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A Map of the Sites in the Polyoma Genome Cleaved by Endonuclease *Alu*I

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The 29 sites in the polyoma genome cleaved by endonuclease (endo) *Alu*I and the site cleaved by endo *Xba*I have been identified. The *Alu*I fragments range in size from 0.9 to 37×10^4 daltons; 17 sites are located in the early region, and 12 are in the late region. The *Xba*I site is located 17% of the genome away from the *Eco*RI site, towards the terminus of DNA replication.

INTRODUCTION

Considerable advances in the specification of biological functions to distinct regions of the polyoma virus genome have been made in the past several years (Condit *et al.*, 1978; Miller and Fried, 1976; Feunteun *et al.*, 1976; Eckhart, 1977; Gibson *et al.*, 1977; Smith *et al.*, 1976). These advances have been possible largely because of the determination of endonuclease cleavage maps of the viral genome. The known physical map of polyoma DNA now includes the sites cleaved by endonucleases *Hpa*II, *Hin*dIII and *Eco*RI (Griffin *et al.*, 1974), *Hin*dII (Folk *et al.*, 1975; Chen *et al.*, 1975), *Kpn*I and *Pst*I (Crawford and Robbins, 1976); *Hae*III (Summers, 1975; Griffin, 1977), *Bam*HI, *Hae*II, and *Hha*I (Griffin and Fried, 1976), *Bum*I (Fried and Griffin, 1977), and *Hga*I (Shishido and Berg, 1976).

In this report, we provide a map of the 29 sites in the polyoma genome cleaved by endonuclease (endo) *Alu*I, and we identify the site cleaved by endo *Xba*I.

MATERIALS AND METHODS

Enzyme preparations and assays. The preparations of endo *Eco*RI and phage T4 polynucleotide kinase have been described previously (Berkner and Folk, 1977a). Endonucleases *Hpa*II, *Hin*dIII, *Hin*dII, and *Bam*HI were purified by published procedures (Sharp *et al.*, 1973; Smith and Wilcox, 1970; Wilson and Young, 1975). Endo *Alu*I was either from New England Biolabs or generously provided by Ron Hart (purified according to the procedure of Bickle *et al.*, 1977). Both preparations had approximately the same activity (5 units/μl), however the activity of the enzyme from Biolabs decreased over several months. The amount of enzyme sufficient to achieve partial or complete digestion was therefore determined prior to most experiments. Endonucleases *Hae*II, *Hha*I, and *Xba*I were purchased from New England Biolabs, and endo *Hae*III was purchased from Bethesda Research Labs.

For all endonucleases, a unit of activity is roughly defined as that amount sufficient to effect complete digestion of 1 μg polyoma DNA after 60 min at 37° in 20 μl of one of the following buffers: A (endonucleases *Bam*HI, *Hin*dII, *Hin*dIII), 6 m*M* Tris–HCl pH 7.5, 6 m*M* 2-mercaptoethanol (2-MSH), 6 m*M* $MgCl_2$; B (endonucleases *Hae*II, *Eco*RI), 6 m*M* Tris–HCl pH 7.5, 6 m*M* 2-MSH, 6 m*M* NaCl, 6 m*M* $MgCl_2$; C (endo *Xba*I), 6 m*M* Tris–HCl pH 7.9, 6 m*M* $MgCl_2$, 6 m*M* 2-MSH, 150 m*M* NaCl; D (endonucleases *Alu*I, *Hae*III), 6 m*M* Tris–HCl pH 7.9, 6 m*M* $MgCl_2$, 6 m*M* 2-MSH; E (endo *Hpa*II), 10 m*M* Tris–HCl pH 7.4, 10 m*M* $MgCl_2$, 1 m*M* dithiothreitol (DDT), 6 m*M* KCl; F (endo *Hha*I), 6 m*M* Tris–HCl pH 7.4,

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6 mM $MgCl_2$, 6 mM 2-MSH, 50 mM NaCl, 100 μg/ml autoclaved gelatin.

DNA preparations and digestions. Pasadena large plaque polyoma DNA uniformly labeled with either [^{3}H]thymidine or with $^{32}P_i$ was purified from infected cells by procedures that have previously been described (Folk and Wang, 1974; Anderson and Folk, 1976). In some cases endonuclease fragments of [^{3}H]DNA were labeled with ^{32}P at 5′-phosphoryl termini using the polynucleotide kinase exchange reaction (Berkner and Folk, 1977a).

*Alu*I-digested polyoma DNA was prepared by incubating 5.4 μg polyoma [^{3}H] DNA in 25 μl buffer D with 5 μl endo *Alu*I (Biolabs) for 90 min at 37°, followed by phenol extraction and ethanol precipitation. For the preparation of *Hin*dIII-digested polyoma DNA, 5.4 μg of polyoma DNA was incubated in 25 μl buffer A with 6 units enzyme for 60 min at 37°. Both DNAs were labeled with ^{32}P using polynucleotide kinase exchange, then dialyzed and ethanol precipitated.

Individual restriction endonuclease fragments of uniformly labeled polyoma [^{32}P] DNA were isolated as follows: *Hpa*II fragments were prepared by incubating DNA (8.4 μg, 1–2 × 10^5 cpm/μg) in 240 μl buffer E with 5 units enzyme for 4 hr at 37°. *Hin*dIII fragments of polyoma DNA were prepared by incubating 3.2 μg of [^{32}P]DNA with 15 units of endo *Hin*dIII in 150 μl buffer A for 60 min at 37°. Polyoma DNA digested with endonucleases *Hae*II and *Bam*HI was prepared by incubating polyoma [^{32}P]DNA (8.5 μg) in 65 μl buffer B with 20 units endo *Hae*II for 2.5 hr at 37°, followed by the addition of 25 units endo *Bam*HI and incubation for another 90 min. All DNAs were then mixed with Ficoll (to 0.25%), EDTA (to 10 mM), and bromphenol blue (to 0.1%), and, in several cases, sodium dodecyl sulfate (to 0.5%). After electrophoresis through a polyacrylamide gel the DNAs were stained with ethidium bromide (5 μg/ml) and visualized by fluorescence under ultraviolet light. Individual fragments were excised, electroeluted, and precipitated with 2 vol of isopropanol.

*Alu*I partial digestion fragments were generated from one preparation of individual *Hpa*II fragments (*Hpa*II-1–4). Each *Hpa*II fragment (1–2 μg) was incubated in 55 μl buffer D with quantities of endo *Alu*I (Biolabs) that gave only partial digestion and subjected to electrophoresis through a polyacrylamide gel, together with *Hpa*II/*Alu*I-digested polyoma DNA as markers. The gels were autoradiographed wet and partial digestion fragments were excised, recovered by electroelution, and precipitated with 2 vol of isopropanol.

Gel electrophoresis. Electrophoresis of DNAs through polyacrylamide gels was performed using 6% acrylamide, 0.15% N,N'-methylenebisacrylamide in 40 mM Tris–HCl pH 7.9, 5 mM sodium acetate, and 1 mM EDTA, unless otherwise stated. The DNA samples were subjected to electrophoresis at 35 mA/gel for 12–16 hr in a vertical slab gel (1.5 mm × 30 cm × 14 cm, Hoefer). For several preparations, DNA fragments were recovered from polyacrylamide gels by electroelution into dialysis bags. Following electrophoresis for 1–4 hr at 80 V in 40 mM Tris–HCl pH 7.9, 5 mM sodium acetate, and 1 mM EDTA, DNA samples were precipitated in 67% isopropanol, 33% 0.2 M NaCl. Agarose gel electrophoresis, performed here at an agarose (Seakem) concentration of 1%, has been described elsewhere (Berkner and Folk, 1977b).

For each gel from which molecular weights were determined, samples of *Hin*dIII-digested and *Hpa*II-digested polyoma DNAs were included for construction of standard curves. Mobilities of restriction endonuclease fragments were measured as a function of molecular weight, using published values (Griffin *et al.*, 1974; Sompayrac and Danna, 1977).

RESULTS

Location of Other Endonuclease Sites in AluI Fragments

Endo *Alu*I cleaves the polyoma genome into 29 fragments (Fig. 1), which range in size from 0.9 to 37 × 10^4 daltons (Table 1). The location of several of these *Alu*I fragments were determined relative to other

restriction endonuclease sites, whose positions have been previously mapped (Fig. 2).

Seven of the fragments from an *Alu*I digest of polyoma DNA were found to contain other endonuclease sites and their approximate locations were thereby specified (Table 2). A precise localization in the polyoma genome for several of these seven *Alu*I fragments was made by considering the size to which each fragment was reduced after cleavage with a second endonuclease (Table 2). Four of the *Alu*I fragments could be correctly oriented with reference to the secondary endonuclease sites. Fragment *Alu*I-1 contains a *Hin*dII and a *Hha*I site, both located near one terminus (Table 2). Only one location on the polyoma genome (between 25 and 35 map units) was consistent with the *Hin*dII and *Hha*I double-digestion products observed and with its not having the *Xba*I site (located at 17 map units; see below). Similarly, fragment *Alu*I-7, which contains a *Hin*dII site near one

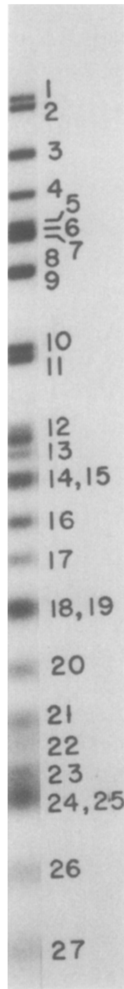

FIG. 1. *Alu*I fragments of polyoma DNA. Polyoma DNA digested with endo *Alu*I was labeled with ^{32}P at 5′-phosphoryl termini using the polynucleotide kinase exchange reaction. The DNA fragments were phenol extracted, ethanol precipitated, and then separated by electrophoresis through a polyacrylamide gel. *Alu*I fragments are numbered in order of decreasing molecular weight. Fragments 28 and 29 were inadvertently run off the gel, but were observed with shorter times of electrophoresis.

TABLE 1

SIZES OF *Alu*I FRAGMENTS

Fragment	Molecular weight × 10^{-4}	Base pairs[a]
1	37.0	565
2	35.8	548
3	30.0	460
4	25.4	388
5	23.1	354
6	22.0	336
7	21.4	327
8	19.1	291
9	18.5	283
10	13.9	211
11	13.3	203
12	9.7	148
13	9.2	141
14,15	8.3	127
16	7.2	110
17	6.0	92
18,19	5.3	81
20	4.2	64
21	3.2	50
22	2.9	44
23,24,25	2.3	36
26	1.7	27
27	1.3	20
28	1.2	17
29	0.9	14

[a] Assuming a molecular weight of 3.5×10^6 (5300 base pairs) for polyoma DNA.

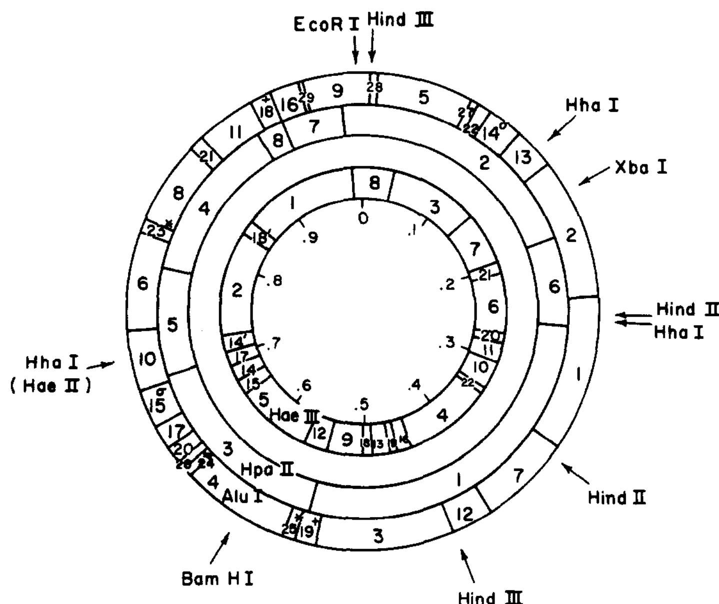

FIG. 2. Map of endonuclease sites in polyoma DNA. The order of *Alu*I fragments within the polyoma genome is illustrated in the outermost circle. Sites cleaved by endo *Hpa*II (Griffin *et al.*, 1974) and endo *Hae*III (Griffin, 1977) are included in the inner circles. The sites for endonucleases *Hae*II, *Bam*HI, and *Hha*I (Griffin and Fried, 1976), *Hin*dII (Folk *et al.*, 1975), *Hin*dIII and *Eco*RI (Griffin *et al.*, 1974), and *Xba*I are indicated by arrows. Fractional map units are indicated within the innermost circle. Those fragments comigrating at the same position are identified by the symbols σ, +, or *.

terminus (Table 2), must be located at 35–41 map units, as an opposite orientation would result in overlap with fragment *Alu*I-1. Fragment *Alu*I-2 is cleaved into products of 26.6×10^4 (and, therefore, 9.2×10^4 daltons by endo *Xba*I (Table 2). Since it does not contain a *Hha*I site, it must be located between 15–25 map units. Fragment *Alu*I-13 has a *Hha*I site approximately in its center (Table 2), and its location must therefore be at 12–15 map units.

Endo *Alu*I cleaves the tetranucleotide

TABLE 2

MOLECULAR WEIGHTS OF PRODUCTS OF SECONDARY ENDONUCLEASE DIGESTION OF *Alu*I FRAGMENTS[a]

*Alu*I fragment	Molecular weight ($\times 10^{-4}$) of *Alu*I fragment	Second endonuclease	Molecular weight ($\times 10^{-4}$) of secondary fragment(s)
10	13.9	*Hae*II	6.0
1	37.0	*Hha*I	31.2; 4.0 or 4.9
10	13.9	*Hha*I	6.0
13	9.2	*Hha*I	4.0 and/or 4.9
1	37.0	*Hin*dII	32.4
7	21.4	*Hin*dII	2.1
2	35.8	*Xba*I	26.6
9	18.5	*Eco*RI	12.1; 5.7
4	25.4	*Bam*HI	14.4

[a] Polyoma DNA was digested with endo *Alu*I and labeled by polynucleotide kinase exchange, as described under Methods. After dialysis, samples of [5'-^{32}P]DNA (20,000 cpm, 0.3 μg in 10 μl) were incubated with a second endonuclease, as indicated above, followed by polyacrylamide gel electrophoresis.

sequence AGCT, which is included within the *Hin*dIII recognition sequence AAGCTT (Old *et al.*, 1975; Jay and Wu, 1976; Roberts *et al.*, 1976). The identification of *Alu*I fragments bordering the *Hin*dIII sites was therefore determined by first labeling *Hin*dIII-generated 5′-phosphoryl termini with ^{32}P, followed by endo *Alu*I cleavage. *Alu*I-3, 9, and 12 and 28 were labeled in such an experiment.

Since *Alu*I-9 contains the *Eco*RI site as well as a *Hin*dIII terminus, it must be located between 97 and 02 map units on the polyoma genome (Fig. 2). This is consistent with the molecular weights of double-digestion products of endonucleases *Eco*RI and *Alu*I (12.1 $\times 10^4$ and 5.7 $\times 10^4$, Table 2) and the known locations of the *Hin*dIII site and *Eco*RI site (Fig. 2).

Identification of AluI Sites in Purified HpaII and HindIII Fragments

To localize several more of the *Alu*I fragments, polyoma [^{32}P]DNA was digested with endonucleases *Hpa*II or *Hin*dIII and the individual fragments were isolated. These were then digested with endo *Alu*I (Tables 3 and 4).

In one instance an *Alu*I/*Hpa*II truncated fragment comigrated with an *Alu*I fragment: *Alu*I-3 and truncated *Alu*I-1, both of which are located in *Hpa*II-1, exhibited the same mobility. The size of the *Hpa*II-truncated *Alu*I-1 fragment is consistent with the position established for *Alu*I-1 in the previous section (Fig. 2). In several instances, an *Alu*I fragment contained in one *Hpa*II fragment comigrated with a truncated *Alu*I fragment present in a different *Hpa*II fragment. The localization of the *Alu*I fragment within a particular *Hpa*II fragment was independently checked by determining in which *Hin*dIII fragment it was located. Thus, while both *Hpa*II-4 and *Hpa*II-6 appear to contain *Alu*I-8 (Table 3), *Alu*I-8 is in *Hin*dIII-1 (Table 4) and must therefore be in *Hpa*II-4 (Fig. 2). The *Alu*I/*Hpa*II-6 fragment, therefore, must be a truncated *Alu*I fragment. Similarly, from Table 3 it appears that *Alu*I-17 is present in both *Hpa*II-3 and *Hpa*II-6. Since *Alu*I-17 is contained in *Hin*dIII-1 (and therefore in *Hpa*II-3) (Table 4), the *Alu*I/*Hpa*II-6 fragment must be a truncated *Alu*I fragment.

TABLE 3

*Alu*I FRAGMENTS AND *Alu*I/*Hpa*II TRUNCATED FRAGMENTS WITHIN INDIVIDUAL *Hpa*II FRAGMENTS[a]

*Hpa*II fragment	1		2		3		4		5		6		7		8	
*Alu*I fragment	3/T1[b]	(30.0)	5	(21.3)	4	(25.4)	8	(19.1)	T6	(12.7)	T2	(18.5)	16	(7.2)	T18	(3.7)
	7	(21.4)	T2	(14.4)	15	(8.3)	T11	(9.7)	T10	(11.0)	T1	(5.3)	T9	(6.6)	T11	(2.3)
	12	(9.7)	T9	(10.4)	17	(6.0)	T6	(7.2)					T18	(1.5)		
	T19	(4.2)	13	(9.2)	20	(4.2)	21	(3.2)					29	(0.9)		
			14	(8.3)	24[c]	(2.3)	23	(2.3)								
			22	(2.9)	25[c]	(2.3)										
			27	(1.3)	26	(1.7)										
			28	(1.2)	T10	(1.3)										
					T19	(1.2)										

[a] Individual *Hpa*II fragments (0.3 μg, 2000–20,000 cpm, prepared as described under Methods), were incubated in 25 μl buffer D with 2 μl endo *Alu*I for 2 hr at 37°, followed by electrophoresis through polyacrylamide. The autoradiogram of *Alu*I-digested *Hpa*II fragments was scanned with a densitometer and fragment areas were quantitated to determine whether comigration of *Alu*I/*Hpa*II truncated fragments and normal *Alu*I fragments had occurred within any of the *Hpa*II fragments. This was also checked by comparing the molecular weight of each *Hpa*II fragment with the summed molecular weights of *Alu*I/*Hpa*II digest products within that fragment. The number in parentheses is the molecular weight $\times 10^4$.

[b] Identification of truncated (T) fragments was facilitated by digesting polyoma DNA with both endonucleases *Hpa*II and *Alu*I, and separating the products by polyacrylamide gel electrophoresis.

[c] Proof that *Hpa*II-3 contains a doublet is provided in the last section of the results.

TABLE 4

*Alu*I FRAGMENTS WITHIN INDIVIDUAL *Hin*dIII FRAGMENTS[a]

*Alu*I fragments	
Within *Hin*dIII-1	Within *Hin*dIII-2
3	1
4	2
6	5
8	7
9	12
10	13
11	14
15	22
16	27
17	28
18,19	
20	
21	
23,24,25	
26	

[a] *Hin*dIII fragments 1 and 2 (0.3 μg; 20,000 cpm; prepared as described under Methods) were incubated in 25 μl buffer D with 2 μl endo *Alu*I for 2 hr at 37°, followed by polyacrylamide gel electrophoresis.

Fragment *Hpa*II-1 contains *Alu*I-3, 7, 12 and two truncated fragments, derived from *Alu*I-1 (*Alu*I-T1) and *Alu*I-19 (*Alu*I-T19) (Table 3). Since *Alu*I-3 and *Alu*I-12 both border a *Hin*dIII site and since *Alu*I-3 is located in *Hin*dIII-1 and *Alu*I-12 is located in *Hin*dII-2 (Table 4), they must be contiguous, spanning 42–53 map units (Fig. 2).

No *Alu*I fragment contains more than one *Hpa*II site, since every *Hpa*II fragment was cleaved by endo *Alu*I. Thus, there are eight *Alu*I fragments with *Hpa*II sites. Seven of these (*Alu*I-1, 2, 6, 10, 11, 18, 19) were readily identified by double digestion with endonucleases *Hpa*II and *Alu*I. The presence of *Hpa*II sites in *Alu*I fragments 1 and 2 and the molecular weights of products of endo *Hpa*II cleavage of these fragments is consistent with their map locations determined in the previous section. *Alu*I-9, whose location has been confirmed both by endo *Eco*RI digestion (Table 2) and by determining that it borders a *Hin*dIII site, must also contain a *Hpa*II site (Fig. 2). Failure to observe a shift in the mobility of *Alu*I-9 upon endo *Hpa*II digestion is probably due to migration of a truncated *Alu*I/*Hpa*II fragment (most likely one from *Hpa*II-6) near the position of *Alu*I-9 (Table 3).

Fragment *Alu*I-10 contains both a *Hae*II/*Hha*I site (Table 2) and a *Hpa*II site. Since its molecular weight is only 13.9 × 10^4, the *Hpa*II site must be that at 70 map units (Fig. 2). Thus, *Alu*I-10 extends from approximately 70–74 map units. Again, the predicted products of *Alu*I/*Hpa*II digestion agree with truncated fragments that are observed (Table 3). The position of *Alu*I-6 can be located adjacent to *Alu*I-10, because *Hpa*II-5 contains only one *Alu*I site, thus requiring the *Alu*I fragment adjacent to *Alu*I-10 to be at least 15 × 10^4 daltons. Of the remaining four possible *Alu*I fragments with *Hpa*II sites (*Alu*I-6, 11, 18 and 19), only *Alu*I-6 is large enough to qualify. In addition, only the positioning of *Alu*I-6 adjacent to *Alu*I-10 is consistent with the *Alu*I/*Hpa*II truncated fragments observed in *Hpa*II-5 and *Hpa*II-4 (Table 3).

Of the remaining three *Alu*I fragments which contain *Hpa*II sites (*Alu*I-11, 18, 19) *Alu*I-19 was determined to contain the *Hpa*II site at map unit 55 by experiments described in the last section of the results. *Alu*I-11 and *Alu*I-18 must include sequences from *Hpa*II-8 and are contiguous since there is only one *Alu*I site in *Hpa*II-8 (Table 3). Only the clockwise order *Alu*I-11 → 18 is consistent with the observed truncated fragments produced by double digestion with *Hpa*II and *Alu*I (Table 3).

In summary, every fragment or truncated fragment observed (Table 3) was identified and assigned a map location. Thus, endo *Alu*I digestion of individual *Hpa*II or *Hin*dIII fragments allowed the following assignments (Fig. 2).

HpaII-1. *Alu*I-12 and *Alu*I-3 are in the clockwise sequence *Alu*I-12 → 3. The clockwise order *Alu*I-1 → 7 → 12 → 3 is suggested from the known positions of *Alu*I-1 and *Alu*I-7, determined in the previous section, and the composition of *Alu*I fragments in *Hpa*II-1 (Table 3).

HpaII-5. *Alu*I-10 and *Alu*I-6 form the clockwise sequence *Alu*I-10 → 6.

HpaII-6. *Alu*I-1 and *Alu*I-2 are known to

TABLE 5

*Alu*I FRAGMENT COMPOSITION OF *Alu*I PARTIAL DIGESTION PRODUCTS[a]

*Hpa*II fragment		Composition of *Alu*I partials	Σ Molecular weight × 10^{-4}	Molecular weight × 10^{-4} for intact partial
1	a	T1,3,7,12	91.1	90.1
	b	T1 or 3,7,12	61.1	63.5
	c	3,12,T19[b]	43.9	45.1
	d	T1 or 3,12	39.7	39.3
2	a	5,T9,22,27,28	38.9	39.4
	b	5,T9,28	34.7	34.5
	c	5,22,27	27.3	28.9
	d	T2,13,14	31.9	34.5
	e	T2,13	23.6	26.0
	f	13,14	17.5	17.9
3	a	4,24 or 25	27.7	28.4
	b	15,17	14.3	14.4
	c	20,26	5.9	6.9
	d	4,T10,15,17,20,24 or 25,26	49.2	51.2
	e	4,17,20,24 or 25,26	39.6	40.7
	f	4,20,24,25,26	35.9	35.8
	g	4,T19,24 and/or 25,26	30.6–32.9	30.6
	h	T10,15,17,20	19.8	21.4
4	a	8,T11,21	32.0	33.0
	b	8,21	22.3	20.3
	c	8,T11,21,23	34.3	37.7
	d	T6,8,23	28.6	33.0
	e	T11,21	12.9	14.4

[a] Undigested *Alu*I partials and limit *Alu*I digests of these partials were electrophoresed in polyacrylamide gels, as described under Methods. Molecular weights were determined using polyoma *Hpa*II fragment standards. Fragments comprising each partial product of *Hpa*II-1 and 3 were quantified by densitometry in order to determine the composition of bands where comigration could occur.

[b] The composition T1, 12, T19 could not occur.

overlap *Hpa*II-6 from experiments described in the previous section. Since *Hpa*II-6 contains only one *Alu*I site, *Alu*I-2 → 1 must be contiguous, in a clockwise order.

HpaII-7 and HpaII-8. Combined information from these *Hpa*II fragments, together with the identification of an *AluI*/*Hpa*II truncated fragment present in *Hpa*II-4, delineated the clockwise order *Alu*I-11 → 18. Furthermore, *Hpa*II-7 contains *Alu*I-16 and *Alu*I-29 as well as a truncated *Alu*I-9 fragment. The clockwise order of fragments in this region is thus most likely *Alu*I-11 → 18 → (16,29) → 9.

Endo AluI Digestion of AluI Partial Products

To help identify or confirm the order of *Alu*I fragments in those *Hpa*II fragments containing several *Alu*I sites (*Hpa*II-1–4), the individual *Hpa*II fragments were isolated and incompletely digested with endo *Alu*I. Partial products were isolated and digested to completion with endo *Alu*I, and the products were separated by polyacrylamide gel electrophoresis (Table 5).

HpaII-1. The positioning of *Alu*I-T19 adjacent to *Alu*I-(3,12) was obtained from

the partial digestion products (Table 5, 1c). It can be determined that *Alu*I-(3 + 12) is also adjacent to *Alu*I-7, which is in turn adjacent to *Alu*I-T1 (Table 5, 1a). The entire clockwise order from 26 to 54 map units is thus *Alu*I-T1 → 7 → (3,12) → T19. This is consistent with the order determined by double digestion with other endonucleases whose sites were previously mapped (first section).

HpaII-2 Since *Alu*I partials composed solely of *Alu*I (T2 + 13) and *Alu*I (13 + 14) were observed (Table 5, 2e and f), the order of fragments must be *Alu*I-14 → 13 → T2. The three fragments *Alu*I-T9, -28, -5 are grouped together, as are *Alu*I-5, -22, -27 (Table 5, 2b, c), indicating the order *Alu*I-T9 → 28 → 5 → (22,27). The entire clockwise order within *Hpa*II-2 from 98 to 19 map units, then, must be *Alu*I-T9 → 28 → 5 → (22,27) → 14 → 13 → T2. The localization of *Alu*I-2 confirms the position of the *Xba*I site in the polyoma genome, which was independently located by digestion of *Hpa*II fragments with endo *Xba*I (Folk and Bancuk, unpublished data).

HpaII-3. *Alu*I-15 and *Alu*I-17 are adjacent (Table 5, 3b) and are next to *Alu*I-20 + T10 (Table 5, 3h). Since *Alu*I-20 is contiguous with fragment *Alu*I-26 (Table 5, 3c), the order must be *Alu*I-26 → 20 → (15,17) → T10. Only the order *Alu*I-26 → 20 → 17 → 15 → T10 is consistent with the products from one of the partials in Table 5 (3e), and additional fragments therein extend the known order to *Alu*I-(24 or 25,4) → 26 → 20 → 17 → 15 → T10. One partial (Table 5, 3f) contains both *Alu*I-24 + 25, lengthening the determined order to *Alu*I-24 or 25 → (24 or 25,4) → 26 → 20 → 17 → 15 → T10. Another partial in Table 5 (3d) is lacking only *Alu*I fragments T-19 and either *Alu*I-24 or *Alu*I-25, thereby completing the order from 54 to 70 map units: *Alu*I-T19 → 25 → (4,24) → 26 → 20 → 17 → 15 → T10. This is consistent with the positions of *Alu*I-10 and *Alu*I-4 determined by double digestion of polyoma DNA with endo *Alu*I and either endo *Hae*II or endo *Bam*HI.

HpaII-4. *Alu*I fragments T11 + 21 were found to be contiguous (Table 5, 4e) and adjacent to *Alu*I-8 (Table 5, 4a). *Alu*I fragment 23 is adjacent to *Alu*I-8 and *Alu*I-T6 (Table 5, 4c and d), which establishes the clockwise order, from 78 to 92 map units, as *Alu*I-T6 → 23 → 8 → 21 → T11.

Endo AluI Cleavage of [5'-^{32}P]DNA Fragments

To solve the order of several *Alu*I fragments and to confirm the localization of many of the fragments ordered by the previous experiments, we employed a procedure originally described by Smith and Birnstiel (1976). Polyoma DNA was cleaved with either endo *Eco*RI or endo *Bam*HI, and 5'-phosphoryl termini were labeled with ^{32}P. A second endonuclease (*Xba*I or *Hae*II, respectively) was used to generate two fragments of 17 and 83% (*Eco*RI/*Xba*I) or 13 and 87% (*Bam*HI/*Hae*II) genome size. The fragments, each containing only one labeled terminus, were separated and partially digested with endo *Alu*I. The partial products were then fractionated by polyacrylamide and/or agarose gel electrophoresis (Fig. 3), and the molecular weights of the partials were determined (Table 6). The difference in molecular weights of these partials, coupled with the known composition of *Alu*I fragments in isolated *Hpa*II fragments (Table 3) allowed the determination or confirmation of the order for most of the *Alu*I fragments (Table 6).

The series of *Alu*I partials of the 13% fragment (*Hae*II/*Bam*HI-2) together with the known composition of *Alu*I fragments in *Hpa*II-1 and 2 is consistent with the counterclockwise order *Alu*I-4 → (two small fragments) → (3,12) → 7 → 1 → 2 → 13 or 14 (Table 6). We were unable to unequivocally detect endo *Alu*I cleavage between *Alu*I fragments 3 and 12, but instead observed a composite fragment whose size (40×10^4) is the sum of *Alu*I-3 (30×10^4) and *Alu*I-12 (9.7×10^4) (Table 6). The *Alu*I site between *Alu*I-3 and *Alu*I-12 is extremely refractory to cleavage, requiring high concentrations of enzyme for complete digestion (our unpublished observations). It is also possible that cleavage between *Alu*I-3 and 12 may go undetected because the resulting partial would comigrate at the position of *Hae*II/*Bam*HI-2,

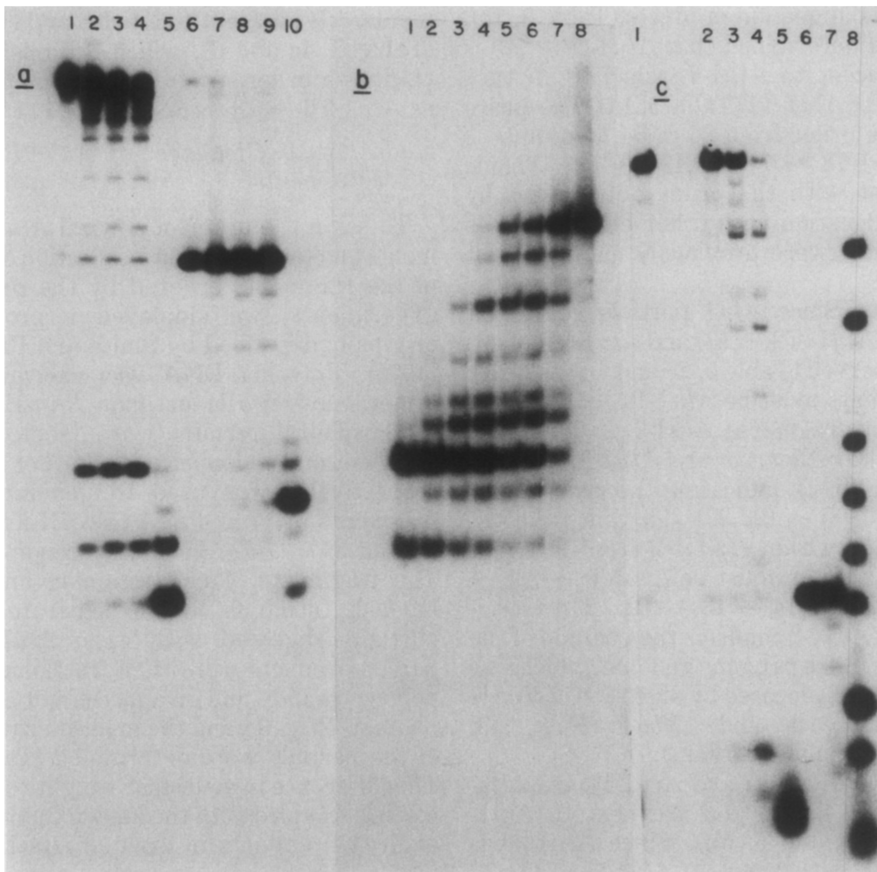

FIG. 3. Endo *Alu*I partial digestion of *Hae*II/*Bam*HI [5′-^{32}P]DNA fragments. (a) For the preparation of *Hae*II/*Bam*HI-digested polyoma DNA, [^{3}H]DNA (41 μg; 3900 cpm/μg) was incubated in 120 μl buffer A with 110 units endo *Bam*HI at 37° for 90 min. The termini were labeled with ^{32}P using polynucleotide kinase exchange, and the DNA was phenol extracted (using distilled phenol freshly equilibrated with 1 *M* Tris), dialyzed, and ethanol precipitated. The DNA (23 μg) was then incubated in 128 μl buffer B with 40 units endo *Hae*II for 4 hr at 37° and then sedimented through a sucrose density gradient (5–20% sucrose in 10 m*M* Tris–HCl pH 7.4, 1 m*M* EDTA) for 5 hr at 45,000 rpm in a SW 50.1 rotor at 20°. Fractions from each *Hae*II/*Bam*HI fragment peak were pooled and the DNAs were ethanol precipitated. DNA (2.5 μg, 5000 cpm) was incubated in 100 μl buffer D with 0.5 μl endo *Alu*I (Biolabs) at 37°, unless otherwise specified. At timed intervals, aliquots (20 μl) were withdrawn and subjected to electrophoresis through polyacrylamide gels. The DNAs are: (1–4) *Hae*II/*Bam*HI-1 incubated for 0, 5, 10, or 15 min or (5) for 30 min with 1 μl endo *Alu*I/20-μl aliquot; (6–9) *Hae*II/*Bam*HI-2 incubated for 0, 5, 10, or 15 min or (10) for 30 min with 1 μl endo *Alu*I/20-μl aliquot. (b) *Hae*II/*Bam*HI-2 (0.7 μg, 2000 cpm) was incubated in 50 μl buffer D with 1–5 μl endo *Alu*I (Biolabs) for 5 or 30 min, followed by polyacrylamide gel electrophoresis. The DNAs are: (1) endo *Alu*I limit digest of *Hae*II/*Bam*HI-2; (2–3) 5 μl endo *Alu*I, 30 or 5 min; (4–5) 2 μl endo *Alu*I, 30 or 5 min; (6–7) 1 μl endo *Alu*I, 30 or 5 min; (8) no enzyme. (c) *Hae*II/*Bam*HI-1 (0.2 μg, 500 cpm) was incubated in 10 μl buffer D with 0–0.3 μl endo *Alu*I (Biolabs) for 5 min or with 3 μl endo *Alu*I for 30 min, followed by electrophoresis through a 1% agarose gel. The DNAs are: (1–5) *Hae*II/*Bam*HI-1 with 0, 0.05, 0.1, 0.3, or 3 μl endo *Alu*I; (6–7) *Hae*II/*Bam*HI-2 (0.2 μg in 10 μl buffer D, 500 cpm); (8) *Hin*dIII polyoma [5′-^{32}P]DNA + *Hpa*II polyoma [5′-^{32}P]DNA. For all gels, autoradiography was performed using a phosphotungstate (Ilford) screen.

TABLE 6

SEQUENCE OF *Alu*I FRAGMENTS DERIVED FROM INCOMPLETE DIGESTION OF *Hae*II/*Bam*HI POLYOMA [5′-^{32}P]DNA OR *EcoR*I/*Xba*I POLYOMA [5′-^{32}P]DNA

Double-digest fragment	Molecular[a] weight × 10^{-4}	Molecular weight[b] difference × 10^{-4}	Deduced *Alu*I fragment[c]	
*Hae*II/*Bam*HI-2				
(13% polyoma DNA)	51	8	Truncated 10	—[d]
	43	9	15	(8.3)
	34	8	17	(6.0)
	26	5	20 or	(4.2 or
	21	4	24/25 or	2.3 or
	17	2	26	1.7)
	15	—	Truncated 4	—[e]
*Hae*II/*Bam*HI-1				
(87% polyoma DNA)	155	11	13 or 14	(9.2 or 8.3)
	144	35	2	(35.8)
	109	33	1	(37.0)
	76	18	7	(21.4)
	58	40	3 + 12	(30.0. + 9.7)
	18	6	19	(5.3)
	12	4	20 or 24/25 or 26	(4.2 or 2.3 or 1.7)
	8	—	Truncated 4	—[e]
*Eco*RI/*Xba*I-2				
(17% polyoma DNA)	65	8	Truncated 2	—[f]
	57	11	13 or 14	(9.2 or 8.3)
	46	9	13 or 14	(9.2 or 8.3)
	37	5	22	(2.9)
	32	2	27 or 28	(1.3 or 1.2)
	30	23	5	(23.1)
	7	1	27 or 28	(1.3 or 1.2)
	6	—	Truncated 9	—[g]
*Eco*RI/*Xba*I-1				
(83% polyoma DNA)	109	9	15	(8.3)
	100	13	10	(13.9)
	87	20	6	(22.0)
	67	5	21 or 23	(3.2 or 2.3)
	62	17	8	(19.1)
	45	5	21 or 23	(3.2 or 2.3)
	40	13	11	(13.3)
	27	6	18	(5.3)
	21	8	16	(7.2)
	13	1	29	(0.9)
	12	—	Truncated 9	—[h]

[a] The molecular weights (averages of at least two gels) were determined using *Hpa*II and *Hin*dIII polyoma DNA fragments as mobility markers.
[b] Difference between two successive fragments in column 1.
[c] Value in parentheses is molecular weight expected of fragment, from Table 1.
[d] A molecular weight of 7.9 × 10^4 or 6.0 × 10^4 is expected from double-digestion experiments (Table 2).
[e] Molecular weights of 11.0 × 10^4 and 14.4 × 10^4 expected (Table 2).
[f] Molecular weight of 9.2 × 10^4 expected (Table 2).
[g] Molecular weight of 5.7 × 10^4 expected (Table 2).
[h] Molecular weight of 12.1 × 10^4 expected (Table 2).

a small amount of which contaminates *Hae*II/BamHI-1. The experiments described in the previous sections make it clear, however, that *Alu*I-3 and 12 are contiguous.

The localization of each *Alu*I/*Bam*HI truncated fragment in either *Hae*II/*Bam*HI-2 or the 87% fragment (*Hae*II/*Bam*HI-1) of the polyoma genome (Table 6) established the exact position of *Alu*I-4 with respect to the *Bam*HI site in the polyoma genome. The identification and orientation of the two small fragments between *Alu*I-4 and *Alu*I-3 (Fig. 2) were determined by several criteria. One of these two fragments must contain a *Hpa*II site, and must therefore be *Alu*I-19, since it is the only fragment, among the eight known to contain *Hpa*II sites, which is small enough to fit between *Alu*I-3 and *Alu*I-4. The known map location of *Alu*I-3, and the known composition of *Alu*I fragments in *Hpa*II-1 are only consistent with the clockwise order *Alu*I-3 → 19. The observed *Hpa*II/*Alu*I-19 truncated products agree with this sequence (Table 3).

To identify the remaining small fragment between *Alu*I-4 and *Alu*I-19, uniformly labeled polyoma [^{32}P]DNA was digested with endonucleases *Hae*II and *Bam*HI, and homogeneous preparations of *Hae*II/*Bam*HI-1 and -2 were obtained by polyacrylamide gel electrophoresis. The *Hae*II/*Bam*HI-2 fragment was digested with endo *Alu*I and the products were resolved by polyacrylamide gel electrophoresis. Comparison of these products with those obtained by endo *Alu*I digestion of *Hpa*II-3 revealed that both DNAs contained *Alu*I fragments 15, 17, 20 and 26 and differed in the truncated *Alu*I fragments at the ends of each DNA. In addition, quantitation of the *Alu*I digestion products from *Hpa*II-3 or *Hae*II/*Bam*HI-2 established that *Hpa*II-3 contains both *Alu*I-24 and *Alu*I-25, while *Hae*II/*Bam*HI-2 contains only one fragment of this size. Therefore, the fragment separating *Alu*I-4 from *Alu*I-19 is *Alu*I-24 or 25, and is arbitrarily given the designation *Alu*I-25. Studies of *Alu*I partials indicated the clockwise order *Alu*I-19 → 25 → (4,24) → 26 (Table 5). As *Alu*I-4 clearly separates *Alu*I-24 from *Alu*I-25, the precise clockwise order must be *Alu*I-19 → 25 → 4 → 24 → 26.

The composition of pure *Hae*II/*Bam*HI-2, as previously mentioned, consists of five *Alu*I fragments (*Alu*I-15, 17, 20, 24 and 26) between *Alu*I-4 and *Alu*I-10. The clockwise order *Alu*I-4 → (20,24,26) → 17 → 15 → 10 was determined from the molecular weights of the partials of endo *Alu*I digestion of 5′-^{32}P-labeled *Hae*II/*Bam*HI-2 (Table 6). This order was additionally checked by digesting uniformly ^{32}P-labeled *Hae*II/*Bam*HI-2 with endonucleases *Hae*III and *Alu*I. As expected, *Hae*III sites were found in *Alu*I fragments T-10, 15, 17 but not in *Alu*I-24, 26 or T4.

To analyze the order of *Alu*I fragments in the remainder of the polyoma genome it was necessary to use *Eco*RI/*Xba*I fragments rather than the *Hae*II/*Bam*HI fragments, since the analysis of partial products labeled at the *Bam*HI site became increasingly inaccurate at *Alu*I cleavage sites distant from the *Bam*HI site. Molecular weight determination of the *Alu*I partials within the 17% fragment (*Eco*RI/*Xba*I-2) (Table 6), together with the known composition of *Alu*I fragments in *Hpa*II-2 (Table 3) gave the following clockwise order: *Alu*I-9 → (27 or 28) → 5 → (27 or 28) → 22 → (14,13) → 2 (Table 6). This is consistent with the locations of *Alu*I-9, 13, and 2 determined by double-digestion studies in a previous section. Furthermore, these results agree with the order determined by endo *Alu*I digestion of *Alu*I partials of *Hpa*II-2: *Alu*I-9 → 28 → 5 → (22,27) → 14 → 13 → 2, and in addition resolves the one ambiguity in this sequence. Since *Alu*I-5 is between *Alu*I-27 and *Alu*I-28 (Table 6), the correct clockwise order is identified as *Alu*I-9 → 28 → 5 → 27 → 22 → 14 → 13 → 2.

Correlation of *Alu*I partial digestion products of the 83% fragment (*Eco*RI/*Xba*I-1) with the previously determined compositions of *Hpa*II fragments 4, 5, 7, and 8 (Table 3) established the counterclockwise order *Alu*I-9 → 29 → 16 → 18 → 11 → (21 or 23) → 8 → (21 or 23) → 6 → 10 → 15. This order is consistent with the known location of *Hpa*II, *Hae*II or *Eco*RI sites in *Alu*I-9, 18, 11, 6, or 10, and is also in agreement with the clockwise order *Alu*I-6 → 23 → 8 → 21 → 11 determined by

endo *Alu*I digestion of *Alu*I partials from *Hpa*II-4.

DISCUSSION

In almost all cases, the locations of the 29 *Alu*I fragments within the polyoma genome have been specified by at least two independent techniques. The order of *Alu*I fragments between 0–25 map units was almost entirely derived from endo *Alu*I digestion of *Alu*I partials isolated from *Hpa*II-2 DNA, as well as from an analysis of the partial *Alu*I digestion products of *Eco*RI/*Xba*I-2[5′-^{32}P]DNA, spanning the genome between the *Eco*RI site (at 0 map units) and the *Xba*I site (at 17 map units). Double digestions with endo *Alu*I and endonucleases *Eco*RI, *Xba*I, *Hha*I, or *Hin*dIII provided independent checks on the positions of several fragments.

Since all of the *Alu*I fragments between 25 and 53 map units contain or border upon previously mapped endonuclease sites, ordering fragments in that region was relatively straightforward. Corroborating evidence was provided by endo *Alu*I digestion of *Alu*I partials isolated from *Hpa*II-1, and by partial endo *Alu*I digestion of *Hae*II/*Bam*HI[5′-^{32}P]DNA. This latter approach was also useful in ordering several of the *Alu*I fragments between 53 and 74 map units. All but two of the *Alu*I fragments in this region were also ordered by endo *Alu*I digestion of *Alu*I partials of *Hpa*II-3. Together, these two approaches provided the entire sequence of *Alu*I fragments between 53 and 74 map units. Digestion of *Alu*I fragments from this region with endonucleases *Hae*II, *Bam*HI, or *Hae*III confirmed several map positions.

Fragment *Hpa*II-5 contains only one *Alu*I site, approximately in its center (Table 3). The location of this site is in agreement with the nucleotide sequence of this region of the polyoma genome (Soeda *et al.*, 1978; T. Friedmann personal communication). The remaining *Alu*I fragments in the polyoma genome (between 74 and 100 map units) could be deduced almost entirely from studies of endo *Alu*I digestion of isolated *Hpa*II fragments (*Hpa*II-4,5,7, and 8). Confirmatory evidence for the order of *Alu*I fragments in this region was provided by endo *Alu*I partial digestion of terminally labeled [5′-^{32}P]DNA spanning the region from 70–100 map units. In addition, the sequence of fragments from 78–92 map units was independently determined by endo *Alu*I digestion of *Alu*I partials isolated from *Hpa*II-4. Since the completion of this work, the nucleotide sequence of the region between 70 and 02 map units has been derived. It confirms the locations of the *Alu*I fragments in this part of the genome (T. Friedmann personal communication).

There are four *Bum*I sites in the polyoma genome, at map positions 8.8, 67.4, 70.2, and 92.4 (Griffin, 1977). The sequence cleaved by endo *Bum*I has recently been determined to be 5′ (p)CAGCTG (R. J. Roberts and M. Mathews, personal communication), which is a subset of the *Alu*I cleavage site (Jay and Wu, 1976). The corresponding *Alu*I sites, then, are most likely those between *Alu*I-5 and 27 or 27 and 22, *Alu*I-15 and 17, *Alu*I-10 and 15, and *Alu*I-11 and 18.

The extensive cleavage of polyoma DNA by endo *Alu*I as well as the distribution of sites within the genome makes this endonuclease valuable for further analysis of polyoma biology. In particular, the early region of the genome is cleaved into 17 fragments, which should be valuable for mapping early gene functions. In recent studies in this laboratory, endo *Alu*I has been employed in the preliminary characterization of mutants of polyoma virus altered at the *Hin*dIII, *Hin*dII, or *Hae*II sites in the polyoma genome (Bendig and Folk, unpublished data).

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Note added in proof. Dr. B. Griffin and Dr. T. Friedmann have established (and we have confirmed) that the polyoma genome contains two endo *Xba*I sites separated by 30–50 nucleotides.

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