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Cleavage Maps of Highly Oncogenic Human Adenovirus Type 12 DNA with Restriction Endonucleases *Bam* HI, *Hin*d III, *Sal* I and *Sma* I

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SUMMARY

Highly oncogenic human adenovirus type 12 DNA (strain Huie) was cleaved into ten, nineteen, three and four specific fragments by the restriction endonucleases *Bam* HI, *Hin*d III, *Sal* I and *Sma* I, respectively. The size of each fragment was estimated from the relative mobility in agarose gel electrophoresis using size markers of known size. These specific fragments were mapped on the adenovirus type 12 genome by analyzing partial digestion products and double digestion products.

INTRODUCTION

Adenovirus type 12 induces tumors in newborn rodents *in vivo* (1), and transforms rodent cells *in vitro* (2). Rat embryo cells (3Y1) have been transformed by virion, whole DNA, *Eco* RI-C fragment DNA, *Hin*d III-G fragment DNA, and *Acc* I-H fragment DNA of adenovirus type 12, and transformed cell lines (W4, WY3, CY1, GY1 and HY1, respectively) have been established (3, 4, 5, 6). These cell lines have offered us an advanced situation to investigate the location and function of the viral genes responsible for cell transformation (7, 8, 9, 10, 11). Cleavage maps of the viral DNA with restriction endonucleases are essential for the analysis of viral DNA integrated in the cellular DNA and viral mRNA transcribed in transformed cells and in lytically infected cells.

In this study, we constructed cleavage maps of adenovirus type 12 DNA with *Bam* HI, *Hin*d III, *Sal* I and *Sma* I by analyzing partial digestion products and double digestion products.

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MATERIALS AND METHODS

Viral DNA

The strain Huie of adenovirus type 12 (Ad12), obtained from Dr. M. Green of St. Louis University, St. Louis, was grown in KB cells in our laboratory. Viral DNA was extracted from purified virions (12, 13). DNA fragments were purified after restriction endonuclease digestion and agarose gel electrophoresis as described (14).

Restriction endonuclease digestion and gel electrophoresis

Bam HI, *Eco* RI and *Sma* I were purchased from Boehringer Mannheim Corp. and Takara Shuzo Co. Ltd. *Hind* III and *Sal* I were prepared according to the procedure described elsewhere (15, 16). Reaction buffers for the enzymes used were as follows: *Bam* HI [6 mM Tris-HCl (pH 7.5), 6 mM $MgCl_2$, 20 mM KCl, 6 mM β-mercaptoethanol]; *Eco* RI [100 mM Tris-HCl (pH 7.5), 10 mM $MgCl_2$, 50 mM NaCl]; *Hind* III [20 mM Tris-HCl (pH 7.4), 7 mM $MgCl_2$, 60 mM NaCl, 7 mM β-mercaptoethanol]; *Sal* I [50 mM Tris-HCl (pH 7.5), 6 mM $MgCl_2$, 6 mM β-mercaptoethanol]. *Sma* I [10 mM Tris-HCl (pH 8.1), 7 mM $MgCl_2$ 20 mM KCl, 7 mM β-mercaptoethanol]. One unit of these enzymes was defined as the minimum amount which was able to digest 1.0 μg of Ad12 DNA at 37°C for 1 hr. Partial digestion was achieved by reducing the amount of enzymes and shortening the reaction period. DNA fragments produced were separated by agarose gel electrophoresis and visualyzed under UV light after staining with ethidium bromide (0.5 μg/ml) or detected by autoradiography. The size of each DNA fragment was calculated from the relative mobility using marker DNA fragments of known size.

Labeling of DNA

Ad12 DNA and its fragments were hydrolyzed at 3′ ends by the 3′→5′ exonuclease activity of exonuclease III (17) or T4 DNA polymerase (18), and then repaired by the 5′→3′ polymerase activity of T4 DNA polymerase (18) in the presence of [α^{32}P] dATP, [α^{32}P] dCTP, dTTP and dGTP. After the labeling reaction was terminated by phenol extraction, unreacted deoxyribonucleoside triphosphates were excluded by gel filtration on a column of Sephadex G-50.

RESULTS AND DISCUSSION

Ad12 DNA was digested with five different restriction endonucleases, *Bam* HI, *Eco* RI, *Hind* III, *Sal* I and *Sma* I, and resulted fragments were identified as shown in Fig. 1. The size of each DNA fragment was calculated

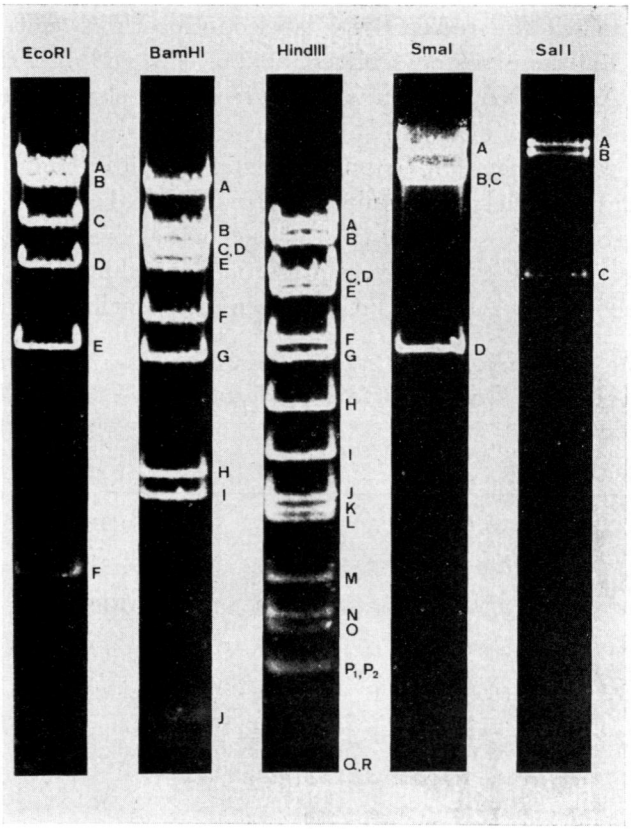

Fig. 1. Agarose gel electropherograms of Ad 12 DNA fragments cleaved by five different restriction endonucleases.

Ad 12 DNA (2 μg) was digested with 2 units of each of *Eco* RI, *Bam* HI, *Hind* III, *Sma* I and *Sal* I at 37°C for 2 hr. The digestion products were separated by 1.4% agarose gel electrophoresis at 60 V (3.5 V/cm) for 4 hr. A small amount of *Sal* I-digested products were electrophoresed to achieve fine separation of two large fragments.

from the relative mobility in agarose gel electrophoresis. At first, *Hind* III fragments were sized sequentially using markers as follows: *Hind* III-G (9, 19) and its recleavage products, *Hind* III-I (T. Kimura *et al.*, unpublished), and then *Hind* III partial digestion products. Fragments produced by other restriction enzymes were sized using *Hind* III fragments as markers. Most of their sizes were confirmed by analyses of recleavage products. Their estimated sizes were summarized in Table 1. The *Eco* RI-C fragment mapped at the left end (20) has been verified to contain transforming ability of Ad12 (4). Recleavage of *Eco* RI-C with *Hind* III yielded *Hind* III-G, *Hind* III-I and another off-sized fragment (Fig. 2 A). Since *Hind* III-G and

Hind III-E retained the radioactivity labeled on both 3′ ends of the viral DNA (Fig. 2 B), these were identified as the left and the right terminal fragments of Ad12 DNA, respectively. It has been also verified that *Hind* III-G has transforming ability (5).

Series of partial digestion fragments containing either end of Ad12 DNA were detected by partial digestion with *Hind* III of the viral DNA labeled at both ends (Fig. 2 C). The number of the partial digestion products with *Hind* III were reduced by recleavage with *Sal* I, resulting in the appearance of *Sal* I-C and *Sal* I-A (Fig. 2 C). Since the smallest partial digestion

Table 1. *Sizes of Ad 12 DNA Fragments Cleaved by Five Different Restriction endonucleases*

Hind III	*Eco* RI	*Bam* HI	*Sal* I	*Sma* I
A 5,300 (15.4)	A 12,180 (35.5)	A 8,020 (23.4)	A 17,760 (51.8)	A 15,030 (43.8)
B 4,700 (13.7)	B 9,280 (27.1)	B 5,210 (15.2)	B 13,050 (38.0)	B 8,620 (25.1)
C 3,650 (10.6)	C 5,570 (16.2)	C 4,590 (13.4)	C 3,500 (10.2)	C 8,290 (24.2)
D 3,600 (10.5)	D 4,060 (11.8)	D 4,540 (13.2)		D 2,370 (6.9)
E 3,350 (9.8)	E 2,500 (7.3)	E 4,040 (11.8)		
F 2,520 (7.3)	F 720 (2.1)	F 2,890 (8.4)		
G 2,320 (6.8)		G 2,370 (6.9)		
H 1,790 (5.2)		H 1,280 (3.7)		
I 1,390 (4.1)		I 1,140 (3.3)		
J 1,100 (3.2)		J 230 (0.7)		
K 1,030 (3.0)				
L 980 (2.9)				
M 680 (2.0)				
N 540 (1.6)				
O 480 (1.4)				
P_1, P_2 2×360 (2×1.0)				
Q 90 (0.3)				
R 70 (0.2)				

The size of each fragment was estimated as described in the text, and presented in nucleotide pairs. The value in percent genome of each fragment was also shown in parenthesis. The size of Ad 12 genome was estimated to be 34, 310 nucleotide pairs as the summation of the sizes of *Hind* III fragmens. Error limit was estimated to be ±50 nucleotide pairs at most for each fragment, and ±500 nucleotide pairs for Ad 12 genome.

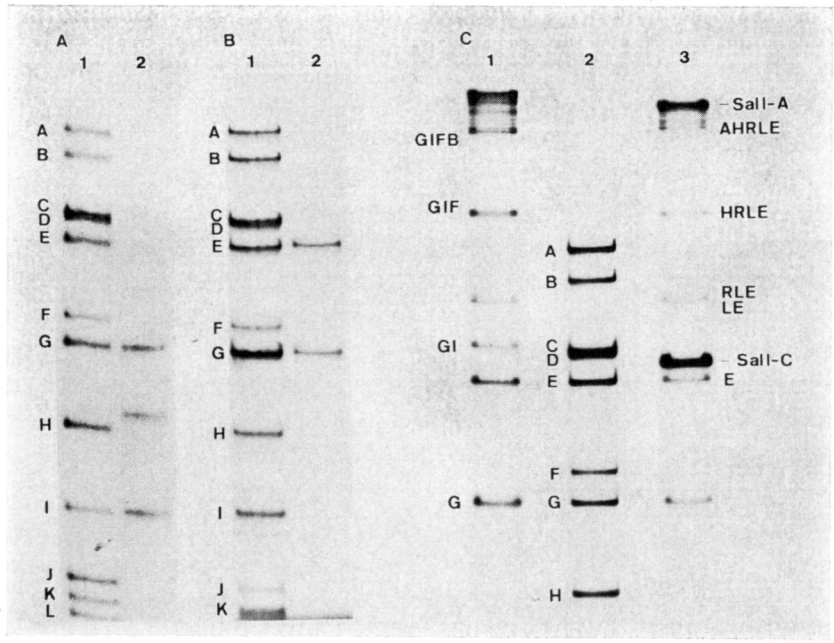

Fig. 2. Characterization and mapping of *Hind* III digestion products of Ad 12 DNA.

(A) *Hind* III digestion products of Ad 12 DNA *Eco* RI-C (lane 2) were labeled and electrophoresed in parallel with *Hind* III digestion products of Ad 12 DNA (lane 1).

(B) Ad 12 DNA labeled at both 3′ ends was digested with *Hind* III (lane 2) and electrophoresed in parallel with *Hind* III digestion products of Ad 12 DNA (lane 1).

(C) Ad 12 DNA labeled at both 3′ ends was digested partially with *Hind* III, and electrophoresed with (lane 3) or without (lane 1) further digestion with *Sal* I. *Hind* III digestion products as markers were in lane 2. Partial digestion fragments were characterized as shown in the figure, sequentially from small ones to larger ones based on the differences of their sizes.

fragment *Hind* III-GI (10.9 map units) from the left end dissappeared by *Sal* I digestion (Fig. 2 C), *Sal* I-C (10.2 map units) were located at the left end, and *Sal* I-A (51.8 map units) was located at the right end of the viral DNA. Thus, the *Hind* III partial digestion products detected after recleavage with *Sal* I originated from the right-hand end, and those dissappeared from the left-hand end of the viral DNA. Comparing the sizes of the partial digestion products in sequence, several *Hind* III fragments were aligned as shown in Fig. 2 C. The results of double digestion experiments summarized in Table II were used to distinguish a fragment from others of similar sizes. 1. *Sal* I severed *Hind* III-D but not *Hind* III-C. 2. *Hind* III-M and *Hind*

Table 2. *Double Digestion of Ad 12 DNA*

Fragment recleaved	Restriction endonuclease for recleavage	Size of recleavage products (nucleotide pairs)
Hind III-I	*Sal* I	1,170, 220
-D		1,870, 1,730
Bam HI-A		3,500, 4,500
-I		800
Hind III-B	*Bam* HI	1,780, 2,370, 540
-C		650, 3,000
-D		1,540, 1,140, 690, 230
-K		830, 200
-A		2,240, 3,060
-L		690, 290
Hind III-B	*Sma* I	2,050, 250, 2,370
-J		110, 990
Bam HI-G		2,070, 280
-H		980, 280
-F		1,700, 1,200
Eco RI-D		2,720, 1,350
-B		1,040, 8,200
-E		360, 2,140
Hind III-F	*Eco* RI	1,860, 660
-B		1,300, 3,400
-O		370
-P_1, P_2		350, 260

Double digestion experiments were carried out by recleavage of purified DNA fragments and by digestion of intact DNA with two different restriction endonuclease in sequence or at the same time. The size of each fragment was estimated as described in the text. Some double digestion products were not detected because of their small size in these experiments.

III-L were produced from *Eco* RI-A by recleavage with *Hind* III. 3. *Bam* HI severed *Hind* III-K but not *Hind* III-J. 4. *Hind* III-J was cleaved by *Sma* I. 5. *Hind* III-P and *Hind* III-O were severed by *Eco* RI. 6. Partial digestion of *Bam* HI-H with *Hind* III showed the presence of a small *Hind* III fragment (about 90 nucleotide pairs) between *Hind* III-B and *Hind* III-C. It was also observed that *Hind* III-D and *Hind* III-N hybridized the same *Acc* I fragment with a size of about 3 map units. Based on the above results, the *Hind* III cleavage map of Ad12 DNA was constructed as shown in Fig. 4.

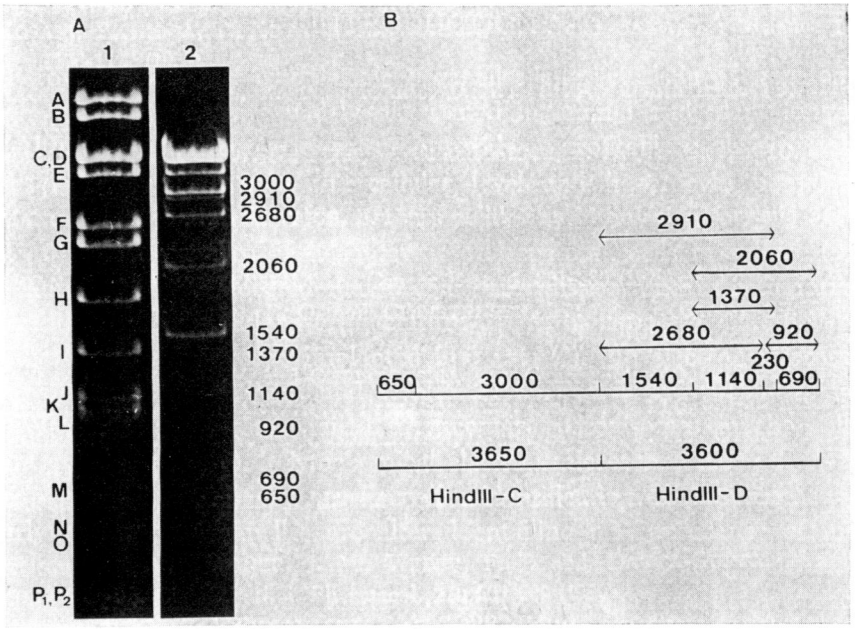

Fig. 3. Partial digestion of *Hind* III-C, D mixture with *Bam* HI.

(A) Mixture of *Hind* III-C and *Hind* III-D (0.68 μg) was digested partially with *Bam* HI (0.15 unit) at 37°C for 20 min, and electrophoresed (lane 2). *Hind* III digestion products of Ad 12 as markers were in lane 1.

(B) *Bam* HI cleavage sites mapped within *Hind* III-C, D and assignment of partial digestion fragments. Partial digestion fragments are represented by lines with arrowheads. Numerics on lines are sizes of respective fragments in nucleotide pairs.

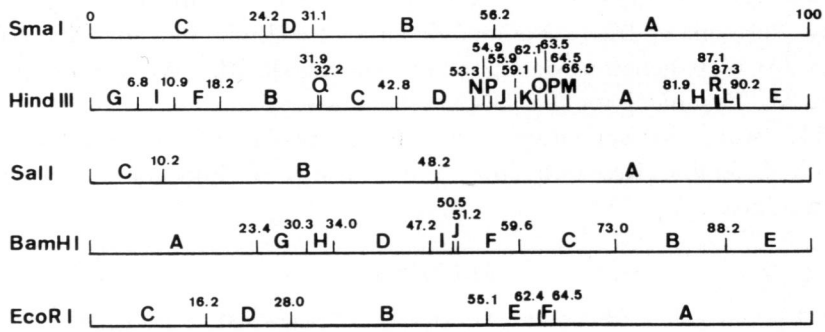

Fig. 4. Cleavage maps of Ad 12 DNA with *Sma* I, *Hind* III, *Sal* I, *Bam* HI and *Eco* RI.

Fragments are located on the Ad 12 genome represented by horizontal lines. Cleavage sites are marked in map units (percent genome length from the left end) over vertical lines.

Three *Sal* I fragments were also aligned along the genome in the order of CBA from left to right by the above results (Fig. 4). The *Sal* I site included in *Hind* III-D and *Bam* HI-I was located at the map position 48.2 by comparing the sizes of recleavage products summarized in Table 2.

It has been reported that *Bam* HI digestion of Ad12 DNA yielded nine fragments (20). We found, however, the presence of a tenth *Bam* HI fragment (Fig. 1). This was located between *Bam* HI-I and *Bam* HI-F by analyzing the partial digestion products of *Hind* III-C and *Hind* III-D with *Bam* HI (Fig. 3). All other *Bam* HI sites were located by analyzing double digestion products shown in Table 2.

Sma I cleaved Ad 12 DNA into four fragments (Fig. 1). By analyzing the double digestion products summarized in Table 2, *Sma* I sites were located at the map positions 24.2, 31.1 and 56.2. The order of the fragments was thus determined as CDBA from left to right (Fig. 4).

The size of Ad12 DNA was estimated to be 34,310±500 nucleotide pairs as the summation of the size of nineteen *Hind* III fragments. Sizes of fragments larger than *Eco* RI-C were also estimated as the summation of those of recleavage products with other enzymes. On the basis of the fragment-sizes so obtained we constructed the cleavage maps of Ad12 DNA accurately. There are some differences between our results and those reported recently by others (21, 22). This suggests, beside experimental errors, that Ad12 virions of strain Huie have some variations in the DNA sequence among laboratories where the strain is maintained.

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