Proc. Nat. Acad. Sci. USA
Vol. 72, No. 12, pp. 4810–4814, December 1975
Biochemistry

Human papillomavirus DNA: Physical map

(human papillomavirus/restriction enzymes/DNA mapping/gene *32* protein of bacteriophage T4)

MICHEL FAVRE*, GÉRARD ORTH*, ODILE CROISSANT†, AND MOSHE YANIV‡

* Unité de Biochimie Enzymologie, Laboratoire de Pharmacologie Moléculaire No. 147 associé au Centre National de la Recherche Scientifique, Institut Gustave-Roussy, 94800, Villejuif, France; and † Département de Virologie, and ‡ Département de Biologie Moléculaire, Institut Pasteur, 75015 Paris, France

Communicated by Jacques Monod, September 16, 1975

ABSTRACT **Human papillomavirus (HPV) DNA form I (supercoiled) was prepared from virions purified from plantar warts. HPV DNA was cleaved with restriction enzymes obtained from the following sources: *Escherichia coli* (*Eco*RI), *Hemophilus influenzae* strain Rd (both unfractionated *Hin*d and separated *Hin*dII and *Hin*dIII enzymes) and *Hemophilus parainfluenzae* (*Hpa*I). The cleavage products were analyzed by polyacrylamide gradient slab gel electrophoresis and electron microscopy. HPV DNA was cleaved into two fragments by *Eco*RI (87% and 13% of the genome) and into six fragments, ranging in size from 33.5 to 1.2% of the genome, by *Hin*d endonucleases. The six *Hin*d fragments result from the cleavage of three sequences recognized by *Hin*dII, two of which are also cleaved by *Hpa*I, and of three sequences recognized by *Hin*dIII. The order of these fragments was determined by comparing their size with that of the fragments obtained with *Hin*dII, *Hin*dIII, *Hpa*I, and the mixture of *Hin*dIII + *Hpa*I. The two *Eco*RI cleavage sites were located on two adjacent *Hin*d fragments and one of these sites has been taken for the zero point to construct a physical map.**

The treatment of superhelical HPV DNA with bacteriophage T4 gene *32* protein yields circular structures with a denaturation loop. The cleavage of these complexes with *Eco*RI and *Hin*dIII has shown two easily denatured regions which were located on the cleavage map.

A papillomavirus, member of the papovavirus group (1), induces different types of human cutaneous warts, anogenital warts, and juvenile laryngeal papillomas (2). The malignant transformation of these benign tumors may be observed under exceptional circumstances (3, 4). The structure of the human papillomavirus (HPV) found in large amounts in some cutaneous warts, and the physicochemical and biochemical properties of its components are well documented (5–7); in particular, the viral genome is constituted of a covalently closed circular double-stranded DNA molecule with a molecular weight of about 5×10^6 and a G+C content of 41% (6). However, the biochemical and genetic analysis of the expression of the viral genome in the infected cells and the study of the role of this virus in human oncogenesis have been impeded since no cell system allows its *in vitro* replication and titration (8).

Recently, the availability of site-specific endonucleases (restriction enzymes) has permitted a rapid advance in the study of the structure and function of the genomes of oncogenic DNA viruses. We report here the specific cleavage map of the HPV DNA with restriction enzymes prepared from *Escherichia coli* (*Eco*RI), *Hemophilus influenzae* (*Hin*d) and *Hemophilus parainfluenzae* (*Hpa*I). Furthermore, two binding sites of the bacteriophage T4 gene 32 protein were located on the physical map.

Abbreviations: HPV, human papillomavirus; *Eco*RI, restriction endonuclease from *Escherichia coli*; *Hin*d, *Hin*dII, and *Hin*dIII, enzymes from *Hemophilus influenzae* strain Rd; *Hpa*I, enzyme from *Hemophilus parainfluenzae*.

MATERIALS AND METHODS

Preparation of HPV DNA. Plantar warts were a kind gift of P. Agache (Clinique Dermatologique, C.H.U. Besançon, France). Full particles of HPV were purified according to a procedure previously reported (7). Viral DNA was extracted from virions and supercoiled molecules (form I) were separated from open circular (form II) and linear (form III) molecules by equilibrium centrifugation in CsCl containing ethidium bromide as previously described (9). Viral DNA solutions (50 μg/ml) in 10 mM Tris·HCl, 1 mM EDTA, pH 7.9, were kept at −20°.

Enzyme Purifications. *E. coli* RI (*Eco*RI) endonuclease was prepared as described by Yoshimori (10). *Hemophilus influenzae* (*Hin*d) endonucleases were prepared from the exonuclease-minus strain Rd by the procedure described by Smith and Wilcox (11). *Hemophilus influenzae* III (*Hin*dIII) endonuclease was further purified as described by Danna *et al.* (12). *Hemophilus influenzae II* (*Hin*dII) endonuclease was a gift of V. Pirrotta (Biozentrum der Universität, Basel, Switzerland). *Hemophilus parainfluenzae I* (*Hpa*I) endonuclease was prepared according to Sharp *et al.* (13).

Enzymatic Digestion. Buffers used for enzyme digestions were: *Eco*RI, 50 mM Tris·HCl (pH 7.5), 10 mM $MgCl_2$, (10); *Hin*d endonucleases, 6.6 mM Tris·HCl (pH 7.5), 6.6 mM $MgCl_2$, 10 mM NaCl, 6.6 mM 2-mercaptoethanol (11); *Hpa*I, 10 mM Tris·HCl (pH 7.5), 10 mM $MgCl_2$, 0.1 mM dithiothreitol (13). HPV DNA form I (2.5 μg) in 0.25 ml of buffer was incubated at 37° for 1 hr with *Eco*RI (2 μl of stock solution) or for 6 hr with the other enzymes (*Hin*d, 5 μl; *Hin*dII, 3 μl; *Hin*dIII, 5 μl; *Hpa*I, 10 μl) or the mixture of *Hin*dIII and *Hpa*I. When the digestion products of HPV DNA by *H. influenzae* or H. *parainfluenzae* endonucleases were further digested with *Eco*RI enzyme, *Eco*RI was added to the mixture 1 hr before the end of the 6 hr incubation. The reaction was stopped by adding 25 μl of 250 mM EDTA. After phenol extraction, the DNA fragments were isolated by ethanol precipitation and centrifugation. The pellets were suspended in 20 μl of buffer containing 4 mM Tris–acetate, 2 mM sodium acetate, 10% sucrose, and 0.02% bromophenol blue as a tracking dye.

Polyacrylamide Gel Electrophoresis. The DNA cleavage products were separated in 2.5–10% polyacrylamide gradient slab gels using the conditions described by Jeppesen (14). The electrophoretic mobility of the fragments was determined from photographic negatives of the stained gels, using a Joyce Loebl double-beam recording microdensitometer. Molecular weights of DNA fragments were estimated by comparing their electrophoretic mobility to that of the λ bacteriophage DNA *Hin*d fragments (15).

Binding of Bacteriophage T4 Gene *32* Protein to HPV DNA. T4 gene 32 protein was purified according to Alberts

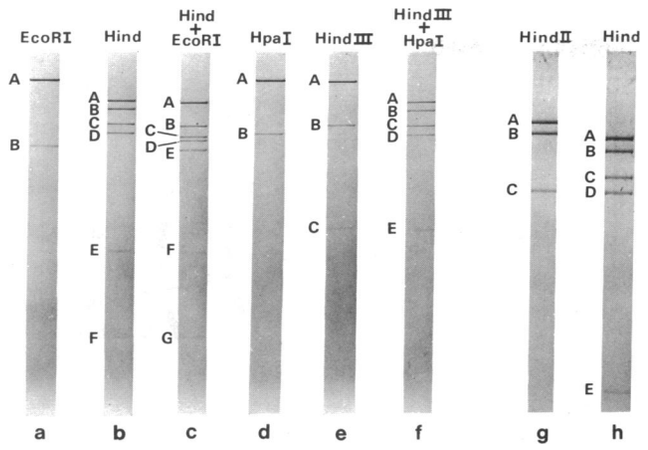

FIG. 1. Polyacrylamide gel electrophoresis of restriction endonuclease cleavage products of HPV DNA. HPV DNA samples (2.5 μg) were incubated as described in *Materials and Methods* with restriction enzymes: (a) *Eco*RI, (b) *Hin*d, (c) *Hin*d + *Eco*RI, (d) *Hpa*I, (e) *Hin*dIII, (f) *Hin*dIII + *Hpa*I, (g) *Hin*dII, (h) *Hin*d. Polyacrylamide gradient slab gels (2.5–10%) were run at 5 V/cm in 40 mM Tris–acetate, 20 mM sodium acetate (pH 7.9) (14) at room temperature for 9 hr (a–f) or for 11 hr (g and h). Bromophenol blue was used as a tracking dye. Gels were stained in 0.02% methylene blue for 2 hr and excess of dye was removed with distilled water.

and Frey (16). Binding of the T4 gene *32* protein on superhelical HPV DNA and fixation with glutaraldehyde were performed as described by Delius *et al.* (17). After dialysis against 10 mM Tris·HCl, pH 7.9, DNA–T4 gene *32* protein complexes were incubated for 1 hr at 37° with *Eco*RI or *Hin*dIII endonucleases and spread for electron microscopy.

Electron Microscopy. HPV DNA, endonuclease DNA fragments, and DNA–T4 gene *32* protein complexes were mounted on collodion-coated grids for electron microscopy using the formamide technique of Davis *et al.* (18). Pt–Pd-shadowed grids were examined and photographed with a Siemens Elmiskop 101 electron microscope. DNA molecules were measured using a coordinatometer connected to a PDP-8 Digital computer. Length calibrations were made with a grating replica [E. F. Fullam, Inc., 54,800 lines per inch (21,600 lines/cm)].

RESULTS

Sensitivity of HPV DNA to *Eco*RI and *Hin*d restriction enzymes

HPV DNA was cleaved with *Eco*RI or with a mixture of *Hin*dII and *Hin*dIII. The digestion products were analyzed by polyacrylamide gradient slab gel electrophoresis and by electron microscopy. As shown in Fig. 1a and b, the *Eco*RI and *Hin*d endonucleases generate two and six fragments, respectively, named by letters according to their increasing electrophoretic mobility. Increasing up to 2-fold the amount of enzyme or the time of incubation did not modify the electrophoretic pattern. A plot of the logarithm of each peak surface, as determined on the scans of the gels, against the electrophoretic mobility of the *Hin*d fragments shows a near-linear relationship (data not shown here). This indicates that every molecule of DNA yields one of each of the six *Hin*d fragments.

The size and the molecular weight of HPV DNA and of the DNA cleavage products are reported in Table 1. The size of HPV DNA (2.48 ± 0.15 μm), as determined by measuring

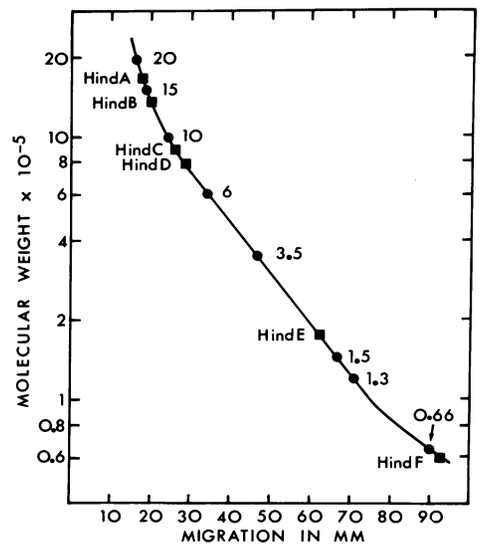

FIG. 2. A semi-logarithmic plot of molecular weights versus electrophoretic mobilities of *Hin*d fragments. The electrophoretic mobilities of the HPV *Hin*d fragments (■) determined from the scan of the photograph of the stained gel were compared to those of the λ phage DNA fragments produced by the *Hin*d endonucleases. Molecular weights of the λ *Hin*d fragments taken as standards (●) were deduced from the size of the fragments given in base pairs (15).

174 form II molecules, corresponds to a molecular weight of 4.80 ± 0.28 × 10^6, which is close to the value reported by Crawford (5.0 × 10^6) (6). The values of the molecular weights obtained by electron microscopy measurements are in good agreement with those determined from the electrophoretic mobility of the fragments, as compared to the mobility of the λ DNA *Hin*d fragments of known molecular weight (0.06 to 2.0 × 10^6) (Fig. 2) (15). The sum of the molecular weights of the two *Eco*RI fragments (4.84 × 10^6, as determined by electron microscopy measurements) or that of the six *Hin*d fragments (4.90 × 10^6, as determined by electrophoresis) equals the molecular weight of HPV DNA.

Table 1. Length and molecular weight of the HPV DNA and of HPV DNA fragments produced by cleavage with *Eco*RI and *Hin*d endonucleases

HPV DNA or fragments	Length (μm)	Molecular weight × 10^{-6}	
		Length*	Mobility†
HPV DNA	2.48 ± 0.15	4.80	ND
*Eco*RI A	2.19 ± 0.09	4.20	ND
*Eco*RI B	0.33 ± 0.04	0.64	0.63
*Hin*d A	0.83 ± 0.04	1.62	1.65
*Hin*d B	0.68 ± 0.05	1.32	1.35
*Hin*d C	0.45 ± 0.03	0.87	0.89
*Hin*d D	0.40 ± 0.03	0.77	0.78
*Hin*d E	0.10 ± 0.02	0.19	0.17
*Hin*d F	ND	ND	0.06

ND, not determined by this method.

* Molecular weights were estimated from the lengths of the molecules determined by electron microscopy, taking as a standard the length of the PM2 bacteriophage DNA (molecular weight, 6.4 × 10^6) (19) measured in the same conditions (3.33 ± 0.22 μm).

† The molecular weights (mean value of three experiments) have been evaluated from the electrophoretic mobilities of the fragments using λ DNA *Hin*d fragments as standards (15).

Table 2. Size of the HPV DNA fragments produced by cleavage with *H. influenzae* and *H. parainfluenzae* restriction endonucleases*

DNA fragments	*Hin*d	*Hpa*I	*Hin*dII	*Hin*dIII	*Hin*dIII + *Hpa*I
A	33.5	84.2	46.2	77.3	33.7
B	27.4	15.8	38.0	17.5	27.0
C	18.0		15.8	5.2	18.5
D	16.0				15.7
E	3.9				5.1
F	1.2				

* The size of the fragments represents their molecular weights expressed as a percentage of the total molecular weight of the fragments (*Hin*d, 4.83 × 10^6; *Hpa*I, 4.78 × 10^6; *Hin*dII, 4.87 × 10^6; *Hin*dIII, 4.91 × 10^6; *Hin*dIII + *Hpa*I, 4.82 × 10^6). Molecular weights were determined by electron microscopy except for *Hin*d F.

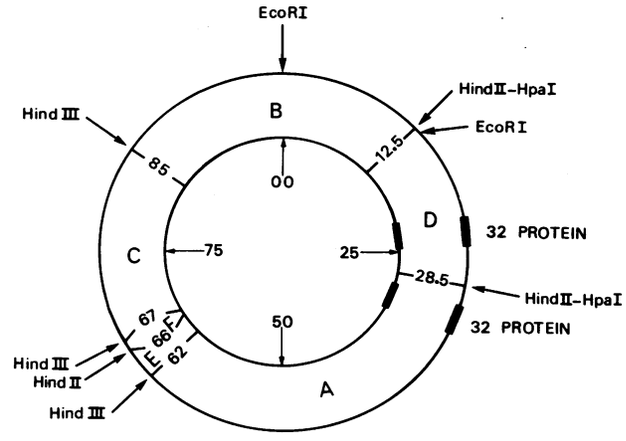

FIG. 3. A physical map of the HPV genome. The map distances from the zero point are given as a percentage of the HPV unit genome length.

Ordering of the *Hin*d fragments

The *Hin*dII and *Hin*dIII endonucleases (12), separated from the *Hin*d mixture, and the *Hemophilus parainfluenzae* *Hpa*I endonuclease, which cleaves one of the palindromic sequences recognized by *Hin*dII endonuclease (20, 21), were used to identify the enzymatic activity producing the *Hin*d fragments and to establish the order of these fragments. As shown in Fig. 1d–g, *Hpa*I, *Hin*dII, *Hin*dIII, and the mixture of *Hin*dIII + *Hpa*I cleave the HPV DNA into two, three, three, and five fragments, respectively, named by letters according to their increasing electrophoretic mobility. The relative sizes of the fragments obtained are reported in Table 2. The results indicate that the six *Hin*d fragments result from the cleavage of three specific *Hin*dII sequences, two of which are recognized by *Hpa*I (referred to later as *Hin*dII-*Hpa*I sites), and of three sequences recognized by *Hin*dIII. Data reported in Fig. 1d–g and in Table 2 further show that: (*i*) the *Hin*d D fragment, analogous to *Hpa*I B and *Hin*dII C fragments, is located between two *Hin*dII-*Hpa*I cleavage sites; (*ii*) the *Hin*d C fragment, analogous to the *Hin*dIII B fragment, is located between two *Hin*dIII cleavage sites; (*iii*) the *Hin*d A and B fragments, analogous to *Hin*dIII-*Hpa*I A and B fragments, are located between an *Hin*dIII and an *Hpa*I cleavage site, on both sides of the *Hin*d D fragment; (*iv*) the size of the *Hin*dIII C and *Hin*dIII-*Hpa*I E fragments corresponds to the sum of the sizes of the *Hin*d E and F fragments; these fragments are thus adjacent and separated by the *Hin*dII cleavage site which is not cleaved by *Hpa*I; (*v*) the size of the *Hin*dII A and B fragments corresponds to the sum of the sizes of the *Hin*d B, C, and F fragments and of the *Hin*d A and E fragments, respectively. This shows that the *Hin*d A and E fragments are adjacent and that the *Hin*d C fragment, located between two *Hin*dIII cleavage sites, lies between the *Hin*d B and F fragments. From these data, the order of the *Hin*d fragments was deduced to be *Hin*d A, E, F, C, B, D.

Table 3. Size of HPV DNA fragments produced by cleavage with *Eco*RI, *Hin*d, and *Hin*d + *Eco*RI restriction endonucleases*

DNA fragments	*Eco*RI	*Hin*d	*Hin*d + *Eco*RI
A	ND	33.6	33.8
B	13.1	27.6	18.5
C		18.2	15.6
D		15.9	15.0
E		3.5	12.5
F		1.2	3.4
G			1.2

ND, not determined by electrophoresis.

* The size of the fragments represents their molecular weights determined by electrophoresis, and expressed as a percentage of the total molecular weight of all the fragments (*Hin*d, 4.90 × 10^6; *Hin*d-*Eco*RI, 4.85 × 10^6), or of the mean value of the HPV DNA molecular weight (4.80 × 10^6) for the *Eco*RI B fragment.

Location of the *Eco*RI cleavage sites on the *Hin*d fragments

The electrophoretic pattern and the size of the fragments obtained after successive cleavage of HPV DNA with *Hin*d and *Eco*RI endonucleases, as compared to the cleavage products obtained with each of these enzymes separately, are reported in Fig. 1a, b, and c and in Table 3. Fig. 1b and c shows that when HPV *Hin*d fragments are further incubated with *Eco*RI, the *Hin*d B and D fragments are lost, showing that the two *Eco*RI sites are located on these fragments. Only three new fragments, *Hin*d-*Eco*RI C, D, and E, are detected, the total size of which nearly corresponds to the sum of the sizes of the *Hin*d B and D fragments; furthermore, Table 3 shows that the smallest of these fragments, *Hin*d-*Eco*RI E (12.5% of the genome length), nearly equals the *Eco*RI B fragment (13.1% of the genome length). This demonstrates that one *Eco*RI site is near a *Hin*d site and confirms that *Hin*d B and D are adjacent. Because of the size of the fragments *Hin*d D and *Hin*d-*Eco*RI C and D, this cleavage site must be located on the *Hin*d D fragment at 0.3–0.6% of the genome from the BD junction, the second *Eco*RI site being located on the *Hin*d B fragment at 12.5% of the genome from the BD junction. The expected fourth fragment, coming from *Hin*d D fragment, is too small to be detected.

Construction of the physical map

The *Eco*RI cleavage site located on *Hin*d B was designated the zero point for the construction of the physical map, since none of the enzymes used gives a unique double-strand break on the circular molecule of HPV DNA. The *Hin*d fragments were arbitrarily mapped clockwise in the order B,

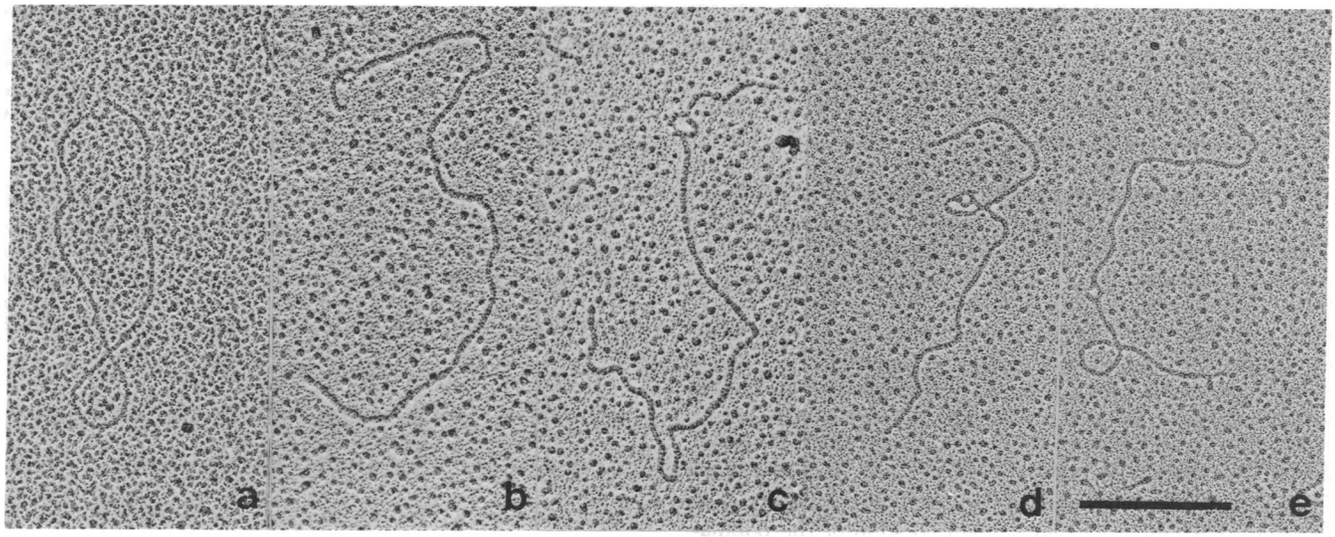

FIG. 4. Electron micrographs of form I HPV DNA complexed to T4 gene *32* protein (a) and of *Eco*RI A fragments–T4 gene *32* protein complexes (b and c) and *Hin*dIII A fragments–T4 gene *32* protein complexes (d and e) obtained after cleavage of circular complexes. Bar represents 0.5 μm.

D, A . . . . The map distances of the cleavages sites, expressed as a percentage of the genome length, were calculated from the distance of the zero point to the BD junction (12.5% genome length) and from the size of the *Hin*d fragments reported in Table 3. The cleavage map is shown in Fig. 3. To further support the location of the cleavage sites, the products of the digestion of HPV DNA with *Hpa*I, *Hin*dII, or *Hin*dIII endonucleases were further incubated with *Eco*RI endonuclease and analyzed by electrophoresis. The number and the size of the fragments reported in Table 4 are consistent with the cleavage map presented in Fig. 3.

Evidence for and location of bacteriophage T4 gene *32* protein binding sites on HPV DNA

The incubation of simian virus 40 (17, 22) or polyoma virus (23) superhelical DNA with T4 gene 32 protein yields circular structures with a denaturation loop, which is located in specific regions as determined by cleavage of the complexes with restriction enzymes. Fig. 4a shows that similar complexes with a denaturation loop are obtained after incubation of form I HPV DNA with T4 gene 32 protein. *Eco*RI cleaves these complexes into two fragments. A denaturation loop has only been observed on fragments with the length of *Eco*RI A fragment (Figs. 4b and c, and 5a). The distance from the midpoint of the loop to the nearer end of the fragment is 0.25 ± 0.03 μm, i.e., 10% of the genome length, for 66% of the complexes, and 0.47 ± 0.03 μm, i.e., 18% of the genome length, for 30% of the complexes (Fig. 5b). Thus the binding sites may be mapped at 23% or 90% (major site) and at 31% or 82% (minor site) of genome length from the zero point. To locate unambiguously these sites, the complexes were cleaved by the *Hin*dIII endonuclease. A denaturation loop is observed only on fragments with the length of *Hin*dIII A which may be separated in two classes, when the distance from the midpoint of the loop to the nearer end of the fragment is measured (Fig. 4d and e). The distances, measured on 158 molecules, were evaluated to be 30 ± 1% (31% of the molecules) and 38 ± 2% (67% of the molecules) of the genome length. Since the ends of *Hin*dIII A fragment (77% of genome length) are located at 85 and 62% of genome length from the zero point, the two binding sites for T4 gene 32 protein may be mapped unequivocally at 23% (major site) and 31% of genome length from the origin (Fig. 3).

Table 4. Size of HPV DNA cleavage products obtained by combination of *Hpa*I, *Hin*dII, or *Hin*dIII endonucleases with *Eco*RI*

DNA fragments	*Hpa*I + *Eco*RI	*Hin*dII + *Eco*RI	*Hin*dIII + *Eco*RI
A	72.7	38.8	49.1
B	14.9	33.7	18.4
C	12.4	15.1	14.5
D		13.0	12.9
E			5.1

* The size of the fragments represents their molecular weight determined by electron microscopy, expressed as a percentage of the total molecular weight of the fragments (*Hpa*I + *Eco*RI, 4.82 × 10^6; *Hin*dII + *Eco*RI, 4.84 × 10^6; *Hin*dIII + *Eco*RI, 4.89 × 10^6).

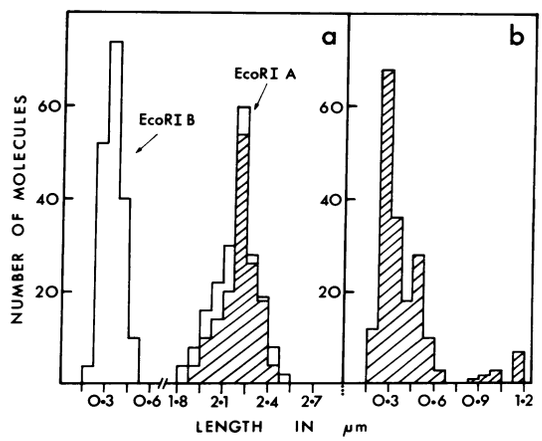

FIG. 5. *Eco*RI cleavage of HPV DNA–T4 gene *32* protein complexes. (a) Histogram of the length (μm) of the *Eco*RI cleavage fragments. Fragments with a denaturation loop are shown in diagonally striped histogram. (b) Histogram of the distance (μm) from the midpoint of the denaturation loop to the nearer end of the *Eco*RI A fragments measured on 195 molecules.

DISCUSSION

*Eco*RI (10) and *Hin*d (11) endonucleases cleave HPV DNA into two and six fragments, respectively. *Hin*dII and *Hin*dIII endonucleases (12) both produce three breaks in viral DNA, while two breaks are produced by *Hpa*I, which cleaves one of the sequences recognized by *Hin*dII (20, 21). The DNA fragments are produced in equimolar amounts and the sum of the molecular weights of the cleavage products corresponds to the HPV DNA molecular weight; this shows the homogeneity of form I DNA preparations purified from virions recovered from human plantar warts. However, when the HPV DNA *Eco*RI fragments (2.5 μg) were electrophoresed, two weak bands, at the detection limit, were evidenced, corresponding to fragments with molecular weight of about 2.3 and 2.6 $\times$ 10^6. Moreover, two minor bands may be detected by electrophoresis of the HPV DNA *Hin*d fragments, one with a slightly lower mobility than *Hin*d B, and the other with a slightly higher mobility than *Hin*d C. This indicates that a small fraction of the population of HPV DNA molecules (1–2%) is constituted of molecules with sequence rearrangements, or originates from an HPV variant. Whether this may be related to the antigenic differences already reported for viruses recovered from papillomas occurring in different locations (cutaneous or anogenital warts) (24) remains to be established.

The small number of breaks generated by the enzymes used in this study has allowed the construction of a cleavage map of HPV DNA using an unlabeled HPV DNA. The specificity of the enzymes producing the *Hin*d fragments and the order of the fragments have been deduced from the comparison of the size of the *Hin*d fragments, as determined by electron microscopy and electrophoresis, with the size of the fragments produced by *Hin*dII, *Hin*dIII, *Hpa*I, and by the *Hin*dIII + *Hpa*I mixture. The two *Eco*RI cleavage sites were located in two adjacent *Hin*d fragments and one of the sites has been taken for the zero point to construct the map shown in Fig. 3.

The bacteriophage T4 gene 32 protein, which binds specifically to single-stranded DNA, has been used to evidence locally melted regions in the superhelical HPV DNA. Two binding sites were detected and located unambiguously on the cleavage map. The relationship between these two sites and the four (AT)-rich regions which have been previously detected after partial thermal denaturation of circular molecules of HPV DNA (6, 25) remains to be established, as well as the functional role of these regions.

The cleavage map of HPV DNA constitutes a useful tool both for the study of the DNA homology between the possibly antigenically distinct papillomaviruses recovered from different types of human warts (2, 24), and for the analysis of the genetic expression of the HPV.

Note Added in Proof: Recent data show that *Bacillus amyloliquefaciens* endonuclease I (*Bam*I) cleaves HPV DNA at a single specific site located in the *Hin*d F fragment. Furthermore, *Hemophilus parainfluenzae* endonuclease II (*Hpa*II) cleaves the HPV DNA into four fragments. However, the results suggest that about 40% of the DNA molecules are partially resistant to this enzyme, although no resistance to *Eco*RI and *Hin*d enzymes was evidenced in the DNA used in all these studies (in preparation).

We thank F. Breitburd for critical reading of the manuscript, and P. Agache for generously providing the human warts. We also thank A. Chestier, C. Dauguet, D. Cany, and N. Jibard for skillful technical assistance. This work was supported by grants from the Centre National de la Recherche Scientifique, the Institut National de la Santé et de la Recherche Médicale, and the Jane Coffin Childs Memorial Foundation for Medical Research.

1. Wildy, P. (1971) in *Monographs in Virology*, ed. Melnick, J. L. (S. Karger, Basel), Vol. 5, pp. 38–39.
2. Rowson, K. E. & Mahy, B. W. (1967) *Bacteriol. Rev.* **31**, 110–131.
3. Jablonska, S., Dabrowski, J. & Jakubowicz, K. (1972) *Cancer Res.* **32**, 583–589.
4. Hudson, H. C., Holcomb, F. L. & Gates, W. (1973) *J. Urol.* **110**, 301–302.
5. Klug, A. & Finch, J. T. (1965) *J. Mol. Biol.* **11**, 403–423.
6. Crawford, L. V. (1969) *Adv. Virus Res.* **14**, 89–152.
7. Favre, M., Breitburd, F., Croissant, O. & Orth, G. (1975) *J. Virol.* **15**, 1239–1247.
8. Butel, J. S. (1972) *J. Nat. Cancer Inst.* **48**, 285–299.
9. Orth, G., Jeanteur, P. & Croissant, O. (1971) *Proc. Nat. Acad. Sci. USA* **68**, 1876–1880.
10. Yoshimori, R. N. (1971) Ph.D. Dissertation, University of California, San Francisco Medical Center.
11. Smith, H. O. & Wilcox, K. W. (1970) *J. Mol. Biol.* **51**, 379–391.
12. Danna, K. J., Sack, G. H., Jr. & Nathans, D. (1973) *J. Mol. Biol.* **78**, 363–376.
13. Sharp, P. A., Sugden, B. & Sambrook, J. (1973) *Biochemistry* **12**, 3055–3063.
14. Jeppesen, P. G. N. (1974) *Anal. Biochem.* **58**, 195–207.
15. Allet, B. & Bukhari, A. I. (1975) *J. Mol. Biol.* **92**, 529–540.
16. Alberts, B. & Frey, L. (1970) *Nature* **227**, 1313–1318.
17. Delius, H., Mantell, N. J. & Alberts, B. (1972) *J. Mol. Biol.* **67**, 341–350.
18. Davis, R. W., Simon, M. & Davidson, N. (1971) in *Methods in Enzymology*, eds. Grossman, L. & Moldave, K. (Academic Press, New York), Vol. 21, pp. 413–428.
19. Franklin, R. M. (1974) *Curr. Top. Microbiol. Immunol.* **68**, 107–160.
20. Smith, H. O. & Nathans, D. (1973) *J. Mol. Biol.* **81**, 419–423.
21. Garfin, D. E. & Goodman, H. M. (1974) *Biochem. Biophys. Res. Commun.* **59**, 108–116.
22. Morrow, J. F. & Berg, P. (1972) *Proc. Nat. Acad. Sci. USA* **69**, 3365–3369.
23. Yaniv, M., Croissant, O. & Cuzin, F. (1974) *Biochem. Biophys. Res. Commun.* **57**, 1074–1079.
24. Oriel, J. D. & Almeida, J. D. (1970) *Br. J. Vener. Dis.* **46**, 37–42.
25. Follett, E. A. C. & Crawford, L. V. (1967) *J. Mol. Biol.* **28**, 461–467.