.070 μm in the sample with intact Ad7 (cl E46-LL) DNA. In experiment 2, PM2 DNA was measured as 3.119 ± 0.063 μm in the sample with fragments and as 3.245 ± 0.142 μm in the sample with intact Ad7 (cl 19) DNA.

TABLE 5. *Properties of endo R·EcoRI fragments from adenovirus type 12 DNA*

DNA fragment	Length (μm)	Mol wt[a] ($\times 10^{-6}$)	Fraction length (%)	Distribution[b] of ^{32}P (%)	Relative[c] mobility	Mol wt[c] ($\times 10^{-6}$)
A	4.261 ± 0.211	7.57 ± 0.37	35.65	35.95 ± 2.96	1.00	(>6)
B	3.273 ± 0.125	5.81 ± 0.22	27.37	25.50 ± 2.44	1.08 ± 0.01	4.0
C	1.953 ± 0.082	3.47 ± 0.15	16.34	16.58 ± 1.45	1.27 ± 0.01	3.15
D	1.375 ± 0.055	2.44 ± 0.10	11.49	12.56 ± 0.946	1.59 ± 0.05	2.3
E	0.857 ± 0.081	1.52 ± 0.14	7.16	7.45 ± 0.31	2.32 ± 0.12	1.45
F	0.236 ± 0.035	0.42 ± 0.06	1.98	1.96 ± 0.13	6.21 ± 0.30	0.3
Total	11.955	21.23				
Intact Ad12	12.206 ± 0.265	21.48 ± 0.47	(100)	(100)		

[a] [b] and [c] As in Table 1. PM2 DNA measured as 3.602 ± 0.154 μm in the sample with fragments and as 3.636 ± 0.091 μm in the sample with intact Ad12 DNA.

phoresis, the fraction of radioactivity found in each band corresponds to the observed fractional length of the viral DNA fragment migrating in that band. Hence, the DNA sequences contained in a particular band must occur once in each viral DNA molecule. Therefore, ^{32}P-labeled DNA from adenovirus serotypes 3, 7, 5, and 12 and Ad2^{+}ND1 was cleaved with endo R·*Eco*RI, deproteinized and analyzed by electrophoresis in cylindrical agarose-acrylamide gels. Time of separation and gel composition was varied for DNA from different serotypes to achieve optimum separation of all fragments. After electrophoresis, the gels were cut into 1-mm slices and the distribution of radioactivity was determined. The patterns obtained for DNA from all serotypes were identical to those obtained with the slab gel technique (Fig. 1). An example is given in Fig. 3 which shows the separation of fragments of adenovirus type 12 DNA into six peaks on a cylindrical gel composed of 0.7% agarose and 1% acrylamide.

In addition, to make sure that a very small fragment was not overlooked or allowed to migrate off the end of the gels, greater than 200,000 counts/min of endo R·*Eco*RI digested [^{32}P]DNA for each serotype was layered onto a 2.2% acrylamide-0.7% agarose gel and electrophoresis was carried out for approximately 4 h. During this brief period of electrophoresis, the bromophenol blue dye marker had only migrated half the length of the gel and a fragment larger than 0.1% (>35 nucleotide pairs) of the adenovirus serotype DNA would have been detected as a band on the gel. However, no bands consisting of small fragments other than those observed in Fig. 1 were detected.

For each adenovirus serotype DNA, the fraction of the total radioactivity migrating in an individual peak was determined by dividing the counts per minute found under that peak by the total radioactivity found in the gel. As is shown in Tables 1 to 5, there was a good correlation for all peaks between the fraction of radioactivity in each peak and the fractional length of the corresponding segment of DNA, as measured by electron microscopy. This indicated that each peak contained only one unique fragment of viral DNA.

The electrophoretic mobility of duplex DNA is inversely related to its molecular weight, and electropherograms can thus be used as an additional method for molecular weight estimations. Gels containing 2.2% acrylamide and 0.7% agarose were used for these experiments and an empirical curve relating the relative mobility of DNA fragments to their measured molecular weight was constructed as described before (18). DNA segments larger than 6×10^6 daltons were not resolved on such gels and the standard curve was thus not applicable to the largest fragments. For all fragments with a molecular weight less than 5×10^6, there was a good correlation between the size estimated by electrophoretic mobility and the size measured by other methods (Tables 1 to 5).

DISCUSSION

All separated fragments are unique. The number and molecular weights of fragments produced by endonuclease R·*Eco*RI cleavage of the DNA from different adenoviruses were determined by two methods, electron microscopic length measurements and the distribution of radioactivity in separated bands after gel electrophoresis. Both methods gave essentially identical results except for endo R·*Eco*RI digested Ad2^{+}ND1 DNA that contained two fragments (C and C′) whose length could not be resolved by the length measurements. Also a molecular weight for all fragments smaller than 4×10^6 was estimated from their mobility during gel electrophoresis relative to those of DNA fragments of known size. Although this method for molecular weight determination can only be applied to fragments small enough to be

resolved in a particular gel system, the ease and reproducibility of the technique justifies its use. The measured length of the fragments agreed well with their observed mobility on 2.2% acrylamide-0.7% agarose gels. We concluded from these results that viral DNA from each serotype was cleaved by endo R·*Eco*RI into a unique set of fragments that could be resolved by gel electrophoresis (see Table 6).

Endonuclease R·EcoRI cleavage pattern of DNA from related adenoviruses. Human adenoviruses can be grouped into three classes by their oncogenic potential in rodents (29). Previous work has shown that adenoviruses within an oncogenic subgroup are related by interserotype neutralization with antisera and by DNA: DNA hybridization (12). However, adenoviruses that are members of different oncogenic subgroups have little cross-neutralization or nucleotide sequence homology. Recently, it was shown (7) by electron microscope examination of heteroduplexes that Ad5 and Ad2 DNA were highly homologous in nucleotide sequence over 85% of their length, whereas Ad2 and Ad7 DNA shared few sequences more than 200 nucleotides long that were completely homologous. Ad12 DNA showed no detectable nucleotide sequence homology to either Ad2 or Ad7 DNA when assayed by the method of DNA filter hybridization (12).

The cleavage of duplex DNA by a restriction endonuclease is an assay for the presence of a specific nucleotide sequence at particular locations. If two segments of DNA have a related

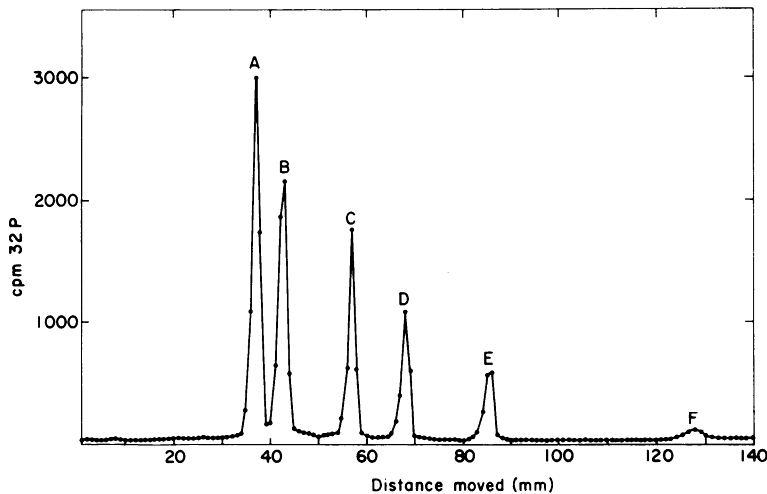

FIG. 3. *Gel electropherogram of [^{32}P]DNA of adenovirus serotype 12 after incubation with endonuclease R·EcoRI. The sample was deproteinized and subjected to electrophoresis on cylindrical gels (6 by 140 mm) made up from 1% acrylamide and 0.7% agarose in E buffer containing 0.5 µg of ethidium bromide per ml. After electrophoresis in the same buffer at 30 V for 16 h at 20 C, the gel was sliced into 1-mm disks. Radioactivity of each slice was measured as Cerenkov radiation. Six peaks, designated A to F, were resolved.*

TABLE 6. *Molecular weights of endo R·EcoRI fragments of DNA of various adenoviruses*[a]

Virus	Intact	A	B	C	D	E	F
Ad2	22.9	13.55	2.80	2.33	1.70	1.40	1.13
Ad2$^+$ND1	22.22	13.51	2.79	2.38	2.48[b]		1.06
Ad5	22.91	17.57	3.66	1.68			
Ad3	22.50	19.23	2.92	0.36			
Ad7 (E46-LL)	22.59	19.33	2.91	0.33			
Ad7 (cl 19)	22.34	19.38	2.96				
Ad12	21.48	7.69	5.68	3.54	2.58	1.57	0.42

[a] The molecular weight values (in 10^6 daltons) in this table were calculated from the average of the fractional length and distribution of radioactivity from Tables 1 to 5. The average percentages thus obtained were multiplied with the molecular weights of intact DNA calculated from direct length measurement of this DNA as given in Tables 1 to 5.

[b] Fragments C and C' of Ad2$^+$ND1 DNA were calculated as 2.43 ± 10^6 daltons and were corrected for the observed differences in their electrophoretic mobilities (Table 2).

nucleotide sequence, then they might be cut by a restriction endonuclease at some positions common to both segments. Ad3 and Ad7 DNA have a great deal of nucleotide sequence homology (7). The three fragments generated by endo R·*Eco*RI from Ad3 DNA appeared to be identical in size to those from Ad7 (cl E46-LL) DNA. Therefore, Ad3 and Ad7 (cl E46-LL) DNA are probably cleaved at identical positions. Ad7 (cl 19) DNA is only cut once by endo R·*Eco*RI and has lost one of the two target sequences found in the DNA from the other strain of Ad7.

Ad12 was the only representative of the highly oncogenic group of adenoviruses used in this study. Neither these viruses nor their DNA shows a close relationship to adenoviruses of either of the other groups. And, indeed, the endo R·*Eco*RI fragmentation pattern of Ad12 was completely unrelated to that of any of the other DNAs studied here (Table 6).

Ad2 and Ad5 DNA have a partially homologous nucleotide sequence over a majority of their length (7) and they appeared to be cut by endo R·*Eco*RI at similar positions. Ad2 DNA is cleaved into six fragments whose order is *ABFDEC* (H. Delius et al., manuscript in preparation). Ad5 DNA was cleaved at two positions yielding three fragments whose molecular weights were: A, 17.6×10^6; B, 3.7×10^6; and C, 1.7×10^6. The order of the Ad5 DNA fragments is *ACB*, and the length of the Ad5 fragment A corresponded to the sum of the length of Ad2 fragments A, B, and F. Ad5 fragment B was the same length as the sum of Ad2-C and Ad2-E; Ad5 fragment C appeared slightly larger than Ad2 fragment D by electrophoretic mobility, but the same size for the two fragments was observed by length measurements and distribution of radioactivity. Therefore, the two target sites in Ad5 DNA for endo R·*Eco*RI were probably identical to the cleavage site between Ad2 fragments F and D and between Ad2 fragments D and E.

$Ad2^+ND1$ is a nondefective hybrid virus whose DNA consists of 95% of the Ad2 genome and 17% of the SV40 genome covalently linked (10). An endo R·*Eco*RI digestion of $Ad2^+ND1$ DNA yielded five fragments. Four of these fragments had molecular weights identical to the Ad2 fragments A, B, C, and F. The $Ad2^+ND1$ DNA fragment C′ that did not correspond to an Ad2 fragment had a molecular weight of 2.5×10^6. If the molecular weights of Ad2 fragments D and E were summed along with the 600,000 daltons of SV40 DNA found in hybrid viral genome and the 1.2×10^6 daltons of Ad2 genome deleted in the $Ad2^+ND1$ DNA was then subtracted, the total equaled the observed molecular weight of $Ad2^+ND1$ fragment C′. Thus, the endo R·*Eco*RI cleavage site between Ad2 fragments D and E was not present in the hybrid virus DNA, and the SV40 DNA sequences were inserted at this site. This interpretation agreed with the heteroduplex mapping of the SV40 sequences in $Ad2^+ND1$ DNA at 13.7% from one end of the adenovirus genome (10), and it was confirmed by studies on heteroduplexes of fragment C′ and SV40 DNA (P. Sharp et al., manuscript in preparation).

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