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Physical Map of the Bacteriophage T5 Genome Based on the Cleavage Products of the Restriction Endonucleases *Sal*I, *Sma*I, *Bam*I, and *Hpa*I†

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A physical map of the bacteriophage T5 genome was constructed by ordering the fragments produced by cleavage of T5 DNA with the restriction endonucleases *Sal*I (4 fragments), *Sma*I (4 fragments), *Bam*I (5 fragments), and *Hpa*I (28 fragments). The following techniques were used to order the fragments. (i) Digestion of DNA from T5 heat-stable deletion mutants was used to identify fragments located in the deletable region. (ii) Fragments near the ends of the T5 DNA molecule were located by treating T5 DNA with λ exonuclease before restriction endonuclease cleavage. (iii) Fragments spanning other restriction endonuclease cleavage sites were identified by combined digestion of T5 DNA with two restriction endonucleases. (iv) The general location of some fragments was determined by isolating individual restriction fragments from agarose gels and redigesting the isolated fragments with a second restriction enzyme. (v) Treatment of restriction digests with λ exonuclease before digestion with a second restriction enzyme was used to identify fragments near, but not spanning, restriction cleavage sites. (vi) Exonuclease III treatment of T5 DNA before restriction endonuclease cleavage was used to locate fragments spanning or near the natural T5 single-chain interruptions. (vii) Analysis of the products of incomplete restriction endonuclease cleavage was used to identify adjacent fragments.

The genome of bacteriophage T5 is a nonpermuted linear DNA duplex (18) with several unique features. It is a relatively large molecule, having a molecular weight of approximately 77 million (7). One strand of the molecule contains a number of interruptions, which occur with varied frequencies at genetically fixed positions (1, 4, 5, 12, 16). The T5 terminal repetition is exceptionally large, consisting of 8.3% of the genome or about 10,000 base pairs (13). Upon infection, only about 8% of the genome is injected; the remainder of the DNA is transferred only after this initial segment is transcribed and translated (8, 9).

To analyze the characteristics and functions of various portions of the T5 genome, we have constructed a fairly detailed physical map by ordering the 28 fragments produced by cleavage of T5 DNA with a restriction endonuclease from *Haemophilus parainfluenzae*—*Hpa*I. Physical maps of the T5 genome have previously been constructed using the cleavage products of *Eco*RI, *Sal*I, *Sma*I, and *Hin*dIII (11, 19). None of these, however, provides as fine resolution as the *Hpa*I map. To facilitate mapping of the *Hpa*I fragments, we have also ordered the T5 fragments produced by the restriction endonucleases *Sal*I, *Sma*I, and *Bam*I.

MATERIALS AND METHODS

Bacteriophage. Wild-type T5 and the heat-stable deletion mutants T5*st*(0) and T5*st*(102) (13, 15) were grown on *Escherichia coli* B23.

***Eco*RI endonuclease.** Purification and assay conditions for *Eco*RI have been described (11).

***Sal*I endonuclease.** *Streptomyces albus* G (from G. Hayward) was grown at 30°C with shaking in a medium containing, in 1 liter: 0.33 g of yeast extract (Difco), 0.33 g of beef extract (Difco), 0.67 g of tryptone (Difco), trace $FeSO_4$, and 3.33 g of glucose. After 2 days of growth, the mycelia were harvested by centrifugation and suspended in 0.01 M Tris, pH 7.9, containing 0.01 M 2-mercaptoethanol (approximately 10 ml per 15 g [wet weight] of mycelia). The cells were disrupted by sonication. Cell debris was removed from the extract by low-speed (8,000 rpm for 15 min in a Sorvall SS34 rotor) and high-speed centrifugation (33,000 rpm for 90 min in a Beckman SW50.1 rotor). The high-speed supernatant was made 1.0 M in NaCl and applied to a 200-ml column

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of Bio-Gel A 0.5m 200 to 400 mesh (Bio-Rad) equilibrated with 0.01 M Tris, pH 7.9, containing 1.0 M NaCl and 0.01 M 2-mercaptoethanol. The restriction activity was recovered in a broad peak from approximately 0.57 to 0.75 column volumes. This partially purified activity was either used directly or dialyzed into 0.02 M potassium phosphate buffer containing 0.1 M NaCl, 0.01 M 2-mercaptoethanol, and 20% (vol/vol) glycerol. Twenty-five microliters of *Sal*I was required to digest 1 μg of T5 DNA. The enzyme preparations were stored at 0°C. One preparation that was stored for 18 months retained about 40% of its original activity. At this stage of purification, *Sal*I is contaminated with a single-strand-specific endonuclease. Attempts to remove this contaminant were unsuccessful; however, its presence did not interfere with the construction of the cleavage maps. Reaction mixtures for *Sal*I contained: 10 mM Tris, pH 7.9, or 20 mM sodium phosphate buffer, pH 6.8; 10 mM $MgCl_2$; 0.1 M NaCl; and 3 μg of T5 DNA per ml. Reactions were incubated at 37°C for 60 min.

***Sma*I endonuclease.** *Sma*I, a restriction endonuclease from *Serratia marcescens*, was a gift from Jose Ramirez. *Sma*I reaction mixtures contained: 30 mM Tris, pH 9.0; 3 mM $MgCl_2$; 15 mM KCl; and 3 μg of T5 DNA per ml. Incubation was for 30 min at 30°C.

***Bam*I endonuclease.** *Bam*I was purified from *Bacillus amyloliquifaciens* H strain RUB 500 (from D. Shortle) according to the procedure of Wilson and Young (20) with the following modifications. (i) The cells were disrupted by sonication instead of grinding with alumina. (ii) Streptomycin sulfate removal of DNA, ammonium sulfate precipitation, and Sephadex G-25 chromatography were omitted; instead, the crude lysate was dialyzed against buffer I (0.01 M sodium phosphate buffer, pH 7.4) and applied directly to the DEAE-cellulose column. Reaction mixtures for *Bam*I contained: 10 mM Tris, pH 7.4; 6 mM $MgCl_2$; 6 mM 2-mercaptoethanol; and 3 to 10 μg of T5 DNA per ml. Reactions were incubated for 1 h at 37°C.

***Hpa*I endonuclease.** *H. parainfluenzae* (from M. Mann) was grown to the late-logarithmic phase of growth in brain heart infusion broth (Difco) supplemented with 2 μg of nicotinamide adenine dinucleotide per ml. Cells were harvested by centrifugation and stored frozen overnight. Sonication and purification through Bio-Gel A 0.5 m (Bio-Rad) were carried out according to Sharp et al. (17). *Hpa*I was further purified by chromatography on DEAE-cellulose. Pooled Bio-Gel fractions were dialyzed into 0.01 M Tris (pH 7.4), 0.01 M 2-mercaptoethanol, 0.1 mM EDTA, and 10% (vol/vol) glycerol. The dialyzed material (10 ml) was loaded onto a 20-ml column of DEAE-cellulose (Whatman) equilibrated with the dialysis buffer. The column was washed with dialysis buffer, and then developed with a 100-ml linear gradient of 0 to 1.0 M KCl in dialysis buffer. The *Hpa*I activity was eluted between 0.05 and 0.15 M KCl. Three microliters of purified *Hpa*I was sufficient to digest 1 μg of T5 DNA. The fractions were made 7 mM in $MgCl_2$ and stored at −60°C. No loss of activity has been observed over a period of 7 months. The DEAE-cellulose chromatography removes a single-strand-specific endonuclease as well as exonuclease and *Hpa*II. The purified *Hpa*I is also free of detectable activities that introduce single-chain interruptions into duplex DNA. The reaction mixture for *Hpa*I assays contains: 20 mM Tris (pH 7.4), 7 mM $MgCl_2$, 50 mM KCl, 3 mM 2-mercaptoethanol, 0.1 mM EDTA, and 10 μg of T5 DNA per ml. Incubations were carried out at 37°C for 60 min.

λ Exonuclease. λ Exonuclease was isolated from *E. coli* 1100 (λ_{T11}) according to the procedure of Radding (10). Reaction mixtures contained either 67 mM glycine-KOH, pH 9.3, or 20 mM Tris, pH 7.8, and 3 mM $MgCl_2$. Reactions were incubated at various temperatures between 25 and 37°C, depending on the rate desired. Reactions were terminated by heating 5 min at 65°C. Extent of hydrolysis was determined by measuring the production of acid-soluble nucleotides according to the method of Kelly and Smith (6).

Exonuclease III. Exonuclease III from *E. coli* 1100 was purified according to Scheible et al. (16). Reaction mixtures for exonuclease III contained 10 mM Tris, pH 8.0, and 10 mM $MgCl_2$. Reactions were incubated at 37°C.

Treatment of DNA with more than one enzyme. When a restriction endonuclease digest was to be treated with another enzyme, it was phenol extracted twice and dialyzed extensively against 10 mM Tris, pH 8.0, containing 0.1 mM EDTA. When DNA was pretreated with λ exonuclease or exonuclease III, the exonuclease reaction was stopped by heating for 5 min at 65°C. The reaction was then diluted, and other reagents were added as necessary for the subsequent restriction digest.

Electrophoresis. DNA was usually analyzed in 10- or 15-cm agarose tube gels according to the procedure of Rhoades (11). In some experiments, Tris-phosphate buffer (5) was used. Gels contained from 0.4 to 2.0% (wt/vol) agarose (Sigma). During purification of restriction enzymes, assays of column fractions were analyzed by electrophoresis on horizontal agarose gel slabs as described by Rogers and Rhoades (14). Bands were visualized by staining in 0.5 μg of ethidium bromide per ml and photographing with Polaroid P/N 105 film (17).

Preparative gels were not stained; instead, they were chilled to 0°C and cut into 1-mm slices. The bands were then located by measuring Cerenkov radiation. DNA was recovered either by electrophoresis (2) or by dissolving the gel slices with KI (3). In the latter case, DNA was separated from the dissolved agarose by chromatography on hydroxyapatite. A 0.5-ml column of hydroxyapatite was equilibrated with 40% (wt/wt) KI in 10 mM Tris (pH 7.8)–0.01 mM EDTA. The sample was loaded, and the column was washed first with 40% KI and then with 0.01 M sodium phosphate buffer, pH 6.8. The DNA was eluted with 0.4 M sodium phosphate buffer, pH 6.8.

Other methods. The procedures for growing phage and extracting DNA and the conditions for electron microscopy have been described (11, 13).

RESULTS

The *Sal*I cleavage map of the T5 genome. Digestion of T5 DNA with *Sal*I—a restriction

enzyme from *Streptomyces albus* – produces four fragments (Fig. 1). The molecular weights of these fragments were estimated from electrophoretic mobility relative to fragments of T5 DNA produced by *Eco*RI (11). The sum of these molecular weights is approximately equal to the molecular weight of the T5 genome (Table 1), indicating that there is only one copy of each fragment per T5 genome. Table 1 also summarizes the experiments performed to determine the *Sal*I cleavage map of T5. The terminal fragments were identified by digestion of T5 DNA with the λ-induced 5′-exonuclease before cleavage by *Sal*I. Digestion to 10% affected only *Sal*I-3 and *Sal*I-4; further digestion to 23% affected all four fragments. These results show that *Sal*I-3 and *Sal*I-4 are located at the ends of the molecule and that *Sal*I-1 and *Sal*I-2 are located in the interior. The order of the two interior fragments was determined by digesting DNA isolated from the heat-stable deletion mutant T5*st*(0). In this mutant, a deletion of approximately 5.7 million extends from 26 to 34% of the wild-type length of the molecule (15, 16). Comparison of the cleavage products of T5*st*(0) DNA with those of T5(+) DNA shows that only *Sal*I-2 is altered by the deletion. *Sal*I-2 must therefore lie to the left of *Sal*I-1 within the interior of the molecule. In order to assign *Sal*I-3 and *Sal*I-4 to the right and left ends of the molecule, the two terminal T5 *Eco*RI fragments, *Eco*RI-1 and *Eco*RI-2, were isolated from agarose gels by electrophoresis and subsequently digested with *Sal*I. *Eco*RI-1 yielded *Sal*I-3 and a new fragment; *Eco*RI-2 yielded *Sal*I-4 and a new fragment. *Sal*I-3 must therefore lie at the right end of the T5 molecule, and *Sal*I-4 must be at the left. The order of the fragments produced by cleavage of T5 DNA with *Sal*I is from left to right: 4-2-1-3, as shown in Fig. 2.

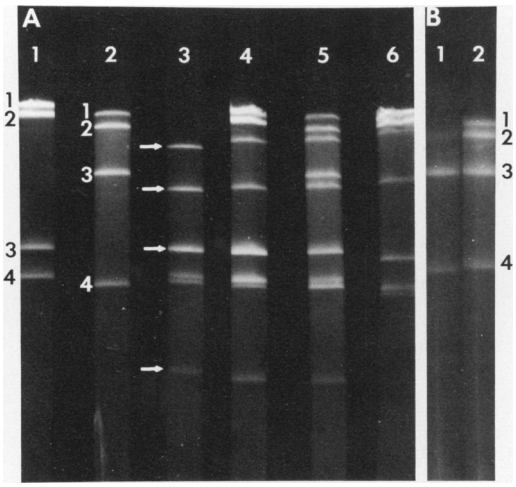


FIG. 1. *Effects of prior treatment with SalI or λ exonuclease on the SmaI pattern of T5 DNA. (A) Electrophoresis on 0.5% agarose gels of T5(+) DNA digested with SalI or SmaI, or both: gel 1, SalI digest; gel 2, SmaI digest; gel 3, SalI-SmaI combined digest (the arrows point to bands not present in the single digests; one new fragment comigrates with SalI-3 producing a bright double band); gel 4, SalI-SmaI combined digest mixed with SalI digest; gel 5, SalI-SmaI combined digest mixed with SmaI digest; gel 6, SalI digest mixed with SmaI digest. (B) Electrophoresis on 0.5% agarose gels of T5(+) DNA digested with λ exonuclease before SmaI digestion: gel 1, 4.5% λ exonuclease digestion (9.2 and 14.1% produced identical patterns); gel 2, no λ exonuclease digestion. In this and subsequent figures the origin is at the top.*

TABLE 1. *Summary of evidence for SalI cleavage map of T5 DNA*

*Sal*I fragments present in[a]:					
T5(+)	T5(+) 10.5% λexo[b]	T5(+) 23% λexo[b]	T5*st*(0)	T5(+) *Eco*RI-1	T5(+) *Eco*RI-2
(76.5)			(71.0)	(28.3)	(19.1)
1 (30.7)	1		1		
2 (26.3)	2		2′(21.7)		
				(19.1)	(10.8)
3 (9.5)			3	3	
4 (8.4)			4		4
Σ 74.9			Σ 70.3	Σ 28.6	Σ 19.2

[a] Numbers in parentheses indicate molecular weights in millions. Electron microscopic measurements were used to determine the molecular weights of T5(+) DNA (16) and T5(+) *Eco*RI fragments (11). The size of the *st*(0) deletion was determined from the sizes of T5*st*(0) *Eco*RI (16) and *Hpa*I fragments. The molecular weights of the *Sal*I fragments were determined from electrophoretic mobility relative to T5 *Eco*RI fragments. Fragments not occurring in *Sal*I digests of T5(+) DNA are listed as molecular weights without fragment numbers.

[b] ^{32}P-labeled T5(+) DNA was digested to varying extents with λ exonuclease (λexo) before cleavage with *Sal*I. The extent of λ exonuclease digestion is indicated as the percentage of radioactivity made acid soluble.

TABLE 2. *Summary of evidence for SmaI cleavage map of T5 DNA*

*Sma*I fragments present in[a]:			
T5(+) (76.5)	T5(+) 4.5% λexo[b]	T5*st*(0) (71.0)	T5(+)·*Sal*I (76.5)
1 (28.4)		1′(23.1)	
2 (23.1)		2	
			(20.1)
3 (15.2)	3	3	
			(13.9)
			*Sal*I-3(9.5)
			(9.5)
			*Sal*I-4(8.4)
4 (8.0)	4	4	4
			(5.8)
Σ 74.7		Σ 69.4	Σ 75.1

[a] Numbers in parentheses indicate molecular weights in millions. The molecular weights of *Sma*I fragments were determined from electrophoretic mobility relative to T5 *Eco*RI and *Sal*I fragments. Other molecular weights were determined as indicated in Table 1. Fragments not appearing in *Sma*I digests of T5(+) DNA are listed as molecular weights without fragment numbers.

[b] ^{32}P-labeled T5(+) DNA was digested with λ exonuclease (λexo) before cleavage with *Sma*I. The extent of λ exonuclease digestion is indicated as the percentage of radioactivity made acid soluble. Identical results were obtained with 9.2 and 14.1% λ exonuclease digestion.

***Sma*I cleavage map of the T5 genome.** Cleavage of T5 DNA with *Sma*I, a restriction endonuclease from *S. marcescens*, also produces four fragments (Fig. 1). The molecular weights of the fragments were estimated from electrophoretic mobility relative to *Eco*RI and *Sal*I fragments of T5 DNA (Table 2). As in the case of *Sal*I, the sum of the molecular weights of the four *Sma*I fragments is equal to the molecular weight of the T5 genome. The evidence used to construct the *Sma*I map is summarized in Table 2. Digestion of T5 DNA with λ exonuclease before cleavage by *Sma*I showed that the largest *Sma*I fragments (*Sma*I-1 and *Sma*I-2) are located at the ends of the molecule (Fig. 1) and that *Sma*I-3 and *Sma*I-4 are interior. The size of *Sma*I-1 is reduced in T5*st*(0) DNA; no other fragments are affected. *Sma*I-1 must therefore be located at the left end of the T5 molecule, and *Sma*I-2 must lie at the right end. To determine the order of *Sma*I-3 and *Sma*I-4, T5(+) DNA was digested first with *Sma*I and then with *Sal*I, and the products were analyzed by electrophoresis on agarose gels. In the combined *Sma*I-*Sal*I digest, *Sma*I-3 is cut by *Sal*I, whereas *Sma*I-4 remains intact (Fig. 1). Thus, *Sma*I-3 covers the *Sal*I site at 46% from the left end of the molecule and must lie to the left of *Sma*I-4. The order of the *Sma*I fragments of T5 from the left end of the molecule is 1-3-4-2, as shown in Fig. 2. The total pattern of the combined *Sal*I-*Sma*I digest is accounted for as follows. *Sma*I-1 is cut by *Sal*I at the 11% cleavage site to produce *Sal*I-4 and a new fragment of molecular weight 20.1 million (the largest fragment in the combined digest). *Sma*I-2 is cut by *Sal*I at the 87.2% site to produce *Sal*I-3 and a new fragment of molecular weight 13.9 million (the second largest fragment in the combined digest). *Sma*I-3 is cleaved, as mentioned above, to produce new fragments of molecular weights 9.5 million (comigrates with *Sal*I-3) and approximately 5.8 million (the smallest fragment in the combined digest). *Sma*I-4 is not cleaved by *Sal*I.

***Bam*I cleavage map of the T5 genome.** Digestion of T5(+) with the restriction endonuclease *Bam*I (from *B. amyloliquifaciens* H) produces five fragments (Fig. 3, Table 3). The arrangement of *Bam*I fragments on the T5 genome (Fig. 2) was resolved by λ exonuclease digestion of the intact molecule followed by *Bam*I digestion, *Bam*I digestion of DNA from heat-stable deletion mutants, and partial *Bam*I digestion of T5(+) DNA. *Bam*I-1 clearly spans the region deleted in T5*st*(0), since it is the only fragment decreased in size by this deletion (Table 3, Fig. 3). Digestion of intact T5(+) DNA with λ exonuclease followed by *Bam*I digestion resulted in the loss of *Bam*I-2 and *Bam*I-3, indicating their terminal positions. *Bam*I-3 was assigned to the left end based upon analysis of DNA from the heat-stable mutant T5*st*(102), in which DNA between 21.6 and 32.0% of the length of the T5(+) genome is deleted (15). When T5*st*(102) DNA was digested with *Bam*I, *Bam*I-1 and *Bam*I-3 were lost, and a new fragment larger than *Bam*I-1 was created; *Bam*I-2, *Bam*I-4, and *Bam*I-5 were not altered (Table 3, Fig. 3). This result requires *Bam*I-3 to be on the left end, next to *Bam*I-1, thereby allowing the *st*(102) deletion to remove the site between them. *Bam*I-2 is then necessarily on the right end of the molecule. *Bam*I-4 and *Bam*I-5 were ordered from the results of partial *Bam*I digests of T5(+) DNA produced by decreasing the incubation time and enzyme dose. Electrophoresis of the partial digests revealed the presence of fragments equal in size to the sum of *Bam*I-4 + *Bam*I-5 + *Bam*I-2 and *Bam*I-5 + *Bam*I-2, but not *Bam*I-4 + *Bam*I-2, indicating that *Bam*I-5 must be next to *Bam*I-2 (Fig. 3, Table 3). Because molecular weights of DNA molecules greater than 30 million cannot be accurately determined on agarose gels, the molecular weight of *Bam*I-1 was estimated from the results of combined digestion of T5(+) DNA with

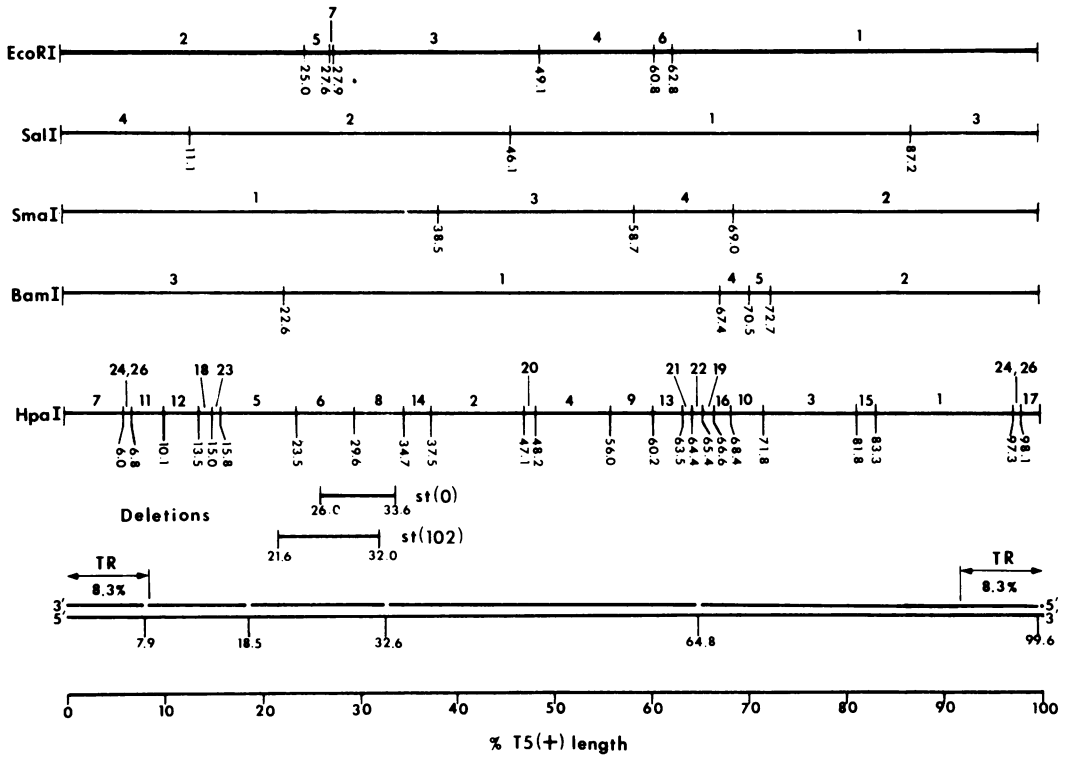
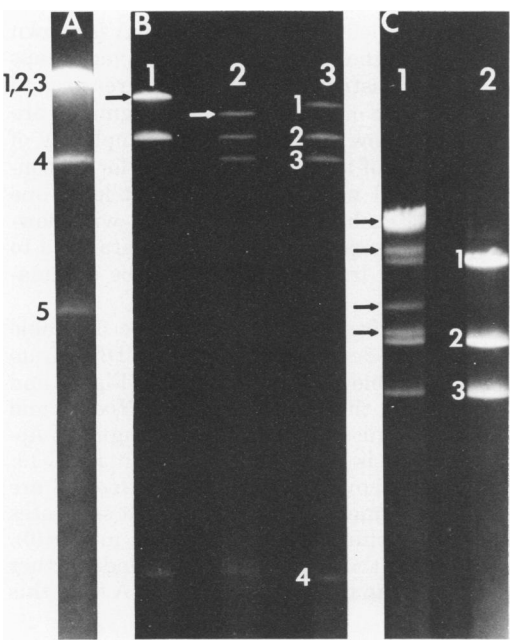


FIG. 2. *Restriction cleavage maps of T5(+) DNA. The numbers above a line are fragment numbers; the numbers below a line are the coordinates of sites expressed as a percentage of the wild-type length from the left end of the molecule. The EcoRI map is from Rhoades (11). The other restriction cleavage maps are discussed in Results. In the case of HpaI, fragments occurring in multiple-fragment bands were arbitrarily numbered from the left end of the molecule. The limits of the st deletions represent minor revisions of the values given in Scheible and Rhoades (15). The locations of the single-chain interruptions are from Scheible et al. (16) and Rhoades (12).*



*Bam*I and *Eco*RI. In the combined digest, *Bam*I-1, *Eco*RI-1, and *Eco*RI-2 are lost, the other *Bam*I and *Eco*RI fragments remain intact, and two new fragments appear (Table 3). Because only *Bam*I-1 is cleaved by *Eco*RI, it must span all of the *Eco*RI cleavage sites and thus comprises *Eco*RI fragments 3, 4, 5, 6, and 7 as well as the two new fragments. *Bam*I-1 was therefore assigned a molecular weight of 34.1 million, which is the sum of the molecular weights of these fragments.

***Hpa*I cleavage map of the T5 genome.** Digestion of T5(+) DNA with the restriction

FIG. 3. *Effects of deletions and partial digestion on the BamI pattern of T5(+) DNA. (A) Electrophoresis in a 1.0% agarose gel of T5(+) DNA digested with BamI. (B) Electrophoresis in 0.5% agarose gels: gel 1, BamI digest of T5st(102) DNA; gel 2, BamI digest of T5st(0) DNA; gel 3, BamI digest of T5(+) DNA. (C) Electrophoresis in 0.5% agarose gels: gel 1, T5(+) DNA partially digested with BamI; gel 2, T5(+) DNA completely digested with BamI. The arrows indicate bands not present in complete BamI digests of T5(+) DNA.*

TABLE 3. *Summary of evidence for BamI cleavage map of T5 DNA*

*Bam*I fragments present in[a]:					
T5(+) (76.5)	T5(+) 10.1% λexo[b]	T5*st*(0) (71.0)	T5*st*(102) (68.8)	T5(+) partial digest[c]	T5(+)·*Eco*RI (76.5)
				(50–76.5)	
			(43)	(37)	
1 (34.1)	1	1′(28.2)			
				(25.2)	
				(22.0)	
2 (21.0)		2	2	2	2
3 (17.3)		3		3	3
					*Eco*RI-3 (16.2)
					*Eco*RI-4 (8.9)
					(3.55)
4 (3.14)	4	4	4		4
					*Eco*RI-5 (2.00)
					(1.83)
					*Eco*RI-6 (1.55)
5 (0.92)	5	5	5		5
					*Eco*RI-7 (0.25)
Σ 76.36		Σ 70.56	Σ 68.06		Σ 76.64

[a] Numbers in parentheses indicate molecular weights in millions. The molecular weight of *Bam*I-1 was calculated from the *Eco*RI-*Bam*I combined digest as discussed in Results. The molecular weights of *Bam*I-2 and *Bam*I-3 were determined from electrophoretic mobility relative to T5*st*(0) *Eco*RI fragments. The molecular weights of *Bam*I-4 and *Bam*I-5 were determined from electrophoretic mobility relative to T5*st*(102) *Hpa*I fragments. Other molecular weights were determined as indicated in Table 1. Fragments not appearing in *Bam*I digests of T5(+) DNA are listed as molecular weights without fragment numbers.

[b] ^{32}P-labeled T5(+) DNA was digested with λ exonuclease (λexo) before cleavage with *Bam*I. The extent of λ exonuclease digestion is indicated as the percentage of radioactivity made acid soluble.

[c] T5(+) DNA was subjected to limited digestion with *Bam*I by decreasing the enzyme dose and incubation time. The presence of *Bam*I-4 and *Bam*I-5 was not determined in this experiment.

endonuclease *Hpa*I (from *H. parainfluenzae*) produces 22 bands, which can be resolved on agarose gels (Fig. 4). The molecular weights of the fragments cover such a wide range – approximately 50-fold – that two gels of different agarose concentrations are required to resolve the bands adequately. Measurement of the amount of radioactivity present in each band when ^{32}P-labeled DNA was cleaved with *Hpa*I showed that several of the bands contained more than one fragment. Bands 2, 18, 20, and 22 each contain two fragments, and band 10 contains three. There are thus a total of 28 fragments detected as products of *Hpa*I cleavage of T5(+) DNA. Fragments smaller than approximately 0.1 million would not have been detected. An *Hpa*I digest of T5(+) DNA was examined electron microscopically, and the lengths of the fragments were measured. Those fragments that were clearly separated in size from others were assigned molecular weights based on these measurements. The other fragments were assigned molecular weights based on their electrophoretic mobility relative to the electron microscopically measured fragments (Table 4). The sum of the molecular weights is again in good agreement with the molecular weight of the whole T5 molecule.

The *Hpa*I cleavage map of T5 DNA is shown in Fig. 2 together with the other cleavage maps used in its construction. The procedures used to determine the order of the *Hpa*I fragments are described below. Because of the complexity of the map, all of the results required for its construction will not be presented. At least one example of each kind of experiment will, however, be discussed, and all of the data used to establish the fragment order will be summarized.

The *Hpa*I fragments covering the deletable region were identified by digesting DNA from two heat-stable deletion mutants (Fig. 4 and Table 4). In the case of T5*st*(0), *Hpa*I-6 and *Hpa*I-8 are missing, and a new fragment appears which is slightly larger than *Hpa*I-19. This result shows that *Hpa*I-6 and *Hpa*I-8 are adjacent fragments, and the site that separates them lies within the region deleted in T5*st*(0). In T5*st*(102), the deleted region extends farther to the left than in T5*st*(0). When DNA from this

mutant is digested with *Hpa*I, *Hpa*I-5 is eliminated as well as *Hpa*I-6 and *Hpa*I-8, and a new fragment is seen between *Hpa*I-3 and *Hpa*I-4. This result indicates that the order of *Hpa*I fragments in the deletable region is 5-6-8 or 5-8-6.

*Hpa*I fragments were assigned to regions of the T5 DNA molecule by locating them at *Eco*RI cleavage sites or within *Eco*RI fragments. When T5(+) DNA is digested with both *Hpa*I and *Eco*RI (Fig. 4), three *Hpa*I fragments are cleaved by *Eco*RI. *Hpa*I-4 is cleaved by *Eco*RI to produce a new fragment that is only slightly smaller than *Hpa*I-4. *Hpa*I-6 and *Hpa*I-13 are also cleaved by *Eco*RI. *Eco*RI-5 and *Eco*RI-6, which are present in the combined digest, must be contained within these two *Hpa*I fragments. Since *Hpa*I-6 is altered by the *st*(0) deletion as well as by *Eco*RI cleavage, it must span *Eco*RI-5 and *Eco*RI-7, leaving *Hpa*I-13 to span *Eco*RI-6. The *Hpa*I fragments that were not cleaved by *Eco*RI were assigned to T5

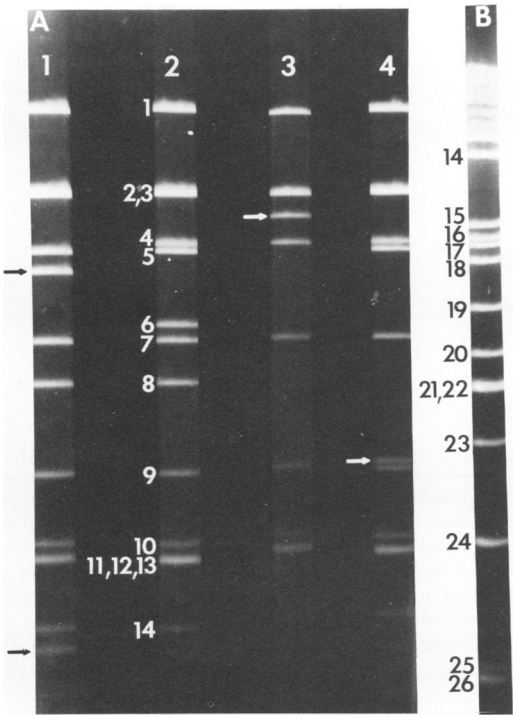


FIG. 4. *Effects of deletions and EcoRI cleavage on the HpaI pattern of T5(+) DNA. (A) Electrophoresis in 0.7% agarose gels: gel 1, HpaI-EcoRI combined digest of T5(+) DNA; gel 2, HpaI digest of T5(+) DNA; gel 3, HpaI digest of T5st(102) DNA; gel 4, HpaI digest of T5st(0) DNA. (B) Electrophoresis in a 2.0% agarose gel of HpaI digest of T5(+) DNA. The arrows indicate fragments not present in HpaI digests of T5(+) DNA.*

*Eco*RI fragments by isolating *Eco*RI fragments from agarose gels by the potassium iodide technique (3) and redigesting them with *Hpa*I. The results of this experiment are summarized in Table 4. Because one member of the pairs of fragments in bands 20 (*Hpa*I-24) and 22 (*Hpa*I-26) appears in *Eco*RI-1 and one appears in *Eco*RI-2, it was assumed that these fragments occur within the terminal repetition. The two members of a pair were given a single number because they should have identical sequences. *Hpa*I-25 was not resolved from *Hpa*I-26 in this experiment, so its location is not known. It is presumably within *Eco*RI-1 or *Eco*RI-2 because no band at its position was observed in *Eco*RI-3 or *Eco*RI-4.

*Hpa*I digests were also made of T5(+) DNA that had been previously digested with *Sal*I, *Sma*I, or *Bam*I. The results are shown in Table 5. The positions of the *Hpa*I fragments that are cleaved by these three enzymes, namely, *Hpa*I fragments 1, 2, 3, 9, 10, 12, and 16, can be established both by the fact of cleavage and by the sizes of the cleavage products. For instance, *Hpa*I-2 is cleaved by *Sal*I and *Sma*I. In both cases, new fragments only slightly smaller than *Hpa*I-3 are produced. Thus, *Hpa*I-2 must span both the *Sma*I site at 38.5% and the *Sal*I site at 46.1% and extend 1% to the left of the *Sma*I site and 1% to the right of the *Sal*I site.

The position of *Hpa*I fragments relative to the ends of the T5 DNA molecule was determined by digesting T5(+) DNA to varying extents with λ exonuclease before digestion with *Hpa*I. The results of this experiment, together with the assignment to *Eco*RI fragments, indicate the approximate distance of *Hpa*I fragments from the right or left end of the molecule (Table 6). The early disappearance of *Hpa*I-24 and *Hpa*I-26 confirms their location within the terminal repetition. Consideration of the results of exonuclease digestion together with the results of cleavage with other restriction endonucleases further identifies the location of some fragments. *Hpa*I-1, for example, is cleaved by *Sal*I at the 87.2% site into pieces of molecular weight 7.9 and 3.0 million. The λ exonuclease results indicate that the larger of these pieces must lie to the right of the *Sal*I site; only *Hpa*I fragments 17, 24, and 26 can lie to the right of *Hpa*I-1, and *Hpa*I-1 is itself affected by 6% digestion with λ exonuclease.

Digestion with λ exonuclease was also used to locate fragments near but not spanning *Eco*RI, *Sal*I, *Sma*I, or *Bam*I cleavage sites. T5(+) DNA was cleaved with one of these enzymes, and the cleavage products were treated with λ exonuclease and then digested with *Hpa*I. For example, the order 21-22-19-16 was

TABLE 4. *Cleavage of T5 EcoRI fragments and T5 deletion mutant DNAs with HpaI*

	*Hpa*I fragments present in[a]:						
T5(+) (76.5)	T5(+) *Eco*RI (76.5)	T5(+) *Eco*RI-1 (28.3)	T5(+) *Eco*RI-2 (19.1)	T5(+) *Eco*RI-3 (16.2)	T5(+) *Eco*RI-4 (8.9)	T5*st*(0) (71.0)	T5*st*(102) (68.8)
1 (10.7)*	1	1				1	1
2 (7.3)*	2			2		2	2
3 (7.3)*	3	3				3	3
							(6.75)
4 (6.05)						4	4
5 (5.85)	5		5			5	
	(5.4)				(5.4)		
6 (4.65)							
7 (4.45)	7		7			7	7
8 (3.90)*	8			8			
						(3.18)	
9 (3.14)*	9				9	9	9
10 (2.64)	10	10				10	10
11 (2.54)	11		11			11	11
12 (2.54)	12		12			12	12
13 (2.54)						13	13
14 (2.15)*	14			14		14	14
	*Eco*RI-5 (2.00)						
	*Eco*RI-6 (1.55)						
15 (1.42)	15	15				15	15
16 (1.32)	16	16				16	16
17 (1.28)	17	17				17	17
	(1.26)			(1.26)			
18 (1.18)*	18		18			18	18
	(1.08)		(1.08)				
19 (0.97)*	19	19				19	19
20 (0.81)*	20			20		20	20
21 (0.71)*	21	21				21	21
22 (0.71)*	22	22				22	22
	(0.62)	(0.62)					
23 (0.56)*	23		23			23	23
	(0.49)			(0.49)			
24 (0.39)[b]*	24	24	24			24	24
	(0.37)				(0.37)		
25 (0.23)	25	?	?			25	25
	*Eco*RI-7 (0.25)						
26 (0.22)[b]*	26	26	26			26	26
Σ 76.16	Σ 75.94	Σ 28.28	Σ 18.81	Σ 15.91	Σ 8.91	Σ 70.79	Σ 68.51

[a] Numbers in parentheses indicate molecular weights in millions. The size of the *st*(102) deletion was determined from the sizes of the T5*st*(102) *Hpa*I fragments. Asterisk indicates that the molecular weight of the *Hpa*I fragment was determined from electron microscopic measurements. The molecular weights of the other *Hpa*I fragments were determined from electrophoretic mobility relative to the electron microscopically measured fragments. Other molecular weights were determined as indicated in Table 1. Fragments not occurring in *Hpa*I digests of T5(+) DNA are listed as molecular weights without fragment numbers.

[b] Because *Hpa*I-24 and *Hpa*I-26 occur within the terminal repetition, there are two copies of each per T5 genome.

established by determining their positions relative to the *Eco*RI, *Bam*I, and *Sma*I cleavage sites at 62.8, 67.4, and 69.0%, respectively. The approximate location of these fragments had been previously established by their assignment to *Eco*RI-1 (Table 4) and their resistance to λ exonuclease (Table 6). As shown in Table 7, *Hpa*I-21 and *Hpa*I-22 are nearest to the *Eco*RI site, *Hpa*I-19 is nearest to the *Bam*I site (excluding *Hpa*I-16, which is cleaved by *Bam*I), and *Hpa*I-16 is nearest the *Sma*I site. Similar reasoning was used to position *Hpa*I fragments 14, 18, 20, and 23. The effect of sequential treatment with *Bam*I and λ exonuclease on the smaller *Hpa*I fragments is shown in Fig. 5.

The location of some fragments was confirmed by analyzing the *Hpa*I pattern produced from T5(+) DNA treated with exonuclease III

from *E. coli*. T5 DNA contains several genetically determined single-chain interruptions. The most prominent of these have been mapped at 7.9, 18.5, 32.6, 64.8, and 99.6% by electron microscopic measurements (16) and by electrophoretic analysis (12). Exonuclease III will initiate at these interruptions as well as at duplex ends, degrading one strand of the duplex DNA as it proceeds in a 3′ → 5′ direction. The *Hpa*I fragments 5, 7, 8, 11, 17, and 22 are affected by exonuclease III digestion, as shown for the larger *Hpa*I fragments in Fig. 6. *Hpa*I fragments 5, 8, 11, 17, and 22 are decreased in size, indicating that they span the major interruptions at 18.5, 32.6, 7.9, 99.6, and 64.8%, respectively. *Hpa*I-7 disappears almost completely because of the high density of minor interruptions near the left end of the molecule. Further digestion with exonuclease III affects fragments near, but not spanning, the single-chain interruptions. The sequential disappearance of *Hpa*I-22, -19, and -16 with increasing amounts of exonuclease III digestion confirmed the order of 22-19-16 to the right of the 64.8% interruption.

TABLE 5. *Combined digests of T5(+) DNA with HpaI and SalI, SmaI, or BamI*

*Hpa*I fragments present in[a]:			
T5(+) (76.5)	T5(+)·*Sal*I (76.5)	T5(+)·*Sma*I (76.5)	T5(+)·*Bam*I (76.5)
1		1	1
	(7.9)		
2			2
3	3	3	
	(6.55)	(6.6)	(6.9)
4	4	4	4
5	5	5	
			(5.1)
6	6	6	6
7	7	7	7
8	8	8	8
9	9		9
	(3.0)		
10	10		
11	11	11	11
12		12	12
13	13	13	13
		(2.16)	
14	14	14	14
	(1.86)	(1.85)	(2.12)
15	15	15	15
16	16	16	
17	17	17	17
		(1.23)	
18	18	18	18
19	19	19	19
	(0.82)		(0.97)
20	20	20	20
21	21	21	21
		(0.71)	(0.71)
22	22	22	22
	(0.67)		
23	23	23	23
		(0.46)	(0.43)
24	24	24	24
			(0.39)
			(0.32)
25	25	25	25
26	26	26	26
Σ 76.16	Σ 76.42	Σ 76.09	Σ 76.09

[a] Numbers in parentheses indicate molecular weights in millions. The molecular weights of fragments that occur only in the combined digests were determined from electrophoretic mobility relative to T5(+) *Hpa*I fragments. These new fragments are listed without fragment numbers. Other molecular weights were determined as indicated in Table 1.

TABLE 6. *Effect of prior digestion with λ exonuclease on HpaI cleavage pattern*

	*Hpa*I fragments absent after[a]:					
No λexo	3% λexo	6% λexo	11% λexo	20% λexo	24% λexo	28% λexo
None	7	1	1	1	1	1
	17	7	7	5	3	3
		17	11	7	5	5
		(24)	12	11	7	(6)
		(26)	17	12	11	7
			24	15	12	(8)
			26	17	15	10
				18	17	11
				23	18	12
				24	23	15
				26	24	17
					—[b]	18
						23
						24
						—[b]

[a] ^{32}P-labeled T5(+) DNA was digested to varying extents with λ exonuclease (λexo) before digestion with *Hpa*I. The extent of λ exonuclease digestion is indicated as the percentage of radioactivity made acid soluble. Parentheses are used to indicate faint bands.

[b] Presence of *Hpa*I-25 and *Hpa*I-26 was not determined in the 24 and 28% experiments. *Hpa*I-25 was present at the lower levels of hydrolysis.

DISCUSSION

There are now available several restriction endonuclease cleavage maps of the bacteriophage T5 genome. Rhoades (11) constructed a physical map of T5 using the seven *Eco*RI cleavage fragments. Von Gabain et al. (19) presented physical maps based on the T5 cleavage products formed by *Eco*RI, *Sal*I (four fragments), and *Sma*I (four fragments) and a partial map of the *Hin*dIII cleavage products. In the study reported here, cleavage maps of T5

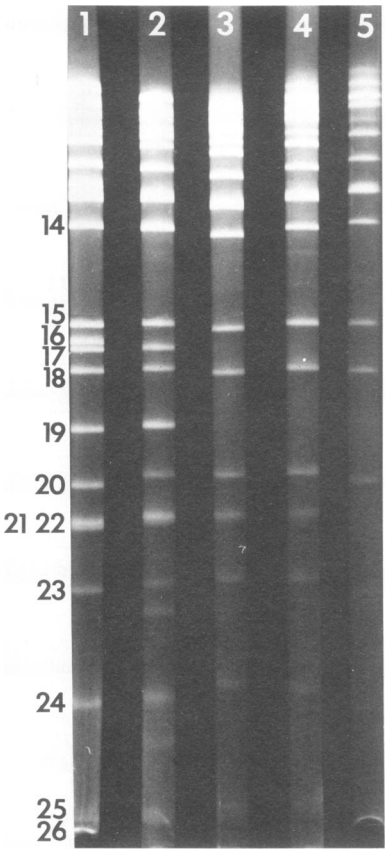


FIG. 5. *Effect of sequential treatment with BamI endonuclease and λ exonuclease on the HpaI pattern of T5(+) DNA. Gel 1, HpaI digest; gel 2, BamI-HpaI combined digest; gels 3, 4, and 5, HpaI digests of λ exonuclease-treated BamI digests (gel 3, 11.4% λ exonuclease digestion; gel 4, 13.9%; gel 5, 18.7%). Electrophoresis was carried out in 1.5% agarose gels. HpaI-16 is cut by BamI; HpaI-19 is not cut by BamI, but is removed by λ exonuclease digestion. The new fragments which appear in the BamI-HpaI combined digest are removed by λ exonuclease because one end is defined by a BamI site.*

DNA were constructed using *Sal*I, *Sma*I, and *Bam*I (five fragments), and *Hpa*I (26 fragments). The *Eco*RI and *Sal*I maps developed in our laboratory are in excellent agreement with those published by von Gabain et al. (19). We therefore feel that these maps are correct and that we are justified in using them to construct the *Hpa*I map. In the case of *Sma*I, however, our cleavage map disagrees with the one published by von Gabain et al. (19). Their arrangement of *Sma*I fragments (4-1-3-2) was based primarily upon the pattern of the combined *Sal*I-*Sma*I digest. They obtained a pattern for the combined digest which they interpreted as consisting of *Sal*I-2, *Sma*I-3, *Sal*I-3, *Sma*I-4 (which they concluded was identical to *Sal*I-4), and the two new fragments of 14 million and 4 million molecular weight. The results reported here, however, clearly show (Fig. 1) that the combined *Sal*I-*Sma*I digest consists of *Sal*I-3, *Sal*I-4, *Sma*I-4, and four new fragments. Moreover, the effect of prior digestion with λ exonuclease on the *Sma*I cleavage pattern unambiguously establishes the terminal position of fragments 1 and 2. We have used our *Sma*I cleavage map in the construction of the *Hpa*I cleavage map. In so doing, we have found our *Sma*I map to be compatible with all other data on the placement of *Hpa*I fragments; the use of the published *Sma*I map would have produced numerous inconsistencies.

The major aim of the study reported here was

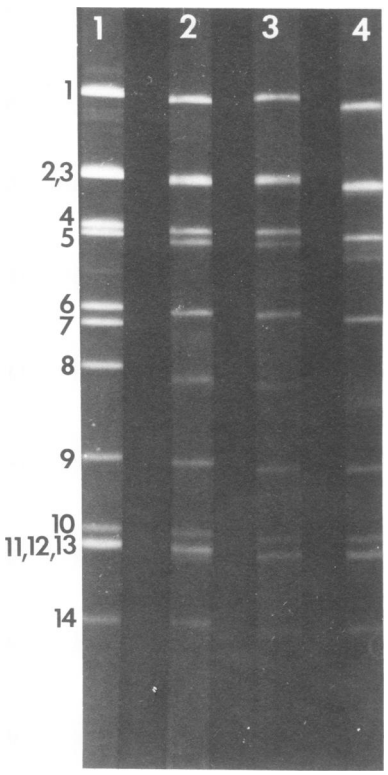


FIG. 6. *Effect of prior treatment with exonuclease III on the HpaI pattern of T5(+) DNA. Gel 1, HpaI digests; gels 2, 3, and 4, HpaI digests of exonuclease III-treated T5(+) DNA (gel 2, 0.8% exonuclease III digestion; gel 3, 1.2%; gel 4, 2.8%). Electrophoresis was carried out in 0.7% agarose gels. HpaI fragments 5, 8, and 11 span three of the major single-chain interruptions and appear to decrease in size with increasing exonuclease III digestion; because HpaI-7 spans several minor interruptions, it disappears almost completely. Faint extra bands appear in gel 1 because the HpaI digestion was not quite complete.*

to obtain a fairly detailed physical map of the bacteriophage T5 genome. The cleavage sites for the restriction endonuclease *Hpa*I were used as the basis for constructing a map, as shown in Fig. 2. This enzyme was chosen because it cleaves T5 at a relatively large, but not unmanageable, number of sites—27. It also proved to be relatively easy to purify. There are still a few ambiguities in the map. The location of *Hpa*I-25 is not known at all. It is not resolved from *Hpa*I-26 in 1.0% agarose gels, which were initially used to analyze the *Hpa*I pattern, and therefore was not detected in the earlier experiments. Because *Hpa*I-25 is so small, it could fit between any two adjacent fragments without changing the map significantly. The order of *Hpa*I-18 and *Hpa*I-23 is not entirely certain. Whereas λ exonuclease treatment of *Bam*I, *Eco*RI, and *Sma*I digests produced clear changes in the *Hpa*I cleavage pattern, λ exonuclease treatment of *Sal*I digests did not (Table 7). The order of *Hpa*I-18 and *Hpa*I-23 is based on the relative intensities of the two bands instead of sequential disappearance. The failure to achieve clear results with *Sal*I is probably due to the presence in *Sal*I of a contaminant that alters the DNA in terms of its properties as a substrate for λ exonuclease. With these two exceptions, the order of the T5 *Hpa*I fragments has been firmly established.

A potentially interesting feature of the *Hpa*I cleavage pattern of T5 DNA is that 11 of 28 fragments appear on agarose gels as pairs or triplets; in other words, some fragments are exactly the same size as others within the resolution of the gel system. Of these eleven, two pairs—*Hpa*I-24 and *Hpa*I-26—are explained by their occurrence within the terminal repetition. Of the remainder, the members of two pairs—*Hpa*I-11, *Hpa*I-12 and *Hpa*I-21, *Hpa*I-22—are adjacent. Such arrangements might be coincidental, or they might reflect tandem duplication that occurred during the evolution of the phage.

A Monte Carlo computer simulation was used to assess the likelihood of such arrangements occurring purely by chance. First, it was found that maps containing 25 randomly distributed restriction sites have, on the average, approximately three pairs of fragments whose sizes are identical within 2%. Thus, the occurrence of multiple-fragment bands in the T5 map is not at all surprising. Second, randomly ordering the actual T5 fragments produces an arrangement having as much apparent order as that actually found with a probability of about 7.5%.

Clearly, if these regions were once duplicated, they have subsequently diverged in sequence, since none of the other T5 restriction maps shows any evidence of duplication in these regions. No attempt has been made to separate these fragments by other means.

Most of the techniques used to produce the *Hpa*I map have been used before. The use of a combination of restriction endonucleases and exonuclease to order fragments near, but not spanning, other restriction cleavage sites is a new extension of these techniques. This application should be of general use in producing physical maps of many DNAs. Another new approach is the use of exonuclease III to order the fragments near the T5 single-chain interruptions. This technique is rather limited in

TABLE 7. *Hpa*I *patterns of restriction digests treated with* λ *exonuclease*

	*Hpa*I fragments absent after[a]:														
T5(+)·*Bam*I				T5(+)·*Eco*RI			T5(+)·*Sma*I			T5(+)·*Sal*I					
No λexo	11.4% λexo	13.9% λexo	18.7% λexo	No λexo	11.8% λexo	20.0% λexo	No λexo	15.1% λexo	18.4% λexo	No λexo	11.1% λexo	13.0% λexo	15.2% λexo	17.7% λexo	18.6% λexo
16	16	16	16	None[b]	17	17	None[b]	14	14	None[b]	17	(15)	(15)	(15)	15
	17	17	17		20	20		16	16		(18)	17	17	17	17
	19	19	19		21	21		17	17		20	(18)	18	18	18
		22	21			22			(24)			20	20	20	20
		(24)	22			(24)			(26)			(23)	(23)	23	23
		(26)	(24)			(26)						(24)	(24)	(24)	24
			—[c]									—[c]	—[c]	—[c]	—[c]

[a] *Bam*I, *Eco*RI, *Sma*I, and *Sal*I digests of ^{32}P-labeled T5(+) DNA were digested to varying extents with λ exonuclease (λexo), as indicated by the percentage of radioactivity made acid soluble. The exonuclease-treated digests were then cleaved with *Hpa*I and subjected to electrophoresis on 1.0 or 1.5% agarose gels to resolve *Hpa*I fragments 14 through 26. New fragments produced in the combined digests (Table 5) are not shown because they are always eliminated by λ exonuclease digestion. Parentheses are used to indicate faint bands.

[b] "None" refers to *Hpa*I fragments 14 through 26.

[c] *Hpa*I-25 and *Hpa*I-26 could not be identified in these gels.

application; most DNAs do not contain genetically fixed interruptions.

The availability of a reasonably detailed physical map of T5 should facilitate analysis of the structure and function of the T5 genome. Studies using the *Hpa*I map to analyze T5 DNA replication and to determine the physical locations of T5 genes are underway.

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