PROPERTIES OF THE TRANSLOCATABLE TETRACYCLINE-RESISTANCE ELEMENT Tn10 IN *ESCHERICHIA COLI* AND BACTERIOPHAGE LAMBDA

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APPENDIX by

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ABSTRACT

A number of independent insertions into bacteriophage λ of the translocatable tetracycline-resistance element Tn10 have been isolated and characterized.—The physical positions and relative orientations of several such insertions were determined. Two independent insertions appear to lie in the same orientation at or very near the same site in the *c*I gene, and two more lie in opposite orientations at or near the same position in or near the *rex* gene.—Insertions in or near genes *c*I, *rex*, and *c*III have been characterized genetically for their effects on expression of nearby genes. Tn10 appears to exert a polar effect on expression of distal genes when it is inserted within an operon, even when expression of that operon is under the influence of λ *N*-function. In addition, Tn10 insertions in *rex* appear to influence in some way expression of an "upstream" gene, *c*I.—Lambda derivatives carrying Tn10 give rise to spontaneously occurring, tetracycline-sensitive deletions at high frequencies. It is likely that formation of these deletions is promoted in some way by the Tn10 element.—Lambda::Tn10 phages carrying a Tn10 element that has undergone several successive cycles of translocation since its first isolation and characterization have been analyzed. The results confirm that Tn10 often retains its physical and functional integrity during many cycles of translocation.—Lambda derivatives carrying Tn10 have been used to generate insertions of Tn10 in the chromosome of *Escherichia coli*. This process is independent of *recA* function, and seems to be quite analogous to the translocation of Tn10 in *Salmonella typhimurium* as studied previously.

TN10 is a translocatable drug-resistance element that carries a tetracycline resistance determinant; it was originally isolated from the drug-resistance transfer factor R222 (WATANABE *et al.* 1972). Tn10 is approximately 9300 base pairs in length and carries a 1400 base-pair inverted repetition at its ends. Tn10 has been shown to translocate from R222 into the genome of the temperate Salmonella phage P22, and thence into the bacterial chromosome of *Salmonella*

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typhimurium LT2 (WATANABE *et al.* 1972; CHAN *et al.* 1972; KLECKNER *et al.* 1975). Basic features of Tn10 translocation in *Salmonella* are described elsewhere (KLECKNER *et al.* 1975; BOTSTEIN and KLECKNER 1977; KLECKNER, ROTH and BOTSTEIN 1977).

The experiments described here concern the translocation of Tn10 in *E. coli*. Insertions of Tn10 into coliphage λ were isolated and characterized. Various approaches for obtaining such insertions and the physical and genetic characteristics of six particular insertions are described. Subsequent translocation of Tn10 from the genome of λ to the *E. coli* chromosome was also studied. The results suggest that the basic features of Tn10 translocation are similar in *E. coli* and in Salmonella. The results also given new information about Tn10-promoted illegitimate recombination and about the effects of the Tn10 insertions in λ on the expression of nearby λ genes.

MATERIALS AND METHODS

Phage and bacterial strains used in this work are described in Table 1.

Isolation of λ::Tn10 phages: Insertions of Tn10 into λ were isolated in many independent experiments following lytic growth of a suitable λ derivative in a bacterial host harboring a chromosomal Tn10 insertion. A number of such experiments are shown in Table 2. Derivatives

TABLE 1A

Bacteriophage strains

Number	Genotype	Source
λ6	*b*221 c1857 *ind*+	This laboratory
λ7	*b*221	This laboratory
λ55	*b*221 cIII167::Tn10 c1857 *ind*+ Oam29	This laboratory
λ70	*b*221 *N*am7 *N*am53 c1857 *ind*+	This laboratory
λ83*	*b*221 cIII167::Tn10 *N*am7 *N*am53 c1857	This laboratory
λ85*	*b*221 c1171::Tn10 *N*am7 *N*am53 c1857	This laboratory
λ86*	*b*221 cIII169::Tn10 *N*am7 *N*am53 c1857	This laboratory
λ87*	*b*221 *rex*173::Tn10 *N*am7 *N*am53 c1857	This laboratory
λ88*	*b*221 *rex*174::Tn10 *N*am7 *N*am53 c1857	This laboratory
λ90*	*b*221 c1172::Tn10 *N*am7 *N*am53 c1857	This laboratory
λ115	*b*221 *red*3 c1857	This laboratory
λ117	*b*221 *red*am270 c1857	This laboratory
λ167†	*b*221 cIII167::Tn10 c1857 *ind*+	This laboratory
λ169†	*b*221 cIII169::Tn10 c1857 *ind*+	This laboratory
λ171†	*b*221 c1171::Tn10 c1857 *ind*+	This laboratory
λ172†	*b*221 c1172::Tn10 c1857 *ind*+	This laboratory
λ173†	*b*221 *rex*173::Tn10 c1857 *ind*+	This laboratory
λ174†	*b*221 *rex*174::Tn10 c1857 *ind*+	This laboratory
λ203‡	*lac-trp* W205 c1857 *ind*+ *nin*5	W. BARNES *via* I. HERSKOWITZ
λ224‡	*lac-trp* W205 cIII224::Tn10 cJ857 *imm*5	This laboratory
λ290§	*lac-trp-imm*λ *imm*21 *nin*5	R. MAURER
	*bio*275 c1857 *ind*+	This laboratory
	*red*am270 *gam*am210 c1857	E. R. SIGNER

TABLE 1B

Bacterial strains

Number	Genotype	Synonyms	Source
KR0	*lac*⁻ *trp*am *rec*A⁻ *su*II+		E. R. SIGNER
NK5002	*gal*1,2= *strA*594 *su*⁻ $T3^RT7^R$	WD5021, 594	E. R. SIGNER
NK5003	*gal*1,2= *strA*594 *su*⁻ $T3^RT7^R$ *rec*A441	WD5259, 594*recA*⁻	E. R. SIGNER
NK5012	*thr*⁻*leu*⁻*thi*+*su*II+ $T1^RT5^R\phi80^R$	Q1,C600*thi*+	E. R. SIGNER
NK5013	W3110 *trpR*⁻ *trpA* am9605 *his*am29 *pol*Ats214	LS799	L. SOLL
NK5014	NK5013 *su*II+		E. SODERGREN
NK5019	*su*⁻	W3110	K. BACKMAN
NK5036	NK5012 *leu*144::Tn10		This laboratory
NK5038	NK5012 *leu*147::Tn10		This laboratory
NK5133	NK5019 *his*::Tn10		This laboratory
NK5151	NK5019 *trp*::Tn10		This laboratory
NK5185	NK5012 (λ*b*515 *b*519 *int*am29 *c*I857 *nin*5)		This laboratory
NK5196	NK5012 (P2)	Q5175	E. R. SIGNER
NK5198	KR0 *su*II+	KR2a	E. R. SIGNER
NK5317	*lac*▽x74 *gal*OP308 str^R	RV308	R. MAURER
NK5324	*ara*⁻ *lacpro*▽ *thi*⁻/F′*laci*^{q}L8 *pro*+	GM1	J. MILLER

* Isolation described in Table 2.

† Derived from corresponding *NN*⁻ derivatives by crosses with λ6 or λ7.

‡ Carries a fusion of *lac*Z·*trp*B·*trp*A to *p*L; the phage is deleted for material in the *b*2 region and for *att*, *int*, *xis*, and *exo* however it is *gam*+; the status of *bet* is not known.

§ Carries a fusion of *lac*Z·*trp*B·*trp*A·λcI within the *b*2 region; functions to the right of and including *att* are intact.

of λ whose genomes are more than about 111% the wild-type length are not efficiently packaged into stable particles (SHULMAN and GOTTESMAN 1971; R. WEISBERG, personal communication). Since the Tn10 element is about 19% the length of normal λ, a λ derivative that is at least 7% shorter than wild type must be used to pick up the Tn10 insertion. In practice, substantially shorter derivatives (—22% to —15%) were used, so that the resulting λ::Tn10 phage had a genome size not too different from that of wild-type λ.

Lambda::Tn10 phages were isolated from suitable lysates, using several different selection procedures. In each case a λ deletion derivative was grown on a host strain harboring the Tn10 element in its chromosome. Specific procedures for the propagation of λ have been described elsewhere (KLECKNER and SIGNER, 1977). The resulting lysate was used to infect tetracycline-sensitive bacteria, and tetracycline-resistant "transductants" were selected under conditions where λ::Tn10 genomes would be stably maintained in the bacterial host.

When the parental λ "vehicle" phage was integration and repression proficient, tetracycline-resistant transductant lysogens were obtained simply by selecting drug resistance following infection of a normal sensitive bacterial host under conditions favoring lysogeny (*i.e.*, high multiplicity of phage per cell). For integration and/or repression-deficient phage vehicles, Tn10-carrying genomes were selected by using a bacterial host harboring a λ prophage. Integration of a λ::Tn10 genome into the resident prophage by homologous recombination produces a tetracycline-resistant "transductant." Finally, λ::Tn10 phages were isolated by taking advantage of the fact that *N*⁻ mutants of λ can stably associate with the bacterial host as unintegrated, autonomously replicating plasmids (SIGNER 1969; KLECKNER and SIGNER 1977). Using a phage vehicle carrying amber mutations in gene *N* and an *su*⁻ bacterial host, tetracycline-resistant

TABLE 2

Insertions of Tn10 into λ

Experiment	Clone	Phage vehicle	State of phage recombination functions	Selection method	Frequency
1	a	*b221 Nam7 Nam53 cI857*	*int⁻ att⁻ exo+ bet+ gam+*	*N⁻* plasmids	1×10^{-8}
	b	*b221 Nam7 Nam53 cI857*	*int⁻ att⁻ exo+ bet+ gam+*	*N⁻* plasmids	8×10^{-9}
	c	*b221 Nam7 Nam53 cI857*	*int⁻ att⁻ exo+ bet+ gam+*	*N⁻* plasmids	7×10^{-9}
	d	*b221 Nam7 Nam53 cI857*	*int⁻ att⁻ exo+ bet+ gam+*	*N⁻* plasmids	5×10^{-9}
2	a	*lac trp cI857 nin5*	*int⁻ att⁻ exo⁻ bet? gam+*	tet^R double lysogens	2×10^{-8}
	b	*lac trp cI857 nin5*	*int⁻ att⁻ exo⁻ bet? gam+*	tet^R double lysogens	4×10^{-8}
	c	*lac trp cI857 nin5*	*int⁻ att⁻ exo⁻ bet? gam+*	tet^R double lysogens	7×10^{-8}
	d	*lac trp cI857 nin5*	*int⁻ att⁻ exo⁻ bet? gam+*	tet^R double lysogens	1.5×10^{-7}
	e	*lac trp cI857 nin5*	*int⁻ att⁻ exo⁻ bet? gam+*	tet^R double lysogens	1.8×10^{-7}
3	a	*lac trp imm21 cI nin5*	*int+ att+ exo+ bet+ gam+*	tet^R lysogens	8.3×10^{-9}
	b	*lac trp imm21 cI nin5*	*int+ att+ exo+ bet+ gam+*	tet^R lysogens	1×10^{-9}
	c	*lac trp imm21 cI nin5*	*int+ att+ exo+ bet+ gam+*	tet^R lysogens	2×10^{-9}
	d	*lac trp imm21 cI nin5*	*int+ att+ exo+ bet+ gam+*	tet^R lysogens	1.2×10^{-8}
	e	*lac trp imm21 cI nin5*	*int+ att+ exo+ bet+ gam+*	tet^R lysogens	1×10^{-9}

For each clone, a plate stock of the phage vehicle was made from a single plaque using as host a *rec+ E. coli* K12 strain carrying a chromosomal Tn10 insertion: Experiment 1, NK5036 *leu*::Tn10; experiments 2 and 3, NK5133 *his*::Tn10 and NK5151 *trp*::Tn10.

For selection of *tet*-resistant "transductants" in each case, host bacteria were grown to late exponential phase and infected with a suitable lysate of the phage vehicle at a multiplicity of ten phage per cell. After adsorption at 32° for 45 minutes, infected cells were plated on broth plates containing tetracycline. For *N⁻* plasmid transductants, the host was a *recA⁻ su⁻* host, KR0; tetracycline was present at 5 to 10 μg per ml. For tet^R lysogens, strain NK5317 was used as host and for tet^R double lysogens, the host was NK5185; tetracycline was present at 15 μg per ml.

plasmid transductants were obtained following growth of the vehicle on an *su*+ bacterial host harboring a chromosomal Tn10 insertion. It should also be possible to obtain analogous plasmid transductants using an *N*+ phage vehicle and a special bacterial host such as *groN* or *Nus* in which *N*-function is not active (FRIEDMAN, JOLLY and MURAL 1973; GEORGOPOULOUS 1971).

When λ::Tn10 phages were obtained as single or double lysogens, the lysogens were induced and individual plaques from the resulting lysate were individually tested for presence of the tet^R determinant by a standard "pick and stab" test (see below). When obtained as plasmid transductants, plaques of Tn10 phage were obtained by plating a culture of the plasmid-containing strain on a lawn of permissive (*su*+) cells. Phage are spontaneously released from such strains at low frequencies; individual plaques were purified, tested, and subsequently grown and maintained in the normal way as phage stocks.

This study was intentionally limited to plaque-forming λ::Tn10 derivatives; insertions in genes essential to phage growth require more elaborate methods of detection and propagation.

Several different λ strains and bacterial hosts carrying Tn10 insertions in several different locations have been used with equal success in the isolation of λ::Tn10 phages. The effects of known bacterial mutations on these particular translocation events have not been tested; however, other experiments (see Section 6 below) have shown that translocation of Tn10 in *E. coli* is independent of the host *recA* function. From the genotypes of the phages in Table 2, it is clear that several known λ-encoded recombination functions (*int, xis* and *exo*) are not involved; the influence of *beta* was not tested.

Testing individual plaques for tet^R: Individual plaques were tested for presence of the tetracycline-resistance determinant by picking and stabbing with a sterile toothpick into a broth plate containing 15 micrograms/ml tetracycline that has been spread with $5 \times 10^7 - 2 \times 10^8$ cells of a tetracycline-sensitive homo-immune λ lysogen. Plates were incubated at 34°–37° for 24 to 48 hours. Plaques of phage carrying the drug resistance determinant give rise to a clump of drug resistant bacteria, presumably *via* integration of the tet^R phage genome into the resident prophage of the homo-immune lysogen to produce a *tet*-resistant double lysogen.

Electron microscope heteroduplex mapping was performed as described in TYE, CHAN and BOTSTEIN 1974.

Geneology of λ224: The Tn10 element in λ224 has undergone five successive translocation events since it was originally found in nature in the genome of the drug-resistance plasmid R222. Step 1: Translocation from R222 to the genome of Salmonella phage P22. Step 2: Translocation from P22 into the *Salmonella leu* operon, as described in KLECKNER *et al.* 1975. This *leu*::Tn10 insertion was subsequently introduced into *E. coli* by conjugation between a Salmonella Hfr *leu*::Tn10 and *E. coli* strain NK5012. Step 3: Translocation from the genome of NK5036 into phage λ, as described in Table 2, Experiment 1, to produce λ83. Step 4: Translocation from an *N*+ Oam derivative of λ83 (λ55) into the *E. coli* chromosome to produce the *trp*::Tn10 strain NK5151. Step 5: Translocation from the *E. coli trp* operon back into the genome of λ, as described in Table 2, Experiment 2. The phenotype of λ224 is indistinguishable from that of λ167 and λ169, and heteroduplex analysis confirms that λ224 contains an insertion of Tn10 in or near the phage *c*III gene (see text).

Nomenclature: All of the insertions of Tn10 into λ have been named in Table 1 according to the recommendations of *Campbell et al.* 1977.

Media: Lambda broth is 10 g tryptone, 2.5 g NaCl per liter; λym broth is λ broth to which yeast extract (0.01% final concentration) and maltose (0.2% final concentration) are added after autoclaving. Lambda plates are λ broth containing 10 g per liter of agar. LB plates contain 10 g tryptone, 5 g yeast extract, 5 g NaCl, and 10 g agar per liter. Phage are routinely diluted and stored in SM buffer: 0.05 M Tris·HCl pH 7.5; 0.01 M $MgSO_4$; 0.1% gelatin.

Isolation of Tn10 insertions in the E. coli *chromosome using* λ55: Recipient bacteria are grown to 6–8 × 10^8/ml in λym broth. Phage are added at a multiplicity of ten or fewer phage per cell, and allowed to adsorb for 45–60 minutes at 34°. If independently arising translocations of Tn10 into the host chromosome are desired, the infected cells can be plated immediately on selective plates: λ agar containing 50 micrograms/ml tetracycline or λ agar containing 25 micro-

grams/ml tetracycline and 0.0025 M sodium pyrophosphate. If a pool of chromosomal insertions is to be subjected to further selection for a particular type of insertion, the infected cells can be diluted several-fold, incubated for two hours more in the absence of tetracyline, then tetracycline added to a concentration of 25 micrograms/ml and the culture grown overnight to saturation.

RESULTS

(1) *Genetic characterization of Tn10 insertions into* λ: Six independent λ::Tn10 phages obtained as derivatives of λ *b*221 *N*am7 *N*am53 *c*I857 *ind*$^+$ in a plasmid selection (experiment 1 of Table 2) have been extensively characterized genetically and physically. After isolation, the corresponding N^+ derivatives of these phages were constructed by genetic crosses and all tests were performed on these N^+ derivatives.

Each of the six N^+ λ::Tn10 phages was tested for plaque phenotype (*i.e.*, clear or turbid) along with suitable control phages. Because the phages carry the thermolabile *c*I857 repressor mutation, phenotypes were tested over a range of temperatures from 32° to 40°. Phage were also tested for expression of several functions that are normally nonessential for phage growth (red and gamma) by testing their relative efficiencies of plating on special bacterial hosts (*recA*$^-$, *polA*$^-$ and a P2 lysogen) that render phage growth sensitive to the presence or absence of these nonessential functions. Lambda *red*$^-$ phage will not grow on a *polA*$^-$ host; *red*$^-$ *gam*$^-$ phages will not grow on a *recA*$^-$ host; and only *red*$^-$ *gam*$^-$ phages will grow on a P2 lysogen (ZISSLER, SIGNER and SCHAEFER 1971a, b).

The results of these tests are shown in Table 3.

None of the λ::Tn10 phages is identical in plaque phenotype to the non-Tn10-carrying parent. The parent makes turbid plaques at or below 37° and clear plaques at temperatures above 37° as a consequence of the *c*I857 mutation, which renders *c*I repressor thermolabile. Phages carrying insertions 171 and 172 make very clear plaques at all temperatures; physical analysis (see below) shows that these insertions lie within or very near to the *c*I gene. Phages carrying insertions 167 and 169 make semi-clear plaques at temperatures at or below 37°; and phages carrying insertions 173 and 174 make clear plaques at 37°, but turbid plaques at lower temperatures. The explanation for these phenotypes is considered below. As expected, all of these phages make very clear plaques at 40° where the *c*I857 repressor is almost totally inactive.

Phages carrying four of the insertions (171, 172, 173 and 174) are normal in their expression of red and gamma functions as judged by their plating on special bacterial hosts. They plate with normal efficiencies on *recA*$^-$ and *polA*$^-$ hosts, producing plaques of normal size; they plate on a P2 lysogen only at drastically reduced efficiencies.

Phages carrying two of the insertions, 167 and 169, plate aberrantly on the special hosts. Their efficiency of plating on *polA*$^-$ is reduced 50- to 100-fold; their efficiency of plating on *recA*$^-$ is nearly normal, but the plaques are drastically reduced in size; their efficiency of plating on a P2 lysogen is increased by several orders of magnitude to nearly one. These properties indicate that these insertions

TABLE 3

Phenotypes of λ b221 c*I857* ind+ *derivatives carrying Tn10 insertions*

Phage genotype	Insertion	Clear/Turbid phenotype* 34	36	37	40	Efficiency of plating on unusual hosts† *polA⁻su⁺*	*polA⁻su⁻*	*recA⁻su⁺*	*recA⁻su⁻*	(P2)*su⁺*
*b*221 c*I857 ind*+	167	semi-C	C	C	C	0.02 very small		0.94 small		0.19
*b*221 c*I857 ind*+	169	semi-C	C	C	C	0.01 very small		0.94 small		0.52
*b*221 c*I857 ind*+	171	C	C	C	C	1.1		0.88		2.6×10^{-5}
*b*221 c*I857 ind*+	172	C	C	C	C	0.95		0.81		2.3×10^{-5}
*b*221 c*I857 ind*+	173	T	C	C	C	1.0		0.91		1.9×10^{-5}
*b*221 c*I857 ind*+	174	T	C	C	C	1.0		0.95		1.9×10^{-5}
*b*221 c*I857 ind*+	none	T	T	C	C	1.6	1.4	1.2		$<3 \times 10^{-8}$
*b*221 c*I857 ind*+ *red*3	none					2×10^{-6}	2×10^{-6}	1.0		7.8×10^{-6}
*b*221 c*I857 ind*+ *red*am270	none					0.65 very small	1.8×10^{-6}	1.3	1.2	3.9×10^{-6}
*bio*275 (*red⁻ gam⁻*)	none					$<3 \times 10^{-7}$	$<3 \times 10^{-7}$	$<3 \times 10^{-7}$	$<3 \times 10^{-7}$	1.5
*b*221 *c*+ *red*am270 *gam* am210	none					$<10^{-3}$	$<1 \times 10^{-7}$	1.2	$<1 \times 10^{-7}$	7.2×10^{-6}

* Clear/turbid phenotype of phages were tested at each temperature by streaking all seven phages on a single plate seeded with a lawn of NK5012.

† Efficiencies of plating on unusual hosts are relative to plating on NK5012. All e.o.p.'s were measured at 37°. Unusual hosts were: *polA⁻su⁺* = NK5014; *polA⁻su⁻* = NK5013; *recA⁻su⁺* = NK5198; *rec⁻su⁻* = KR0; (P2) *su⁺* = NK5196.

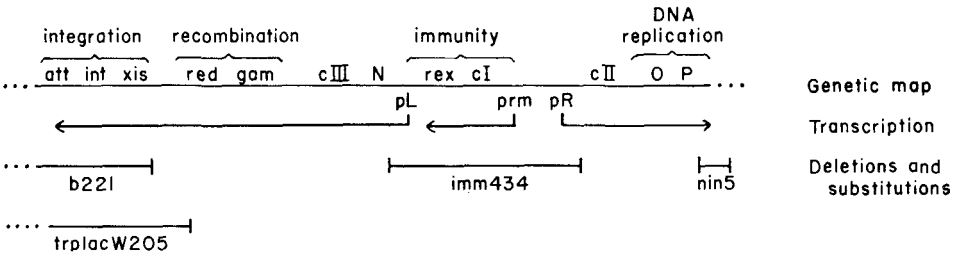


FIGURE 1.—Genetic map of the early region of bacteriophage lambda showing the location of the promoters pL, pR and *prm* and the direction and extent of normal transcription from them. Also shown are the genetic positions of deletion and substitution mutants used in this study.

somehow affect expression of both red and gamma. A partial map of the λ genome is shown in Figure 1. Genes *c*III, red and (*exo* and *beta*), and gamma lie in a single transcription unit to the left of the immunity region. The phenotypes of phages carrying insertions 167 and 169 sugggested that they might carry a Tn10 insertion upstream of these genes. Tn10 insertions are known to be polar in other operons (KLECKNER *et al.* 1975; FOSTER 1977), and an insertion mapping between *N* and *c*III should be totally viable and might be expected to exert a pleiotropic effect on expression of *red* and *gam*.

(2) *Physical mapping of λ::Tn10 insertions*: The six λ::Tn10 derivatives were subjected to electron microscope heteroduplex analysis to determine the approximate positions of the insertions on the phage genome.

The phages were first compared directly with λ *imm*434, a phage totally homologous to wild-type λ except that the immunity region of the related coliphage 434 has been substituted for that of λ. In heteroduplexes between a λ*b*221 *imm*λ Tn10 phage and a λ*imm*434 phage, regions of nonhomology corresponding to the *b*221 deletion, the *imm*434 substitution, and the Tn10 insertion are expected. From the distance of these nonhomologous regions from each other and from the ends of the molecules, the positions of the Tn10 insertions on the physical map of λ were determined. In some of the cases where two insertions seemed to lie near one another, the two were heteroduplexed against each other in order to assess directly their relative positions.

Heteroduplexes of λ::Tn10 phages carrying insertions 167 and 169 directly confirm the predictions of the functional analysis: both of these phages carry insertions in the region immediately to the left of the immunity region as shown in Figure 2a and 2b. Measurements of several molecules place both insertions very near the physical location of the *c*III gene reported by DAVIDSON and SZYBALSKI (1971). A direct comparison between phages carrying insertions 167 and 169 shows that the two insertions are separated by a short but readily measurable distance of approximately 300–400 basepairs and thus are not at the same site.

The physical positions and genetic properties of insertions 167 and 169 are consistent with their lying either within the *c*III gene or just to the right of *c*III,

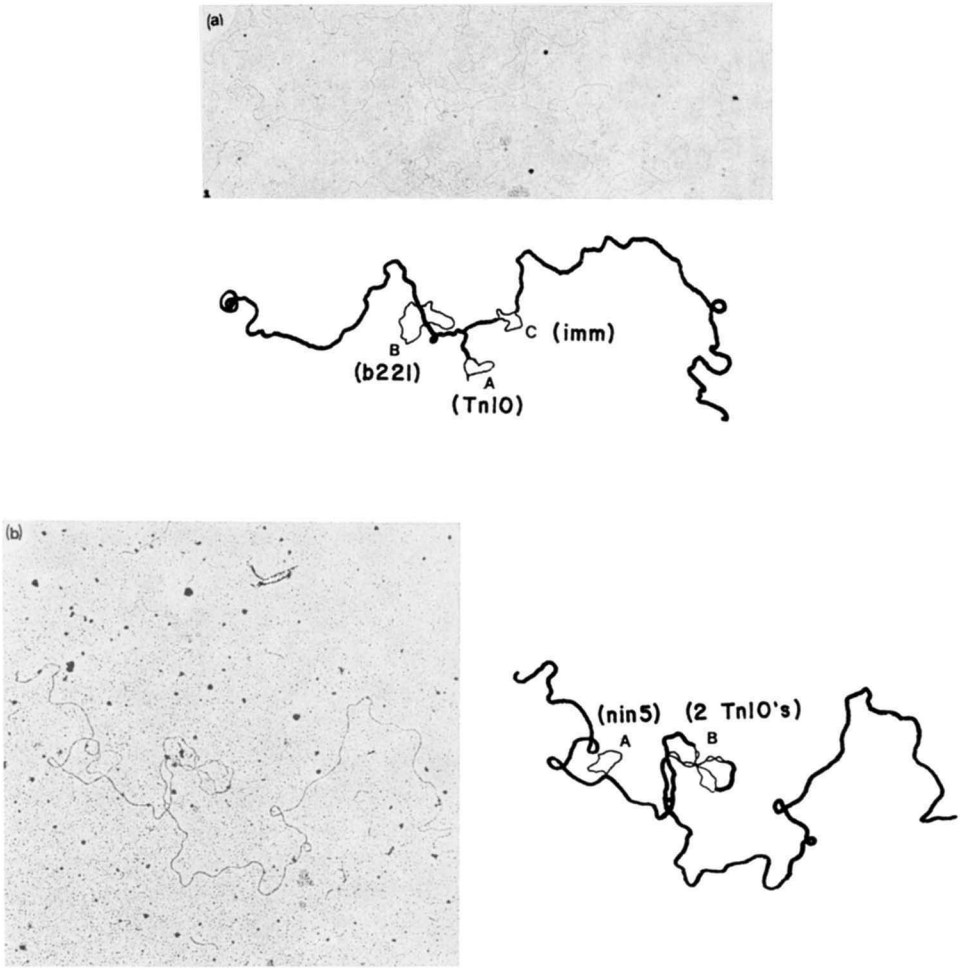


FIGURE 2.—(a) λ*imm*434/λ*b*221 *c*III167::Tn10 heteroduplex
(b) λ*b*221 *c*III167::Tn10 *nin*5/λ*b*221 *c*III169::Tn10 heteroduplex
A = *nin*5 deletion; *B* = pair of Tn10 insertions.

but to the left of *N*. Additional genetic evidence suggests that at least one of these insertions probably lies outside of the *c*III gene itself. Lambda::Tn10 insertion 167 gives rise to *Red*$^+$ *Gam*$^+$ *c*III$^-$ revertants at very high frequencies (10^{-3} in plate lysates). (V. LUNDBLAD, unpublished). Previous experiments with Tn10 insertions in the Salmonella histidine operon have shown that precise excision of Tn10, resulting in *his*$^+$ revertants that have lost the entire Tn10 insertion, is a rare event (10^{-7} to 10^{-10} in a culture). However, at much higher frequencies, *his*::Tn10 insertions give rise to derivatives in which the polar effects of the Tn10 insertion have been alleviated without proper reconstruction of the gene interrupted by the insertion. A similar situation exists for Tn10 insertions in the lambda immunity region: insertions 171 and 173 give rise to imprecise excision

derivatives at high frequencies (10^{-4}) in plate lysates (see below), while precise excision derivatives (c^+ revertants of a *c*I::Tn10 insertion) arise at much lower frequencies (10^{-7} to 10^{-8}, N. KLECKNER, unpublished). We therefore suspect that the *Red*$^+$ *Gam*$^+$ *c*III$^+$ revertants thrown off by insertion 167 at high frequencies are likely to be "polarity relief" imprecise excision derivatives, and hence that the insertion itself lies "upstream" of the *c*III gene rather than within it.

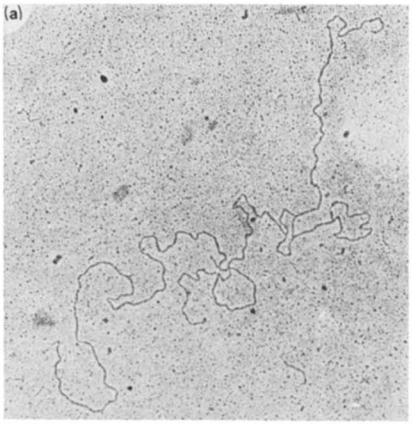
The fact that insertions 167 and 169 exert polar effects on expression of distal genes has an additional interesting implication. Expression of *red*, *gam*, and *c*III from pL is under the influence of λ *N*-function. *N*-function is known to alleviate polar effects of nonsense and insertion mutations in other situations (FRANKLIN 1974; ADHYA, GOTTESMAN and DE CROMBRUGGHE 1974). It is therefore noteworthy that *N*-function does not fully alleviate polarity as conferred by Tn10. However, since expression of *red* and *gam* is not totally abolished (as shown, for example, by residual plating on *recA*$^-$ hosts), it may be that *N*-function partially alleviates Tn10 polarity. Alternatively, *N* may be totally irrelevant to the residual expression observed; such residual expression could instead reflect partial polarity of the Tn10 element itself or the presence within Tn10 of a suitable promoter.

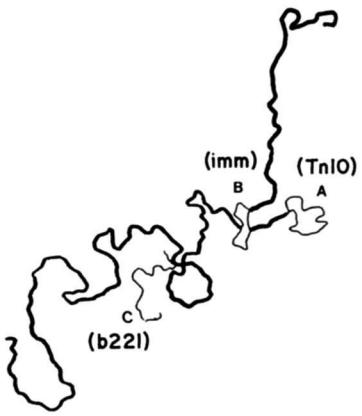
Heteroduplex comparisons between λ *imm*434 and phages carrying the other four insertions revealed (Figure 3) that in each case the Tn10 insertion, with its characteristic stem-and-loop structure, is located within the *imm*λ/*imm*434 substitution bubble; thus each of these insertions lies within the λ immunity region. Superficial examination of these heteroduplexes suggested that insertions 173 and 174 are located in the left half of the immunity region, while insertions 171 and 172 are roughly in the middle of the immunity region.

The positions of these insertions within the immunity region were more precisely determined by analysis with site-specific restriction endonucleases. Insertions 173 and 174 lie well to the left of the structural gene for *c*I; insertions 171 and 172 lie in the distal third of the *c*I structural gene. The orientations of these four insertions with respect to the λ genome have also been determined by restriction enzyme analysis (171, 173 and 174) and heteroduplex analysis (171 and 172, see below). The details of analysis with restriction endonucleases is described in the accompanying APPENDIX. The available information on the physical positions and orientations of these four insertions is summarized in Figure 4.

The position of insertions 171 and 172 within the *c*I gene explains simply their clear-plaque phenotype. The positions of insertions 173 and 174 suggested that they might lie in the *rex* gene, which lies to the left of the *c*I gene in the immunity region. Both insertions 173 and 174 do, in fact, confer Rex$^-$ phenotype

FIGURE 3.—(a) λ*imm*434/λ*b*221 *c*I171::Tn10 heteroduplex
(b) λ*imm*434/λ*b*221 *c*I172::Tn10 heteroduplex
(c) λ*imm*434/λ*b*221 *rex*173::Tn10 heteroduplex
(d) λ*imm*434/λ*b*221 *rex*174::Tn10 heteroduplex
A = Tn10 insertion; B = *imm*434/*imm*λ substitution; C = *b*221 deletion


(a)


(imm)
B
(TnlO)
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C
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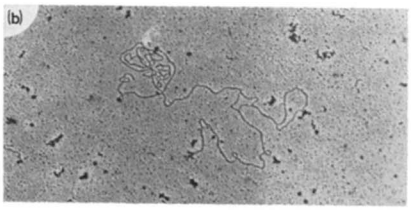
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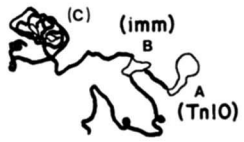
(C)
(imm)
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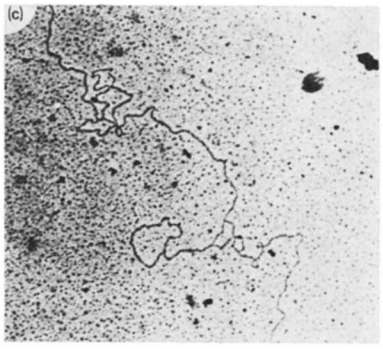
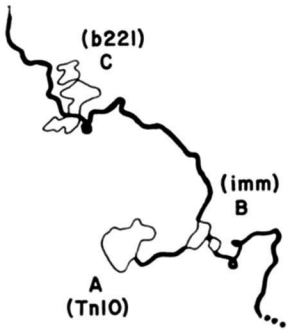

(c)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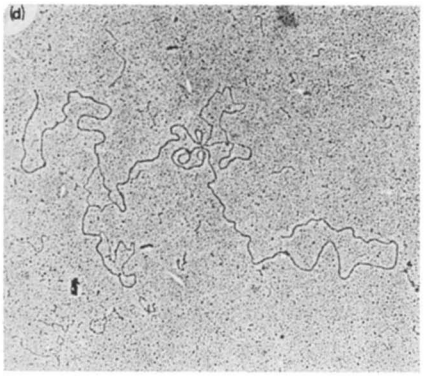
(d)


C
(b221)
(imm)
B
(TnlO)
A

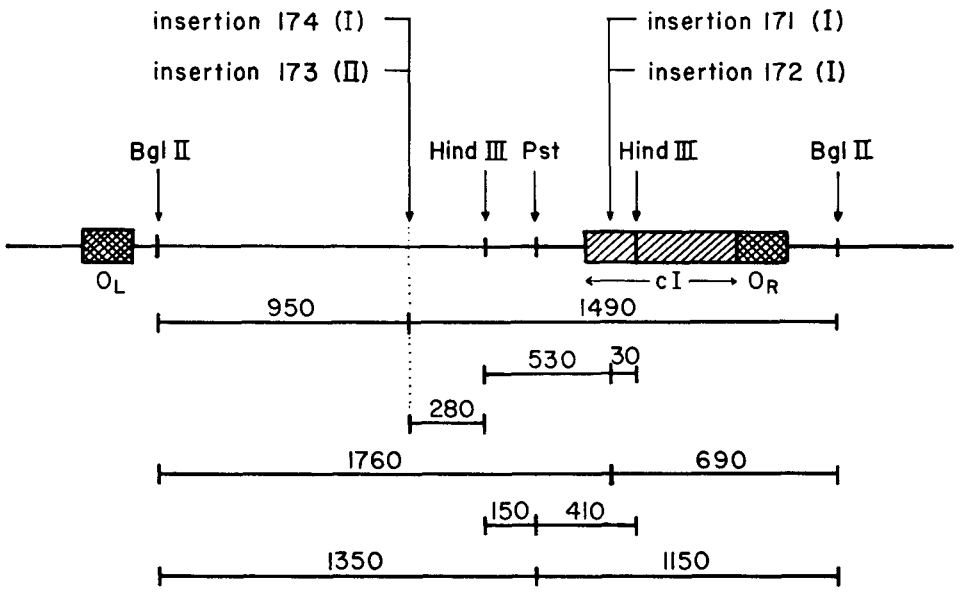

FIGURE 4.—Positions and orientations of Tn10 insertions in λ immunity region. (I) and (II) are opposite orientations. Positions of known restriction endonuclease sites in λ and distances between these sites and the Tn10 insertions are given in base pairs.

(D. Ross, unpublished results). However, the physical position of the *rex* gene is not known accurately, and we cannot yet tell whether the insertions actually lie within the *rex* structural gene or upstream of it, producing Rex$^-$ phenotype by virtue of polar effects.

(3) *Repeated insertion of Tn10 at (or very near) the same position*: Insertions 171 and 172 are two independently isolated Tn10 insertions in the *c*I gene. Direct heteroduplex comparisons between phages carrying these insertions suggest that both insertions lie in the same orientation with respect to the genome and that they lie at or within a few basepairs of the same position in the phage immunity region.

Figure 5 shows micrographs of a number of heteroduplex molecules containing one strand from a phage carrying insertion 171 and one strand from a phage carrying insertion 172. In this experiment, the phage carrying insertion 171 also carries an additional deletion, *nin5*. Heteroduplex molecules 172/171*nin*) can thus be identified by virtue of the *nin5* nonhomology without reference to the insertions themselves. Several features of these heteroduplexes are noteworthy: (a) The two insertions always appear to emanate from the same position in the duplex. We estimate that these experiments would probably have resolved insertions separated by 100 basepairs, but might not have resolved insertions separated by less than this distance. (b) The inserted Tn10 material in the two complementary strands appears to be interacting in an unusual way. These interactions have been described previously (KLECKNER *et al.* 1975) and are thought to arise because of partial pairing between the complementary "loop" portions of

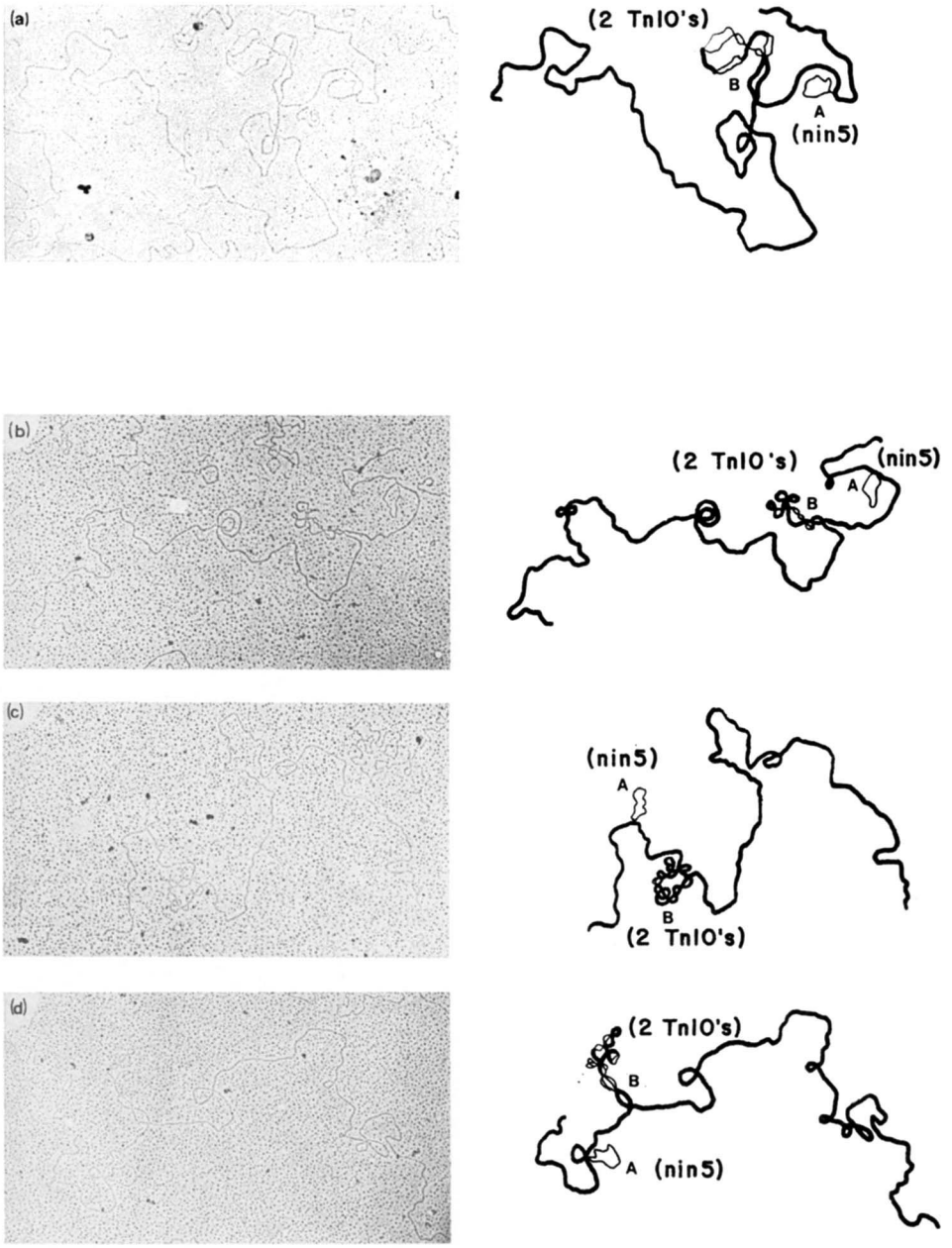

FIGURE 5.—(a) to (d): λ*b*221 *c*I171::Tn10 *nin5*/λ*b*221 *c*I172::Tn10 heteroduplex. Molecules in (b) to (d) exhibit branch migration around the "stems" of the Tn10 insertions.

A = *nin5* deletion; B = pair of Tn10 insertions.

the Tn10 "stem-and-loop" structures on each strand. (Since intra-molecular reannealing of each Tn10 inverted repeat will precede intermolecular reannealing between complementary λ strands, the "loop" portions of each strand will remain free to interact with one another.) If this interpretation is correct, it implies that the Tn10 elements in these phages are both inserted in the same orientation with respect to the λ genome. If they were in opposite orientations, the opposing loops would not be complementary and could not interact. (c) The lengths of the Tn10 "stems" in these heteroduplexes can be measured and compared directly to stem lengths of single-stranded molecules in the same field (or, in a few cases, molecules from the same grid in a different field). These measurements reveal that for several molecules, such as those shown in Figure 5 b-d, the "stems" are substantially shorter than the normal full length (Figure 5a). It appears that in these cases branch migration has occurred at the junction of the two "stems" with the λ duplex. Branch migration of this type is analogous to that described by LEE, DAVIS and DAVIDSON (1970) and BROKER and LEHMAN (1971) and has already been documented for heteroduplex molecules in which both strands carry the same Tn10 insertion (KLECKNER *et al.* 1975).

For heteroduplex molecules in which both strands carry the same Tn10 insertion, branch migration is possible because the two regions of Tn10 on each strand, which are inverted repetitions of each other (and thus form a stem), are not only homologous to each other, but are also homologous to the same region on the other strand of the heteroduplex. Therefore, during branch migration the breakage of two basepairs in the two stems is compensated for by the formation of two basepairs between the two strands of the heteroduplex. Thus, migration within the stem regions is not energetically expensive.

However, if the two strands of a heteroduplex contain Tn10 insertions at different positions, initiation of branch migration would be more difficult, since the breakage of the first n basepairs (where n is the distance between the two insertion points) would first be required before the breakage of stem basepairs can be compensated by formation of interstrand basepairs in the heteroduplex. This consideration leads us to the conclusion that insertions 171 and 172 must be very close to each other if branch migration is observed. Since regions of homology of a few as 20 basepairs are very stable (for example, the cohesive ends of λ: WANG and DAVIDSON 1968) we argue that this might be a conservative upper limit for the distance between the two insertions 171 and 172 which show branch migration. It is certainly clear that the two insertions could not be as much as 150 basepairs apart, since the strand-migrating structures would, in this case, show two visible loops at the ends of the insertions, corresponding to the 150 basepairs that must be broken before migration could begin.

Restriction endonuclease analysis of phages carrying insertions 171 and 172 also shows that these insertions must lie within about 25 basepairs of one another. In addition, restriction endonuclease analysis of phages carrying insertions 173 and 174 suggests that these two insertions also lie very close together, fewer than 50 basepairs apart in this case. The data supporting these conclusions are discussed in the APPENDIX.

Since 2/2 insertions in *c*I and 2/2 insertions in *rex* appear to lie extremely close together, it appears that Tn10 preferentially inserts at certain positions, and that the element can repeatedly insert into the same position in a particular target. This physical evidence is wholly consistent with the results of genetic analysis of Tn10 insertions in the Salmonella *his* operon. 133 independent *his*::Tn10 insertions lie at 22 discrete loci in the *his* operon, a region of about 10,000 basepairs (N. KLECKNER, D. STEELE, K. REICHARDT and D. BOTSTEIN, in preparation).

The fact that insertions 173 and 174 appear to lie at the same position is additionally interesting because these insertions lie in opposite orientations with respect to the phage genome (see above and APPENDIX). This result suggests that the Tn10 element may be able to insert into the same site in both orientations. Genetic analysis of Tn10 insertions at one position in *hisG* suggested that Tn10 could insert in both orientations at that site as well. Taken together, then, these results begin to suggest that the ability to insert at a given position in either orientation is a general property of Tn10 insertion at any position.

(4) *Maintenance of the integrity of Tn10 during translocation*: All of the Tn10 insertions we have isolated in λ are descended from one original Tn10 insertion in P22, P22Tc10. For the phages described thus far, two translocation steps separate the Tn10 element in λ from that in P22. For the insertion in another phage, λ 224, five successive translocation events have occurred since the element was isolated from nature on the drug-resistance (R)-factor R222. These five steps are described in MATERIALS AND METHODS.

In order to determine whether Tn10 normally retains its size and structural integrity during successive cycles of translocation, careful measurements were made of heteroduplexes between λ 173 (carrying insertion 173) and λ 224. Since the Tn10 insertions in these two phages are inserted at different positions in the phage genome, the lengths of the inverted repetitions ("stems") and the intervening nonrepeated "loop" for both insertions can be compared directly in the same heteroduplex molecule. The stem/loop ratios for these insertions can also be compared to the stem/loop ratio obtained for P22Tc10 in earlier experiments under the same conditions. Representative λ 173/λ 224 heteroduplexes are shown in Figure 6; measurements of such heteroduplexes are summarized in Table 4. Within the experimental error of these experiments, the size and structure of Tn10 in the two λ insertions and in P22Tc10 are identical. It appears that Tn10 usually maintains its structural integrity during several successive cycles of translocation.

Phages λ 224 and λ 173 have both been used to generate auxotrophs in *E. coli* in experiments analogous to those described below (data not shown). These experiments demonstrate that the Tn10 element has also retained its functional integrity in these phages.

(5) *Tn10-promoted deletions*: Deletion variants of a given λ phage can be selected by treating phage particles with heat plus a Mg^{++}-chelating agent such as EDTA. When such a selection is carried out on λ::Tn10 phages, an unusual result is obtained, as shown in Table 5. Prior to selective treatment, a stock of

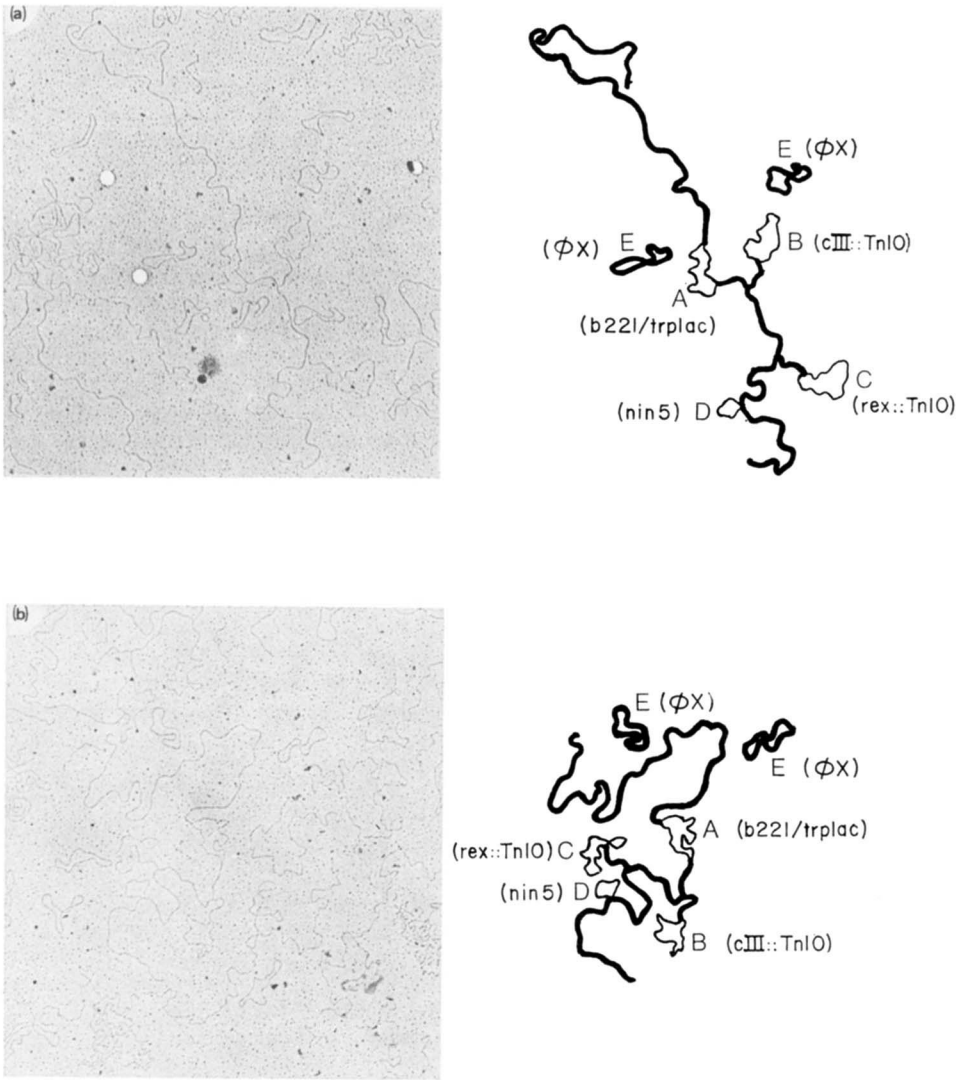

FIGURE 6.—(a) and (b) are two examples of heteroduplexes between λ173/λ224. λ173 is λ*b*221 *rex*173::Tn10 *c*I857 and λ224 is λ*trp lac*W205 *c*III224::Tn10 *c*I857 *nin*5. A indicates the *b*221/*trp lac*W205 substitution loop, B the *c*III224::Tn10 insertion, C the *rex*173::Tn10 insertion, D the *nin*5 deletion loop, and E the φX174 RF DNA standard.

λ::Tn10 phages contains fewer than 1% tetracycline-sensitive derivatives (Table 5 and unpublished). Among survivors of selective treatment, the proportion of tetracycline-sensitive phages is roughly 30%. This result suggested that many of the tetracycline-sensitive derivatives present in such a stock are in fact deletions. *Tet*-sensitive derivatives occur at frequencies of 1–6 × 10^{-4}, well above the expected frequency for point mutations. The deletional nature of several variants was confirmed directly by heteroduplex analysis, as shown in Figure 7. All three

TABLE 4

Measurements of representative λ173/λ224 heteroduplexes

Insertion	[A] Stem length(kb)	[B] Loop length(kb)	Total length of Tn10 insertion ([B] + 2[A])	Ratio loop:stem ([B]/[A])
λ *c*III224::Tn10	1.39 ± 0.02	6.56 ± 0.14	9.34 ± 0.18	4.72
λ *rex*173::Tn10	1.38 ± 0.03	6.57 ± 0.09	9.33 ± 0.15	4.76
P22 Tc10::Tn10	1.41 ± 0.02	6.62 ± 0.5	9.44 ± 0.54	4.69

Each length determination is based on measurement of 15–23 molecules.
For λ::Tn10 phages: All stem measurements are relative to φX174 RF DNA; all loop measurements are relative to φX174 SS DNA. φX174 DNA has a length of exactly 5.375 kb (SANGER *et al.* 1977).
For P22::Tn10 phage: All measurements are relative to P22 DNA, which was in turn measured relative to φX174 SS DNA and corrected for length difference between single and double-stranded φX174 DNA under similar conditions. Data from TYE, CHAN and BOTSTEIN (1974).
Abbreviations used: kb, kilobases; RF, double-stranded replicative form of φX174 DNA; SS, single-stranded virion DNA of φX174.

of the variants tested remove substantial portions of Tn10 material; two of them appear to remove portions of the lambda genome as well.

It is likely that the formation of these deletions is in some way promoted by the Tn10 element itself. The strongest argument for this view is the high frequency with which these deletions arise. Comparable experiments in two different laboratories (HENDERSON and WEIL 1975; PARKINSON and HUSKEY 1971) have established that the normal frequency of deletion mutants in λ is 5×10^{-6} or less, roughly 100-fold lower than that observed with λ::Tn10 phages. The argument for Tn10-generation of these deletions is also strengthened by observations in other systems where Tn10 has been shown to generate deletions.

(6) *Use of λ::Tn10 phages to generate Tn10 insertions in the* E. coli *chromosome*: Translocation of Tn10 from the genome of Salmonella phage P22 to the chromosome of Salmonella has been described previously (KLECKNER *et al.*

TABLE 5

Selection for Tn10-promoted deletions by heat plus EDTA

Phage vehicle	Insertion	w/o EDTA *tet*^{s}/Total	After EDTA EDTA^R/Total	After EDTA *tet*^{s} EDTA^R/EDTA^R	After EDTA *tet*^{s} EDTA^R/Total
*b*221 *c*I857 *ind*+	171	nt	3.5×10^{-3}	55/115	1.5×10^{-4}
*b*221 *c*I857 *ind*+	172	nt	1.6×10^{-3}	24/87	4.4×10^{-4}
*b*221 *c*I857 *ind*+	173	nt	2.2×10^{-3}	29/101	6.3×10^{-4}
*b*221 *c*I857 *ind*+	174	0/131	1.6×10^{-3}	20/96	3.3×10^{-4}

A single plaque of each phage was suspended in 1 ml of SM buffer + chloroform and titered. The suspension was then diluted 1/20 into 0.01 M EDTA prewarmed to 42°. After incubation for 20 min at 42°, $MgSO_4$ was added to a final concentration of 0.1 M and the suspension cooled and plated on NK5012. Plaque-forming survivors were then tested for presence of the *tet*^{R} determinant (see MATERIALS AND METHODS).

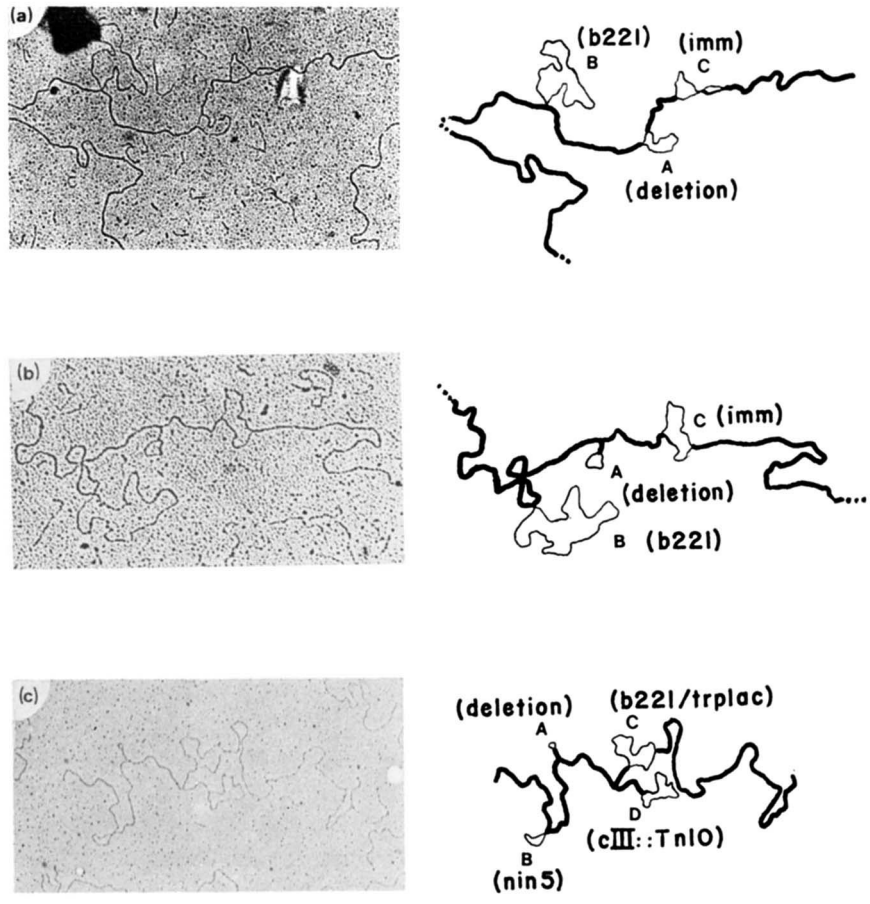

FIGURE 7.—Tn10-promoted deletions of lambda::Tn10 phages

Deletions of λ*b*221 *c*I857*ind*+ Tn10 phages were isolated by the procedure described in Table 5.

(a) λ*b*221 *c*III167::Tn10 *del*-DB1/λ *imm*434 heteroduplex
A = Deletion of material in and around Tn10 insertion; B = *b*221 deletion; C = *imm*434/*imm*λ substitution.

(b) λ*b*221 *c*III167::Tn10 *del*-DB2/λ*imm*434 heteroduplex
A = deletion within Tn10 element; B = *b*221 deletion; C = *imm*434/*imm*λ substitution.

(c) λ*b*221 *rex*173::Tn10 *del*-KR267/λ *trp lac*W205 *c*III224::Tn10 *nin*5 heteroduplex
A = deletion within *rex*173::Tn10 element; B = *nin*5 deletion; C = *b*221 *trp lac*W205 substitution; D = *c*III224::Tn10 insertion.

1975; KLECKNER, ROTH and BOTSTEIN 1977). It is accomplished by selecting tetracycline-resistant bacteria following infection of a suitable bacterial host with a P22::Tn10 phage under conditions where the phage genome cannot perpetuate itself and does not kill the host cell. The results of analogous experiments using an *O*am derivative of λ 167 and an *E. coli* host are shown in Table 6. As in

TABLE 6

Translocation of Tn10 from λ to the E. coli *chromosome*

Experiment	Bacterial host	*b221 cI857 ind+* phage vehicle	Multiplicity of infection	*tet*^{R} "transductants"/ infecting phage	*tet*^{R} auxotrophs/total *tet*^{R} "transductants"
1	NK5324 *su⁻recA+*	*c*III167::Tn10Oam29	10	1.3×10^{-7}	nt
2	NK5019 *su⁻recA+*	*c*III167::Tn10Oam29	1	5×10^{-7}	43/6314 = 0.7%
3a	NK5002 *su⁻recA+*	*c*III167::Tn10Oam29	1	3.5×10^{-7}	14/670 = 1.6%
3b	NK5003 *su⁻recA⁻*	*c*III167::Tn10Oam29	1	8.7×10^{-7}	39/2486 = 1.6%
3c	NK5003 *su⁻recA⁻*	*c*III167::Tn10Oam29	0.2	1×10^{-6}	14/755 = 1.8%
3d	NK5003 *su⁻recA⁻*	*c*III167::Tn10Ots21	1	1.4×10^{-6}	6/677 = 0.8%
3e	NK5003 *su⁻recA⁻*	*c*III167::Tn10Ots21	0.2	2.6×10^{-6}	12/1018 = 1.2%

The bacterial host was grown to 5×10^8 per ml in λym broth. Phage were added in a small volume of SM buffer to give the desired multiplicity of infection, and the mixture incubated at 40° for 60 minutes (experiments 2 and 3) or 37° for 45 minutes (experiment 1) to allow time for phage adsorption and for expression of tetracycline resistance. The mixture was then plated on λ plates containing either tetracycline at 50 μg/ml or tetracycline at 15 μg/ml plus 0.0025 M sodium pyrophosphate (equivalent results are obtained with either method). Plates were then incubated at 40° for 24 to 36 hours. Auxotrophs were identified by replica-plating the resulting colonies onto λ plates and minimal glucose plates both of which contained 15 μg/ml tetracycline. Experiment 1 was performed by D. GALBREATH.

Salmonella, the frequency of translocation is quite low, approximately one translocation event per 5×10^7 infecting phage genomes; the frequency of translocation is independent of the multiplicity of infection used, and is the same in *recA*$^+$ and *recA*$^-$ hosts.

The assumption that tetracycline-resistant bacteria have acquired insertions of Tn10 within the bacterial chromosome is confirmed by the finding that approximately 1% of these bacteria have also acquired new auxotrophic insertion mutations. As in Salmonella, a wide variety of different auxotrophic mutations can be isolated from such an experiment. In one such experiment, the following distribution of 43 independent auxotrophs was obtained: seven phenylalanine, six arginine, four tryptophan, three proline, two guanine, two methionine, two thiamin, two histidine, one leucine, one purine, one lysine, one tyrosine, one glutamate and ten not identified.

A number of these tetracycline-resistant auxotrophs were further examined to confirm that they arose by insertion of Tn10 within a structural gene and to examine certain features of the translocation process. If these mutations have arisen by insertion of Tn10 in such a way that the structural integrity of a gene has been damaged, then restoration of structural gene function in such a situation should inevitably be accompanied by excision of the Tn10 element and concomitant lost of the tetracycline-resistance determinant. Table 7 shows the results of reversion tests on seven independent insertion auxotrophs. The vast majority of all prototrophic revertants obtained from these strains are tetracycline sensitive, confirming that the mutations arose by insertion of Tn10.

The fact that six of seven auxotrophic insertion mutations reverted to prototrophy confirms another important feature of Tn10 translocation as described in

TABLE 7

Revertant frequencies of seven independent insertion auxotrophs

Strain†	Auxotrophic requirement	Reversion* frequency	tet-sensitive revertants / total revertants
5121	methionine	1.6×10^{-9}	3/3
5123	leucine	1.5×10^{-9}	2/2
5135	tryptophan	5×10^{-6}	41/41
5145	tryptophan	5.3×10^{-7}	36/36
5146	histidine	1.6×10^{-7}	153/156
5151	tryptophan	1×10^{-6}	2/2
5167	arginine	$<5.3 \times 10^{-10}$	—
		Total	237/240

* Based on analysis of five to ten independent clones of each strain.
† All strains obtained by isolation of chromosomal Tn10 insertions in NK5019, as described in Table 6.

Salmonella: insertion of Tn10 is precise. That is, insertion of Tn10 has not resulted in any damage to the target chromosome at the site of insertion that is massive enough to preclude subsequent reconstruction of a functional gene. As in Salmonella, reversion frequencies are low, ranging from 5×10^{-6} to $<10^{-9}$ in a saturated culture.

Insertions of the Tn10 element within the bacterial chromosome can be very useful tools for performing genetic analysis of bacteria (see discussion in KLECKNER, ROTH and BOTSTEIN 1977). In performing such manipulations, it is often

TABLE 8

P1-mediated transduction of Tn10

Donor strain	P1 phage	Lysate No.	tet^R transductants Proto	Auxo	Total	Frequency prototrophic transductants
Nk5133=*his*::Tn10	*clr*100 Cm	1	21	15	36	0.58
Nk5133=*his*::Tn10	*clr*100 Cm	2	14	27	41	0.34
Nk5133=*his*::Tn10	*clr*100 Km	1	19	19	38	0.5
Nk5133=*his*::Tn10	*clr*100 Km	2	21	14	35	0.6
Nk5133=*his*::Tn10	*clr*100 Km	3	21	25	46	0.46
NK5151=*trp*::Tn10	*clr*100 Cm	1	0	11	11	<0.09
NK5151=*trp*::Tn10	*clr*100 Cm	2	6	25	31	0.19
NK5151=*trp*::Tn10	*clr*100 Cm	3	5	35	40	0.12
NK5151=*trp*::Tn10	*clr*100 Km	1	7	37	44	0.16
NK5151=*trp*::Tn10	*clr*100 Km	2	3	28	31	0.10
NK5151=*trp*::Tn10	*clr*100 Km	3	3	33	36	0.08

P1 lysates were made by heat induction of appropriate lysogens. Transductants were obtained by mixing 0.02 ml of phage lysate with 0.1 ml of NK5317 in late exponential phase, adsorbing for 60 minutes at 32° in the presence of 0.005 M $CaCl_2$ and plating at 32° on LB plates containing 15 μg/ml tetracycline and no added Ca^{++}. Transductants were then tested for growth on LB tetracycline, minimal glucose tetracycline plates supplemented with histidine (NK5133) or tryptophan (NK5151).

convenient to move a Tn10 insertion from one strain to another by phage-mediated generalized transduction. In the case of *E. coli*, phage P1 is frequently employed for this purpose.

In the simplest case, one would expect that transduction of the tetracycline-resistance element to a new strain would inevitably be accompanied by co-transduction of the associated auxotrophic insertion mutation. This appears to be the case for P22-mediated transduction of Tn10 mutations in Salmonella (KLECKNER *et al.* 1975). The data in Table 8, however, show that this need not be the case in P1-mediated transduction in *E. coli*. In some cases, in fact, the majority of tetracycline-resistant transductants are not auxotrophs. Since reversion analysis strongly argues that the original auxotrophic mutations have in fact arisen by insertion of Tn10, we believe that these unusual results reflect features of P1-mediated transduction, rather than aberrant behavior of the Tn10 element in *E. coli*. The basis of these results is still under study; however, this problem is one that must be properly anticipated during use of Tn10 (and other translocatable drug resistance elements) in *E. coli*. Recently L. CSONKA (personal communication) has found that some P1 derivatives transduce Tn10 insertion mutations faithfully.

DISCUSSION AND SUMMARY

The results presented above demonstrate that insertions of Tn10 into the genome of phage λ can readily be isolated, and that λ::Tn10 phages can be used as vehicles for the isolation of Tn10 insertions in the *E. coli* chromosome. Several other features of Tn10 translocation have also been documented:

(1) The Tn10 element usually maintains both its physical and functional integrity through successive cycles of translocation. One particular Tn10 element was shown to have retained its integrity after five successive cycles of translocation.

(2) Insertions of Tn10 in or near the *c*III gene exert polar effects on the expression of the promoter-distal genes *red* and *gam*. Both insertions tested lie in the same orientation relative to the phage genome, although the actual orientations relative to insertions 171, 172, 173 and 174 have not been determined (KLECKNER, unpublished). Since expression of the *c*III-*red-gam* region normally occurs under the influence of phage *N*-function, and since *N*-function has been shown to alleviate the polar effects of nonsense and insertion mutations in other systems (FRANKLIN 1974; ADHYA, GOTTESMAN and DECROMBRUGGHE 1974) it is noteworthy that Tn10-mediated polarity is still observed in this situation. Further experiments will be required to determine whether the observed low-level residual expression of *red* and *gam* originates from the normal phage promoter, pL, or from promoter(s) within Tn10 and to determine whether or not this expression is sensitive to *N*-function.

(3) Two sets of Tn10 insertions in the immunity region affect gene expression there. Two insertions in the *c*I gene abolish *c*I expression; the effects of these insertions on the distal gene *rex* have not been tested. Two other insertions in the

immunity region lie distal to the *c*I gene and abolish *rex* function. They probably lie within the *rex* gene, although it is still possible that they lie just "upstream" of *rex* and exert a polar effect on *rex* expression.

Certain properties of the *rex*::Tn10 insertions 173 and 174 have yet to be explained. Despite the fact that these insertions unambiguously lie well to the left of the *c*I structural gene, and phages carrying them make turbid plaques and stable lysogens at temperatures below 37°, phages carrying insertions 173 and 174 make clear plaques at 37°, while the non-Tn10-carrying parent phage makes turbid plaques at this temperature. Apparently these insertions affect the ability of the phage to establish repression at this critical temperature, which is the highest temperature at which the non-Tn10 parent still makes a turbid plaque.

This unusual phenotype is not simply a consequence of the *rex*$^-$ phenotype conferred by insertion. Deletion derivatives of the λ *rex*173::Tn10 *c*I857 phage have been isolated, and they have regained the plating properties of the non-Tn10 parent, but are still *rex*$^-$. It seems likely, therefore, that the unusual phenotype is a direct consequence of the presence and/or position of the Tn10 insertion itself. In this connection it should be noted that the unusual phenotype does not depend on the orientation of the *rex*::Tn10 insertion, since insertions 173 and 174 lie in opposite orientations, but confer indistinguishable phenotypes.

There is no obvious explanation for the effects of insertions 173 and 174 on plaque phenotype. One possibility is that the insertions exert some type of "antipolar" effect on expression of the nearby (albeit "upstream") *c*I gene. Alternatively, the insertions might conceivably be affecting expression of other λ genes which influence the decision between lysis and lysogeny.

(4) The two insertions in *c*I appear to lie within a few base pairs of one another in the same orientation. The two insertions in or near *rex* lie in opposite orientations within 50 basepairs of one another. These results strongly suggest that Tn10 insertion is nonrandom along the λ genome, *i.e.*, Tn10 prefers to insert at particular positions ("sites"). If the two *rex*::Tn10 insertions lie at the same site, these results suggest that Tn10 is capable of inserting at a single site at either orientation. Genetic analysis of Tn10 insertions in the Salmonella *his* operon has also shown a strong Tn10 preference for insertion at particular positions and has shown that insertions at one such position lie in both orientations. Taken together, these results suggest that insertion in either orientation at particular sites may be a general property of Tn10.

(5) Experiments with λ::Tn10 phages provide additional evidence that the Tn10 element can promote the formation of deletions.

(6) Translocation of Tn10 from the genome of λ to the *E. coli* chromosome is qualitatively and quantitatively the same as translocation of Tn10 from the genome of Salmonella phage P22 to the chromosome of *Salmonella typhimurium*. In particular, translocation from the λ genome to the *E. coli* chromosome occurs at low frequency, is independent of *recA* function, and produces approximately 1% auxotrophic insertion mutations. As previously noted in Salmonella, insertion of Tn10 in *E. coli* is usually precise with respect to the target chromosome.

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APPENDIX

RESTRICTION ENZYME ANALYSIS OF Tn10 INSERTIONS IN THE IMMUNITY REGION OF BACTERIOPHAGE LAMBDA

Four λ::Tn10 phages carrying Tn10 insertions in the phage immunity region were subjected to restriction enzyme analysis with 14 different sequence-specific endonucleases ("restriction enzymes"). From analysis of the results, we have determined the positions and orientations of these four Tn10 insertions with respect to known endonuclease cleavage sites in the λ genome and have also determined the positions of certain cleavage sites within the Tn10 element itself. This information is summarized in Figures 8 and 9 and Table 9. The results strongly suggest that Tn10 preferentially inserts at particular sites and that Tn10 can insert in either orientation at a given site.

The properties of the four phages used are described fully in the text and are summarized briefly in Table 10. DNA from the relevant phages was digested using restriction enzymes singly or in pairs; the resulting fragments were displayed on agarose gels stained with ethidium bromide. The methods used are those of SUGDEN *et al.* (1975) and ENDOW and ROBERTS (1977); these methods are briefly described in the legend to Figure 10. All enzymes were from the collection of R. J. ROBERTS.

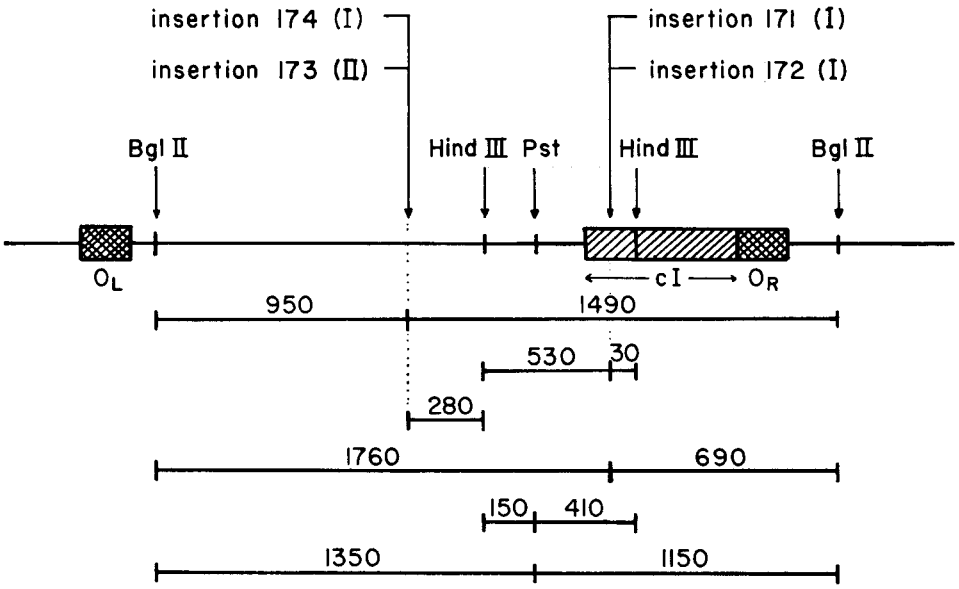

FIGURE 8.—Positions and orientations of four λ::Tn10 insertions with respect to known restriction enzyme cleavage sites in the λ immunity region. Positions of sites in λ are known from ALLET and BUKHARI (1975), SMITH, BLATTNER and DAVIES (1976) and PTASHNE and co-workers (unpublished). All of the distances shown are those determined in this work. The positions of each of these λ sites can, in fact, be independently derived solely on the basis of experiments described here.

TABLE 9

Endonuclease cleavage sites in λ+ and Tn10

Enzyme	*Number of cleavage sites in λ+	Number of cleavage sites in Tn10
Bal	15	0
Bam	5	1
*Bgl*I	22	1
*Bgl*II	5	2
Eco RI	5	1
*Hin*dIII	6	3
*Hpa*I	11	3
Kpn	2	0
Pst	18	0
*Sal*I	2	0
*Sst*II	0	2
*Acc*I	0	3
*Xho*I	1	0
*Xma*I	3	0

The properties of these enzymes are reviewed in ROBERTS (1977).

* These numbers are provided to give an idea of the relative degrees of specificity of these enzymes. The number of cleavage sites in λ+ is generally larger than the number in the non-Tn10-containing λ parent used in this work, which carries a deletion of 18% of the λ genome (*b*221).

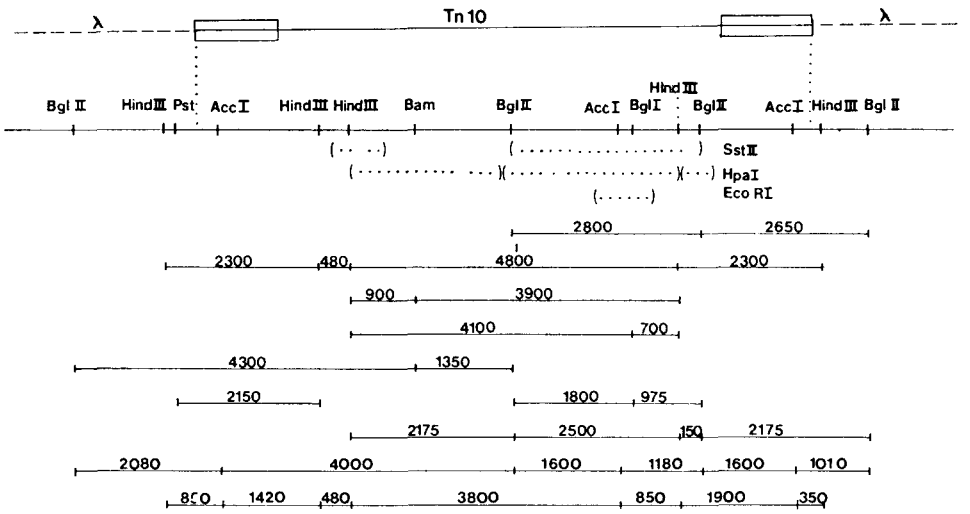

FIGURE 9.—Positions of restriction enzyme cleavage sites within the Tn10 element and the surrounding λ immunity region of λ *b*221 *c*I857 *c*I171::Tn10. Distances given are the lengths of fragments obtained in digests using the appropriate enzyme or pair of enzymes. All lengths were determined by comparison to fragments of known size present in each gel. In most experiments, size markers were *Eco* RI fragments of adenovirus DNA of 4270, 3605, 2625, 2205, and 1820 base pairs in length. In *Acc*I/*Bgl*II and *Acc*I/*Hin*dIII digests, the size markers are a mixture of *Acc*I and *Hae*III fragments of φX174 of 3030, 2345, 1342, 1078, 872, and 606 basepairs in length. Relative to these standards, the lengths of unknown fragments between 500 and 4000 base pairs can generally be determined to an accuracy of ± 5%.

Throughout this analysis, all references to "left" or "right" refer to the physical maps of λ and of Tn10 shown in Figures 8 and 9.

Approximate positions of each Tn10 insertion in lambda

The first step in this analysis was to determine the approximate positions of each of the four Tn10 insertions with respect to known cleavage sites in the immunity region. For any particular enzyme or pair of enzymes, a direct comparison of the digestion patterns obtained from a λ::Tn10 phage and from its non-Tn10-containing parent immediately reveals which of the normal λ

TABLE 10

Properties of the four λ::*Tn10 phages*

Strain	Genotype	Name of insertion	Location of insertion	Phenotype*	Orientation of† insertion
λ171	*b*221 *c*I857 *c*I171::Tn10	171	*c*I	*c*I⁻ all temperatures	I
λ172	*b*221 *c*I857 *c*I172::Tn10	172	*c*I	*c*I⁻ all temperatures	I
λ173	*b*221 *c*I857 *rex*173::Tn10	173	*rex*	*c*I ts *rex*⁻	II
λ174	*b*221 *c*I857 *rex*174::Tn10	174	*rex*	*c*I ts *rex*⁻	I

* *c*I857 renders the *c*I product thermolabile; phages carrying *c*I857 as the only *c*I mutation make clear plaques at temperatures above 37° and turbid plaques at temperatures below 37°.
† As determined here.

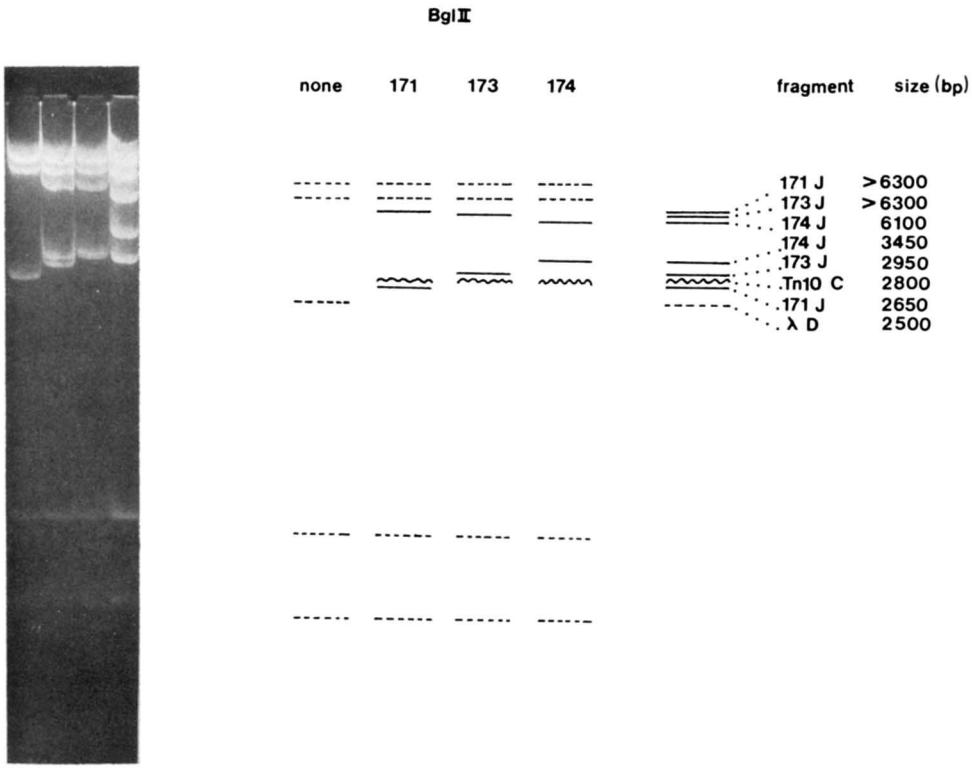

FIGURE 10.—*Bgl*II digests of λ *b*221 *c*I857 derivatives carrying no Tn10 insertion, or insertions 171, 173, and 174. ----- λ fragments: contain only λ sequences ~~~~ Tn10 "common" fragments: contain only Tn10 sequences. ——— Junction fragments: contain both Tn10 and λ sequences. D = λ donor fragment destroyed by Tn10 insertion.

DNA was extracted from intact phage particles by three successive extractions with cold phenol equilibrated in 0.01 M Tris pH 7.5 and 0.001 M EDTA. Phenol extraction was followed by several extractions with ether and overnight dialysis against 0.01 M Tris pH 7.5 and EDTA 0.001 M. Standard enzyme reaction mixture contained 2 μg DNA; 0.0006 M Tris pH 7.9, 0.006 M $MgCl_2$, 0.006 M β-mercaptoethanol, and a suitable amount of enzyme in a total volume of 50 μl. Reactions were carried out at 37° for one to 16 hours as needed. Reactions were stopped by addition of EDTA to 0.01 M.

In most experiments, the mixture was also heated to 65° for 10 min and rapidly cooled in an ice/water mixture to eliminate re-annealing of fragments containing the λ sticky ends. Reactions were layered onto 1.4% agarose slab gels made and run in 0.04 M Tris, 0.001 M EDTA, and 0.05 Sodium acetate pH 7.8. Details of the gel procedures are given in SUGDEN *et al.* (1975).

fragments carries the Tn10 insertion: since the integrity of that fragment has been destroyed by the insertion of new sequences, that fragment will be present in the digest of the parent and absent in the digest of the λ::Tn10 derivative. Comparisons of this kind were carried out using the enzymes *Bgl*II, *Hind*III, and *Pst*. The results are shown in Figure 8 and are based on the following observations:

(1) Each of the four insertions destroys a 2500 bp λ *Bgl*II fragment that contains most of the λ immunity region (Figure 10).

(2) Insertions 171 and 172 destroy a known 560 bp λ *Hind*III fragment from the immunity region (Figure 11). Insertions 173 and 174 are known to lie to the left of this position from

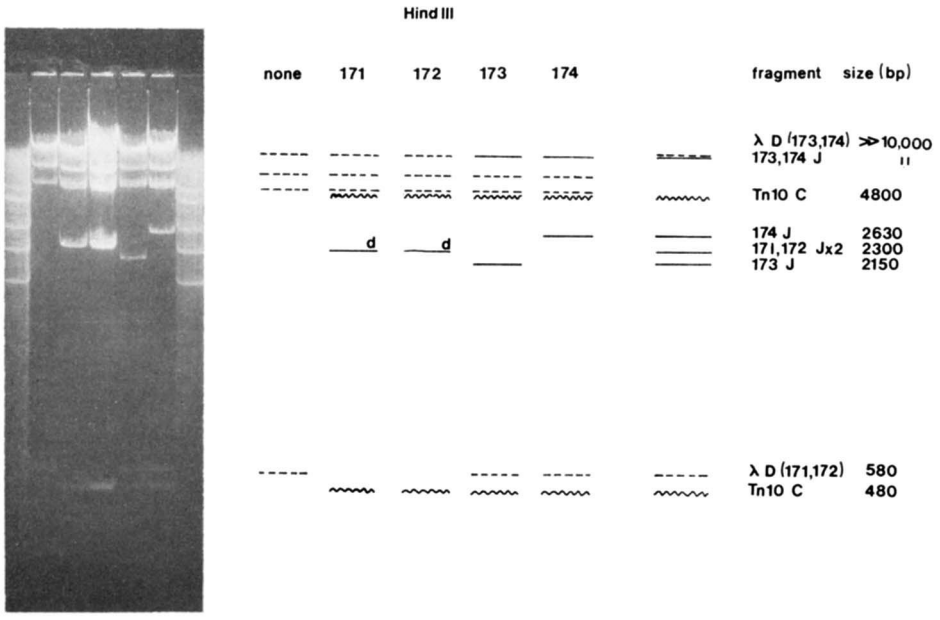

FIGURE 11.—*Hind*III digests of λ *b*221 *c*I857 derivatives carrying no Tn10 insertion or insertions 171, 172, 173, or 174. Size markers shown in end slots are 4270, 3605, 2625, and 1820 basepairs. ----- λ fragments; ~~~~ Tn10 common fragments; ——— λ/Tn10 junction fragments; D = λ donor fragment; d = "doublet" band where two fragments co-migrate.

electron microscopy (KLECKNER, ROTH and BOTSTEIN 1977) and are presumed to lie within the very large λ *Hin*dIII fragment that contains the entire left end of the λ genome. Since the Tn10 element itself contains *Hin*dIII cleavage sites, cleavage of a λ genome carrying Tn10 inserted very near the end of the large λ *Hin*dIII fragment would generate a new large fragment whose mobility would not be distinguishable from that of the original λ fragment; the data are in accord with this prediction (Figure 11).

(3) The 2500 bp λ *Bgl*II fragment is cleaved once by *Pst*. Insertions 173 and 174 are in a 1350 bp λ *Bgl*II/*Pst* fragment; insertion 171 is in a 1150 bp λ *Bgl*II/*Pst* fragment (Figure 12). Although the relative positions of these two λ fragments within immunity was not previously known, we can infer that the 1350 bp fragment lies to the left, and the 1150 bp fragment lies to the right on the basis of the heteroduplex analysis showing that insertions 173 and 174 lie to the left of insertions 171 and 172. We conclude, therefore, that insertions 173 and 174 lie to the left of the *Pst* site in the λ immunity region, and that insertion 171 lies to the right of the *Pst* site. These assignments are consistent with *Hin*dIII mapping cited above.

(4) This analysis places insertion 171 to the right of the *Pst* site and to the left of the rightmost *Hin*dIII site in the λ immunity region. This assignment has been confirmed by *Hin*dIII/*Pst* double digests: insertion 171 destroys a 410 bp *Hin*dIII/*Pst* λ fragment (data not shown).

Orientations of Tn10 insertions and cleavage sites within Tn10

In order to determine the orientations of the four Tn10 insertions, it was necessary to determine a certain number of restriction enzyme cleavage sites within the Tn10 element itself. The principles by which Tn10 cleavage sites were analyzed and the relevant data are described below.

Mapping principles: For any given restriction enzyme or combination of enzymes, a comparison between digests of a λ::Tn10 phage and its parent will also reveal fragments that are present only in the digest of the Tn10 phage. These fragments presumably all contain Tn10 sequences, and will be of several types:

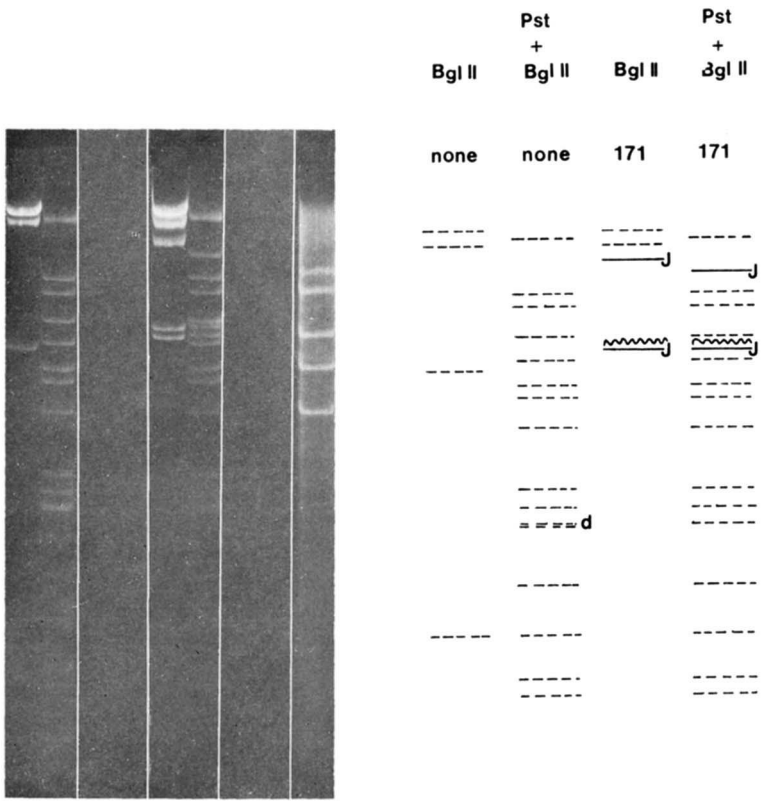

FIGURE 12.—*Bgl*II and *Bgl*II/*Pst* digests of λ *b*221 *c*I857 derivatives carrying no Tn10 insertion or insertion 171. ------ λ fragments; ~~~~~ Tn10 common fragments; ——— λ/Tn10 junction fragments; d = doublet band.

(1) If the enzyme in question does not cleave Tn10, only one new Tn10-specific fragment will be seen; this fragment will be larger than the original λ fragment affected by the insertion by an amount equal to the size of the Tn10 element, roughly 9300 base pairs.

(2) If the enzyme in question does cleave Tn10, some of the Tn10-specific fragments will carry both λ and Tn10 sequences. These fragments contain the junctions between Tn10 and the λ genome. There should be two and only two such junction fregments as long as Tn10 has inserted into the λ genome as a single discrete unit.

(3) If the enzyme in question cleaves Tn10 more than once, fragments containing only Tn10 sequences will be generated. The number of such fragments generated by any given enzyme(s) will be one less than the number of cleavage sites for that enzyme within the Tn10 element.

Internal fragments containing only Tn10 sequences can be distinguished from "junction" fragments. Since the lengths of the junction fragments depend on the position of the Tn10 element with respect to cleavage sites in the λ genome, digestion of DNAs from different Tn10 insertions will yield junction fragments of different sizes. By contrast, fragments containing only Tn10 sequences will be present at the same position in digests of any λ::Tn10 phage regardless of the position of the Tn10 insertion. "Common" internal fragments and position-specific "junction" fragments can be distinguished by comparing analogous digests of two or more different λ::Tn10 phages carrying insertions in different positions.

The positions of various restriction enzyme cleavage sites within Tn10 and the relative orientations of different insertions with respect to the λ genome have been determined by iden-

tifying and determining the lengths of "common" and "junction" fragments from various single and double digests of λ::Tn10 phages.

All of these approaches make the assumption that the Tn10 insertions in independently isolated λ::Tn10 phages are identical in nucleotide sequence. Since the analysis described here gave completely consistent results after analysis of several different insertions with many different restriction enzymes, we have every reason to believe that this assumption is correct.

Orientations of Tn10 insertions: The orientations of insertions 171, 173, and 174 were deducted on the basis of digests with enzymes *Bgl*II and *Pst*.

*Bgl*II cleaves Tn10 at two sites to produce one 2800 bp common fragment (Figure 10). *Pst* does not cleave Tn10 (Figure 12). *Bgl*II digestion of phage carrying insertion 171 produces two junction fragments of 2650 and greater than 6300 base pairs. A double digest of the same phage with *Bgl*II plus *Pst* reveals that the larger of these two junction fragments is cleaved by *Pst* (Figure 12). Since *Pst* does not cleave Tn10, this cleavage must occur at a *Pst* site in lambda. In particular, it must occur at the *Pst* site in the middle of the immunity region because all other *Pst* sites in the λ lie outside of the two *Bgl*II sites in the immunity region. Since we know (see above) that insertion 171 lies to the right of this *Pst* site, we can conclude that the larger *Bgl*II junction fragment on insertion D carries the left junction of Tn10 and λ sequences while the smaller junction fragment contains the right junction. This establishes the positions of *Bgl*II cleavage sites in the Tn10 sequences of insertion 171 as shown in Figure 9 and thereby defines an orientation for this particular insertion with respect to the λ genome, which we will call orientation I.

Since electron microscope heteroduplex evidence suggested that insertions 171 and 172 are in the same orientation, we conclude that insertion 172 is also in orientation I.

Analogous arguments can be used to determine the orientations of insertions 173 and 174. *Bgl*II cleavage of insertion 173 produces λ/Tn10 junction fragments of 2950 bp and greater than 6300 bp, and the larger of these two junction fragments is cleaved by *Pst* (data not shown). Since we have previously determined that insertion 173 lies to the left of the λ *Pst* cleavage site, this result implies that the larger junction fragment contains the right junction, and the smaller junction fragment the left junction. Since the distance between insertions 171 and 173 is small compared to the difference in size of the large and small junction fragments, these results permit us to place insertion 173 in orientation II. *Bgl*II cleavage *of* insertion 174 produces junction fragments of ~ 6100 bp and 3450 bp. The larger is not cleaved by *Pst*. The smaller is cleaved by *Pst* to 2320 bp (data not shown). This change of 3450 to 2320 = 1130 corresponds exactly to the distance from the λ *Pst* site to the right λ *Bgl*II site in immunity. We conclude therefore that the small *Bgl*II junction fragment from this insertion is the right junction, and thus that insertion 174 is in orientation I.

Further mapping of cleavage sites within Tn10: The positions of a number of other cleavage sites for a variety of restriction enzymes are shown in Figure 9. The positions of these sites were determined as follows: Comparisons among *Bgl*II and *Bgl*II/*Bam* digests of the non-Tn10 parent and of phages carrying insertions 171 and 173 reveal that *Bam* does not cleave the 2500 bp *Bgl*II λ immunity fragment and that it cleaves Tn10 only once (Figure 13). In a phage carrying insertion 171, this cleavage occurs within the largest (left) *Bgl*II junction fragment to produce fragments of greater than 5000 and 1350 base pairs. The 1350 bp fragment is found in *Bgl*II/*Bam* digests of both phages 171 and 173 and is thus a common internal Tn10 fragment; similarly, the 4300 bp fragment is unique to digests of phage 171 and is thus a junction fragment. This assessment permits localization of the *Bam* cleavage site to a position approximately 1350 base pairs to the left of the left-most *Bgl*II cleavage site in Tn10.

*Hin*dIII cleaves Tn10 three times to produce common fragments of 450 and 4800 base pairs (Figure 11). *Bam* cleaves only the 4800 bp common fragment (Figure 14). Thus, at least one of the *Hin*dIII cleavage sites in Tn10 must lie to the left of the Tn10 *Bam* site. Similarly, *Hin*dIII cleaves the Tn10 2800 bp *Bgl*II common fragment (Figure 14), and there must therefore also be at least one *Hin*dIII cleavage site between the two Tn10 *Bgl*II sites. These two *Hin*dIII sites must be separated by at least 1350 base pairs, the distance from the Tn10 *Bam* site to the

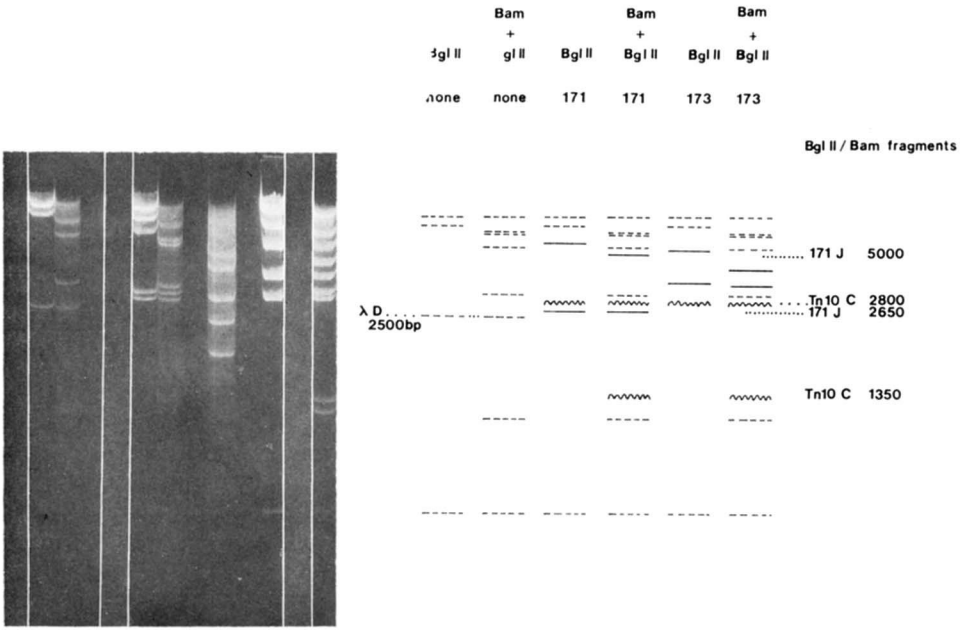

FIGURE 13.—*Bgl*II and *Bgl*II/*Bam* digests of λ *b*221 *c*I857 derivatives carrying no Tn10 insertion or insertions 171 or 173. ----- λ fragments; ~~~~ Tn10 common fragments; ——— λ/Tn10 junction fragments; D = λ donor fragment.

nearest Tn10 *Bgl*II site. Since these two sites cannot generate the Tn10 450 bp *Hin*dIII common fragment, the third Tn10 *Hin*dIII cleavage site must lie 450 base pairs away from one or the other of these two sites. If this third site lies close to the site previously assigned between the *Bgl*II sites, the third site must also lie between the *Bgl*II sites; this constraint follows from the observation that *Bgl*II does not cleave the 450 bp *Hin*dIII common fragment. Thus, we can establish two alternative arrangements of *Hin*dIII sites in Tn10 that are compatible with data presented thus far: (a) two closely spaced sites to the left of the *Bam* site with one site between the *Bgl*II sites, or (b) two closely spaced sites between the *Bgl*II sites and one site to the left of the *Bam* site.

These two alternatives can be distinguished as follows: *Bam*/*Hin*dIII double digest of phage carrying insertion 171 produces Tn10 common fragments of 3900 bp and 900 bp, which result from *Bam* cleavage of the 4800 bp Tn10 *Hin*dIII common fragment (Figure 14). Since the distance from the Tn10 *Bam* site to the nearest Tn10 *Bgl*II site is 1350 base pairs, and since any *Hin*dIII site that lies to the right of the *Bam* site must lie beyond the aforementioned *Bgl*II site, the minimum distance from the *Bam* site to the nearest *Hin*dIII site in the rightward direction is 1350 base pairs. Thus, the 3900 base pair *Hin*dIII/*Bam* fragment must lie rightward from the *Bam* site and the 900 base pair fragment must lie leftward from the *Bam* site.

These observations permit us to assign reasonably precise positions to two of the three *Hin*dIII sites: site 1 lies 900 base pairs to the left of the *Bam* site, and site 2 lies ~ 3900 base pairs to the right of the *Bam* site. Since the distance from the *Bam* site to the first *Bgl*II site is 1350 base pairs, and the calculated distance from the *Bam* site to the second (right-most) *Bgl*II site is 1350 + 2800 = 4150 base pairs, these comparisons place *Hin*dIII site 2 very close to (approximately 250 bp to the left of) the right-most *Bgl*II site. From other observations (above), we know independently that this *Hin*dIII site must lie between the *Bgl*II sites and thus to the immediate left of the right-most *Bgl*II site.

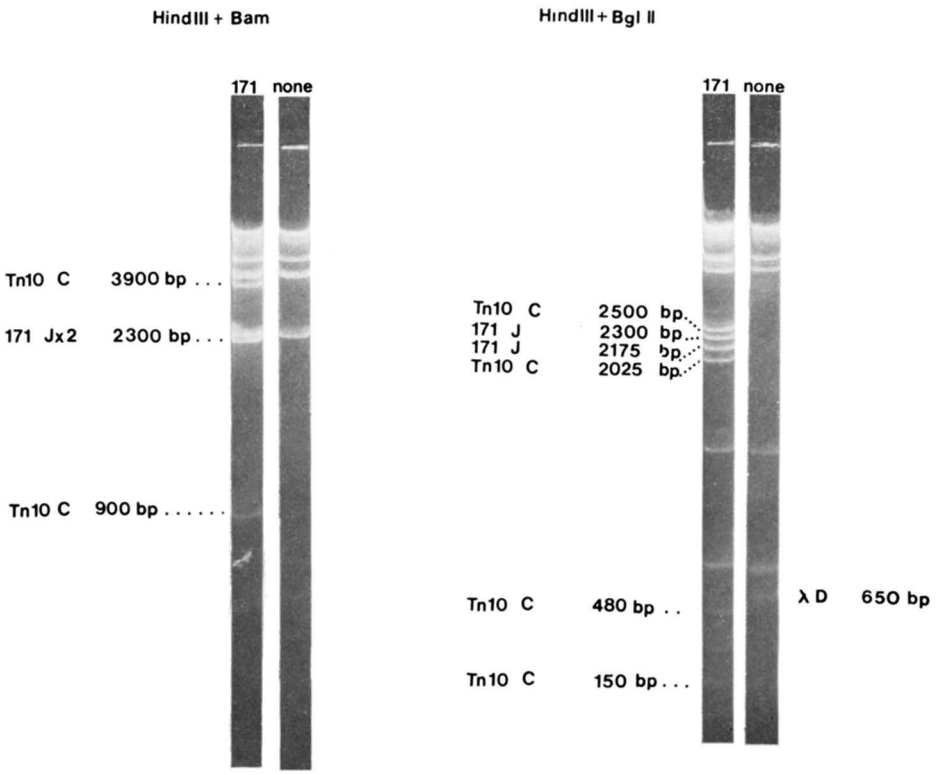

FIGURE 14.—*Hin*dIII/*Bam* and *Hin*dIII/*Bgl*II digests of λ *b*221 *c*I857 derivatives carrying no Tn10 insertion or insertion 171. C = Tn10 common fragments; J = λ/Tn10 junction fragments; D = λ donor fragment. All digests shown were run on the same gel together with appropriate size markers.

If *Hin*dIII site 2 is only 250 bp from the right-most *Bgl*II site, it would not be possible to have the third Tn10 *Hin*dIII site 450 bp to the right of site 2 and still to the left of the right-most Tn10 *Bgl*II site.

An analogous argument can be made on the basis of *Hin*dIII/*Bgl*I and *Bgl*II/*Bgl*I double digests of insertion 171. *Bgl*I cleaves Tn10 only once. It cleaves the Tn10 *Bgl*II 2800 bp common fragment to produce fragments of 1800 and 975 base pairs (data not shown), and the *Bgl*I cleavage site must therefore lie between the two Tn10 *Bgl*II sites. *Bgl*I cleaves the Tn10 *Hin*dIII common fragment to produce fragments of 4100 and 700 base pairs (data not shown). Since the distance from *Hin*dIII site 1 is 900 + 1350 = 2250 base pairs to the left of the left-most *Bgl*II site, the 4100 bp *Bgl*I/*Hin*dIII common fragment must lie to the left of the *Bgl*I site. These assignments make it possible to order the 1800 bp and 975 bp fragments generated by cleavage of the *Bgl*II 2800 bp common fragment by *Bgl*I and thus to assign a position to this Tn10 *Bgl*I site. In order to provide a distance of 4100 base pairs from *Hin*dIII site 1 to the *Bgl*I site, the 1800 bp fragment must lie to the left of the *Bgl*I site and the 975 bp fragment must lie to the right. (The opposite arrangements provide a distance of only 3225 bp from *Hin*dIII site 1 to the *Bgl*I site.)

Since the distance from the *Bgl*I site rightward to the nearest *Bgl*II site is 975 bp and the distance from the *Bgl*I site rightward to the nearest *Hin*dIII site (site 2) is 700 bp, we are again forced to conclude that there would not be room for another *Hin*dIII site 450 bp to the right of site 2 and yet still to the left at the right-most Tn10 *Bgl*II site.

We conclude that *Hin*dIII sites in Tn10 are arranged as described by array (a). This conclusion is further supported by the results of a *Hin*dIII/*Bgl*II double digest of insertion 171. The fragments produced by such a digest are quite compatible with the proposed array of sites.

Tn10 is not cleaved by the following enzymes: *Bal*, *Xma*I, *Sal*I, *Kpn*, *Xho*I, or (as described above) *Pst*. These conclusions are based on comparison among digests of λ::Tn10 and non-Tn10-carrying phages using each of these enzymes alone in single digests and in combination with at least one other enzyme (usually *Bgl*II or *Hin*dIII) in double digests (data not shown).

Analysis comparable to that described above for *Hin*dIII, *Bgl*II, *Pst*, *Bam*, and *Bgl*I have also revealed the presence within Tn10 of cleavage sites for *Eco* R1, *Sst*, and *Hpa*I. Approximate positions of these cleavage sites are shown in Figure 9.

Exact positions of Tn10 insertions within λ *immunity and analysis of* Acc*I cleavage sites within Tn10*: Extensive analysis of all four insertions was also carried out with one additional enzyme, *Acc*I. *Acc*I is unique among the enzymes tested in that it cleaves Tn10 within the terminal inverted repetition; it also cleaves once within the nonrepeated portion of Tn10.

Comparison of *Bgl*II and *Bgl*II/*Acc*I digests of insertions 171 and 173 and of the non-Tn10 parent (Figure 15) show that *Acc*I clearly cleaves all of the *Bgl*II Tn10 fragments in phages carrying either insertion; that is, *Acc*I cleaves both *Bgl*II junction fragments and the 2800 bp Tn10 *Bgl*II common fragment. The products of such *Bgl*II/*Acc*I digests of insertions 171 and

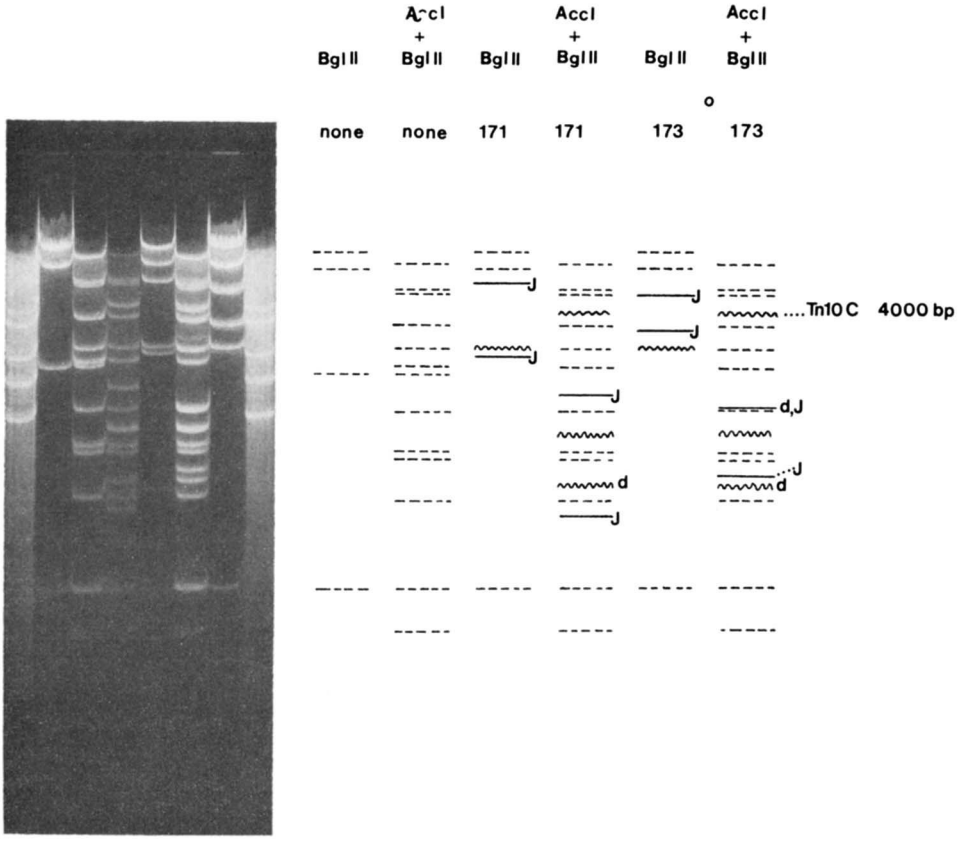

FIGURE 15.—*Bgl*II and *Bgl*II/*Acc*I digests of λ *b*221 *c*I857 derivatives carrying no Tn10 insertion or insertions 171 or 173. ----- λ fragments; ~~~~ Tn10 common fragments; ——— λ/Tn10 junction fragments; d = doublet band.

173 include a very large 4000 bp common fragment. Given the already established positions of *Bgl*II cleavage sites in Tn10 and their positions relative to surrounding *Bgl*II cleavage sites in λ (see Figure 9), the only way of generating such a 4000 bp fragment in insertion 171 is to have a *Acc*I cleavage site within the left inverted repetition, and this site must lie within a few hundred base pairs of the left junction between Tn10 and λ sequences in this insertion.

Since *Acc*I appears to cleave both junction fragments of phages carrying either insertion, the simplest idea is that the inverted repetitions of Tn10 are in fact exactly homologous at the *Acc*I site, and that *Acc*I cleaves the inverted repetitions in homologous positions near the ends of Tn10. Since the 2800 bp *Bgl*II common fragment is also cleaved, there must be at least one additional *Acc*I cleavage site that is internal to Tn10.

If these are all of the *Acc*I cleavage sites, one can predict the number and sizes of common and junction fragments that should be produced by *Bgl*II/*Acc*I cleavage of phage carrying insertion 171. The observed fragments are in good agreement with these predictions, as can be seen in Figure 9. These assignments for *Acc*I cleavage sites can also be derived independently from comparisons among *Hin*dIII and *Hin*dIII/*Acc*I digests of phages carrying insertion 171 or 173. Both the Tn10 *Hin*dIII 4800 bp common fragment and the two *Hin*dIII junction fragments of insertions 171 and 173 are cleaved by *Acc*I and the five *Hin*dIII/*Acc*I Tn10 common fragments observed have the sizes expected from the *Bgl*II/*Acc*I digests (partial data in Figure 16).

*Bgl*II/*Acc*I and *Hin*dIII/*Acc*I digests of phages carrying Tn10 insertions provide the most sensitive way of determining accurately the positions of these insertions with respect to known cleavage sites in the λ immunity region. If one knew the distance from the *Acc*I site in the inverted repetition to the end of Tn10, the size of a *Acc*I/*Hin*dIII or *Acc*I/*Bgl*II junction fragment tells the distance from the corresponding *Hin*dIII or *Bgl*II site to the Tn10 element. In addition, for two insertions that lie close to one another, comparison of the sizes of the junction fragments provides information as to exactly how close together the insertions lie. Since the *Acc*I site is presumably symmetrically placed on either end of the Tn10 insertion, such comparisons can be carried out even if the two insertions lie in opposite orientations.

This type of analysis has been applied to phages carrying each of the four Tn10 insertions under study here: 171, 172, 173 and 174. The results are shown in Figure 16. Two important conclusions emerge:

(1) Digests of phages carrying insertions 171 and 172 are identical for both *Acc*I/*Bgl*II and *Acc*I/*Hin*dIII. This is certainly consistent with the electron microscope heteroduplex evidence, which suggested that these two insertions lie very near or at identical positions. Figure 16 shows, however, that digests of phages carrying insertions 173 and 174 are also identical. That is, both insertions give rise to *Acc*I/*Bgl*II and *Acc*I/*Hin*dIII junction fragments indistinguishable in size for the two insertions. The smallest of these fragments (the right *Acc*I/*Hin*dII junction fragment) is only 610 base pairs long. Had the junction fragments of these two insertions differed by as much as 50 base pairs, we certainly would have observed that the junction fragments migrated to different positions in the gel. It is highly likely that even a difference of 25 base pairs would have been detected. This result strongly suggests that these two insertions are inserted either very near to one another or at the identical position. This result is particularly interesting because insertions 173 and 174 lie in opposite orientations, suggesting that Tn10 may insert in either orientation at a given site.

(2) We can calculate the distance from the *Acc*I sites in the inverted repetition to the end of Tn10 from the data in Figure 16 as follows: the sum of the two *Hin*dIII/*Acc*I junction fragments in insertion 171 is $850 + 350 = 1200$ base pairs. Of this 1200 base pairs, 560 are lambda material lying between the two lambda *Hin*dIII sites. This leaves $1200 - 560 = 640$ base pairs of Tn10 material. If the *Acc*I sites are symmetrically disposed in Tn10, then the distance from the *Acc*I sites to the ends of Tn10 is $640/2 = 320$ base pairs.

Using this estimate and the sizes of the *Acc*I/*Bgl*II and *Acc*I/*Hin*dIII junction fragments, we can determine the positions of insertions 171/172 and 173/174 with respect to the *Bgl*II and *Hin*dIII cleavage sites in the phage immunity region. These estimates are shown in Figure 8.

(Note added in proof: direct DNA sequence analysis of insertion 171 has confirmed that this insertion lies within the *cI* structural gene; the insertion lies at a position exactly 41 base pairs to the left of the right-most *Hin*dIII cleavage site in the phage immunity region.)

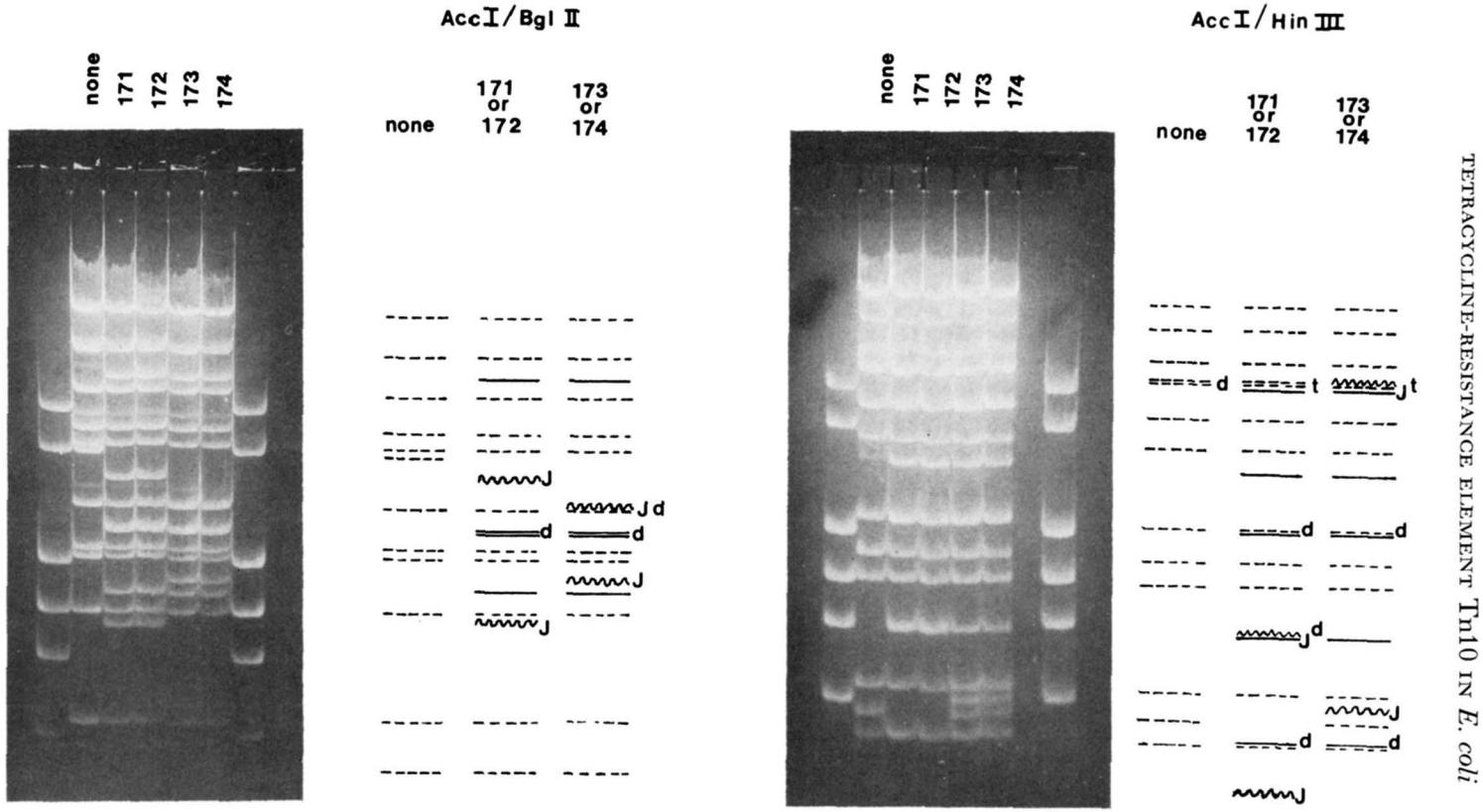

FIGURE 16.—*Acc*I/*Bgl*II and *Acc*I/*Hin*dIII digests of λ *b*221 *c*I857 derivatives carrying insertions 171, 172, 173, or 174. ----- λ fragments; Tn10 common fragments; ——— λ/Tn10 junction fragments; d = "doublet" band; t = triplet band. Size markers are *Acc*I fragments of φX174 (3030 and 2345 basepairs) and *Hae*III fragments of φX174 (1342, 1078, 872, and 606 base pairs). Sizes of insertion 171 fragments containing Tn10 sequences as calculated from these gels are shown in Figure 9.