DNA RESTRICTION SITE MAPPING OF ADENOVIRUS TYPE 16 WITH *Bam*I, *Eco*RI, *Hpa*I AND *Sal*I

Tamas M. VARSANYI, Gösta WINBERG and Göran WADELL
Department of Virology, Karolinska Institutet, S 105 21 Stockholm, Sweden

Received 4 February 1977

1. Introduction

Human adenoviruses are divided into four subgroups. DNA restriction (RE) site mapping of Ad 2 (member of subgroup III) has been performed with *Bal*I, *Bam*I, *Eco*RI, *Hind*III, *Hpa*I and *Sal*I [1]. The availability of these maps has enabled localization of *ts*-mutations [2], determination of the fraction of integrated viral genome in transformed cells [3] and mapping of early and late Ad 2 transcripts [4]. The moderately oncogenic adenovirus types belonging to subgroup I differ in several biological aspects from members of other subgroups [5–7]. Furthermore, members of subgroup I, represented by Ad 16, produce more incomplete virus-particles (i.e., virus-particles with DNA of less than unit size) than members of other subgroups [8]. Access to DNA restriction site maps is a prerequisite for analysis of the structure of these DNA molecules.

The aim of this communication is to present the RE site maps of Ad 16 with *Eco*RI, *Bam*I, *Hpa*I and *Sal*I since no RE site map of Ad-types belonging to subgroup I has been reported.

2. Materials and methods

2.1. *Production and purification of virus*

The prototype strain of Ad 16 was propagated in Hela cells as previously described [8]. Unless otherwise stated three-times plaque-purified virus, passaged between each plaque isolation at a m.o.i. of 10^{-4} p.f.u. in A-549 cells (cell-line established by Dr W. A. Nelson-Rees) was used. Virus-particles produced for extraction of DNA were obtained after infection of Hela cells at a m.o.i. of 50 p.f.u. with purified virions.

Harvests of infected cells were suspended in 20 mM Tris–HCl (pH 8.0) sonicated for three 30 s intervals in an ice-bath and extracted twice with an equal volume of freon. The supernatant was layered on a CsCl-gradient (1.27–1.37 g/ml) in 20 mM Tris–HCl (pH 8.0) and centrifuged at 25 000 rev./min, using a Beckman Model L5-65 ultracentrifuge and SW 27 rotor, for 2 h at +4°C. The virions were sedimented to equilibrium in CsCl, at 35 000 rev./min for 24 h at +4°C, using a type-65 rotor. In vivo labeling with ^{32}P was performed in roller cultures (1650 cm^2) at 4 h post-infection (p.i.), 5 mCi ^{32}P (carrier-free $Na^{32}PO_4$, Amersham) was added and cells were harvested at 68 h p.i. Viral DNA was purified as described below. ^{32}P samples were counted in water (Cerenkov-effect).

2.2. *Extraction of viral DNA*

The DNA was extracted from purified virions according to Tibbetts et al. [9] precipitated by adding 0.1 vol. 3 M Na-acetate (pH 5.5) and 2 vol. isopropanol, stored at −20°C overnight and centrifuged at 100 000 × *g*, 0°C. The pellet was taken up in 10 mM Tris–HCl (pH 7.5) 15 mM $MgCl_2$ and stored at +2°C over a drop of $CHCl_3$. The DNA concentration was determined from absorbance (*A*) at 260 nm taking 1 A_{260}-unit as equivalent to 48 μg/ml [10].

2.3. *Enzyme reactions*

*Eco*RI restriction endonuclease, obtained from *Escherichia coli* RY13, was purified according to the method of Yoshimori [11]. The *Bam*I restriction endonuclease (from *Bacillus amyloliquefaciens* H), the *Sal*I restriction endonuclease (from *Streptomyces*

albus G) and the *Hpa*I restriction endonuclease (from *Haemophilus parainfluenzae*) were kindly provided by Drs Ulf Pettersson, Uppsala, Walter Doerfler, Cologne and Göran Magnusson, Stockholm, respectively. As mol. wt reference Ad 2 DNA (mol. wt 23.0×10^6) [12], cleaved with *Eco*RI and/or *Hpa*I was included. The reference sizes have been adopted after R. J. Roberts [1]: seven fragments appear after cleavage of Ad 2 DNA with *Hpa*I, 6.992, 6.302, 4.554, 3.243, 1.010, 0.552 and 0.345×10^6. Ad 2 DNA is cleaved with *Eco*RI into six fragments, 13.45, 2.80, 2.369, 1.725, 1.450 and 1.196×10^6. All enzyme reactions were carried out at 37°C in 10 mM Tris–HCl (pH 7.5) 15 mM $MgCl_2$ for 90 min. The amount of enzyme necessary for complete cleavage was determined by incubating 1 μg DNA with varying amount of enzyme. Reactions were stopped by addition of EDTA to 50 mM final concentration. Partial cleavage was obtained by incubation for 8 min at room temperature.

2.4. *Agarose slab-gel electrophoresis*

The agarose solution (0.5–1.2%, w/v) was prepared by dissolving agarose (SeaKem HGT-S type) in the electrophoresis buffer, 89 mM Tris, 89 mM Boric-acid, 2.5 mM EDTA and 0.5 μg/ml ethidium bromide [13]. The electrophoresis apparatus was a slightly modified version of Studier's [14] with a $4 \times 130 \times 170$ mm slab-gel. Electrophoresis was performed at 4.25 V/cm (constant voltage) for 16–22 h at +5°C. DNA fragments were visualised using an ultraviolet lamp (Chromato-Vue Transilluminator C-61, Ultraviolet Products Inc., San Gabriel, California, USA) and photographed with a Nikon F camera, using Kodak Wratten 29 filter and black and white negative film.

Autoradiography of ^{32}P-labeled DNA was performed by exposing dried gels to Kodak RP Royal X-Omat Medical X-ray film. After exposure the ^{32}P-labeled fragments were excised from the gel and the amount of radioactivity in the fragments was counted.

2.5. *Isolation of the DNA fragments*

The agarose gel-slices were dissolved in 1 vol. 5 M sodium perchlorate (pH 7.0) at 60°C and passed through a 1.0 ml column of hydroxylapatite in 1 mM sodium phosphate (pH 7.1) at 60°C. The column was washed with 1 ml 5 M perchlorate, 2 ml 10 mM phosphate and finally 4 ml 0.4 M phosphate. The peak fractions were pooled, dialyzed against 10 mM Tris–HCl (pH 7.5) over 24 h with three changes of buffer. The DNA was precipitated and centrifuged in an SW 40 rotor, at 23 000 rev./min for 30 min and the pellet resuspended in 10 mM Tris–HCl (pH 7.5), 15 mM $MgCl_2$.

2.6. *Preparation of the Ad 16 DNA–protein complex*

The procedure of Robinson et al. [15] was slightly modified. ^{32}P-Labeled virion was dialyzed against 0.01 M Tris–HCl (pH 8.0), 0.001 M EDTA, 0.1 M NaCl. The virus was mixed with equal vol. 8 M guanidine–HCl (Schwartz-Mann, Ultra Pure grade), 0.01 M Tris–HCl (pH 8.0), 0.001 M EDTA in an ice-bath at 0°C for 2–3 min and extracted with chloroform. The aqueous-phase was chromatographed on a Sepharose 4B column (5×40 mm) equilibrated with 4 M guanidine–HCl, 0.01 M Tris–HCl (pH 8.0), 0.001 M EDTA. Fractions containing the peak of radioactivity were pooled and dialyzed against 10 mM Tris–HCl (pH 7.5), 15 mM $MgCl_2$.

3. Results and discussion

3.1. *Cleavage with* Eco*RI*

The mol. wt of the complete genome, 21.9×10^6 was obtained by summing the *Bam*I restriction fragments (table 1). This value agreed well with length measurements in the electron microscope. The *Eco*RI restriction enzyme cleaves Ad 16 DNA into four fragments (table 1 and fig.1a). The size of A fragment was calculated by subtracting the sum of mol. wt of fragments B, C and D from 21.9×10^6. The end fragments can be identified by cleaving adenovirus DNA complexed with terminal protein, since these terminal RE fragments do not migrate into the agarose-gels [16]. After cleavage of the Ad 16 DNA–protein complex, fragments C and D migrated into the gel while only 15% of fragment A and no detectable amount of fragment B appeared (fig.1c). In accordance with published maps of Ad 2 DNA [17], the A and B fragments were placed at the left and right ends of the genome, respectively. Partial cleavage reaction was carried out (fig.1b) and it was found that the major partial fragment had a

Table 1
Restriction cleavage fragments of adenovirus 16 DNA

Fragment	*Bam*I fragments of Ad 16 DNA			Fragment	*Eco*RI fragments of Ad 16 DNA	
	Distribution of ^{32}P (%)	Electrophoresis Mol. wt[a] ($\times 10^{-6}$)	Relative % of genome		Electrophoresis Mol. wt[a] ($\times 10^{-6}$)	Electron microscopy[b] Mol. wt ($\times 10^{-6}$)
A	25.1	5.57 ± 0.01	25.5	A	18.38	18.70 ± 0.4
B	21.6	4.90 ± 0.10	22.4	B	1.87 ± 0.07	1.80 ± 0.07
C	13.0	2.93 ± 0.03	13.4	C	1.27 ± 0.05	1.20 ± 0.07
D	11.7	2.37 ± 0.03	10.8	D	0.38 ± 0.01	0.36 ± 0.04
E	10.0	2.20 ± 0.02	10.0			
F	6.8	1.61 ± 0.01	7.4			
G	5.6	1.21 ± 0.01	5.5			
H	4.5	1.10 ± 0	5.0			
	*Hpa*I fragments of Ad 16 DNA				*Sal*I fragments of Ad 16 DNA	
A		14.9	68.1	A	10.94	
B		7.0 ± 0.15	31.9	B	7.23 ± 0.2	
				C	3.73 ± 0.07	

[a] Measured in 0.5% agarose slab-gels, with Ad 2 DNA–*Eco*RI and Ad 2 DNA–*Hpa*I restriction fragments as reference
[b] ΦX 174-RFII DNA was used as internal standard – its size was taken as 3.4×10^6 daltons

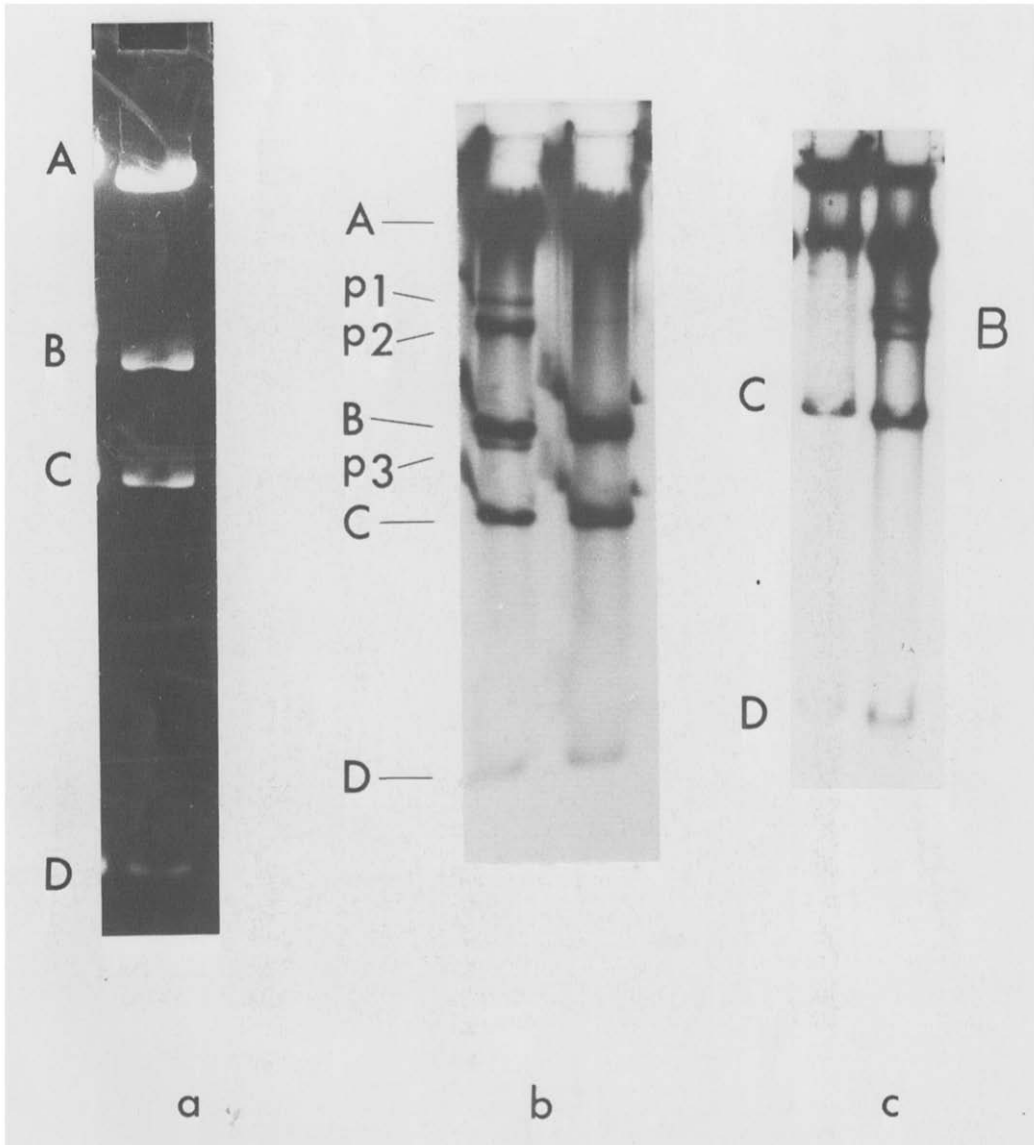

Fig. 1. Cleavage of Ad 16 DNA with endonuclease *Eco*RI. (a) 1 μg DNA was incubated for 90 min at 37°C with 3 μl of *Eco*RI and the products electrophoresed in a 0.5% agarose slab-gel (4.25 V/cm, 18 h). (b) Partial cleavage was carried out at room temperature for 8 min using 1 μl *Eco*RI. Partial fragmentation product p1 is D + C + B, p2 is B + C and p3 is C + D. (c) The ^{32}P-labeled DNA–protein complex was digested with *Eco*RI and after separation fragments C, D and 15% of fragment A were detected. In a parallel run DNA–protein complex was digested with *Eco*RI and subsequently with pronase. DNA from non-plaque purified virus was used in this experiment, containing a 1.1% insertion at the right molecular end (Winberg and Wadell, 1977, submitted to Cell). This explains the heterogenity seen in the region of fragment B.

size of 3.15×10^6, which is very close to the sum of the B and C fragments, and a fragment of 3.50×10^6, corresponding to D + C + B. The sequence of the *Eco*RI restriction fragments was found to be A–D–C–B (fig.5).

3.2. *Cleavage with* Hpa*I*

The *Hpa*I cleaves the Ad 16 DNA in two fragments (table 1 and fig.2). After incubation of the

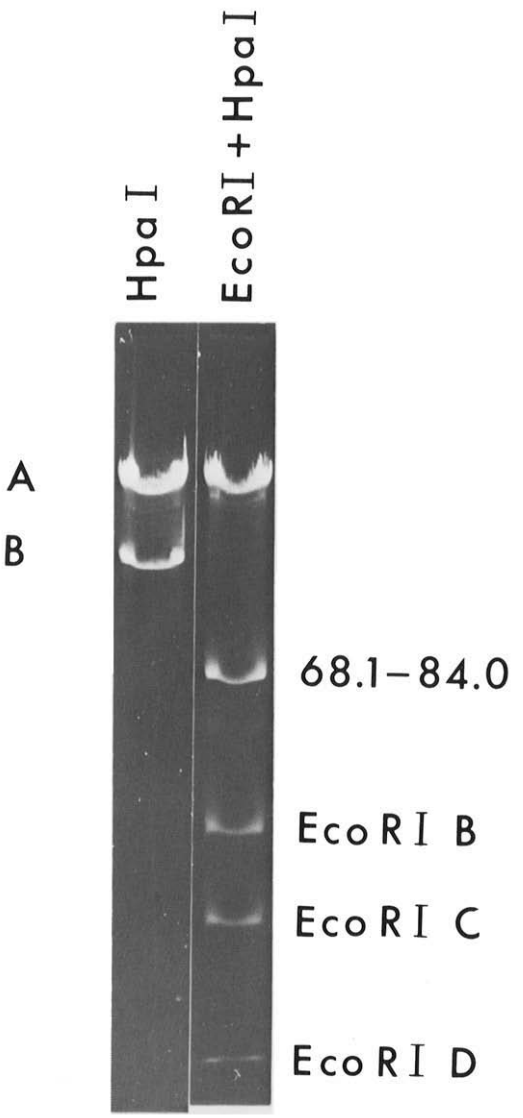

Fig.2. Cleavage of Ad 16 DNA with *Hpa*I endonuclease. 1 μg DNA was digested with *Hpa*I and a mixture of *Hpa*I + *Eco*RI, respectively, fragments were separated in a 0.5% agarose slab-gel.

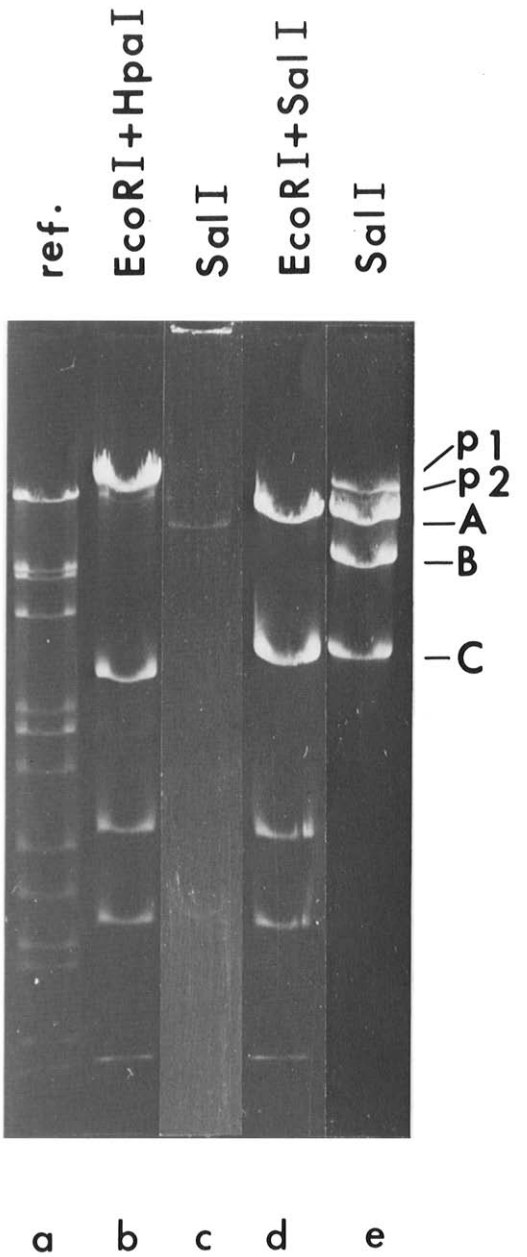

Fig.3. Cleavage of Ad 16 DNA with *Sal*I endonuclease. (e) 1 μg DNA was digested with *Sal*I and separated in a 0.5% agarose slab-gel. Three fragments were seen (and two partial fragments, p1 and p2 were observed as well). (c) Cleavage of the DNA–protein complex with *Sal*I revealed A fragment, while digestion with *Eco*RI and *Sal*I showed (d) intact A and C fragments as well as appearance of *Eco*RI-B, *Eco*RI-C and *Eco*RI-D fragments. (a) The reference used was a mixture of Ad 2 DNA–*Eco*RI and Ad 2 DNA–*Hpa*I fragments.

DNA with a mixture of *Eco*RI and *Hpa*I, the *Hpa*I-A fragment remained intact and the *Hpa*I-B fragment was cut into four fragments, 3.5, 1.87 (*Eco*RI-B), 1.27 (*Eco*RI-C) and 0.38 × 10^6 (*Eco*RI-D). The sum of the four fragments, 7.0 × 10^6 is the size of the *Hpa*I-B fragment. Consequently the *Hpa*I-A fragment is 14.9 × 10^6 and the order of the two fragments from left to right is A–B (fig.5).

3.3. *Cleavage with* SalI

The *Sal*I restriction enzyme cuts the Ad 16 DNA into three fragments (table 1 and fig.3e). After cleavage of the DNA–protein complex with *Sal*I, only fragment A migrated into the agarose-gel. Thus fragments B and C are terminal. Cleavage of the DNA with a mixture of *Eco*RI and *Sal*I revealed that on electrophoretic separation the *Sal*I-B fragment was missing and *Eco*RI-B, *Eco*RI-C and *Eco*RI-D fragments had appeared (fig.3d), suggesting that *Sal*I-B fragment represents the right end of the genome. The order of *Sal*I RE fragments is C–A–B (fig.5).

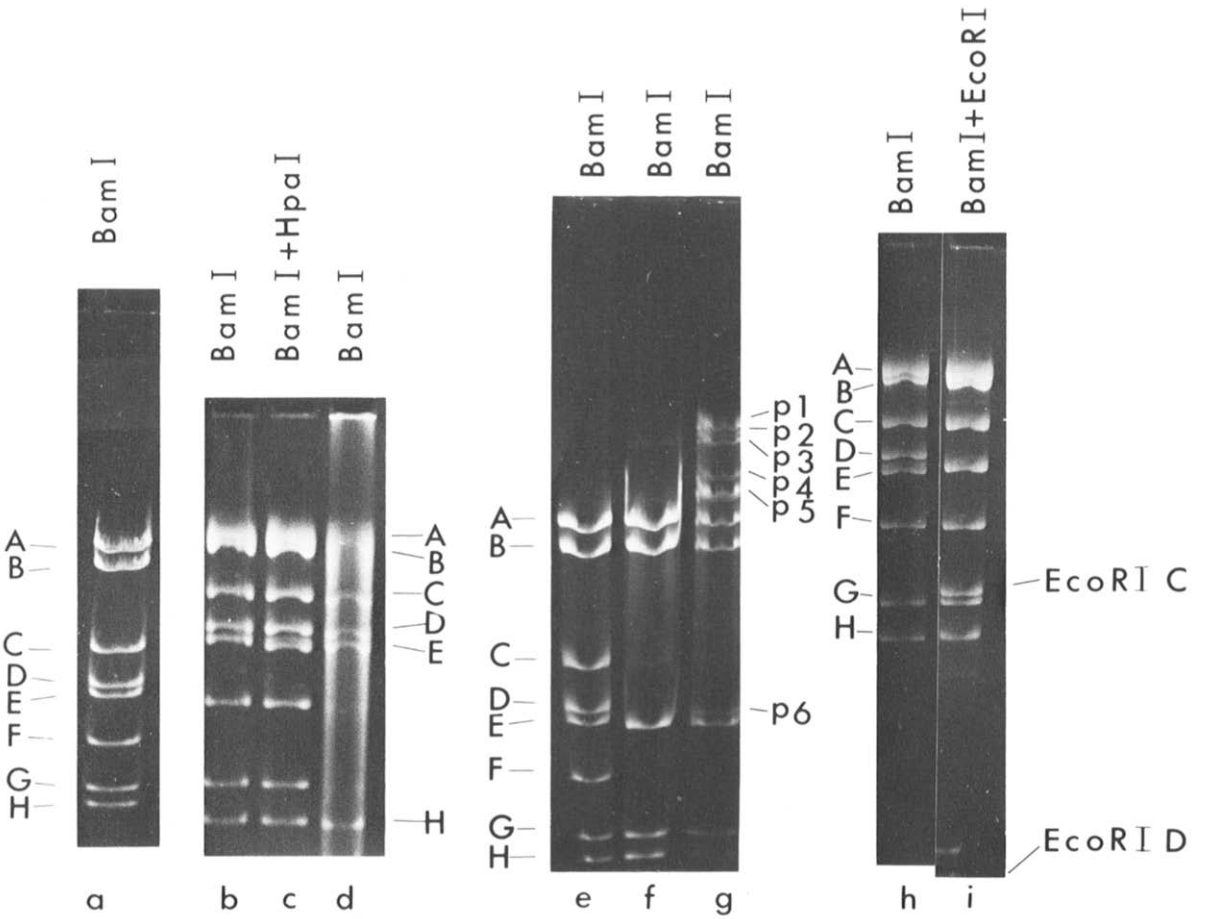

Fig.4. Cleavage of Ad 16 DNA with *Bam*I endonuclease. (a) 1 μg DNA was cleaved with *Bam*I and the products were separated into eight fragments in a 0.5% agarose slab-gel. (d) The DNA–protein complex digested with *Bam*I showed that the terminal fragments F and G were missing. (f) Cleavage of the *Hpa*I-A fragment with *Bam*I yielded five fragments. (g) Partial digestion of the *Hpa*I–A fragment with *Bam*I gives six partial fragments, where p1 is 13.0 × 10^6 (≃ G + H + A + B), p4 is 8.0 × 10^6 (≃ G + H + A), p5 is 7.15 × 10^6 (≃ B + E) and p6 is 2.32 × 10^6 (≃ G + H). (i) Cleavage with a mixture of *Bam*I and *Eco*RI showed that the *Bam*I-D fragment was missing while *Eco*RI-C, *Eco*RI-D appeared and also a 0.48 × 10^6 fragment, positioned between 81.8–84.0% of the genome.

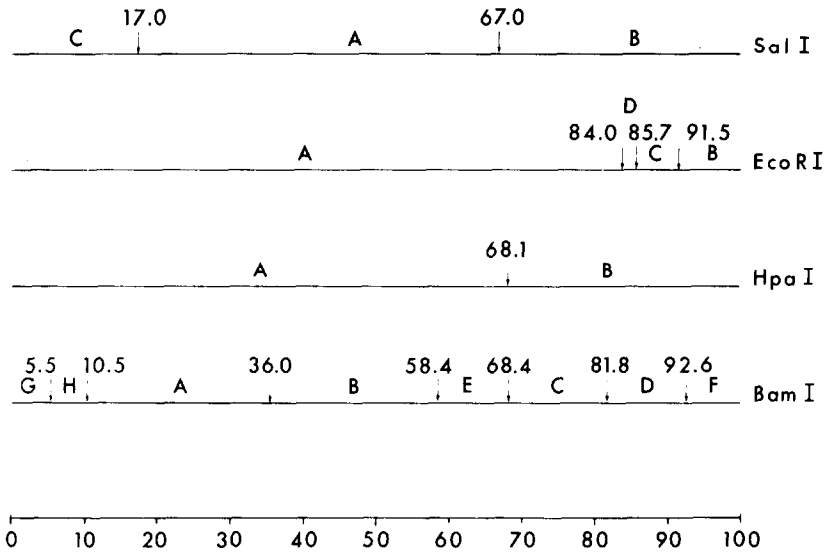

Fig.5. Ad 16 DNA restriction site maps by *Bam*I, *Hpa*I, *Eco*RI and *Sal*I.

3.4. *Cleavage with* Bam*I*

The *Bam*I restriction enzyme cleaves the Ad 16 DNA into eight fragments, varying between $1.10–5.57 \times 10^6$, making *Bam*I a useful tool for more detailed analysis of the genome (table 1 and fig.4a). Cleavage of the DNA–protein complex with *Bam*I revealed that F and G fragments are terminally located (fig.4d). The *Hpa*I-A fragment, corresponding to 0–68.1% of the genome, was isolated as described in Materials and methods and recleaved with *Bam*I. Electrophoresis revealed that C, D and F fragments were missing (fig.4f). The F fragment is consequently the terminal fragment at the right-end. The other end-fragment, G is thus located at the left-end. Cleavage of the complete genome with a mixture of *Eco*RI and *Bam*I showed (fig.4i) that the *Bam*I-D fragment contains all *Eco*RI RE sites, since *Eco*RI-C, *Eco*RI-D and *Bam*I-F fragments could be detected whereas the *Bam*I-D fragment was missing. The order of *Bam*I fragments within the *Hpa*I-B fragment is thus C–D–F. Cleavage of the DNA with a mixture of *Sal*I and *Bam*I showed that the *Bam*I-A fragment was missing, demonstrating that this fragment contains the *Sal*I cleavage site at 17.0% of the genome. After partial cleavage of the isolated *Hpa*I-A fragment with *Bam*I, partial cleavage products of 2.32 and 13.0×10^6 were observed, corresponding to G + H and G + H + A + B, respectively (fig.4g). The order of the *Bam*I RE fragments is G–H–A–B–E–C–D–F.

Acknowledgements

This work was supported by the Swedish Cancer Society grant 137-B76. We thank Marianne Peterson and Ingrid Rehnlund for excellent technical assistance.

References

[1] Roberts, R. J., Cold Spring Harbour Lab., personal communication.
[2] Sambrook, J., Williams, J., Sharp, P. A. and Grodzicker, T. (1975) J. Mol. Biol. 97, 369–390.
[3] Gallimore, P. H., Sharp, P. A. and Sambrook, J. (1974) J. Mol. Biol. 89, 49–71.
[4] Pettersson, U., Tibbetts, C. and Philipson, L. (1976) J. Mol. Biol. 101, 479–501.
[5] Philipson, L., Pettersson, U. and Lindberg, U. (1975) Virol. Monogr. 14, 1–115.
[6] Wadell, G. and Norrby, E. (1969) J. Virol. 4, 671–680.
[7] Norrby, E. and Wadell, G. (1969) J. Virol. 4, 663–670.
[8] Wadell, G., Hammarskjöld, M.-L. and Varsanyi, T. (1973) J. Gen. Virol. 20, 287–302.

[9] Tibbetts, C., Johansson, K. and Philipson, L. (1973) J. Virol. 12, 218–225.
[10] Wetmur, J. G. (1976) Ann. Rev. Biophys. Bioeng. 5, 337–361.
[11] Yoshimori, R. N. (1971) Ph. D. Thesis, Univ. of California, San Francisco.
[12] Pettersson, U., Mulder, C., Delius, H. and Sharp, P. (1973) Proc. Natl. Acad. Sci. USA 70, 200–204.
[13] Peacock, A. D. and Dingman, C. W. (1968) Biochemistry 7, 668–674.
[14] Studier, F. W. (1973) J. Mol. Biol. 79, 237–248.
[15] Robinson, A. J., Younghusband, H. B. and Bellet, A. J. D. (1973) Virology 56, 54–69.
[16] Sharp, P. A., Moore, C. and Haverty, J. L. (1976) Virology 75, 442–456.
[17] Mulder, C., Arrand, J. R., Delius, H., Keller, W., Pettersson, U., Roberts, R. J. and Sharp, P. A. (1974) Cold Spring Harbour Symp. Quant. Biol. 39, 397–400.