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Cleavage Maps of Weakly Oncogenic Human Adenovirus Type 7 DNA by Restriction Endonuclease *Bam* HI and *Sal* I

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SUMMARY

Weakly oncogenic human adenovirus type 7 DNA (Grider strain) was cleaved into ten and three specific fragments by the restriction endonuclease *Bam* HI and *Sal* I, respectively. These specific fragments were mapped on the adenovirus type 7 genome by analyzing partial digestion products and by the DNA-DNA hybridization.

INTRODUCTION

Restriction endonuclease (EndoR) have been used to obtain specific fragments of the human adenovirus (Ad) genome and study the transcription and translation of adenovirus DNA, and cell transformation by these fragments (1).

We previously reported that EndoR *Hind* III and *Eco* RI cleaved Ad 7 DNA into 10 and 2 specific fragments, respectively (10), and the *Hind* III-I•J fragments was used to transform rat embryo cells (9). These *Hind* III fragments of Ad 7 DNA have been also used to construct transcriptional maps of "early" mRNA in lytically infected cells and viral specific RNA in transformed cells on the genome of Ad 7 (manuscript in preparation).

In this study, the fragments of Ad 7 DNA cleaved by *Bam* HI and *Sal* I were mapped on the genome of Ad 7 by the analyzing partial digestion products and by the DNA-DNA hybridization.

METHODS AND MATERIALS

Virus and viral DNAs

The Grider strain of Ad 7 was grown in KB cells and virions were purified as described previously (3). Viral DNA was extracted from purified

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virion by the method of Green and Pina (3, 4). To prepare ^{32}P-labeled Ad 7 DNA, KB cells were infected in phosphate-free Eagle's minimum essential medium containing 100 μCi/ml [^{32}P] orthophosphate and 5% dialyzed calf serum, as described previously (10). The specific activity of Ad 7 [^{32}P]-DNA was $1-2\times10^6$ cpm/μg.

Restriction endonuclease digestion and gel electrophoresis

Eco RI and *Bam* HI were purchased from Boehringer Mannheim. *Sal* I was obtained from New England Biolabs. *Hind* III was purified from *Haemophilus influenzae* Rd strain by the method of Lai and Nathans (6). Reaction buffers for each of the enzymes were as follows: *Bam* HI [6 mM Tris-HCl (pH 7.4), 6 mM $MgCl_2$, 6 mM β-mercaptoethanol]; *Sal* I [6 mM Tris-HCl (pH 7.9), 6 mM $MgCl_2$, 6 mM β-mercaptoethanol]; *Hind* III [10 mM Tris-HCl (pH 7.6), 7 mM $MgCl_2$, 7 mM KCl, 7 mM β-mercaptoethanol]; *Eco* RI [0.1 M Tris-HCl (pH 7.6), 10 mM $MgCl_2$, 50 mM NaCl]. One unit of these enzyme was defined as the minimum amount which could digest 1.0 μg of Ad 7 DNA at 37°C for 1 h. EndoR digestion and agarose gel electrophoresis were carried out as described previously (9, 10).

Hybridization and labeled probes

Hybridization was performed as follows: *Sal* I fragments of Ad 7 DNA were transferred to a nitrocellulose membrane filter after electrophoresis by the Southern's blotting technique (11) as described by Fujinaga *et al* (2). The Ad 7 *Hind* III fragment A and J were prepared as decribed previously (9) and labeled *in vitro* by nick translation (5) as described by Mackey *et al* (7). ^{32}P-labeled probes were annealed to Ad 7 *Sal* I fragments on a filter in 1 ml of 0.2 M Tris-HCl (pH 7.9), 0.6 M NaCl, 0.02 M EDTA, 0.5% SDS, and 50% (v/v) Formamide at 37°C for 40 h as described by Fujinaga *et al* (2). The filter was washed, air-dried, and autoradiographed.

RESULTS AND DISCUSSION

Ad 7 DNA was cleaved by *Bam* HI and fractionated by electrophoresis in 1.4% agarose. Figure 1 B shows a photograph of an ethidium bromide-stained slab gel containing nine distinct Ad 7 *Bam* HI fragment bands. Molecular sizes were estimated from electrophoretic modility of each fragment using Ad 7 DNA fragments produced by *Eco* RI and *Hind* III, and Ad 12 DNA fragments produced by *Eco* RI as size markers (8, 10). Percentages of each fragment to the whole genome of Ad 7 were calculated from their molecular weights as follows: A (27.4%), B (22.6%), C (12.7%), D (9.3%), E (7.2%), F (3.4%), G (2.7%), H (2.0%), and I (1.4%). The plot of the relative [^{32}P] radioactivity versus percentages of each fragment to the whole genome

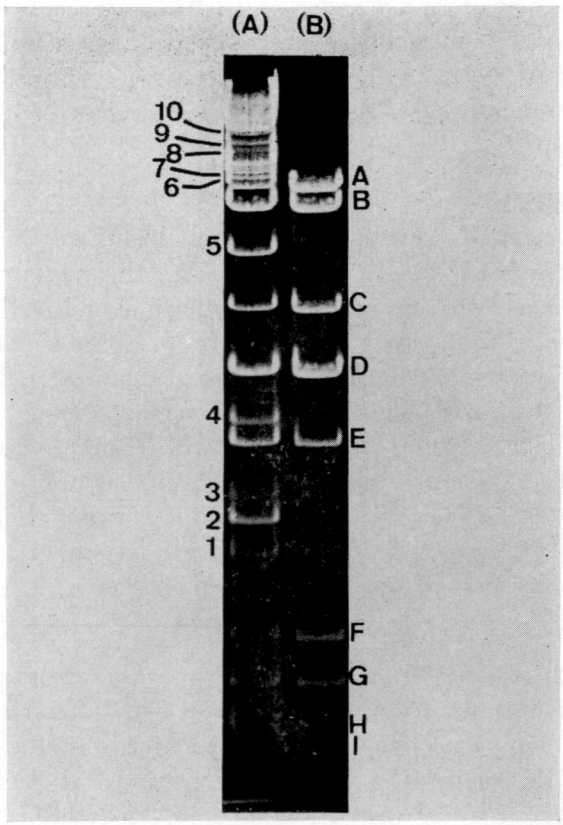

Fig. 1. *Partial digestion of Ad 7 DNA by EndoR Bam HI.*

Ad 7 [^{32}P] DNA (13 μg) was partially cleaved with 6 units of *Bam* HI for 10 min at 37°C. The digestion products were electrophoresed in a 1.4% agarose slab gel (0.5×16×22 cm) at 50 V for 20 h. Five μg of Ad 7 DNA was digested with 5 units of *Bam* HI for 1 h at 37°C and the digestion products were coelectrophoresed. Bands were labeled as A, B, C. D, E, F, G, H, and I for complete digestion fragments and as 1 to 10 for the partial digestion products.

Slot A.: Partiol digestion. Slot B: Complete digestion.

showed that fragment D was present in two-time molar amounts as the other fragments (data not shown).

The order of Ad 7 *Bam* HI fragments along the viral genome was determined by the analysis of partial digestion products. Ten partial digestion products (numbered in Fig. 1.) were recovered from the gel and purified by phenol extraction. The DNA was redigested with an excess amount of *Bam* HI and the digestion products were resolved by electrophoresis, followed by autoradiography (data not shown).

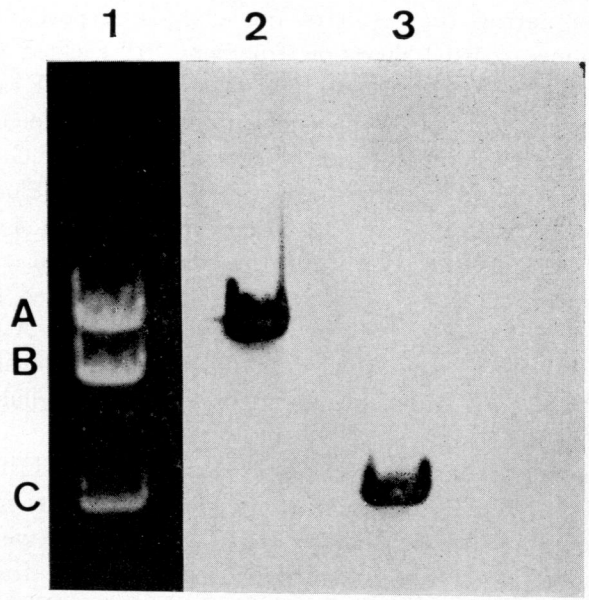

Fig. 2. *Hybridization of ^{32}P-labeled Ad 7 Hind III fragment A and J with Ad 7 Sal I fragments.*

Nick-translated Ad 7 *Hin*d III fragment A (map position : 29.2–51.4) and J (4.3–8.1) were annealed to Ad 7 *Sal* I fragments on the nitrocellulose filter. Slot 1 represents 1.0 μg of Ad 7 *Sal* I fragments on 1.0% agarose gel and stained with ethidium bromide. The same amount of DNA was present in Slot 2 and 3. Slot 2 represents Ad 7 *Sal* I fragment hybridized with [^{32}P] *Hin*d III fragment A (5×10^4 cpm). Slot 3 represents Ad 7 *Sal I* fragments hybridized with [^{32}P] *Hin*d III fragment J (5×10^4 cpm).

Table 1. *Cleavage of Partial Digestion Products with Bam HI*

Partial digestion products	Complete cleavage products
1	G I
2	H F
3	F G
4	H F G
5	D E
6	I A
7	H F G I A
8	D C D E
9	I A B
10	B D C D
	H F G I A B D C D E

Ten bands containing partial digestion products were cut from the gel shown in figure 1. The DNA was extracted from these gels as described previously (9) and redigested with *Bam* HI. The digestion products were resolved by electrophoresis and autoradiographed.

Table 1 summarizes the result of the analysis of partial digestion products. For instance, partial digestion fragment "1" yielded G and I; "2" yielded H and F; "3" yield F and G; "4" yielded H, F and G. From their overlappings in each partial digestion product, the order of the *Bam* HI fragments along the viral genome can be determined as H F G I A B D C D E.

Sal I digestion of Ad 7 DNA yields three fragments (Slot 1 in Fig. 2), and their molecular weights are 11.4×10^6, 7.59×10^6, and 4.00×10^6 corresponding to 49.6, 33.0, and 17.4% of the whole genome. The order of Ad 7 *Sal* I fragments along the viral genome was determined by filter hybridization (Slot 2 and 3 in Fig. 2). Ad 7 *Hind* III fragments A (map position: 29.2–51.4) and J (4.3–8.1) were labeled *in vitro* by nick-translation and hybridized with three *Sal* I fragments immobilized on nitrocellulose membrane filter.

Sal I fragments A and C hybridized only with *Hind* III fragments A and J, respectively. Thus, the order of the *Sal* I fragments must be C A B.

Cleavage maps of Ad 7 DNA by *Bam* HI and *Sal* I were constructed from above results, as shown in figure 3. Ad 7 *Bam* HI fragment H was located at the left hand end of the genome, because its DNA sequence was homologous with the Ad 7 *Hind* III fragment I (0.0–4.3). Similary, Ad 7 *Sal* I fragment C was located at the left hand end of the genome, because its DNA sequence was homologous with the Ad 7 *Hind* III fragment J (4.3–8.1) (Figure 2). The *Sal* I cleavage map of Ad 7 Grider strain thus determined agrees with that of Ad 7 Gomen strain reported by Tibbetts (12), but the *Bam* HI cleavage map does not. Some differences were observed between cleavage maps of above two strains of Ad 7 by *Hind* III and *Eco* RI (10).

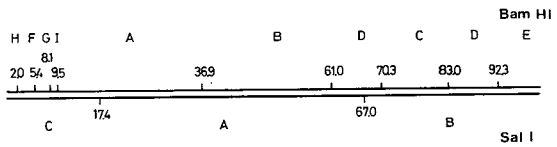

Fig. 3. *Restriction endonuclease Bam HI and Sal I cleavage maps of Ad 7 DNA.*

Bam HI and *Sal* I cleavage sites on Ad 7 DNA were shown on the horizontal line. Sites of cleavage are indicated by vertical lines and numerinl coordinates, and letters give the nomenclature of each cleavage pattern.

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