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Restriction Enzyme Cleavage Map of Tn*10*, a Transposon Which Encodes Tetracycline Resistance

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A cleavage map of a recombinant plasmid carrying Tn*10* was constructed for 13 different restriction enzymes. The Tn*10* region of this plasmid contains cleavage sites for *Bam*HI, *Ava*I, *Bgl*I, *Bgl*II, *Eco*RI, *Xba*I, *Hin*cII, *Hin*dIII, and *Hpa*I. Restriction enzymes *Pst*I, *Sma*I, *Kpn*I, *Xho*I, *Sal*I, and *Pvu*I do not cleave within the Tn*10* element. This map confirms the previously reported structure of this transposon; it is composed of a unique sequence (~6,400 base pairs long), which in part codes for the tetracycline resistance functions and is bounded by inverted repeats (~1,450 base pairs long).

Plasmid-mediated tetracycline resistance in bacteria is, in some instances, encoded within a 9,300-base pair long transposon termed Tn*10* (12). Electron microscope examination of denatured DNAs containing Tn*10* reveals a characteristic 1,400-base pair double-stranded segment, terminated at one end by a 6,500-base single-stranded loop and at the other end by the site of insertion (8, 12). The double-stranded segment indicates that sequences at the ends of Tn*10* are repeated in reverse orientation. Sequences within the 6,500-base pair long central, or loop, region of Tn*10* are known to encode tetracycline resistance (12). We are studying the location, organization, and regulation of those genes that mediate tetracycline resistance in Tn*10* and have reported the generation of deletion mutations defining these genes by standard recombinant DNA techniques (6). We report here the construction of a restriction enzyme cleavage map of Tn*10* for enzymes that cleave at infrequently occurring DNA sequences (primary sequences 6 base pairs in length).

The study of gene organization in a particular region of a genome is facilitated by the isolation of a fragment of DNA carrying the region of interest with little extraneous DNA. Such a fragment is generated by any restriction enzyme that does not cleave within the region of interest but that does cleave near its boundaries. Since Tn*10* is a transposable element, it is possible to obtain an enriched source of Tn*10* DNA by isolating λ::Tn*10* transducing phages. Identification of restriction fragments carrying Tn*10* can be accomplished by comparison of independent λ::Tn*10* phages with the parental phage. The isolation and characterization of two such phages [called λ::Tn*10*(1) and λ::Tn*10*(2)] is described elsewhere (R. Jorgensen, Ph.D. Thesis, University of Wisconsin, Madison, 1978).

To generate a DNA fragment containing Tn*10*, we screened parental λ and λ::Tn*10*(1) DNAs with a number of different restriction enzymes (data not shown) to determine the frequency of each enzyme's cleavage sites in Tn*10* DNA relative to λ DNA. This analysis indicated that the *Pst*I enzyme does not cleave Tn*10* DNA, whereas it cleaves λ DNA at more than 15 sites (14), several of which are located in the *b*2 region, where Tn*10* is inserted in λ::Tn*10*(1). To purify and obtain sizable quantities of the λ::Tn*10*(1) *Pst*I fragment carrying Tn*10*, we decided to incorporate it into a small multicopy plasmid by recombination in vitro. The plasmid vector chosen for this experiment was a *Pst*I fragment from the multi-copy plasmid ColE1. ColE1 DNA possesses two *Pst*I cleavage sites; the larger (3.35 megadaltons [Mdal]) of the two fragments produced by *Pst*I cleavage of ColE1 carries both the ColE1 origin of replication and the colicin E1 immunity function (3). This 3.35-Mdal ColE1 *Pst*I fragment was joined to the Tn*10*-containing *Pst*I fragment from λ::Tn*10*(1), and the resulting plasmid, called pRT44, was used to map restriction enzyme cleavage sites in Tn*10*.

The number of cleavage sites in pRT44 for each of a variety of restriction enzymes was determined by electrophoresis of cleavage prod-

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ucts on 0.7% agarose gels and 4% polyacrylamide gels (data not shown). The results of such experiments are presented in Table 1 (lines a and b). In addition to the enzymes listed here, we have found that *Sal*I and *Xho*I do not cleave pRT44.

The relative locations of restriction enzyme cleavage sites were determined by digestion of pRT44 DNA with combinations of various enzymes followed by analysis of digestion products by gel electrophoresis. Cleavage sites for restriction enzymes *Kpn*I, *Bam*HI, *Sma*I, *Ava*I, *Pst*I, and *Eco*RI were mapped by analysis of the double digestion data presented in Table 2. The cleavage map derived from these data is drawn in Fig. 1. Regions of pRT44 originating from ColE1, λ, or Tn*10* sequences were determined from knowledge of the distribution of cleavage sites in ColE1 (3; Jorgensen and Reznikoff, unpublished data) and λ and λ::Tn*10* phages (1, 7, 9, 10; R. Jorgensen, Ph.D. thesis).

By a similar series of double digestion experiments, pRT44 cleavage sites for restriction enzymes *Bgl*I, *Bgl*II, *Hin*dIII, *Hin*cII, *Hpa*I, *Pvu*I, and *Xba*I were mapped. Tables 3, 4, and 5 present the double digestion data used to construct the final restriction enzyme cleavage map (which is presented in Fig. 2). The map was derived from the double digestion data as outlined below (detailed arguments for the construction of the map are presented elsewhere [R. Jorgensen, Ph.D. thesis]).

First, *Hpa*I cleavage sites were located on the map in Fig. 1 by means of data obtained from double digestions of pRT44 with *Hpa*I and other enzymes (Table 3). These data also give accurate

TABLE 1. *Frequency and distribution of cleavage sites in pRT44*[a]

Sites	No. of sites detected by restriction enzyme:												
	*Kpn*I	*Bam*HI	*Sma*I	*Ava*I	*Pst*I	*Bgl*I	*Bgl*II	*Eco*RI	*Hin*dIII	*Hpa*I	*Hin*cII	*Xba*I	*Pvu*I
(a) Detected on 0.7% agarose	1	1	1	2	2	3	2	3	3	3	8	ND[b]	2
(b) All detected sites	1	1	1	2	2	4	2	3	4	4	12	ND	2
(c) Tn*10* region	0	1	0	1	0	1	2	1	3	4	9	1[c]	0
(d) λ region	1	0	1	1	1[d]	3	0	1	1	0	3	ND	1
(e) ColE1 region	0	0	0	0	1[d]	0	0	1	0	0	0	0	1

[a] Plasmid DNA was prepared by the method of Humphreys et al. (5). All enzymes were obtained from New England Biolabs except *Eco*RI, a gift from J. Gardner and S. Hardies. All digestions were done in 0.05 ml of 60 mM NaCl–7 mM $MgSO_4$–10 mM tris(hydroxymethyl)aminomethane-hydrochloride (pH 7.9)–10 mM β-mercaptoethanol. Agarose and polyacrylamide gel electrophoresis were done essentially as described by Shinnick et al. (13) and Blakesley and Wells (2), respectively. DNA fragments were stained for UV photography by immersing the gels in ethidium bromide solution (10 μg/ml) for 10 min, followed by destaining in water for 20 min.

[b] ND, Not determined.

[c] The *Xba*I cleavage site in Tn*10* was identified in pRT61 (see text).

[d] The *Pst*I cleavage sites in pRT44 are the junctions between λ and ColE1 DNAs. Thus one-half of each site originates from λ DNA and one-half from ColE1 DNA.

TABLE 2. *Molecular weights in Mdal of pRT44 restriction fragments*[a]

*Kpn*I + *Sma*I	*Kpn*I + *Bam*HI	*Bam*HI + *Sma*I	*Pst*I	*Pst*I + *Kpn*I	*Pst*I + *Bam*HI	*Pst*I + *Ava*I
>10.0 (>17,000)	7.6 (12,700)	8.1 (13,500)	7.7 (12,900)	7.0 (11,700)	4.00 (6,650)	5.20 (8,650)
0.45 (750)	3.50 (5,850)	2.95 (4,900)	3.35 (5,575)	3.35 (5,575)	4.00 (6,650)	3.35 (5,575)
				0.60 (1,000)	3.35 (5,575)	1.50 (2,500)
						1.08 (1,800)

*Ava*I	*Ava*I + *Bam*HI	*Eco*RI	*Eco*RI + *Kpn*I	*Eco*RI + *Bam*HI	*Eco*RI + *Pst*I
5.85 (9,750)	5.85 (9,750)	5.45 (9,100)	5.45 (9,100)	5.45 (9,100)	3.78 (6,300)
5.20 (8,650)	2.95 (4,900)	3.78 (6,300)	3.78 (6,300)	2.14 (3,550)	3.30 (5,500)
	2.56 (4,075)	2.14 (3,550)	1.27 (2,125)	1.90 (3,150)	2.25 (3,750)
			0.63 (1,050)	1.70 (2,825)	2.05 (3,425)

[a] Fragment molecular weights were determined relative to λ *Eco*RI fragments (4) on agarose gels and are reported as Mdal to avoid confusion with molecular weights in base pairs, determined relative to φX174 fragments on polyacrylamide gels, which are given in parentheses. Methods for restriction enzyme analysis are described in Table 1.

(reproducibility = 3%) distances between *Hpa*I cleavage sites and the *Bam*HI, *Eco*RI, and *Ava*I cleavage sites. The *Hpa*I map aids in determination of the *Hinc*II map, since *Hinc*II cleaves all *Hpa*I cleavage sites (*Hinc*II cleaves the sequence $\frac{\text{GTPyPuAC}}{\text{CAPuPyTG}}$, where *Hpa*I cleaves the sequence $\frac{\text{GTTAAC}}{\text{CAATTG}}$). Thus, of the 12 *Hinc*II sites in pRT44, 4 must also be *Hpa*I sites. By comparison of the *Hpa*I double digestion data (Table 3) with similarly obtained *Hinc*II double digestion data (Table 4), it is possible to identify which *Hinc*II fragments lie within which *Hpa*I fragments on the map and thus to map all the *Hinc*II cleavage sites within *Hpa*I fragments 2025, 1775, and 675. In addition, the *Hinc*II and *Hpa*I double digestion data are useful for mapping *Bgl*I, *Bgl*II, *Xba*I, and *Hin*dIII cleavage sites in the Tn*10* region of pRT44 when considered in conjunction with the pRT61 double digestion data presented in Table 5. (pRT61 is a deletion derivative of pRT44 constructed by in vitro deletion of the 2.14-Mdal *Eco*RI fragment [see Fig. 1].) Next, all but one of the remaining *Hinc*II cleavage sites can be mapped, along with the *Bgl*I and *Hin*dIII sites in the 2.14-Mdal *Eco*RI fragment, by considering together three sets of data: (i) *Hinc*II digestion patterns of two λ::Tn*10* transducing phages and the parental λ phage (data not shown), (ii) pRT44 double digestion data in Table 5, and (iii) the *Hpa*I and *Hinc*II double digestion data in Tables 3 and 4. This analysis yields a map with a single discontinuity, between *Hpa*I fragment 675 and *Hinc*II fragment 940. *Hinc*II fragments 1050 and 420, which carry no cleavage sites for the enzymes tested here and so could not be mapped by

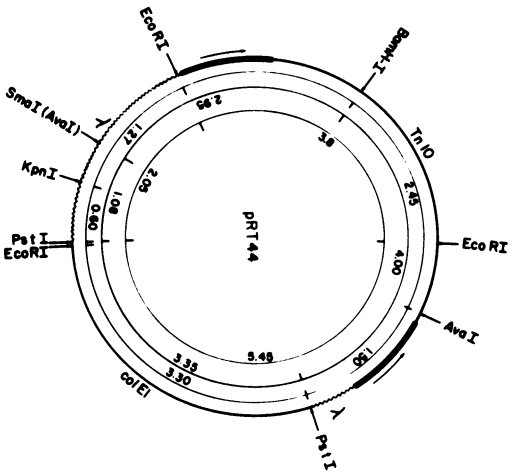

FIG. 1. *Cleavage map of pRT44 determined from data in Table 2. Numbers indicate distances in Mdal. λ, ColE1, and Tn10 regions of pRT44 are indicated on the map. The heavy lines indicate the inverted repetitions in Tn10; their relative orientation is indicated by the arrows. The cleavage site indicated as SmaI (AvaI) is cleaved by both enzymes, since SmaI cleaves at* $\frac{\text{CCCGGG}}{\text{GGGCCC}}$ *and AvaI cleaves at* $\frac{\text{CPyCGPuG}}{\text{GPuGCPyC}}$.

TABLE 3. *Sizes in base pairs of cleavage products of pRT44 HpaI restriction fragments*[a]

Fragment size	HpaI+ fragments cleaved by second enzyme:									
	*Eco*RI	*Pst*I	*Bam*HI	*Kpn*I	*Sma*I	*Ava*I	*Bgl*I	*Bgl*II	*Hin*dIII	*Xba*I
>5,000 (9 Mdal)	−	−	+	−	−	−	−	+	−	+
2,025	−	+	+	+	+	+	−	+	+	−
1,775	+	+	−	+	+	+	+	−	+	+
675	+	+	+	+	+	−	+	−	−[b]	+
	Sizes of new fragments cleaved by:									
	*Eco*RI	*Pst*I	*Bam*HI	*Kpn*I	*Sma*I	*Ava*I	*Bgl*I	*Bgl*II	*Hin*dIII	*Xba*I
	>5,000	>5,000	1,630	>5,000	>5,000	>5,000	>5,000	1,600	>5,000	1,300
	~3,250	>5,000	160	>5,000	~4,600	~4,600	~4,150	640	2,800	655
	2,800	2,500				~630	1,640	225	685[b]	
	1,400						1,250		515	
	595						435		400	
							370		310	

[a] *Hpa*I fragments cleaved by a second enzyme are indicated by a minus sign (−). Fragments not cleaved are indicated by a plus (+) sign. Novel fragments produced by digestion of *Hpa*I fragments are listed in the appropriate columns. Methods for these restriction enzyme analyses are described in Table 1. Fragment sizes were determined by comparison with φX174 restriction fragments whose exact lengths are known from sequencing studies (11).

[b] *Hpa*I fragment 675 was shown to be different from *Hpa*I;*Hin*dIII fragment 685 by coelectrophoresis (data not shown).

TABLE 4. *Sizes in base pairs of cleavage products of pRT44 HincII restriction fragments*[a]

Fragment size	*Hinc*II[+] fragments cleaved by second enzyme:									
	*Eco*RI	*Pst*I	*Bam*HI	*Kpn*I	*Sma*I	*Ava*I	*Bgl*I	*Bgl*II	*Hin*dIII	*Xba*I
>5,000 (4.2 Mdal)	−	−	+	−[b]	+	+	−	+	+	+
2,525	+	+	+	+	+	+	+	+	−	+
1,600	+	+	−	+	+	+	+	−	+	+
1,525	−	+	+	+	+	+	+	+	+	+
1,275	−	+	+	+	+	+	−	+	+	+
1,175	+	+	+	+	−	−	−	+	−	+
1,050	+	+	+	+	+	+	+	+	+	+
940	+	+	+	+	+	+	+	+	+	+
695	+	+	+	+	+	+	+	+	+	−
675	+	+	+	+	+	−	+	−	−[c]	+
420	+	+	+	+	+	+	+	+	+	+
195	+	+	+	+	+	+	+	+	+	+

	Sizes of new fragments cleaved by:									
	*Eco*RI	*Pst*I	*Bam*HI	*Kpn*I	*Sma*I	*Ava*I	*Bgl*I	*Bgl*II	*Hin*dIII	*Xba*I
	>5,000	>5,000	1,530	<5,000	580	580	>5,000	1,590	1,375	655
	1,460	1,360	155	195	550	580	860	620	1,160	
	990	175				550	650		685[c]	
	660						615		515	
	590						575		400	
	515						435		310	
							370			

[a] *Hinc*II fragments cleaved by a second enzyme are indicated by a minus sign (−). Fragments not cleaved are indicated by a plus (+) sign. Novel fragments produced by digestion of *Hinc*II fragments are listed in the appropriate columns. Methods for these restriction enzyme analyses are described in Tables 1 and 3.

[b] Slight mobility changes in this fragment are difficult to detect in this gel system. Cleavage of this fragment was confirmed by other evidence.

[c] *Hinc*II fragment 675 was shown to be different from *Hinc*II;*Hin*dIII fragment 685 by coelectrophoresis (data not shown).

TABLE 5. *Sizes in base pairs of cleavage products of the indicated restriction enzyme digests*[a]

Plasmid	Restriction enzyme	Restriction fragments
pRT44	*Bgl*I	435
	*Bgl*I;*Pst*I	1,225, 435, 245
	*Bgl*I;*Kpn*I	750, 475, 435
	*Pst*I;*Eco*RI	112
	*Pst*I;*Kpn*I	1,125
pRT61	*Hin*dIII	515
	*Hin*dIII;*Ava*I	515, 530
	*Hin*dIII;*Bgl*II	515, 335
	*Hin*dIII;*Bgl*I	645, 615
	*Hin*dIII;*Bam*HI	850
	*Hin*dIII;*Eco*RI	900
	*Bgl*I;*Ava*I	925
	*Bgl*I;*Eco*RI	185
	*Bgl*I;*Bgl*II	1,850, 1,050

[a] Only those restriction fragments less than 2,000 base pairs long are listed here. Methods for these analyses are described in Table 1.

double digestion data, lie in this gap. The relative orientation of these two fragments, as we have drawn it in Fig. 2, is based on the assumption that the inverted repeats of Tn*10* are identical with respect to their *Hinc*II cleavage sites. This assumption may not prove valid; however, of 12 restriction enzyme cleavage sites mapped in each inverted repeat of the Tn*5* transposon, all sites are, in fact, found in both repeats (Jorgensen and Reznikoff, manuscript in preparation).

In summary, a two-step procedure was used to obtain purified Tn*10* DNA from which a restriction enzyme cleavage map was constructed. The first step involved transposition of Tn*10* to a bacteriophage genome and the second in vitro recombination of a Tn*10*-containing restriction fragment from the phage into a multicopy plasmid. This plasmid (pRT44) was found to carry 34 nonidentical cleavage sites for 13 different enzymes. The Tn*10* region of the plasmid carries 19 of these sites for 9 different enzymes. The final map is shown in Fig. 2. The length of Tn*10*, as well as the lengths of the central region and the inverted repeats, can be calculated from the sizes of restriction fragments shown on the map in Fig. 2. These sizes are 9,300 ± 300 base pairs for the entire Tn*10* element, 6,400 ± 300 base pairs for the central region, and 1,450 ± 100 base pairs for the inverted repeats,

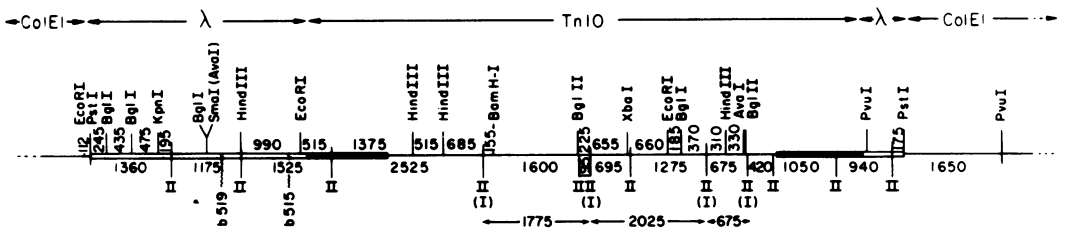

FIG. 2. *Cleavage site map of pRT44. Numbers refer to distances in base pairs. II refers to HincII cleavage sites. (I) indicates those HincII cleavage sites that are also HpaI cleavage sites. b515 and b519 indicate the positions of these two deletions in λ. Note that BglI and SmaI do not cleave at the same site in HincII fragment 1175; rather, the BglI and SmaI cleavage sites lie very close together approximately in the middle of fragment 1175, such that their relative locations could not be determined from the available data. The PvuI sites in pRT44 were mapped by HincII;PvuI and PstI;PvuI double digestions (data not shown).*

and are in close agreement with the lengths measured in the electron microscope (central region, 6,400 ± 300 base pairs; inverted repeats, 1,400 ± 200 base pairs; 8, 12).

The cleavage map presented here confirms and extends our preliminary Tn*10* cleavage map presented earlier (6), as well as the physical analysis of a series of recombinant plasmids carrying deletions defining and altering the tetracycline resistance genes in Tn*10* (6). The physical maps, tetracycline resistance phenotypes, and polypeptide products of these plasmids have led to the mapping of a tetracycline resistance gene and its controlling elements in *HpaI* fragments 2025 and 675 (6; Jorgensen and Reznikoff, manuscript in preparation).

ADDENDUM IN PROOF

The results reported here confirm and extend those recently described by N. Kleckner, D. F. Barker, D. G. Ross, D. Botstein, J. A. Swan, and M. Zabeau. (Genetics **90**:427–461, 1978). In addition, the observation that the restriction sites in the unique region of Tn*10* are located in one orientation vis-a-vis the sites in adjoining λ and ColE1 DNA suggests that the unique region does not undergo recombinational rearrangement or does so very infrequently.

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