REGIONAL SPECIFICITY OF ILLEGITIMATE RECOMBINATION BY THE TRANSLOCATABLE AMPICILLIN-RESISTANCE ELEMENT Tn*1* IN THE GENOME OF PHAGE P22

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ABSTRACT

Insertions of the translocatable ampicillin-resistance element Tn*1* were selected in the genome of the temperate Salmonella phage P22 by growing the phage on hosts carrying the resistance plasmid RP4. Insertions of Tn*1* into phage P22 are rare (10^{-10} per phage) and nonrandomly distributed in the P22 genome. They are found mainly in the vicinity of the P22 *ant* gene. Insertions within the *ant* gene are found at many (at least 15) genetically separable sites, are found equally frequently in both orientations and cause irreversible loss of gene function. Some insertions in *ant* appear to be associated with an adjecent deletion.——Prophage deletions were derived from P22::Tn*1* phages by two methods. Low multiplicity transductants have nonrandomly distributed endpoints. One end is at or very near the site of the Tn*1* insertion, and the other is in the vicinity of gene *12*; however, there are many genetically distinguishable endpoints within gene *12*. Prophage deletions selected as survivors of induction of a P22Ap *mnt-ts* lysogen have similarly nonrandom endpoints, with the Tn*1*-distal end frequently near the *ant* gene, as well as gene *12*. Physical analysis of several prophage deletions suggests that the Tn*1* is intact to the resolution of DNA electron microscopy and that the deletions begin at the end of the Tn*1* insertion.——These results suggest that illegitimate recombination associated with Tn*1* shows regional specificity (*i.e.*, preference for some large areas of the P22 genome over other areas), but that within these regions is quite nonspecific.

THIS paper presents genetic studies of illegitimate recombination associated with the translocatable ampicillin-resistance element, Tn*1*. We studied Tn*1* insertions and deletions in the genome of the temperate Salmonella phage P22 because the genetic and physiological properties of this phage offer several important advantages for the study of translocatable elements. Therefore, this introduction is in two parts. First, we briefly summarize what is known about the translocatable drug-resistance elements, especially the closely related elements Tn*1*, Tn*2*, and Tn*3* (previously all called TnA). This subject has been reviewed several times recently (STARLINGER and SAEDLER 1976; COHEN 1976; KLECKNER 1977; NEVERS and SAEDLER 1977; see also the book edited by BUKHARI, SHAPIRO

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and ADHYA 1977). Second, we briefly introduce the relevant features of P22 genetics and physiology, for these are essential for understanding the design of the selections for Tn*1* insertions and deletions that were used to investigate the properties of this genetic element. P22 genetics has recently also been reviewed (SUSSKIND and BOTSTEIN 1978).

Translocatable drug-resistance elements are discrete segments of DNA that can integrate into other DNA molecules in the absence of any obvious sequence homology. The integration of such an element is an example of illegitimate recombination; as might be expected, integration occurs normally in bacteria (*recA*$^-$) defective in their ability to carry out homologous recombination. Another type of illegitimate recombination is the formation of deletions. Some translocatable elements have been associated with this kind of illegitimate recombination event as well. These properties are shared with the translocatable drug-resistance elements by some bacteriophages and a few short DNA sequences known as IS elements.

The closely related translocatable elements, Tn*1*, Tn*2* and Tn*3*, are about 4.8 kilobases (kb) in length, carry a gene encoding the TEM-β-lactamase, which confers resistance to ampicillin, and have a short (about 100 base pair) terminal inverted repetition (HEDGES and JACOB 1974; HEFFRON, RUBENS and FALKOW 1975; HEFFRON *et al.* 1975; BENNETT and RICHMOND 1976; KRETSCHMER and COHEN 1977). Previous studies established that these elements can integrate into a recipient plasmid chromosome by a process that is independent of the host's *recA* gene (RUBENS, HEFFRON and FALKOW 1976). HEFFRON *et al.* (1977) showed that translocation of Tn*2* requires intact terminal sequences, as well as a diffusible product encoded by the element. Integration can occur at a large number of nonrandomly distributed sites (HEFFRON *et al.* 1975; RUBENS, HEFFRON and FALKOW 1976; KRETSCHMER and COHEN 1977; NISEN *et al.* 1978). Integration within a gene inactivates the gene, and these elements can be strongly polar on gene expression, but only when integrated in a particular orientation (RUBENS, HEFFRON and FALKOW 1976).

Many translocatable elements have been associated with the production of deletions (λ: DAVIS and PARKINSON 1971; Tn*10*: CHAN and BOTSTEIN 1972, CHAN *et al.* 1972; IS*2*: AHMED and JOHANSEN 1975; IS*1*: REIF and SAEDLER 1975). Tn*3* has also been implicated in deletion formation (NISEN *et al.* 1978). One of the most persuasive arguments supporting an active role of the elements in deletion formation is the characteristic structure of many of these deletions: one endpoint located at or very near the terminus of the element and the other endpoint located more or less randomly to one side or the other of the insertion element. In simple elements (*e.g.*, IS*1*, REIF and SAEDLER 1975), the deletions usually retain the apparently intact element at one end. This is also apparently true of some deletions associated with the larger compound element, Tn*10* (CHAN *et al.* 1972). Tn*10* also produces deletions with more complicated structures, most of which could be reconciled with the idea that the IS-like terminal repetition (SHARP, COHEN and DAVIDSON 1973) is the active agent and remains at one end of these deletions (BOTSTEIN and KLECKNER 1977; KLECKNER *et al.* 1979; Ross,

SWAN and KLECKNER 1979). Finally evidence from DNA heteroduplex studies (NISEN *et al.* 1978) and from direct nucleotide sequencing (OHTSUBO, OHMORI and OHTSUBO 1979) shows that one of the endpoints in Tn*3* deletions is exactly at the end of the element.

The salient features of the life cycle and genetic map of P22 are shown in Figure 1. The mature P22 chromosome is a linear double-stranded DNA molecule 42 kb in length. It has a short terminal repetition and displays limited circular permutation of the nucleotide sequence (TYE, CHAN and BOTSTEIN 1974; TYE, HUBERMAN and BOTSTEIN 1974). As a consequence, the genetic map is circular. Encapsidation of P22 DNA occurs by a headful packaging mechanism: DNA molecules about 2% longer than the P22 genome are packaged sequentially from a multimeric precursor starting from a unique site (called *pac* in Figure 1) (TYE, CHAN and BOTSTEIN 1974; JACKSON, JACKSON and DEANS 1978). Since the multimeric precursor (called a concatemer) consists of phage genomes tandemly repeated, the packaging mechanism results in each phage chromosome having a 2% terminal repetition. The terminal repetition is essential both for phage growth and lysogeny, presumably because circularization of the DNA after infection is required for both of these processes (BOTSTEIN and MATZ 1970; WEAVER and LEVINE 1977). P22 genomes containing insertions are still packaged efficiently by this process, but the resulting phage chromosomes are incomplete and lack the terminal repetition when the insertion makes the composite genome larger than the P22 headful (CHAN *et al.* 1972; TYE, CHAN and BOTSTEIN 1974). Phage particles containing chromosomes lacking terminal repetition are defective in single infections since the DNA cannot circularize. However, as shown by CHAN *et al.* (1972), these phages can grow at high multiplicities of infection (where permuted chromosomes can recombine with each other to generate a circular molecule) or upon infection of a host carrying a nonimmune prophage deletion (where recombination with the prophage can usually generate a circular molecule).

The study of insertions is aided by the above properties in two important respects. First, insertions can be recovered and propagated regardless of size by using high multiplicity of infection, using hosts with nonimmune prophage deletions, or by induction of lysogens made by multiple infection. Insertions of drug-resistance elements can be isolated simply by looking for high-frequency transduction of drug resistance at high multiplicity of infection. Second, deletions can be selected from oversize genomes containing insertions simply by demanding growth or lysogenization after single infection.

P22 can lysogenize its host, a process that involves repression of the lytic phage functions and the integration of the P22 genome into the Salmonella chromosome. The lysogen is immune to superinfection because the repression active on the resident prophage also acts upon homologous superinfecting phage. However, the repression and immunity system of phage P22 is bipartite: two repressors are required to maintain repression and thus immunity (BOTSTEIN *et al.* 1975; LEVINE *et al.* 1975). One of the repressors (the *c2* repressor) represses directly the early lytic phage functions, which indirectly results in the failure of the late

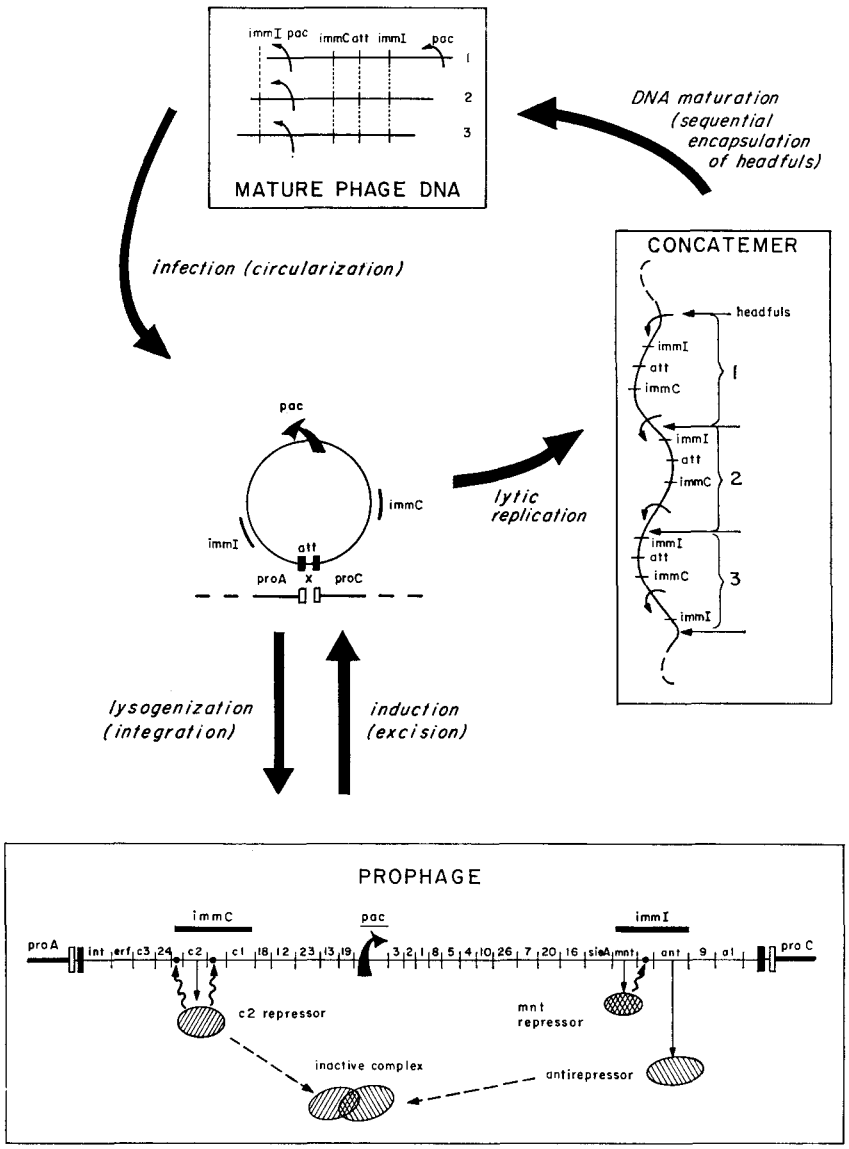

FIGURE 1.—Schematic diagram of the DNA rearrangements during the lifecycle of bacteriophage P22. The symbols are gene names; *pac* (JACKSON *et al.* 1978) indicates the unique initiation site at which unidirectional headful packaging begins on a concatemer (TYE, HUBERMAN and BOTSTEIN 1974). The detailed genetic map is given at the bottom of the diagram in the prophage orientation along with a pictorial representation of the functional relationship between the two immunity regions (BOTSTEIN *et al.* 1975; LEVINE *et al.* 1975). The lifecycle of P22 has recently been reviewed by SUSSKIND and BOTSTEIN (1978).

genes to be expressed. The other repressor (the *mnt* repressor) acts only to prevent synthesis of an antirepressor that, when present, binds the *c2* repressor and inactivates it, resulting in derepression of the lytic functions (SUSSKIND and BOTSTEIN 1975). Since the repressors are both linked to their sites of action, removal of either repressor region (*immC*, where the *c2* gene is located, or *immI*, where the *mnt* and *ant* genes are located) by deletions results in a lysogen not immune to superinfection (CHAN and BOTSTEIN 1972; BOTSTEIN *et al.* 1975). The antirepressor is required only for growth on deletion lysogens that are missing *immI*; in nonlysogens, antirepressor is not required in any way. Similarly, the *mnt* repressor is required only when the *ant* gene is intact; in *ant*$^-$ mutants, it is dispensible for lysogeny (BOTSTEIN *et al.* 1975; LEVINE *et al.* 1975). In fact, deletion of the entire *immI* region (both *mnt* and *ant*) results in a well-regulated temperate phage: the entire region is dispensible; in fact, close relatives of P22 have been found that are missing *immI* entirely (SUSSKIND and BOTSTEIN 1978).

The relevance of these properties to the study of Tn*1* lies first in the fact that many insertions occur in the *immI* region; very many were conveniently recovered in the *ant* gene. Second, deletions that remove *immI* are easily identified because such lysogens are sensitive to *ant*$^+$, but not *ant*$^-$ superinfecting phage; their endpoints are easily mapped because they are sensitive to superinfection by most of our P22 mutants. Third, the incorporation of a temperature-sensitive mutation in the *mnt* gene made possible a selection for deletion mutations based on the necessity of removing the *ant* gene in order for such temperature-sensitive *mnt* lysogens to survive at nonpermissive temperatures.

MATERIALS AND METHODS

Bacteria

The bacterial strains used are listed in Table 1. *Salmonella typhimurium* strains are derivatives of LT2. The source of Tn*1* was RP4 (DATTA *et al.* 1971), a 34×10^6 d (MEYERS *et al.* 1976) self-transmissible R factor conferring resistance to tetracycline, ampicillin, kanamycin, and neomycin. RP4 was transferred by conjugation from its original host, the *E. coli* strain DB6292, to DB7011, or a lysogenic derivative of DB7011, to produce DB7189, DB7222, and DB7226, the strains used for the isolation of P22Ap phages.

All lysogens used in superinfection experiments were *sieA*$^-$ (by point mutation or deletion) to prevent exclusion (SUSSKIND, WRIGHT and BOTSTEIN 1971). DB5057 and DB7283, which contain *c2*$^+$ *mnt*$^-$ prophage deletions (*i.e.*, deletions of *immI*) were used to test superinfecting phages for *ant*. DB147 contains a short *c2*$^-$ *mnt*$^+$ prophage deletion (*i.e.*, a deletion of *immC*) and was used in a mixed indicator with DB53 to titer P22Ap phages. DB5000 was the source of P22Tc10 phage, which were obtained by induction with UV (CHAN *et al.* 1972). Other prophage deletions, used for mapping, are described in the text.

Phage

The following derivatives of P22 were used:

P22 *sieA*-44: The parent of most P22Ap phages. The *sieA*-44 mutation eliminates the major superinfection exclusion system active on P22 (SUSSKIND, WRIGHT and BOTSTEIN 1971; SUSSKIND, BOTSTEIN and WRIGHT 1974). The presence of the *sieA*-44 mutation in P22Ap phages is necessary in order to map by superinfection with amber mutants.

P22 *virB*-3: A double mutant (*Vx* and *K5*) that is insensitive to *c2* repression (BRONSON and LEVINE 1971). Phages containing *virB*-3 and two amber mutations (*virB am am* phages) were

TABLE 1

Bacterial strains

Strain	Genotype	Source/Reference
Salmonella typhimurium:		
DB21	*su*⁻ prototroph	BOTSTEIN and MATZ 1970
DB53	*su*⁻ *hisC-am527 cysA-am1348*	BOTSTEIN and MATZ 1970
DB5000	DB21 (P22Tc10)	WATANABE *et al.* 1972
DB7000	*su*⁻ *leuA-am414*	SUSSKIND *et al.* 1974
DB7004	*su2 leuA-am414*	SUSSKIND *et al.* 1974
DB7011	*su*⁻ *leuA-am414 hsd*R	D. BOTSTEIN
DB7136	*su*⁻ *leuA-am414 hisC-am527*	D. BOTSTEIN
DB7189	DB7011 (P22)/RP4	This work
DB7222	DB7011/RP4	This work
DB7226	DB7011 (P22 *sieA*-44)/RP4	This work
Prophage deletions:		
DB147*	DB53 (P22 prophage deletion *proA-c1*: retains *imm1* but not *immC*)	CHAN and BOTSTEIN 1972
DB5057*	DB21 (P22Tc10 deletion *a1*-gene *20*: retains *immC* but not *imm1*)	CHAN and BOTSTEIN 1972
DB7283	DB7000 (P22Ap2 *sieA*-44 *mnt-ts*1, deletion *Ap2*-gene *20*)	This work
Escherichia coli:		
DB6292	J53 *F*⁻ *pro met* (λ)/RP4	HEDGES and JACOB 1974; E. SIGNER

* See Figure 1.

constructed by sequential crosses between *virB*-3 and P22 amber mutants and identified by spot complementation (see below) against the parents. These phages were used to map Tn*1* insertions.

P22Tc10: A specialized transducing phage for tetracycline resistance containing a Tn*10* insertion (CHAN and BOTSTEIN 1976). DNA from this phage was used as a standard in heteroduplex analysis of P22Ap phages because of the lariat structure of Tn*10* in single strands (TYE, CHAN and BOTSTEIN 1974a).

The amber mutations used in this work are described in BOTSTEIN, CHAN and WADDELL (1972) and POTEETE and KING (1977), and the *ant*⁻ mutations were those of BOTSTEIN *et al.* (1975).

Media and solutions: Phages were diluted in buffered saline (BS) or dilution fluid (DF) and plated in soft agar on λ agar plates. Plating cultures were grown in LB broth. These solutions are described in EBEL-TSIPIS and BOTSTEIN (1971).

M9CAA (SMITH and LEVINE 1964) is a phosphate buffered mineral medium (M9) containing glucose and a charcoal-clarified amino acid mixture. M9CAA was used for crosses and complementation tests and for procedures involving UV irradiation of lysogens. Lysogens can be induced with relatively low doses of UV in M9CAA, since UV-absorbing material has been removed from the medium by adsorption to charcoal.

Green indicator plates (CHAN *et al.* 1972) were used for single colony isolation of bacteria, as well as for streak tests for immunity, exclusion, and other prophage markers (SUSSKIND, WRIGHT and BOTSTEIN 1971). Green/amp plates, containing 20 μg/ml of ampicillin trihydrate (generously donated by Bristol Laboratories, Syracuse, New York), were used to select ampicillin-resistant cells.

General methods

Concentrated phage stocks were prepared by infection (BOTSTEIN and MATZ 1970) or, for phages containing an insertion, by UV induction of lysogens (CHAN and BOTSTEIN 1972).

Lysates made by induction are base-plate deficient (ISRAEL *1967*) and were treated with at least 1×10^{10} phage equivalent per ml of base-plate parts (the product of gene *9*, p9; generously supplied by A. POTEETE) at 37° for one hr prior to concentration. Phages were purified in either discontinuous CsCl gradients (BOTSTEIN 1968) or equilibrium CsCl gradients consisting of nine parts phage stock layered on 11 parts 65% (w/v) CsCl (in 10 mM Tris buffer, pH 8) and centrifuged at least 12 hr at 22,000 rpm, 20° in an SW50.1 rotor.

UV irradiation to destroy immunity of lysogens, either in liquid culture or on plates, was performed at a dose of 200 ergs/mm^2.

Phage crosses and complementation tests were performed in M9CAA at 25° by the method of BOTSTEIN, CHAN and WADDELL (1972).

Spot tests, for mapping insertions with P22 *virB am am* phages and spot complementation tests, used to identify P22 *virB am am* phages, were performed as described in BOTSTEIN, CHAN and WADDELL (1972).

Conjugation of RP4 was accomplished by making intersecting lines of donor and recipient cells on a λ plate and incubating overnight at 37°. Cells from the point of intersection were streaked directly on selective medium.

Isolation of insertions

Transducing lysates were prepared by UV induction of DB7189 or DB7226 or single-cycle infection of DB7222. For induced lysates made with helper, P22 *sieA*-44 was added to a multiplicity of ten immediately after irradiation. These lysates were used to infect DB7000 or DB7136 at high multiplicity (> 5 phage/cell). About 10^9 infected cells were spread on a green/amp plate and incubated at 30°. Under these conditions, most cells become lysogens.

The *amp*^{R} transductants were screened for production of HFT lysates by the method of KAYE, BARRAVECCHIO and ROTH (1974). Unpurified transductants were transferred to a λ agar plate and a master green/amp plate with a sterile wooden dowel and incubated until patches of cells were visible. Then the λ agar plate was UV irradiated and incubated at 30° for five hr. Cells were lysed by inverting the plate over a metal planchet containing $CHCl_3$ for 20 min. DB7000 and p9 (10^{11} phage equivalents) were spread on a green/amp plate and the lysed cells were immediately replica-plated onto this lawn. After overnight incubation, an HFT lysate produces a heavy patch of *amp*^{R} transductants while generalized transduction usually produces no *amp*^{R} cells.

Several precautions are necessary in this protocol: (1) transduction for *amp*^{R} is most reliably accomplished on green/amp plates; LB/amp plates allow many *amp*^{S} cells to survive; (2) it is important that transducing lysates be made at 30° or lower temperatures; lysates made at 37° mainly yield transductants that do not produce HFT lysates; (3) it is important to perform the HFT screening on unpurified transductants since contaminating wild-type phage help defective phage to transduce.

Phenotypes of P22Ap phage

Each colony that produced an HFT lysate was purified by repeated single-colony isolation. Since double lysogens of P22 are unstable, purification yields mainly single lysogens. Purified *amp*^{R} colonies were picked onto four λ plates with a sterile wooden dowel. The plates were incubated until patches of cells were visible. The plates were then UV irradiated and each patch overlayed with a drop of either DB7000, DB147 + DB53, DB147 + DB53 plus 4×10^{10} eq/ml of p9, or DB5057 plus p9. After overnight incubation, the spots were scored as ++, +, or —, depending on the degree of clearing.

The Tn*1* insertions were classified according to the following rationale (see Table 2): (1) Insertion in a nonessential region of the genome (other than *ant*): The particles lack terminal repetition and their growth in a nonlysogen (DB7000) requires multiple infection and biparental recombination during each cycle. Growth is therefore less efficient than wild type. However, in a nonimmune prophage deletion (DB147 or DB5057) recombination with the prophage usually permits growth. Thus, these particles grow poorly in a nonlysogen but grow quite well in a prophage deletion strain. (2) Insertion inactivating essential gene (other than gene *9*): The

TABLE 2

Scheme for classification of P22Ap phage phenotypes

	Phenotype	DB7000	DB147	DB147 + p9	DB5057 + p9
WT	wild type	++	++	++	++
NE	mutation in nonessential gene (other than *ant*)	+	++	++	++
E	mutation in essential gene (other than gene *9*)	—	—	—	—
A	*ant*⁻	+	++	++	—
T	*9*⁻	—	—	++	++
AT	*ant*⁻ and *9*⁻	—	—	++	—

Lysogens of P22Ap phages were induced on plates, then overlayed with various indicators as described in MATERIALS AND METHODS. Here and throughout the paper, "essential" gene refers to a gene required for production of infectious particles other than gene *9*, and "nonessential" gene or region refers to a region, other than *ant*, not required for production of infectious particles.
++ Means a large spot of clearing occurred.
+ Means a small spot of clearing occurred.
— Means no more than a few isolated plaques were seen.

lysogens do not produce infectious particles and thus there is no growth on any indicator. (3) Insertions inactivating gene *9*: The particles grow as in (1) above as long as p9 is added to the plates. (4) Insertions inactivating *ant*: The particles do not grow in DB5057, a prophage deletion strain lacking *imml* (*i.e.*, *mnt*) but retaining *immC* (*i.e.*, *c2*).

Mapping insertions with P22 virB am am *phages*

A set of phages containing *virB*-3 and two linked amber mutations (*virB am am* phages) were used to map insertions in P22Ap phages. The amber alleles *12-am*N11, *23-am*H316, *13-am*H715, *19-am*N111, *3-am*N6, *2-am*H200, *1-am*N10, *8-am*H202, *5-am*N114, *10-am*N107, *26-am*H204, *20-am*N20, *16-am*N121 and *9-am*N9 were used to construct the following double amber mutant *virB*-3 phages: *12⁻23⁻*, *23⁻13⁻*, *13⁻19⁻*, *19⁻3⁻*, *3⁻2⁻*, *2⁻1⁻*, *1⁻8⁻*, *8⁻5⁻*, *5⁻10⁻*, *26⁻20⁻*, *20⁻16⁻* and *16⁻9⁻*. To map Tn*1* insertions, these phages were spotted on lysogens of *sieA*⁻ P22Ap phages. Failure of a *virB am am* phage to grow indicated that the Tn*1* insertion in the prophage affected the region of the genome spanned by the two amber mutations.

Manipulations with P22Ap phages

Growth and titering: Stocks of P22Ap phages were prepared by UV induction of lysogens and titered on a mixture of DB147 and DB53 by the method of CHAN *et al.* (1972). The principle of this method is that the short prophage deletion provides P22 homology to the incomplete, permuted genome fragments allowing circularization of the DNA and phage growth. The ratio of titer on DB147 to particular titer, estimated from absorbance at 260nm ($A_{260} = 1$ means 5×10^{11} particles/ml) for P22Ap2, Ap4, Ap5, Ap7 and Ap9 (purified in discontinuous CsCl gradients) was 1/4, 1/8, 1/7, 1/9 and 1/5, respectively. Thus, to approximate the number of particles, observed titers on DB147 were multiplied by seven.

Crosses between different P22Ap phages: Crosses between different *ant*⁻ P22Ap phages, performed to determine if the Tn*1* insertions were separable by recombination, were done by the standard method used for phages with normal-sized genomes (BOTSTEIN, CHAN and WADDELL 1972). The titer of infectious particles of each parental phage was estimated from its titer on DB147 (see above). The progeny of the cross were plated on DB147 to titer total particles and DB5057 to titer *ant*+ recombinants.

Heteroduplex analysis of DNA

Electron microscopy of heteroduplexes was performed by a modification of the method of TYE, CHAN and BOTSTEIN (1974). Purified phages in CsCl were incubated in 0.1 M NaOH, 10

mM Tris (pH 8.5 at 1 M), 5 mM EDTA for one hr at 37°, then neutralized, renatured in 50% formamide and processed further according to TYE, CHAN and BOTSTEIN (1974). Lengths are expressed as (mean) ± (standard error), where mean $= \bar{x} = (\Sigma x)/n$ and the standard error is $[\Sigma(x-\bar{x})^2]^{1/2}/(n-1)$ when $\bar{x}$ represents one set of measurements or $[\Sigma(x-\bar{x})^2/(m-1)]^{1/2}$ when $\bar{x}$ is taken from m sets of measurements. For heteroduplexes involving Tc*10* DNA, the double-stranded standard was the Tn*10* stem (1390 base pairs) and the single-stranded standard was the Tn*10* loop (6560 bases) (D. Ross, personal communication). These values for the stem and loop were obtained by comparison with double- and single-stranded ϕX174 DNA.

Isolation of prophage deletions as low multiplicity transductants

Deletions selected as restoring terminal repetition were obtained as amp^R transductants from low multiplicity ($\leq 10^{-2}$ phage/cell) infections as described in CHAN and BOTSTEIN (1972) and CHAN *et al.* (1972).

Isolation of prophage deletions as survivors of mnt-ts*1 induction*

Deletions obtained without requiring restoration of terminal repetition were selected as survivors of heat induction of a lysogen of P22Ap2 *sieA*-44 *mnt-ts*1. The temperature-sensitive mutation in the *mnt* gene (GOUGH 1968) causes antirepressor to be produced at high temperature, inactivating the *c2* repressor and causing cell death. To isolate deletions in this genome, 10^6 to 10^7 cells were spread on a green plate containing 20 μg/ml ampicillin and 10 mM [ethylenebis (oxyethylenenitrilo)] tetraacetic acid (EGTA). After incubation at 42°, colonies that appeared were purified once on green/amp/EGTA plates, then streak tested (SUSSKIND, WRIGHT and BOTSTEIN 1971) for gene *9* against P22 *virB*-3 *9-am*N9. Only cells lacking the *9* allele were further characterized.

Mapping deletions

Mapping of nonimmune prophage deletions by spot tests or efficiency of plating was as described in CHAN and BOTSTEIN (1972). For fine-structure mapping of gene *12*, the criterion for rescue was at least a three-fold increase in efficiency of plating over that on the nonlysogenic strain DB7000. Mapping of immune prophage deletions was performed with virulent phages. To map immune deletions in *ant*, the prophage was induced with UV, the tester phage adsorbed or ten min, and then the infected cells were plated with DB7283 to assay selectively for any *ant*+ recombinants that might have been formed.

RESULTS

Isolation and characterization of P22Ap phages

P22 phages containing insertions of Tn*1* in their genomes (P22Ap phages) were identified as specialized transducing phages capable, upon lysogenization, of conferring resistance to ampicillin (amp^R). P22Ap phages were generated by growing P22 in strains carrying the R-plasmid, RP4, and using the resulting lysates to transduce cells to ampicillin resistance. Since there are ways other than formation of composite specialized transducing genomes in which the *amp* gene can be transduced, the amp^R transductants were screened for their ability to produce high-frequency transducing (HFT) lysates, since only composite genomes will yield transducing titers even remotely comparable to the number of particles in the lysate.

P22Ap phages were recovered at a frequency of about one in 10^{10} phages by this procedure, regardless of whether growth through the RP4-containing strain was by infection or by UV induction of lysogens. When growth was by induction, the addition of helper phage immediately after irradiation did not alter the fre-

TABLE 3

Phenotypes of P22Ap phages

Method of lysate preparation	Number of lysates	Total number of transductants	Phenotypes* % NE	E	A	T	AT	WT
Induction	7	51	39	8	8	24	12	10
Induction with helper	4	102	50	11	6	24	7	3
Infection	5	34	50	15	3	29	3	–
All methods	16	187	47	11	6	25	7	4

Method of preparation of the lysates and the plate screening method for determining the phenotypes are described in the MATERIALS AND METHODS.

* Phenotype designations (see Table 2): NE = insertion in nonessential gene other than *ant*. E = insertion in essential gene other than gene *9*. A = *ant*$^-$ phenotype. T = *9*$^-$ phenotype. AT = *ant*$^-$ and *9*$^-$ phenotype. WT = wild type.

quency, suggesting that pre-existing insertions of Tn*1* into phage genes essential for growth did not contribute significantly to the yield of P22Ap phages.

The growth properties of particles in HFT lysates are shown in Table 3. Virtually all (96%) of the *amp*^{R} transductants that produced HFT lysates contained a mutant prophage. Those few (4%) transductants with a wild-type prophage probably are double lysogens containing a wild-type and a P22Ap prophage; they were not tested further. Most insertions affect gene *9* (32%), *ant* (13%) or other nonessential regions (47%).

Transductants that produce HFT lysates were presumed to be lysogens of P22Ap specialized transducing phages consisting of Tn*1* inserted into P22 DNA. Since the composite P22-Tn*1* genome is larger than a P22 headful, these lysogens should produce particles that are defective because their DNA lacks terminal repetition. In addition, Tn*1* insertion might inactivate one or more genes. Relatively few (11%) of the Tn*1* insertions were found to affect essential genes. This distribution was essentially the same with lysates made by induction (with or without helper) or infection. Some insertions appear to affect *ant* and *9* concomitantly. As shown elsewhere (WEINSTOCK and BOTSTEIN, in preparation), these insertions are located in *ant* and are polar on *9*.

Thus, the distribution of Tn*1* insertions in P22, as judged by the phenotypes of P22Ap phages, is nonrandom, since a disproportionately large number of insertions affect the *ant* and *9* genes and relatively few affect essential genes.

*Mapping Tn*1 *insertions in P22Ap phages*

To map the site of Tn*1* insertion in P22Ap phages, a set of virulent P22 phages, each containing two amber mutations in neighboring genes, was constructed. The two amber mutations in each phage define a short interval of the P22 genetic map and the set of intervals spans much of the P22 chromosome. Growth of the virulent phages on a lysogen requires rescue, into the virulent phage, of both wild-type alleles from the prophage. The presence of an intervening Tn*1* insertion drastically reduces the frequency of rescue, allowing the site of insertion to be localized.

The results obtained by testing the growth of the *virB* double amber phages

TABLE 4

*Mapping Tn*1 *insertions with* virB *double-amber phages*

Intervals tested (in prophage map order)*	Phenotypic class†				
	NE	E	A	T	AT
12–23	—	1	—	—	—
23–13	—	—	—	—	—
13–19	—	—	—	—	—
19–3	—	—	—	—	—
3–2	—	—	—	—	—
2–1	—	—	—	—	—
1–8	1	—	—	—	—
8–5	—	—	—	—	—
5–4–10	—	—	—	—	—
26–7–20	—	4(3)	—	—	—
20–16	—	7	—	—	—
16-ant-9	1	5(4)	11(9)	13	14(13)
outside tested intervals	12	3(2)	—	3	—

P22 *virB* double-amber mutant phages were constructed as described in MATERIALS AND METHODS. These were used in plating tests of P22Ap lysogens as described in the text. The numbers in parentheses indicate the number of P22Ap insertions in the class which were isolated from different lysates.

* See Figure 1; an interval is defined by the two amber mutations in a particular *virB* double-amber phage. When these span a third gene, this is indicated.

† Phenotypic classes (see Tables 2 and 3) are: NE = insertion in nonessential gene other than *ant*. E = insertion in essential gene other than gene *9*. A = *ant*$^-$ phenotype. T = *9*$^-$ phenotype. AT = *ant*$^-$ and *9*$^-$ phenotype.

on each of the P22Ap lysogens are summarized in Table 4. Of the 75 P22Ap lysogens tested, 57 supported poorly the growth of at least one virulent phage. In general, the locations of the insertions agreed with phenotypes described in the preceding section. Thus, all (25/25) *ant*$^-$ P22Ap phages and most (13/17) *9*$^-$ P22Ap phages have mutations between genes *16* and *9*. Those *9*$^-$ P22Ap phages not mutant in this interval probably have insertions in gene *9* to the right of the *9*$^-$ mutation used in the *virB 16*$^-$ *9*$^-$ phage. Of the 14 insertions affecting nonessential regions, only two map near any of the essential genes tested, and may be in nonessential intergenic regions. Lastly, of the 20 insertions affecting essential genes, 16 were localized, while three must lie in regions not spanned by the *virB* tester phages.

Some insertions affecting essential genes gave unexpected results in that they reduced rescue from more than one interval. Thus, the insertion in P22Ap34 affected the *26–20*, *20–16*, and *16–9* intervals, while other insertions (as in P22Ap27, see next section) affected only the *26–20* interval. Where this kind of rescue occurred, it always affected a set of contiguous intervals. These insertions are polar (WEINSTOCK 1977; WEINSTOCK and BOTSTEIN, in preparation), indicating that complementation is important in this mapping procedure; complementation tests with P22 amber mutations (not shown, WEINSTOCK 1977) confirm these results. Therefore, insertions affecting multiple intervals have been placed in the promoter-proximal interval affected.

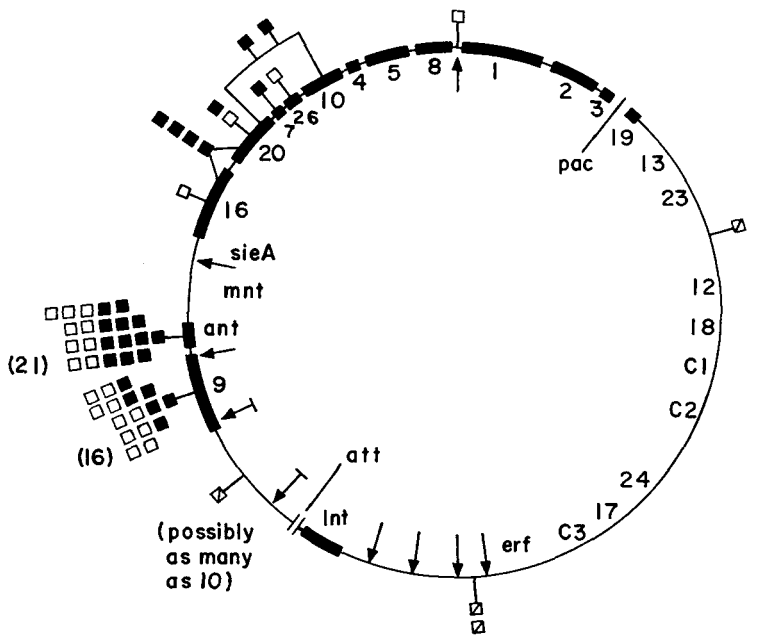

FIGURE 2.—Distribution of Tn*1* insertions in the genome of phage P22. The methods used to determine the positions of Tn*1* insertions are described in the text. Filled boxes indicate insertions in the orientaton that is polar on downstream genes; open boxes indicate nonpolar insertions. Boxes with a diagonal line indicate that orientation is not known. The arrows inside the circle represent restriction endonuclease cleavage sites (JACKSON, MILLER and ADAMS 1978; WEINSTOCK 1977); ordinary arrows are EcoR1 sites and the modified arrows indicate BamHI sites.

Figure 2 summarizes the overall distribution of Tn*1* insertions in P22. It is clear that most of the insertions are clustered in the vicinity of the *ant* gene. The distribution of insertions in essential genes supports this idea: most of these insertions are in genes nearest to the *ant* gene.

*Fine-structure mapping of Tn*1 *insertions in the* ant *gene*

Because of the uneven distribution of Tn*1* insertions in P22, we recovered many independent insertions in the *ant* gene. These insertions were mapped genetically in order to determine whether they were randomly distributed within the *ant* gene. To construct such a fine-structure map, we relied on two properties of translocatable elements (KLECKNER, ROTH and BOTSTEIN, 1977). First, integration of a translocatable element within a gene creates a mutation. The mutant phenotype (ant⁻) can thus be used genetically to map the position of the insertion. Second, translocatable elements stimulate adjacent deletion formation

(KLECKNER 1977; see also below). Insertions within a gene can therefore be used to obtain partial deletions of the gene. These deletions then can be used to construct a deletion map of the gene, which can serve to order the insertions.

As described above, a functional *ant* gene is required for P22 to grow on a strain carrying a deletion prophage that is missing the *imm1* region (*i.e.*, missing the *mnt* repressor) but that retains the *immC* region (*i.e.*, retains the *c2* repressor). If phages carrying an insertion in the *ant* gene are crossed with one another, progeny able to grow on such a deletion lysogen can be produced only by recombination between the two insertions. Reversion of *ant*::Tn*1* under these conditions has never (less than 10^{-10}) been observed.

In order to make infectious P22Ap particles, P22Ap lysogens were induced with UV. Lysates were treated with purified p9 *in vitro* to compensate for the possible polarity of the Tn*1* insertions on gene *9*. Although the P22Ap particles in these lysates have incomplete permuted fragments of the composite genome, there are two circumstances under which they can produce progeny. One is infection of a prophage deletion strain that provides most of the particles with DNA sufficient to allow circularization and subsequent growth. The other is infection at high multiplicity of infection, under which circumstance recombination between different permutations results in circularization and subsequent growth.

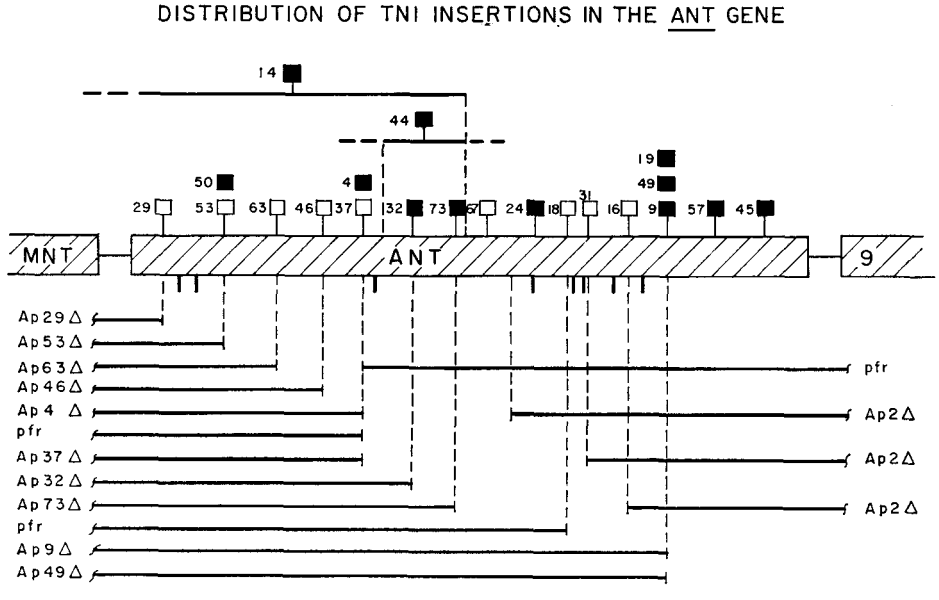

FIGURE 3.—Fine-structure map of distribution of Tn*1* insertions in the *ant* gene. The open and closed symbols follow the same convention as in Figure 2. The extent of the deletions associated with P22Ap14 and P22Ap44 is indicated by the bar; the squiggles indicate that the deletion continues farther. Dotted line means "not determined further." The extent of deletions used to map the insertions is indicated below by the solid bars; the notations next to each indicate the source of the deletion. "pfr" indicates a plaque-forming revertant of a P22Ap phage; Δ means a prophage deletion (see below) derived from a particular P22Ap phage. The vertical ticks pointing down indicate *ant*⁻ point mutations.

The infectious P22Ap particles were used in two kinds of genetic crosses. One kind involved infection of strains carrying deleted prophages with a deletion endpoint within the *ant* gene. The other kind was infection of nonlysogenic cells with a high multiplicity (7) of each of two different P22Ap particles. In both cases, the P22Ap particles produce progeny, which are assayed for Ant$^+$ phenotype by plating on strain DB5057, whose prophage has a deletion of *immI*, but which retains *immC*.

The crosses of P22Ap particles with prophage deletions yielded the result that 19 *ant*::Tn*1* insertions fell into 13 deletion intervals (Figure 3) spanning the length of the gene as determined from the location of *ant*$^-$ mutations. The results of crosses among the insertions falling into the same intervals are shown in Table 5. In some cases (*e.g.*, Ap50 and Ap53; Ap4 and Ap37; Ap9, Ap19 and Ap49), no recombinants were found among 10^8 progeny particles, indicating that the sites of insertion are inseparable by recombination. In other cases (*e.g.*, Ap57 and Ap45; Ap19 and Ap16), recombination was observed, indicating that these pairs of insertions occupy different sites. It should be noted that the sensitivity of this test is very high and that "negative" results are at least three orders of magnitude less than the lowest "positive" value found. Finally, two insertions (Ap14 and Ap44) failed to recombine with at least two others that do recombine with each other. These are most simply interpreted by assuming that they contain, in addition to the Tn*1* insertion, an adjacent deletion, as shown in Figure 3.

The aggregate data from the fine-structure mapping can be summarized by saying that the 21 Tn*1* insertions include two deletions and that the 19 remain-

TABLE 5

Cross	Frequency of *ant*$^+$ recombinants	Conclusion
Ap50 × Ap53	$< 10^{-8}$	Same "site"
Ap4 × Ap37	$< 10^{-8}$	Same "site"
Ap9 × Ap49	$< 10^{-8}$	Ap9, 49, 19
Ap9 × Ap19	$< 10^{-8}$	same "site"
Ap14 × Ap49	$< 10^{-8}$	
Ap19 × Ap16	1.4×10^{-4}	Different site
Ap19 × Ap57	3.2×10^{-3}	Different site
Ap57 × Ap45	1.2×10^{-3}	Different site
Ap14 × Ap29	$< 10^{-8}$	Ap14 carries
Ap14 × Ap32	$< 10^{-8}$	a deletion
Ap14 × Ap73	$< 10^{-8}$	Ap29–Ap73
Ap14 × Ap67	6.9×10^{-4}	inclusive
Ap14 × Ap49	3.0×10^{-2}	
Ap44 × Ap73	$< 10^{-8}$	Ap44 carries
Ap44 × Ap32	$< 10^{-8}$	a deletion
Ap32 × Ap73	1.1×10^{-2}	Control

P22Ap particles were grown by induction of lysogens and titered on strain DB147, as described in the MATERIALS AND METHODS. Crosses were at a nominal m.o.i. of seven of each phage. Total yield was calculated from the titer on DB147; *ant*$^+$ was titered on DB5057.

ing insertions occupy 15 genetically separable sites of insertion. With respect to the insertions occupying genetically inseparable sites, it is important to emphasize that in each case the insertions are of independent origin and that in two cases the two genetically inseparable insertions are in opposite orientations with respect to one another (WEINSTOCK 1977). These data do not, of course, exclude the possibility that one or more of the insertions occupying the same site might be short deletions, particularly since two deletions were observed among the 21 insertions studied. We conclude that insertions are quite evenly spread across the *ant* gene.

Orientation of insertions in ant

As described earlier, some P22Ap phages containing Tn*1* insertions in *ant* were found to be phenotypically also *9*$^-$ (Table 3). In a subsequent publication (WEINSTOCK and BOTSTEIN), we will show by direct measurement of p9 production and complementation tests that these phages contain insertions in *ant* that are polar on gene *9*. Since the Tn*1* element is polar in one orientation but not in the other orientation (RUBENS, HEFFRON and FALKOW 1976), the orientation of a Tn*1* insertion in *ant* can be determined from its effect on expression of gene *9*. As summarized in Table 3 and Figure 3, about half the Tn*1* insertions in *ant* are in the polar orientation, and insertions in either orientation are found throughout the gene. Thus, there is no apparent orientation specificity among Tn*1* insertions in *ant*.

*Reversion of Tn*1 *insertion mutations*

None of the Tn*1* insertions in *ant* reverts to *ant*$^+$, as judged from plating tests capable of detecting one revertant in 10^{10} phages. The sensitivity of the plating procedure was verified in reconstruction experiments in which a few wild-type phages were plated in the presence of large numbers of P22Ap phages. Thus, Tn*1* integration within a gene causes irreversible loss of function.

On the other hand, Tn*1* insertions in *ant* that are polar on gene *9* revert to *9*$^+$ at a frequency of at least one in 10^6 phages. Thus, reversion of Tn*1* insertion mutations is a convenient way of determining whether insertion mutations are within or simply polar upon a structural gene.

Another kind of "reversion" can be selected from P22Ap phages containing Tn*1* inserted in genes nonessential for growth. Such phages (*e.g.*, P22Ap2) will not grow after single infection due to the lack of terminal repetition in the phage DNA. Plaque-forming revertants (pfr's) arise by deletion of some nonessential DNA that restores terminal repetition for the P22Ap phages; such pfr's occur at frequencies of about 10^{-5} to 10^{-6} and usually have lost all or part of the Tn*1* element and the ability to transduce *amp*^{R}. As was found previously for analogous Tn*10* insertions (CHAN *et al.* 1972; TYE, CHAN and BOTSTEIN 1974) and *pro*-transducing phages with oversize genomes (CHAN and BOTSTEIN 1976), the pfr derivatives of P22Ap phages often have deletions of P22, as well as Tn*1*, DNA. They were not studied further.

Isolation of prophage deletions from P22Ap phages by transduction at low multiplicity

As described above (see also Figure 1), the fact that the P22-Tn*1* composite genome is larger than a headful of P22 DNA means that circularization, which is prerequisite to integration of phage DNA into the chromosome to form a prophage, can occur only at high multiplicity of infection where several of the permuted incomplete genomes can recombine to form a complete circular genome. This situation forms the basis of a selection for deletions in the composite genome: transduction at very low multiplicity yields transductants that do not contain complete composite genomes, but instead are prophage deletions (CHAN *et al.* 1972; TYE, CHAN and BOTSTEIN 1974). These shortened genomes presumably were able to circularize unaided because they are short enough to fit into a head with enough terminal repetition to permit circularization.

Such prophage deletions were isolated from P22Ap phages after low multi-

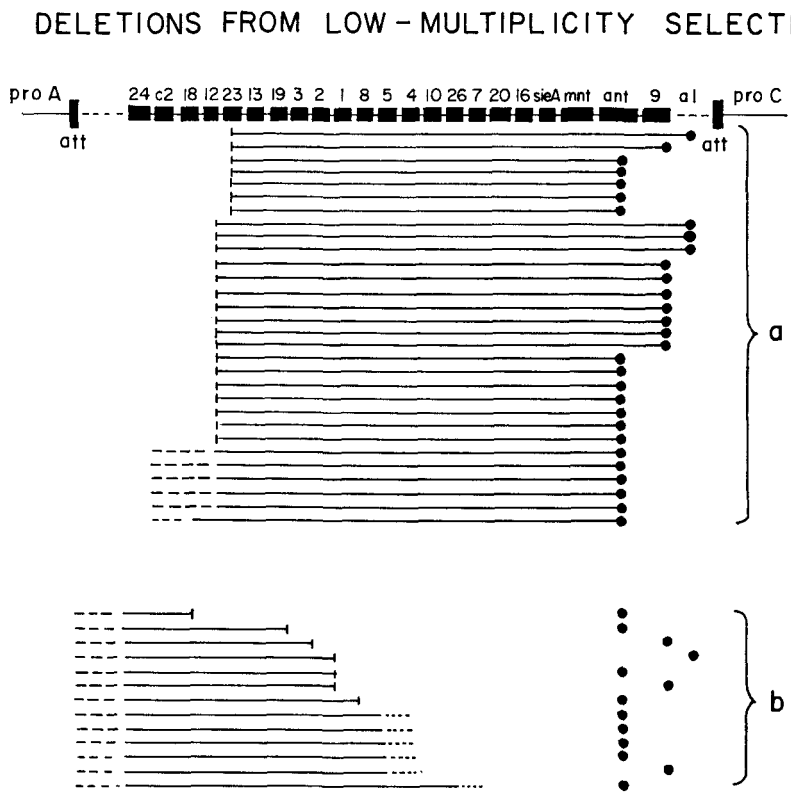

FIGURE 4.—Prophage deletions derived by the low multiplicity selection. The rationale and methods are described in the text. The solid lines indicate deleted material; the solid dots indicate the genetic position of the Tn*1* insertion in the parent P22Ap phage. Dotted lines mean the exact extent of the deletion was not determined. (a) shows deletions with endpoints at or near Tn*1*; (b) shows all the remaining deletions, which appear to begin and end far from the Tn*1* insertion.

plicity transduction for ampicillin resistance. The frequency at which these deletions were recovered was about one in 10^5 particles.

The results of genetic mapping of the prophage deletions in 43 independent transductants obtained from 15 different P22Ap phages are shown in Figure 4. Two types of deletions were found. One class, shown in Figure 4a, have one endpoint tightly linked genetically to the site of the Tn*1* insertion in the parent P22Ap phage. Thus, deletions in P22Ap2 have an endpoint in the *a1* region (the site of Tn*1* insertion in P22Ap2), while deletions from ten *ant*⁻ P22Ap phages all have an endpoint in *ant* that is not separable by genetic tests from the site of Tn*1* insertion. The observation that deletions have an endpoint very near or within the Tn*1* insertion, suggests that their formation actively involves Tn*1*.

The other endpoints of these insertion-linked deletions are not randomly located, but limited to the vicinity of gene *12*. This is true of deletions generated from Tn*1* insertions at several different sites representing both orientations of the Tn*1* element. A number of the endpoints in gene *12* have been mapped with respect to 42 independent amber mutations and one frameshift mutation within this gene. The results (Figure 5) show that the 14 deletions examined have at least eight genetically distinct endpoints. The fact that these deletions end in the same region, around gene *12*, is an example of *regional specificity*. But since there are many different endpoints within gene *12*, there is also *local non-specificity*. This distribution is strongly reminiscent of that found above for Tn*1* translocations.

A second class of deletions obtained from the low multiplicity transduction selection is shown in Figure 4b. These deletions are not linked to the site of Tn*1* insertion in the parent P22Ap phage, and they all have one endpoint to the left

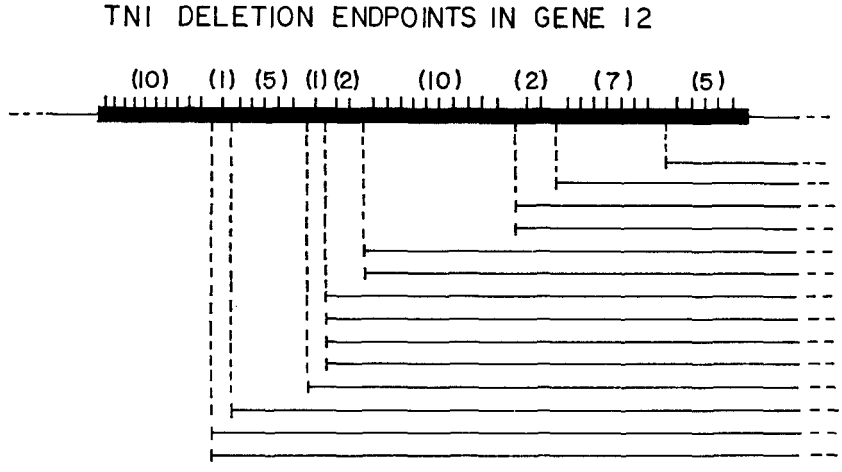

FIGURE 5.—Fine-structure mapping of the deletions from the low multiplicity selection that end in gene *12* (from Figure 4a). Each of the vertical ticks indicates a point mutation (amber or frameshift). These are all independent, but not all have been shown to be different. The numbers in parentheses indicate the number of such point mutations falling in each deletion interval.

of gene *24* and extend beyond *c2* to at least seven different sites. It is possible these deletions are generated not by Tn*1* but by phage functions since they are adjacent to the phage attachment site. However, in this case they should have deleted the *int* gene and therefore should not have been able to integrate. Therefore their nature is still obscure and they were not studied further.

Isolation of prophage deletions as survivors of heat induction of a P22Ap mnt-ts *lysogen*

The deletions described in the previous section involved selection for restoration of terminal repetition and thus had to be large enough to compensate for the size of the Tn*1* insertion. Because the nonrandomness of the prophage deletions could conceivably be related to this size requirement, a second, unrelated procedure was used to obtain amp^R prophage deletions from P22Ap phages. DB7273, a lysogen of P22Ap2 *sieA*-44 *mnt-ts*1 (containing an insertion in the *a1* region; see Figure 1) was constructed. This strain is temperature-sensitive since at high temperature the *mnt* repressor is inactivated, allowing antirepressor to be synthesized, which induces the prophage and kills the cell. Temperature-resistant survivors are expected to include ant^- mutants, mnt^+ revertants, deletions removing the entire *immI* region and deletions removing the killing functions near the *immC* region (CHAN and BOTSTEIN 1972; BOTSTEIN *et al.* 1975). Survivors were screened by marker rescue for the presence of gene *9*, and only 9^- prophages were characterized. Deletions associated with the P22Ap2 insertion must extend from the *a1* region to *ant* and will thus be 9^-; whereas ant^- point mutations, mnt^+ revertants or deletions not linked to the Tn*1* insertion will be 9^+.

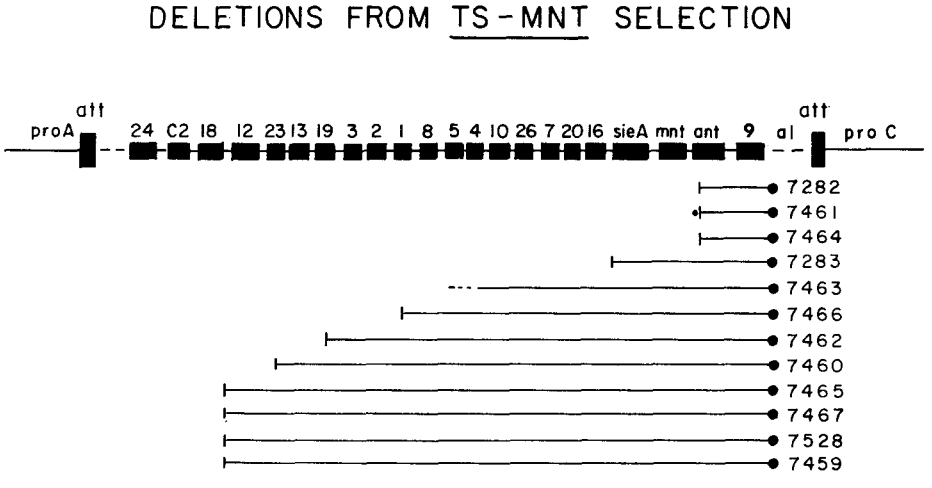

FIGURE 6.—Prophage deletions derived by the *mnt-ts* selection. The rationale and methods are described in the text. The parent lysogen was DB7273, a lysogen of P22Ap2 *sie*A-44 *mnt-ts*1. amp^R survivors at 42° were tested only when they had lost gene *9*, as determined by streak-testing with P22 *virB*-3 *9-am*N9. The conventions are the same as in Figure 4. A single mutation in each of genes *12* and *18* was used; the last four deletions shown were missing the 12^- marker but retained the 18^- marker.

The *ant*⁻ *9*⁻ *amp*^{R} prophage deletions were recovered at a frequency of about one per 10^7 induced cells. The results of genetic mapping are shown in Figure 6. All deletions have an endpoint in the *a1* region, where the Tn*1* insertion was. The other endpoint is distributed more randomly than was found in low multiplicity transductants. Nevertheless, there is a bias to the vicinity of gene *12*, supporting the notion that the regional specificity of deletion formation is not an artifact of the selection procedure. Deletions also appear to end frequently in the *mnt-ant* region, which coincides with the preferred location for Tn*1* integration described above. Deletions in this region would have been missed in the selection for low multiplicity transductants since they would have been immune (*c2*⁺ *mnt*⁺) and possibly might not have had their genomes shortened enough to restore terminal repetition. Three of these deletions (DB7282, DB7461, DB7464) end in *ant*, but each has a genetically different endpoint. Thus, like deletions in gene *12*, the endpoints of these deletions appear to be locally nonspecific.

Those deletions with an endpoint in the central region of the prophage, between genes *12* and *ant*, were not observed in the selection for low multiplicity transductants, and appear to be randomly distributed. Since they occur at about 1% of the frequency of low multiplicity transductants, it is likely that they would have been missed previously. The frequency is low enough so that they could even be spontaneous deletions. Alternatively, there may be differences in the behavior of Tn*1* in replicating DNA as opposed to repressed prophage DNA. This question has not been studied further.

Physical structure of P22Ap insertion and deletion derivative genomes

DNA heteroduplex analysis of P22Ap phage DNA was carried out as described in the MATERIALS AND METHODS. As expected from the published studies of Tn*1* (RUBENS, HEFFRON and FALKOW 1976; HEFFRON *et al.* 1977), all the P22Ap phages examined had an insertion of about 4.8 kb in their DNA, which had a small (less than 100 bases) inverted repetition at the ends (Figure 7 and Table 6). As was expected from similar studies with Tn*10* insertions (TYE, CHAN and BOTSTEIN 1974), the DNA molecules in P22Ap particles were incomplete permuted fragments of the P22-Tn*1* composite genome and were visibly lacking terminal repetition. Since, as was shown above, many of the Tn*1* insertions have well-defined genetic positions as inferred from the mutations they cause, heteroduplex comparisons with respect to a distinguishable marker (the Tn*10* insertion) makes possible correlation of the genetic and physical maps of P22. As described in detail elsewhere (WEINSTOCK 1977; WEINSTOCK and BOTSTEIN, in preparation), the physical position of the insertions is consistent with the genetic lesions they cause.

The structure of four of the deletion derivatives of P22Ap2 obtained by the *mnt-ts* selection scheme described above was also examined by heteroduplex analysis. These deletion derivatives were chosen because they do not remove any of the genes required to encapsulate DNA (BOTSTEIN, WADDELL and KING 1973) and induction of these lysogens results in a yield of particles that are defective because they are missing proteins essential for injection of phage DNA. These

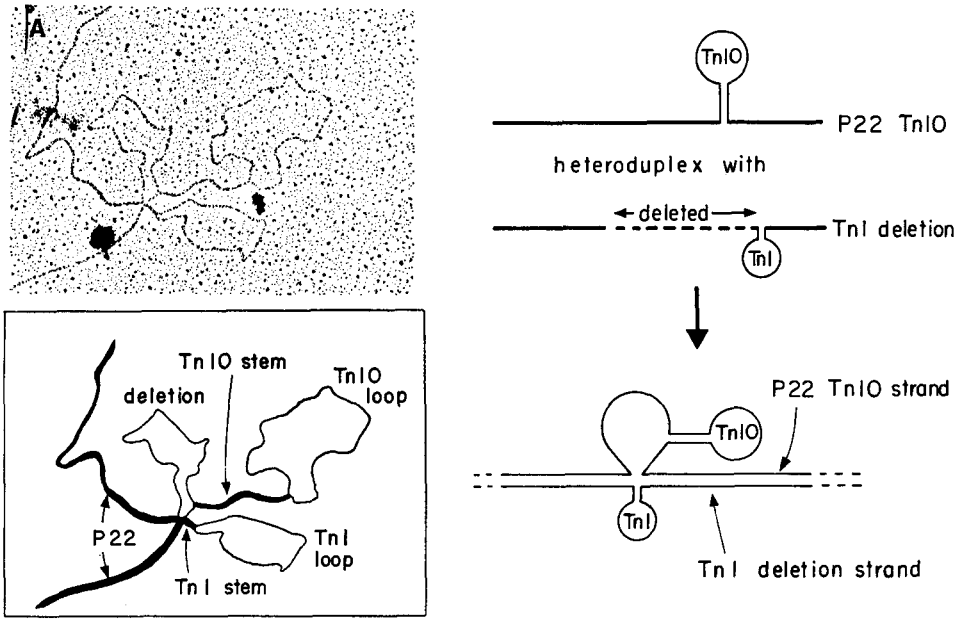

FIGURE 7.—Heteroduplex analysis of a Tn*1*-associated prophage deletion. DNA from an induced lysate of strain DB7283, a deletion derived from P22Ap2 by the *mnt-ts* selection (Figure 6), was annealed with DNA from P22Tc10 (TYE, CHAN and BOTSTEIN 1974; CHAN and BOTSTEIN 1976) and prepared for electron microscopy as described in the MATERIALS AND METHODS. The box marked A shows the actual electron micrograph; the box below, a drawing showing regions of single-stranded DNA (thin line) and double-stranded DNA (thick line). The diagram on the left shows a schematic interpretation of the micrograph.

particles were purified, and DNA heteroduplexes were prepared with DNA from P22Tc10 (TYE, CHAN and BOTSTEIN 1974), which is a specialized transducing derivative of P22 that contains the Tn*10* element inserted at a site between the Tn*1* insertion in Ap2 and the *imm1* region. Thus the deletions (which, by the

TABLE 6

Heteroduplex analysis of Tn1 insertions in P22Ap phages

Phage	Site of Tn*1* insertion	Size of Tn*1* insertion	Size of Tn*1* stem
P22Ap2	*a1* region	4720 ± 212	93 ± 13
P22Ap7	*9*	4610 ± 138	42 ± 13
P22Ap4	*ant*	4770 ± 140	160 ± 68
P22Ap38	*16*	4680 ± 172	160 ± 82
P22Ap12	*20*	5030 ± 228	48
Mean values		4760 ± 160	100 ± 58

Heteroduplexes between the indicated phage DNA and P22Tc10 DNA were formed and visualized as decribed in the MATERIALS AND METHODS. The measurements were related to the Tn*10* stem (taken as 1400 bases) and Tn*10* loop (taken as 6300 bases) for double- and single-strand standards, respectively (KLECKNER and ROSS 1978). The errors are standard deviations of the means of the measurements.

nature of the selection used to obtain them, at least enter the *imm1* region as far as the *ant* gene) must include the site of the Tn*10* insertion in P22Tc10. As shown in Figure 7, the expected heteroduplex will include a region of nonhomology corresponding to the Tn*10* DNA. The 1.4 kb inverted repetition in Tn*10* causes it to have a clearly identifiable stem and loop structure (SHARP, COHEN and DAVIDSON 1973; TYE, CHAN and BOTSTEIN 1974). The other strand in this region of nonhomology should contain whatever is left of the Tn*1* insertion in P22Ap2. As shown in Figure 7, the entire Tn*1* insertion is apparently still present, as judged from its size and, more significantly, the fact that in most of the heteroduplexes examined the short stem (caused by the inverted repetition) is still observed. This means that the Tn*1* insertion is intact, to the level of heteroduplex analysis, in these deletion derivatives. The presence in these heteroduplexes of the Tn*10* marker near the deletion endpoint allows another check (*i.e.*, distance) of the idea that the endpoint is at the end of the Tn*1* insertion. Measurement of the distance between the deletion endpoint and the Tn*10* marker gives an average of 200 bp, which is not statistically different from the value of 160 bp obtained from P22Ap/P22Tc10 heteroduplex molecules (WEINSTOCK 1977). Similar results have recently been reported by NISEN *et al.* (1978) for Tn*3*.

The apparent retention of an intact Tn*1* in the four deletions examined supports the idea that the deletions are generated in some way by Tn*1*.

DISCUSSION

The results presented in this paper can be summarized as follows: (1) Insertions of the translocatable ampicillin-resistance element Tn*1* into phage P22 are rare (10^{-10}/phage) and nonrandomly distributed in the P22 genome. They are found mainly in the vicinity of the *ant* gene. (2) Insertions within the *ant* gene are found at many (at least 15) genetically separable sites, are found equally frequently in both orientations, and cause irreversible loss of gene function. Some insertions in *ant* appear to be associated with an adjacent deletion. (3) Prophage deletions selected as low-multiplicity transductants have nonrandomly distributed endpoints. One end is at or very near to the site of the Tn*1* insertion and the other is in the vicinity of gene *12*; however, there are many genetically distinguishable endpoints within gene *12*. Prophage deletions selected as survivors of induction of a P22Ap *mnt-ts* lysogen have similarly nonrandom endpoints, with the Tn*1*-distal end frequently near the *ant* gene, as well as gene *12*. (4) Physical analysis of several prophage deletions suggests that the Tn*1* is intact to the resolution of DNA electron microscopy and that the deletions begin at the end of the Tn*1* insertion.

*Specificity of illegitimate recombination associated with Tn*1

The most striking feature of illegitimate recombination emerging from these results is that Tn*1* appears to have specificity of an unusual kind: Tn*1* shows some preference for inserting into particular regions of the P22 genome (near the *ant* gene for insertion; near gene *12* and, probably again, the *ant* gene for

deletion). Yet mapping of insertion sites and deletion endpoints to higher resolution (*i.e.*, less than one gene, *ca.* 1 kb) shows a rather even distribution of events, with independent events generally having genetically distinguishable sites. In other words, Tn*1* seems to display regional specificity, but not local specificity, with respect to both insertion and deletion formation. Similar conclusions have been reached by others from experiments involving Tn*2* and Tn*3*, elements nearly identical to Tn*1* (HEFFRON *et al.* 1977; RUBENS, HEFFRON and FALKOW 1976; KRETSCHMER and COHEN 1977; NISEN *et al.* 1977).

On the other hand, a quite different picture has emerged from studies of specificity of insertion and deletion formation by the tetracycline-resistance element, Tn*10*. In that case, very strong preference for "hot spots" (*i.e.*, sites at most a few nucleotides in length) rather evenly distributed over bacterial genomes has been observed (BOTSTEIN and KLECKNER 1977; KLECKNER *et al.* 1979; NOEL and AMES 1978).

An analogy might be proposed between the kind of specificity displayed by Tn*1* and the specificity exhibited by type-I restriction endonucleases, which recognize specific nucleotide sequences, but which cleave DNA at points a variable distance away (NATHANS and SMITH 1975; see also STARLINGER and SAEDLER 1976 and NISEN *et al.* 1977 for similar arguments). A parallel analogy could be drawn between the kind of specificity displayed by Tn*10* and the type-II restriction endonucleases, which recognize and cleave the same sequences.

Although these analogies might serve to clarify the distinction between local and regional specificity, it should not be assumed that there is direct evidence for any analogy in the actual molecular mechanism. It is not known, for example, that either Tn*1* or Tn*10* actually recognizes particular DNA sequences: the possibility remains (especially for Tn*1*) that some more general feature (base composition, for example) is being recognized. Nevertheless, it is tempting to speculate that the specificity of illegitimate recombination that we have observed is the result of some aspect of the mechanism of this recombination that recognizes sequence; in the case of Tn*1*, this recognition occurs at a variable distance from the site of recombination; whereas, in the case of Tn*10*, the recognition and recombination occur very close to each other. The analogies might be imperfect in another way: our data suggest that several independent insertions of Tn*1* into the *ant* gene are inseparable by recombination. Given that the sensitivity of crosses in P22 is very high (BOTSTEIN, CHAN and WADDELL 1972) and that very closely linked insertions seem to behave well in transductional crosses (KLECKNER, ROTH and BOTSTEIN 1977; KLECKNER *et al.* 1979), it seems possible that despite the relatively even distribution of Tn*1* insertions within *ant* there might still be some residual preference for particular sites.

Relation between insertion and deletion formation

The second striking feature of our results is that both deletion formation and insertion exhibit regional specificity and local nonspecificity. This can be interpreted most simply by assuming that there is a common element in the molecular mechanism involved in these two kinds of illegitimate recombination. It has been

shown by HEFFRON *et al.* (1977) that the ends of Tn*2* (an element closely related to Tn*1*) are involved in transposition by this element, and that Tn*2* specifies a diffusible factor (most likely a protein) essential to transposition. It seems reasonable to suppose that one or both of these essential features might be the element common to the two kinds of illegitimate recombination.

Our finding that most of the deletions derived from Tn*1* insertion phages end at or very near the Tn*1* element supports the idea that the deletions are somehow caused by the element. The simplest hypothesis is that the illegitimate joints necessarily involved in both translocation and deletion formation must involve the ends of the element.

One question, which remains to be addressed, is our failure to find insertions in the region of gene *12*, an region obviously favored by Tn*1* for deletion formation. Although no definitive explanation can be offered, one possibility is that insertions in this region [which involves DNA replication and contains the origin of replication (BOTSTEIN and HERSKOWITZ 1974; HILLIKER and BOTSTEIN 1976)] might be lethal and not complementable by a helper phage. If this were so, our methods of isolating insertion mutants would not have recovered these. On the other hand, insertions may actually not occur in this region. This would imply that the mechanisms of translocation and deletion generation have different detailed site specificities, although they are otherwise related (*i.e.*, regional specificity, local nonspecificity and an essential role for the Tn*1* termini).

The nature of the preferred regions

One simple explanation for the existence of preferred regions for illegitimate recombination associated with Tn*1* is that some specific nucleotide sequence is recognized, as suggested by the restriction endonuclease analogy given above. However, some alternative possibilities also must be considered. One of these is that some general feature (possibly base composition) is involved. This possibility is made somewhat more plausible by the observation that both preferred regions (near *imm1* and near gene *12*) are known to be rich in AT base pairs (TYE, HUBERMAN and BOTSTEIN 1974; SKALKA and HANSON 1972). Another alternative possibility might be that certain regions of the DNA are more accessible for insertion and/or deletion because of structural reasons, such as binding of proteins, transcription apparatus, DNA packaging proteins, etc. This kind of model would require further, relatively unattractive assumptions because genetic recombination is quite uniform across the genome, and it seems likely that this kind of recombination might require the same kind of accessibility.

Usefulness of P22 in studying insertion elements

It is probably worth emphasizing that certain features of the life cycle of phage P22 (Figure 1) have been particularly useful in analyzing Tn*1* as well as Tn*10* (CHAN and BOTSTEIN 1976). Foremost among these is the possibility of recognizing, propagating and manipulating P22 genomes that are too large to fit into a phage capsid. The requirement for terminal repetition is facultative, since permutation can compensate for lack of terminal repetition in high-multiplicity

infection, and induction of lysogens is normal even when genomes are grossly oversize. The may well-characterized genes (*e.g., ant, 9*) that are not absolutely esssential for propagation of phage and, of course, the ability to perpetuate massive deletions as prophages also have been useful.

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