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Genetic Engineering *in Vivo* Using Translocatable Drug-resistance Elements

New Methods in Bacterial Genetics

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A number of translocatable drug-resistance elements have recently been described which are able to insert themselves into a large number of different sites in prokaryotic genomes. These elements cause recognizable mutations when insertion occurs within a structural gene or an operon. Drug-resistance elements are also associated with other kinds of illegitimate recombination events, notably deletions and inversions.

This paper summarizes uses to which these properties of translocatable drug-resistance elements can be put in genetic manipulations of bacteria. Translocatable drug-resistance elements are useful in isolation of mutants (even where the mutant phenotype is not easily scored), in the construction of strains and other genetic manipulations (even when selection is difficult or impossible), in localized mutagenesis, in chromosomal mapping, in construction of Hfr strains with known origin and direction of chromosome transfer, in complementation tests, in construction of new F′ plasmids, in construction of new specialized transducing phages, in isolation of deletions with one or both endpoints specified, in construction of gene and operon fusions, and in the selection and maintenance of chromosomal duplications. Experiments are described which illustrate many of these techniques.

1. Introduction

A number of reports have been published recently which describe the translocation, from one replicon to another, of genetic elements which carry genes conferring resistance to various antibiotics (Hedges & Jacob, 1974; Heffron *et al.*, 1975; Berg *et al.*, 1975; Kleckner *et al.*, 1975; Gottesman & Rosner, 1975). For a number of these elements, translocations into known structural genes of bacterial and phage genomes have been identified. These events cause recognizable insertion mutations. The drug-resistance elements have in common their ability to insert at a large number of sites.

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The elements differ in the degree of specificity which they display; some appear to insert randomly, whereas others have distinct preferences for particular sites. In all the cases examined, insertion occurs even in mutant (*rec*A$^-$) bacteria which cannot carry out homologous recombination.

All the translocatable drug-resistant elements thus far described ultimately derive from drug-resistance plasmids (R-factors), which are widely transmissible among bacteria. The demonstration that many of the drug-resistance determinants on these plasmids have the capacity to be translocated *in vivo* to a large number of sites on virtually any DNA in the cell has done much to explain the extensive and rather confusing literature on drug-resistance plasmid recombination. In addition, a clear relationship has emerged between drug-resistance elements and the previously described IS sequences (Ptashne & Cohen, 1975; MacHattie & Jakowski, 1977). The translocatable IS sequences were detected by virtue of the polar mutations (Starlinger & Saedler, 1976) which they cause and by their frequent appearance in plasmids of bacteria, particularly the fertility factor (F) and the R factors. The IS sequences are shorter than the drug-resistance elements and, in one case, an inverted repetition at the ends of a drug-resistant element conferring tetracycline resistance (Tn10) has been shown to be homologous to the known IS sequence IS3 (Ptashne & Cohen, 1975). Similarily, Tn9 is flanked by copies of IS1 in direct orientation (MacHattie & Jakowski, 1977).

The IS sequences (either alone, or as part of drug-resistance elements) have been implicated in a variety of unusual (often "illegitimate" and *rec*A-independent) recombination phenomena; deletions, inversions, fusions, integration of F factors and amplification of R factors (Starlinger & Saedler, 1976). It seems likely that the capacity of the IS elements and the translocatable drug-resistance elements to insert into non-homologous DNA may play a major role in the gross rearrangements of segments of DNA within a particular species and (by virtue of their transfer among diverse bacterial species on plasmids) between organisms quite distantly related.

Just as translocatable drug-resistance elements appear to have played a part in naturally selected rearrangements of bacterial and plasmid genes, the bacterial geneticist can use these elements to manipulate the genes of bacteria, phage and plasmids in ways which are otherwise difficult or impossible.

Any genetic element capable of inserting itself in many different sites is useful in genetic manipulation. Experiments taking advantage of multiple possible insertion sites of the F factor (Signer & Beckwith, 1966), of bacteriophage Mu-1 (Faelen & Toussaint, 1976; Faelen *et al.*, 1975; reviewed by Howe & Bade, 1975) and of bacteriophage lambda (under special circumstances; Shimada *et al.*, 1973) have become classic in modern molecular genetics. The drug-resistance elements offer the geneticist an additional, and equally important advantage: an inserted drug-resistance element can act as a positive selective marker by virtue of its drug resistance, thus permitting selection for inheritance of the insertion and any mutation caused by it. The drug-resistance elements have thus made possible a new technology of manipulation of genes *in vivo*.

Since starting to use insertion elements in genetic manipulations, we have continually encountered new situations where the elements could be used to advantage. So many new possibilities have arisen and so many old tasks have become easier, that we feel that anyone interested in the genetics of bacteria should seriously consider use of these elements. In this paper we review the properties of translocatable drug

resistance elements which are useful in genetic manipulations (section 2), and review many specific uses of these elements which seem feasible (section 3). Data are presented for the possibilities that have been experimentally realized. We also discuss general principles and particular methods for the construction of suitable vehicles carrying translocatable drug-resistance elements which can be used to introduce insertions into prokaryotic chromosomes. Our own work has dealt mainly with the tetracycline-resistance element Tn10 but the general principles described here should apply to all similar translocatable elements.

Many of the data presented were obtained by colleagues and students in the course of work on a variety of genetic projects not directly related to translocatable elements. Their work will be described later in more detailed publications concerning their individual projects. The following have contributed to the data presented: Philip Anderson, David Barker, Christoph Beck, Donald Biek, Russell Chan, Forrest Chumley, Laszlo Csonka, Ann Gauger, Paula Grisafi, Susan Gottesman, Ingrid Hoppe, Mark Johnston, Rolf Menzel, Miriam Susskind, George Weinstock, Fred Winston and Katherine Reichardt.

2. Properties of Translocatable Drug-resistance Elements

In order to use translocatable elements in bacterial genetics, one needs a simple way of introducing the elements into the bacterial chromosome. In a later section of this paper, we describe in some detail several ways in which this can be done. For the present, a summary of the principles behind these methods will suffice. One needs a vehicle (usually a plasmid or a phage) to carry the desired drug-resistance element, and a means of selecting against the maintenance of the intact vehicle in the recipient bacteria. If these conditions are met, the only way the recipient bacteria can become stably drug-resistant is for the drug-resistance element to leave the vehicle and to become inserted in the bacterial genome.

Once one has suitable vehicles, one can select arbitrarily many independent insertions into the bacterial chromosome. These insertions will result in detectable mutations when the continuity of a gene whose mutant phenotype is recognizable is interrupted. The most convenient kinds of mutations to work with are auxotrophs; by studying the distribution of new nutritional requirements among insertion mutants a great deal has been learned about the genetics of a few of the translocatable drug-resistance elements.

The properties of drug-resistance elements which are particularly important for their usefulness in bacterial genetics are the following.

(a) Translocatable drug-resistance elements can be found inserted at a large number of sites on the bacterial chromosome.
(b) Interrupted genes suffer complete loss of function.
(c) The phenotype of the insertion mutation is completely linked to drug-resistance in genetic crosses.
(d) Insertion mutants can be recovered at high frequency after low level "mutagenesis" by exposure to a translocatable element.
(e) Insertion mutations revert by precise excision with concomitant loss of drug-resistance.
(f) Insertions in operons are strongly polar.
(g) Drug-resistance insertions can generate deletions nearby.

(h) Drug-resistance elements can provide a portable region of homology.
(i) Insertions behave as point mutations in fine-structure mapping.
(j) Insertions can be specifically obtained which are *near* but not *within* a gene of interest.

Each of these properties (with relevant data) are discussed in the remainder of this section of this paper. The following section gives examples of the uses which we have been able to make of these elements based on these ten properties.

(a) *Translocatable drug-resistance elements can be found inserted at a large number of sites on the bacterial chromosome*

Recognizable insertion mutations have been obtained in our laboratories and elsewhere with several drug-resistance elements (Table 1). We have concentrated on mutagenesis of the chromosomes of *Salmonella typhimurium* and *Escherichia coli* using the translocatable tetracycline-resistance element Tn10 (Kleckner *et al.*, 1975), although we have made some *E. coli* auxotrophs using the translocatable kanamycin-resistance element Tn5 (first described by Berg *et al.*, 1975). Virtually all these insertions were made by selecting for translocation of the element from a bacteriophage into the bacterial chromosome.

The most important conclusion to be drawn from the distribution of auxotropic mutations (Table 2) is that apparently most genes in *Salmonella* or *E. coli* can be inactivated by insertion of Tn10 or Tn5.

However, this conclusion must be somewhat modified after one examines more closely the distribution of Tn10 insertion events at a scale smaller than whole genomes. Within the histidine operon, for example, it seems that there are particular genes in which Tn10 insertions occur more frequently than others. Thus, in the case of Tn10 insertion, mutagenesis might not yield mutations in all genes with equal probability.

Figure 1 shows the distribution of 55 independent insertions into the *his* operon of *S. typhimurium*. This distribution is not random: more than half of the insertions are

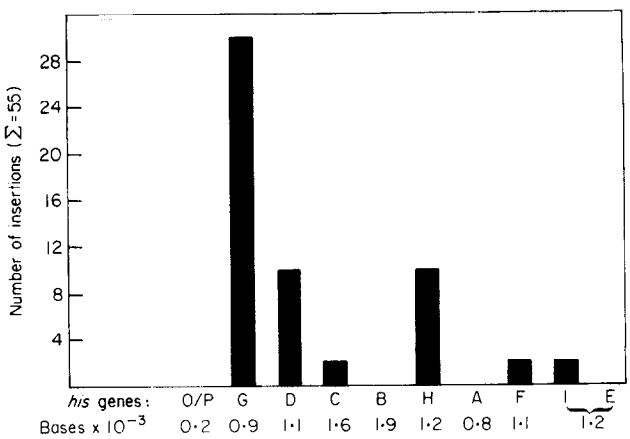


FIG. 1. Distribution of Tn10 insertions in the *Salmonella* histidine operon. 55 independent *his*:: Tn10 insertion auxotrophs were isolated following infection of *S. typhimurium* LT2 with a suitable P22 Tn10 vehicle (see section 4). The positions of these insertions within the *his* operon were determined by deletion mapping and complementation tests as described elsewhere (Kleckner *et al.*, 1975).

TABLE 1

Translocatable drug-resistance elements

Element†	Original R-factor	Phage vehicles	Approx. size (base-pairs)	Terminal repetition (base-pairs)	Polarity: Yes/no	Polarity: One/both orientations	Translocation‡: Specificity	Translocation‡: Frequency	Deletion generation	References
Tn1(*amp*^{R})	RP4	P22, λ	4800	140, inverted	Yes	One	Uncertain	Variable	Yes	Hedges & Jacob (1974); Bennett & Richmond (1976); Weinstock, Kleckner & Botstein, unpublished work.
Tn2(*amp*^{R})	RSF1030	λ	4800	140, inverted	Yes	One	Low	Variable	—	Heffron *et al.* (1975); Reubens *et al.* (1976).
Tn3(*amp*^{R})	R1	—	4600	140, inverted	—	—	Low	—	—	Kopecko *et al.* (1976)
Tn4(*amp*^{R}, *sul*^{R},*str*^{R})	R1	—	20,500	140, inverted contains Tn3	—	—	Low	—	—	Kopecko *et al.* (1976)
Tn5(*kan*^{R})	JR67	λ, P22	5200	1460, inverted	Yes	Both§	Low	Medium	Yes	Berg *et al.* (1975); Berg (1977).
Tn6(*kan*^{R})	JR72	λ	4100	?, perhaps direct	—	—	—	—	—	Berg *et al.* (1975)
Tn7(*str*^{R} *tmp*^{R})	R483	—	—	—	—	—	High	Low	—	Barth *et al.* (1976)
Tn9(*cam*^{R})	pSM14	P1, λ	2500	800, direct homologous to IS1	Yes§	Both§	Uncertain	Low	Yes	Gottesman & Rosner (1975); Rosner & Gottesman (1977); Kondo & Mitsuhashi (1964); MacHattie & Jakowski (1977).
Tn10(*tet*^{R})	R222 (R100)	P22, λ	9300	1400, inverted homologous to IS3	Yes	Both	Medium	Low	Yes	Kleckner *et al.* (1975); Botstein & Kleckner (1977).

This Table lists the known translocatable drug-resistance elements and certain of their properties relevant to their possible use in bacterial genetics. They are described in several recent reviews (Starlinger & Saedler, 1976; Cohen, 1976; Kleckner, 1977; Bukhari *et al.*, 1977).

† Elements are shown in the newly accepted nomenclature (Bukhari *et al.*, 1977); drug-resistances are abbreviated as follows: *amp*, ampicillin; *cam* chloramphemicol; *kan*, kanamycin; *str*, streptomycin; *sul*, sulfonamide; *tmp*, trimethoprim.

‡ Translocation specificity: *high* means most insertions are found at one or a few sites in a bacterial chromosome; *medium* means that many sites are found in a bacterial chromosome but that the distribution among and within genes is clearly not random and that "hot spots" are easily observed; *low* means that almost every independent event (even within a single gene, as with Tn5) is at a demonstratably different site.

Translocation frequency: *medium* means more than 10^{-4} translocation events per survivor of a phage-infection or conjugation experiment; *low* means less than 10^{-5} translocation per survivor of such an experiment.

§ These are likely inferences although direct demonstrations have not been published.

TABLE 2

Distribution of independently isolated auxotrophs generated by insertion of Tn10 and Tn5 in E. coli *and* Salmonella

Nutritional requirement	Tn10 *Salmonella* *recA*$^+$	Tn10 *Salmonella* *recA*$^-$	Tn10 *E. coli* *recA*$^+$	Tn5 *E. coli* *recA*$^-$
Alanine	2	1	0	0
Arginine	31	9	6	6
Aromatic amino acids	4	0	0	0
Aspartate	16	0	0	0
Biotin	14	0	0	0
Cysteine	10	11	1	10
Glutamine	12	0	0	0
Glutamate	0	0	1	0
Glycine	2	0	0	0
Guanine	9	3	2	1
Histidine	56	34	2	7
Isoleucine/valine	72	13	0	2
Leucine	18	2	1	0
Lysine	7	2	1	2
Methionine	83	35	2	7
Nicotinic acid	3	0	0	0
Pantothenic acid	5	0	0	0
Pyridoxine	1	0	0	0
Phenylalanine	5	2	7	2
Proline	34	1	0	0
Purine	71	5	0	3
Pyrimidine	21	0	0	12
Serine	18	3	0	1
Thiamin	13	0	2	0
Threonine	23	7	3	0
Tryptophan	44	9	4	0
Tyrosine	10	2	1	0
Not identified	47	71	10	1
Total	631	210	43	54

in the *his*G gene, and Tn10 insertion mutants in three of the *his* genes (*his*B, *his*A and *his*E) have not yet been found. Even within a gene (such as *his*G) there appear to be "hot spots" at which insertion events have occurred repeatedly (Botstein & Kleckner, 1977; Roth & Kleckner, unpublished results). These insertions are inseparable in fine-structure genetic mapping crosses and may well be at exactly the same point. The question whether the nature of the vehicle and the conditions of mutagenesis affect the frequency and distribution of mutations obtained is still under investigation.

Berg (1977) in a study of Tn5 (*kan*^{R}) insertion mutations in the *lac* operon of *E. coli*, found a much more uniform distribution than that found with Tn10 mutations in the *his* operon.

In conclusion, the translocatable drug-resistance elements are capable of insertion at a very large number of sites in the bacterial chromosome. They can therefore be used as genetic tools for the study of virtually any locus or region of the chromosome.

(b) *Drug-resistance is completely associated with insertion mutations*

Each mutation made by insertion of a drug-resistance element carries the drug-resistance determinant intimately associated with the new mutation. Such mutations

will result not only in the appropriate deficiency phenotype (e.g. auxotrophy) but also in the drug-resistance phenotype specified by the inserted DNA. These two phenotypes are thus completely associated, both are transferred together in genetic crosses and lost together in reversion events (Kleckner *et al.*, 1975). Since inheritance of drug-resistance necessitates inheritance of the functional defect, the deficiency mutation (plus nearby genetic material) can be selectively transferred into a new genetic background.

(c) *Interrupted genes suffer complete loss of function*

In mutations caused by insertion of a drug-resistance element, the linear integrity of the affected gene has been disrupted. Insertion mutations therefore will normally result in complete loss of the function encoded by the damaged gene. Insertion mutations will not be susceptible to the processes which are normally responsible for residual function in point mutations (e.g. low level suppression or partial function of a protein in which only one amino acid is altered). Insertion mutations thus provide a way of completely eliminating a function without actually deleting the relevant gene; mutations of this kind are useful in assessing unambiguously the role of the corresponding function *in vivo*. Genes encoding multifunctional proteins might be an exception to the above rule, since it is conceivable that insertions in the distal portion of such a gene might not completely eliminate the function(s) encoded in the proximal portion.

(d) *Mutants are recovered at high frequency following low level mutagenesis*

Use of translocatable drug resistance elements permits efficient recovery of mutants following very low levels of mutagenesis. The procedures for selecting mutants induced by insertion involve selection for a spontaneously occurring, rare event (translocation of Tn10 from P22 or lambda occurs at a frequency of 10^{-6}, see below). Thus the probability of double events is vanishingly small, and every tetracycline-resistant clone which arises in a translocation experiment is a clone of cells which has suffered a single mutational event. In a collection of such clones, the level of mutants for a particular gene is thus governed only by the probability of the single insertion having occurred in that gene; this will be a function of the size of the gene in question and whether it possesses sites of high frequency integration. In general one expects mutations in a particular gene at a level of about one in 5000 mutagenized, selected cells. This frequency approaches the level found following heavy mutagenesis by nitrosoguanidine; yet, in the case of insertion mutations, there is virtually no problem of multiple mutational events.

The drug-resistance phenotype associated with insertion mutations makes it possible to verify unambiguously that a mutant strain carries only one insertion. This can be done by two sorts of crosses. First, upon transfer of the drug-resistance to a new genetic background, drug-resistant recombinants will invariably have acquired the mutant phenotype if and only if there is only a single insertion in the donor. Second, if one transfers into a mutant strain the wild-type allele of the insertion mutation, then all the recombinants will be drug-sensitive if and only if there is a single insertion of the drug-resistance element. Finally, the singleness of the insertion mutation can be verified by reversion; strains carrying only one insertion will invariably lose their drug-resistance phenotype when they lose the mutation. It should be noted that reversion of some mutations can occur by secondary mutation at "suppressor" or "bypass" loci: such pseudo-revertants retain their drug-resistance. This

property of insertion mutations can be used to detect simply and unambiguously such bypass or suppression phenomena.

(e) *Insertion mutations revert by precise excision with concomitant loss of drug-resistance*

Virtually all of the Tn10- and Tn5-generated mutations thus far analyzed are capable of reversion to non-mutant phenotype with concomitant loss of the associated drug-resistance determinant (Kleckner *et al.*, 1975). Figure 2 shows the distribution of reversion frequencies of 28 different Tn10 insertion auxotrophs in *Salmonella*. Although these frequencies vary over a range of at least 50-fold, most reversion frequencies are at the lower end of this range, about 10^{-9}. As discussed in detail elsewhere (Kleckner, Reichardt and Botstein, manuscript in preparation), reversion rate is characteristic of the particular insertion: repeated measurements on many clones of the same mutant give the same result. Reversion frequencies of Tn10 (tet^R) and Tn5 (kan^R) mutations in *E. coli* are comparable to those found for Tn10 in *Salmonella*.

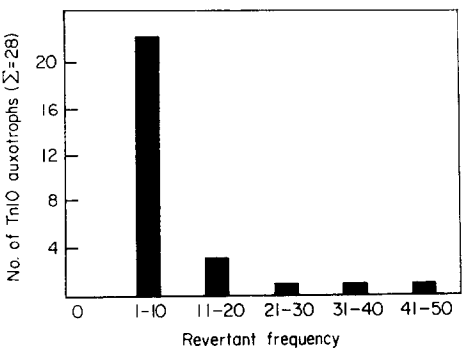


FIG. 2. Distribution of reversion frequencies of Tn10 insertion auxotrophs. Twenty-eight independent Tn10-generated auxotrophic insertion mutations were tested for reversion to prototrophy. Three independent clones of each auxotroph were grown to saturation in nutrient broth to a titer of approx. 2×10^9 cells/ml. To select His^+ revertants, 0·1 ml of each culture was then spread on a minimal glucose agar plate without additional nutrients; the plates were incubated for 3 days at 37°C. Revertant frequency presented is total revertants on the 3 plates or revertants/6×10^8 cells plated. Many of the revertants apparently do not pre-exist in the culture, but arise during incubation of cells on the selective plates; therefore, meaningful calculation of frequency is difficult.

The unusual stability of insertion mutations is a clear advantage. However, the revertibility of these mutations can, in some circumstances, also be an advantage. As mentioned above, this property can be used to verify that a mutation is caused by insertion of the drug-resistance element and to verify that the identified mutation is the only such insertion in the genome. In addition, the revertibility of these mutations means that when such a mutation is used in a strain construction to bring in a nearby marker, the insertion mutation can subsequently be eliminated precisely, leaving the nearby marker as the only alteration in the newly constructed strain.

(f) *Insertions in operons are strongly polar*

The insertion of a drug-resistance element not only inactivates the gene into which insertion occurs, but it also has a strong polar effect on expression of operator-distal

TABLE 3

Secondary promoters in the his *operon revealed by the complementation behavior of* his::*Tn10 insertions*

Location of insertion	Number of insertions tested	Complementation of F'*his* episomes carrying mutations								
		G⁻	*D*⁻	*C*⁻	*B*⁻	*H*⁻	*A*⁻	*F*⁻	*I*⁻	*E*⁻
G	29	−	−	−	+	n.t.	+	+	+	+
D	10	+	−	−	+	n.t.	+	+	+	+
C	2	+	+	−	+	n.t.	+	+	+	+
H	10	+	+	+	+	n.t.	−/+	−/+	+	+
F	2	+	+	+	+	n.t.	+	−	+	+
I	2	+	+	+	+	n.t.	+	+	−	−
Location of promoters: →					→				→	

Strains carrying Tn10 insertions were tested for complementation using a series of characterized F'*his* mutant episomes as described by Kleckner *et al.* (1975). The deduced promoters correspond to those discovered by Atkins & Loper (1970) using deletions instead of insertions. No F'*his*H⁻ episome is available, therefore this complementation test was not done (n.t.).

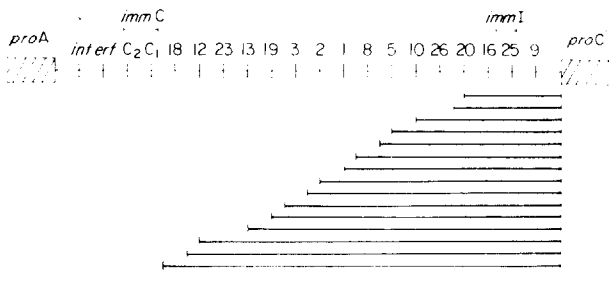


FIG. 3. Map of P22 prophage showing extent of tetracycline-resistant prophage deletions isolated from P22Tc-10. A P22Tc-10 lysogen carries the Tn10 insertion at the extreme right-hand end of the prophage genome (between gene *9* and the attachment site). Each line represents the material deleted in an individual strain. These deletions are described in more detail by Chan *et al.* (1972), from which the Figure is reproduced.

genes in the same operon. Insertion mutations can be used to determine the extent of transcription units in uncharacterized gene clusters. Preliminary data using insertions of the Tn10 element in the isoleucine-valine gene cluster in *Salmonella* suggest that transcription unit may be *ilv*A *ilv*D *ilv*E *ilv*O with transcription proceeding leftward. This contradicts the established order (Ramakrishnan & Adelberg, 1965; Pledger & Umbarger, 1973) but agrees with newer data on this gene cluster (Cohen & Jones, 1976).

However, caution should be exercised if direction of polarity is to be determined based only on complementation behavior of polar Tn10 insertions. While polarity effects are easy to detect using enzyme assays, the existence of low level internal promoters can provide weak expression of some distal genes. In some cases, this weak expression is sufficient to permit growth in the complementation test. This was found to be the situation for the *his* operon where use of Tn10 permitted identification of the internal promoter sites (Table 3; Kleckner *et al.*, 1975). A number of other insertion elements (phage Mu and the IS insertion sequences) have been used in an analogous fashion to determine polarity in uncharacterized gene clusters (e.g. Jaskunas *et al.*, 1975; Toussaint, 1969; Nomura & Engbaek, 1972; Silverman & Simon, 1973).

Of course, the property of strong polarity means that when a new insertion mutation is isolated, it is necessary to determine whether its phenotypic effects derive from inactivation of a structural gene directly by insertion or whether the observed phenotype might be due entirely or in part to the indirect polar effect of the insertion.

(g) *Drug-resistance insertions can generate deletions nearby*

The tetracycline-resistance element Tn10 appears to be capable of generating deletions which extend into the chromosomal regions to one side or the other (but not both) of a Tn10 insertion. The ability to generate deletions of this form seems to be a general property of translocatable elements (Reif & Saedler, 1975; Chan *et al.*, 1972; Botstein & Kleckner, 1977). For Tn10, some of these deletions retain the tetracycline-resistance determinant and some do not. The distribution of deletion endpoints within the chromosome appears to be random (Chan, 1974). The distributions of deletion endpoints for tetracycline-resistant deletions in prophage P22 and for tetracycline-sensitive deletions in *Salmonella* are shown in Figures 3 and 4.

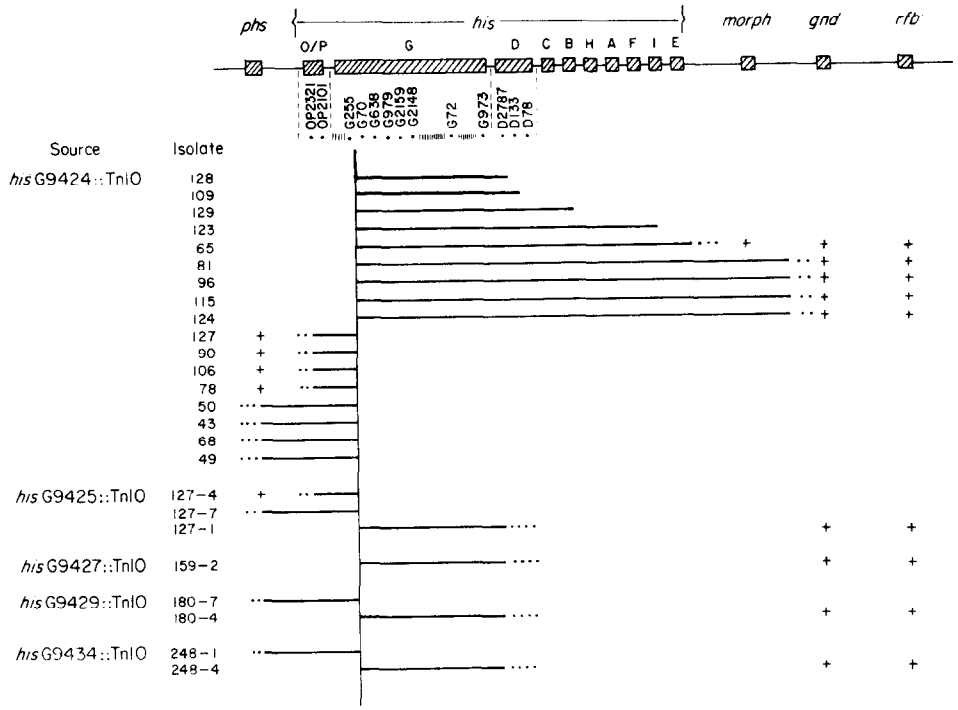


FIG. 4. Tetracycline-sensitive deletions. Deletions were obtained among tetracycline-sensitive derivatives of several independent *his*G::Tn10 insertions at the apparent hot spot. These deletions were tested, both as donors and as recipients, in P22-mediated transductional crosses with known *his* mutations as described elsewhere (Kleckner *et al.*, 1975). The known *his* mutations used which map closest to the common endpoint of these deletions are given. Several other mutations mapping through the operon were also used. Each vertical "tick" represents 2 additional point mutations in the *G* gene whose positions have been mapped by Hoppe & Roth (unpublished results). Each deletion was also tested for other markers in the *his* region (see text).

General methods for using Tn10 to generate deletions in the chromosomes of bacteria and bacterial viruses are described in section 3. Many types of deletions can be obtained without direct selection for loss of any chromosomal function; only scoring of relevant chromosomal markers is required. Like the simple insertion mutations, deletions which retain the resistance determinant can easily be transferred from strain to strain by virtue of the associated drug-resistance.

(h) *Drug-resistance elements can provide a portable region of homology*

Wherever a translocatable element inserts, an extensive sequence of base-pairs is added to the chromosome. In the case of the Tn10 element, approximately $9{\cdot}3 \times 10^3$ bases or the equivalent of nine genes is added (Sharp *et al.*, 1973; Tye *et al.*, 1974*a*). This new sequence can be placed, at the experimenter's option, virtually anywhere on the bacterial chromosome, and provides a site of genetic homology with which other copies of the insertion element will recombine.

This portable region of homology can be used, for example, to direct the integration of phage genomes or F′ plasmids at particular specified sites (for construction of Hfr

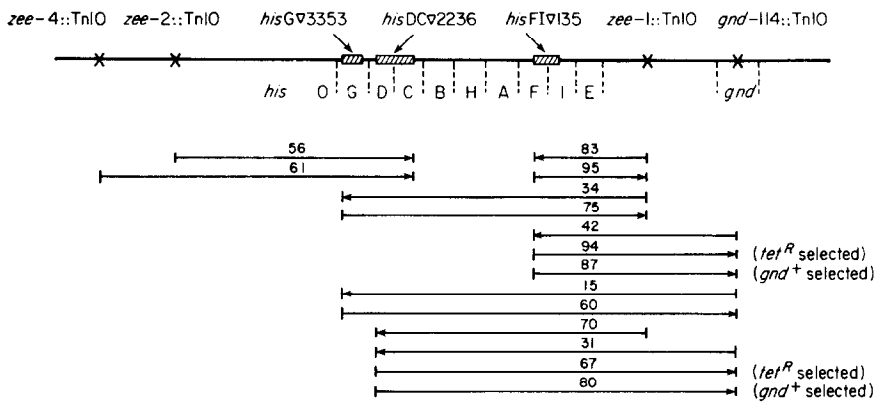


FIG. 5. Two-point crosses involving the Tn10 translocatable element. Arrows point to selected marker. Numbers indicate % cotransduction. Insertion *gnd*114::Tn10 is within the structural gene for 6-phosphogluconate dehydrogenase. Thus this marker can be used as a donor selective marker (selecting tetracycline-resistance) or as a recipient marker selecting for inheritance of a donor *gnd*$^+$ allele. The selective marker used is indicated at the head of the appropriate distance arrows. Whenever *gnd*$^+$ was used as the selective marker, the recipient carried both an *edd* mutation and the *gnd*::Tn10 insertion. Insertion mutations not in known structural genes (such as *zee*-1::Tn10) are given a 3-letter designation based on their position in the chromosome; see legend to Table 4 for details.

and F′ derivatives, specialized transducing phages, etc.). The only precondition is that one translocatable sequence be placed at the desired site and another copy be present on the extra chromosomal element to be integrated. Alternatively, recombination between two homologous drug-resistance elements at different sites in the same bacterial chromosome can be used to generate deletions, duplications and inversions, all having predetermined endpoints specified by the positions of the original insertions.

(i) *Insertions are well-behaved markers in fine structure mapping*

A growing body of data based on many sorts of genetic crosses indicates that Tn10 insertions behave as standard genetic markers. The tetracycline-resistance determinant can be used in two-point crosses to estimate distances, and in three-point crosses

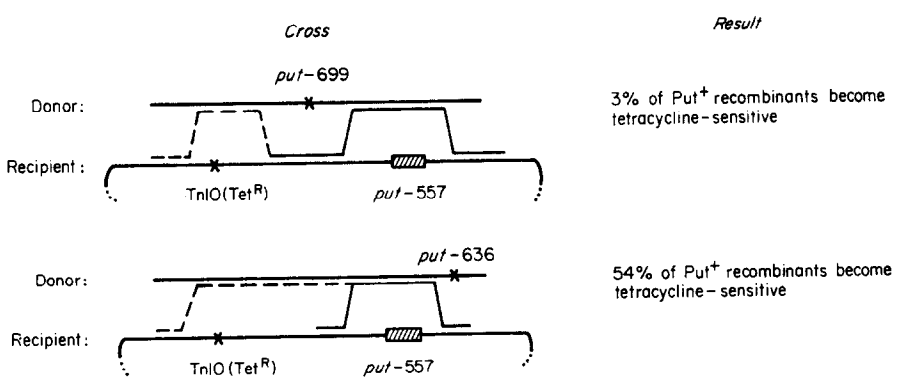


FIG. 6. Three-point crosses involving Tn10 mediated by P22 generalized transduction.

to determine gene order. In crosses with deletions, the insertion element maps as a point. In crosses between two strains each carrying a Tn10 insertion, the frequency of recombination between the Tn10 element seems roughly equivalent to that expected for deletion or point mutations separated by the same genetic distance. Sample data for the sorts of crosses mentioned above are presented in Figures 5 and 6.

In Figure 5 are presented results of two-point crosses between *his* mutations and several Tn10 elements inserted near the histidine operon. These crosses were performed by Forrest Chumley and Mark Johnston. In general, it can be seen that for a given Tn10 insertion, the cotransduction frequency increases as the *his* mutation chosen is closer to the Tn10 insertion site. Linkages are generally greater in crosses were Tet^R is the selected phenotype as opposed to the unselected marker. This difference could be due to transduced fragments with endpoints within the Tn10 element.

In Figure 6 are presented three-point test results involving several *put* mutations and a Tn10 insertion linked to the *put* region. The order of the *put* mutations has been established by deletion mapping. Only if the Tn10 insertion is located to the left on the Figure can one account for the data presented. These crosses were performed by Rolf Menzel.

Many Tn10 insertions in the histidine operon have been mapped by deletion mapping (Hoppe, Biek, Johnston & Roth, manuscript in preparation). In these crosses, the insertion behaved much like a point mutation. In no case did the insertion behave as a multi-site mutation. This suggests that there is no severe depression of recombination near the insertion site. Insertions mapping in a single small region of the *his*G gene were tested for recombination with each other; in no case was recombination detected. This failure to recombine reinforces the conclusion of deletion mapping and suggests that the insertions may be located at the same or very closely linked sites.

(j) *Insertions can be obtained for use as linked markers near but not within any gene of interest*

In many situations it is advantageous to have a drug-resistance element inserted near but not within a gene of interest. Such a closely linked insertion provides a genetic marker that is useful both in fine structure and chromosome mapping and which provides a convenient means of introducing characterized mutations of a variety of types into new genetic backgrounds. Techniques for isolating insertions near genes of interest and some uses of such insertions are discussed in section 3.

3. Uses of Translocatable Drug-resistance Elements in Genetic Manipulations

Translocatable drug-resistance elements can be used to advantage in virtually every type of manipulation performed by the bacterial geneticist. In many cases the use of such elements makes possible operations which could not be performed at all using conventional tests. In this section we describe those uses of drug-resistance elements which have thus far occurred to us. Data are given in support of the possibilities that have been experimentally realized. The reader is invited to use his imagination in formulating further applications.

The uses of translocatable drug-resistance elements which are discussed in this section can be summarized as follows.

(a) Strain constructions: transfer of otherwise unselectable markers by virtue of the drug-resistance associated with insertion mutations.

(b) Isolation of mutants which lack an enzymatic function but have no readily scorable phenotype.

(c) Obtaining and exploiting drug-resistance insertions near but not within regions of interest:
  (i) manipulation of markers with no selectable phenotype;
  (ii) localized mutagenesis;
  (iii) chromosomal mapping;
  (iv) F′ mapping and complementation tests;
  (v) "cloning" of prokaryotic genes using *in vitro* recombination techniques.

(d) Drug-resistance insertions as a portable region of homology:
  (i) construction of Hfr strains with origin and direction of transfer specified;
  (ii) construction of new F′ episomes;
  (iii) construction of specialized transducing phages;
  (iv) deletions and duplications with predetermined endpoints specified by positions of insertions.

(e) Generation of deletions with one endpoint at an insertion and the other endpoint unspecified.

(f) Selection of chromosomal duplications with translocatable drug-resistance elements and their use in chromosomal mapping.

(a) *Strain construction: transfer of markers with no selectable phenotype*

The association of the mutational and drug-resistance phenotypes in a drug-resistant insertion mutation can be extremely useful in strain building. Auxotrophic mutations that normally could not be transferred selectively can now easily be introduced into any new genetic background, even into strains carrying other mutations with the same auxotrophic phenotype. For example, a *met*A::Tn10 mutation could be transduced into a *met*E recipient by selecting tetracycline resistance on medium containing methionine. The transductants then all carry both the original *met*E mutation and the new *met*A::Tn10 mutation.

Genetic manipulation of the *Salmonella rec*A and *E. coli sup*F alleles with Tn10 provide good examples of uses of insertion elements in strain construction.

The *rec*A allele destroys a cell's capacity for recombination and must frequently be introduced into strains to stabilize plasmids carrying genetic material homologous to the chromosome. It was recently found that genes controlling sorbitol utilization (*srl*) are closely linked to the *rec*A gene (McEntee, 1976). This fact has been used by Phil Anderson to construct the following strains useful in transferring the *rec*A mutation.

A mutant was isolated which carries a Tn10 insertion in one of the *srl* genes. A *rec*A$^-$ mutation was transferred into this mutant by conjugation and a recombinant of the genotype: *srl*::Tn10, *rec*A$^-$ was recovered. Generalized transducing phage prepared on this strain can be used to transduce the *rec*A$^-$ mutation into any strain; selection is made for tetracycline resistance and the recombinants are scored for acquisition of the donor's *rec*A mutation. Approximately 50% of the tetracycline-resistant recombinants are *srl*::Tn10 *rec*A$^-$. For most purposes these transductants

are satisfactory *rec*A$^-$ strains. If more perfectly isogenic (*rec*A$^+$ and *rec*A$^-$) pairs are desired, *srl*$^+$ revertants of the *srl*::Tn10 *rec*A$^-$ strain can be selected by demanding growth on sorbitol as sole carbon source. These revertants have lost the *srl* mutation and the tetracycline-resistance element but retain the *rec*A$^-$ mutation. They should be perfectly isogenic with the *rec*A$^+$ parent used as recipient in the original transduction.

An analogous *srl*::Tn10 *rec*A$^-$ strain has been constructed in *E. coli* by Laszlo Csonka. Susan Gottesman and Phil Leder (personal communication) have used a similar approach to move the *trp*-linked *E. coli* amber suppressor *sup*F into a strain already containing an amber suppressor, *sup*E. A *trp*::Tn10 *sup*F strain was constructed and used as donor in a transductional cross. Tetracycline-resistant transductants of the *sup*E recipient were screened (with suitable lambda amber mutants) for those which had acquired the unique suppression spectrum conferred by *sup*F. The Tn10 insertion was then removed by demanding growth in the absence of tryptophan, producing a *sup*E *sup*F strain isogenic to the original *sup*E parent.

The low reversion frequencies of drug-resistance insertion auxotrophs also make them very convenient markers for recipients in Hfr-mediated conjugational crosses. Several different insertion auxotrophs can be introduced into a single strain if auxotrophs generated by several elements carrying different resistance phenotypes are used.

Similarly, chromosomal drug-resistance insertions (with or without associated mutational defects) can conveniently be introduced into a recipient strain as counterselectable markers at any convenient position on the map. If a sufficiently high concentration of antibiotic is used, no drug-resistant mutants of the donor strain will arise.

(b) *Isolation of mutants which lack an enzymatic function but have no readily scorable phenotype*

Drug-resistance insertions provide potential advantages in hunts for mutants which lack a particular enzymatic function but have little or no phenotypic consequences for cell growth. Mutants of this type are laborious to isolate and difficult to manipulate genetically, but they have proven valuable in unraveling cellular processes. Examples of such mutants are those lacking DNA polymerase I (*pol*A), various RNA and DNA nucleases and methylases and the relaxed control (*rel*) gene. In each case experimental use of the mutation frequently involves its introduction into strains carrying a variety of other mutations.

If such mutants were isolated as insertion mutants, several advantages would result. The strains isolated will carry single mutations (see above). The mutants recovered will most likely be completely deficient for the activity in question. A scoreable phenotype (drug-resistance) will be associated with the mutation; this makes genetic mapping extremely easy. The mutation can be selectively transduced into any new genetic background yielding mutant strains very nearly isogenic with the parent.

(c) *Obtaining and exploiting drug-resistance insertions near but not within regions of interest*

In many situations it is advantageous to have a drug-resistance element inserted near but not within a gene of interest. Such a closely linked insertion provides a

genetic marker that is useful both in fine structure and chromosome mapping and which provides a simple means of introducing characterized mutations of a variety of types into new genetic backgrounds. Techniques for isolating insertions near genes of interest are outlined below. Some uses of such insertions are also described.

(i) *Isolation of insertion mutations in specific regions*

The selection of insertions near genes of interest involves generation of a collection of random insertions by a translocation experiment such as those outlined in section 4 (vehicles). Many such clones (500 to 2000) are mixed together; in practice this has been done by scraping all colonies from the surface of selection plates in the translocation experiment. Once a mixed pool of insertion clones is made, a generalized transducing phage lysate is grown on the mixed pool. This lysate is used to transduce a recipient strain selecting for inheritance of a bacterial gene of interest in the donor population (i.e. a *his*$^-$ recipient could be transduced to prototrophy). The resulting transductants are screened for tetracycline resistance. The resistant clones should be those that have received from the donor population in a single transduced fragment both the selected gene *and* the inserted tetracycline element. Since a minority ($\sim 10^{-3}$) of the donor cells will happen to have the element close enough to the selected gene to give a high frequency of cotransduction, it is necessary to use a random donor pool of at least 1000 clones. If the random donor pool contains 1000 clones and one is looking for an insertion marker one need not look at many more than several thousand transductants. If the desired insertion is present at all, it must be present at a frequency close to 1/1000 in the population of transductants.

In practice, the following results have been obtained using a donor phage preparation grown on a pool of approximately 2000 clones of random Tn10 insertions. In *Salmonella*, insertions have been sought near the *his* control region (by Forrest Chumley), near the *pyr*B gene (by Donald Biek) near the *put* genes and near a minor proline permease gene (both by Rolf Menzel). Representative data are presented in Table 4. It should be noted that this general method is an adaptation of the method described by Hong & Ames (1971) for obtaining point mutations linked to particular genes. Their system of nomenclature has been modified slightly for naming insertion mutations outside known genes.

(ii) *Uses of linked insertions for manipulating mutations*

Drug-resistance insertions linked to otherwise non-selectable mutations can obviously be used to transfer those mutations from strain to strain.

Linked insertions also are extremely useful in construction of strains with multiple mutations in a particular small region. Strains can be simply constructed in situations where previously no selective means was available for bringing the desired mutations together. The possibilities are best illustrated by an example. A strain was recently constructed by Mark Johnston in a study of the histidine biosynthetic pathway. The strain carries three separate lesions in the *his* operon. To construct this strain, a Tn10 insertion linked to the histidine operon was transduced into a *his*B mutant by selection for tetracycline resistance. Among the recombinants it was simple to identify the recombinant type carrying both Tn10 and *his*B$^-$. Transducing phage grown on this double mutant was then used to transduce a *his*D$^-$ mutant to tetracycline-resistance. This cross yielded recombinant types which have one or both of the *his*

TABLE 4

Selection for insertion of Tn10 near specific genes

Recipient mutation	Selected recombinant type†	Tetracycline-resistant transductants (% of total)	Cotransduction frequency of particular Tn10 insertions to selective marker (%)
*his*Δ*0G203*	His$^+$	3%	*zee*-2::Tn10 60 *zee*-3::Tn10 60
*put*Δ*557*	Put$^+$‡	~0·05%	*zcc*-4::Tn10 20 *zcc*-5::Tn10 50 *zcc*-6::Tn10 50 *zcc*-7::Tn10 80
*pyr*B	Pyr$^+$	0·25%	*zje*-28::Tn10 11 *zje*-36::Tn10 21 *zje*-40::Tn10 69 *zje*-35::Tn10 97
*pro*P	ProP$^+$§	0·1%	*zjd*-27::Tn10 70

† In all cases, donor phage used was grown on a pool of approx. 2000 random Tn10 insertions.

‡ The recipient strain (*put*-557) is defective in proline utilization. Selection is made for growth on proline as sole nitrogen source.

§ The recipient strain is TR1995 (*pro*AB47, *put*P639, *pro*P673); selection was made for growth on 0·02 mM-proline to satisfy the auxotrophic requirement. Among the colonies arising on the selection plate are *pro*AB$^+$ and *put*$^+$ recombinants in addition to *pro*P$^+$ types. Among the *pro*P$^+$ recombinants approx. 0·01% were tetracycline-resistant.

Notation such as *zij*::Tn10 indicates the position of an insertion with no mutant phenotype according to its position on the genetic map. Letters i and j indicate the approximate location in minutes (i.e., aa is 0, ab is 1; bb is 11; ij indicates location at minute 89) (Hong & Ames, 1971).

mutations in addition to the selected insertion marker. Using F′-mediated complementation tests, it was simple to screen recombinants and identify the desired triple mutant: Tn10 *his*D *his*B. Phage on the triple mutant was then used to repeat the cycle using a *his*G mutant as recipient. The strain finally constructed carried three separate mutations in the *his* region: *his*G, *his*D and *his*B in addition to the linked Tn10 element. The construction of this strain would have been prohibitively difficult without use of the translocatable element.

(iii) *Use of insertions in localized mutagenesis*

A technique for mutagenesis of specific small regions of the chromosome was described by Hong & Ames (1971). This method can be made more generally useful through use of translocatable drug-resistance elements. The original method involves treatment of a transducing lysate with a chemical mutagen and subsequent use of that lysate to transduce a recipient strain. Recombinants are selected which have acquired a specific selective marker from the donor phage lysate. The genetic material received by these recombinants has been heavily mutagenized, so there is a high probability of finding recombinants which carry mutations linked to the selective marker. If the transduction is done at low temperatures and recombinant clones are checked at high temperatures for mutant phenotypes, one can isolate temperature-sensitive mutations affecting the selected gene or genes closely linked to it.

Through use of drug-resistance elements one need not be limited to isolation of conditional mutants. A resistance element is inserted very near a wild-type gene of interest. A generalized transducing lysate is prepared and mutagenized. The mutagenized lysate can then be used to transduce any recipient to drug-resistance. The recombinants will inherit the drug element and adjoining chromosomal material from the mutagenized lysate. Any sort of mutation affecting a linked gene can be recovered, including those with a complete, unconditional loss of function.

(iv) *Uses of translocatable elements in chromosome mapping*

Many mutations of interest have a detectable phenotype only in strains carrying one or more additional mutations; examples are informational suppressors, mutations in secondary recombination pathways, and mutations affecting interconversion of pyrimidine nucleotides (for examples see Hartman & Roth, 1973). Mutations such as these are difficult to map by conventional methods because of the strain building required to permit detection of recombinants. Using drug-resistance elements, this procedure can be simplified. A Tn10 insertion is selected which is cotransducible with the gene of interest, using methods described in section (c)(i), above. This is done in a strain carrying any additional mutations needed to provide the mutation of interest with a selective phenotype. To map the mutation of interest, one merely maps the nearby tetracycline-resistance determinant. This is an easy task, since the resistance determinant can be transduced selectively into any Hfr strain or multiply marked recipient strain useful in mapping. Rolf Menzel has used this method in mapping a minor proline permease gene (*pro*P). To detect the minor permease defect, one must use strains carrying a *pro* (A, B or C) mutation and a defect in the major proline uptake system (*put*P). Strains carrying *pro*A and *put*P can still grow using low exogenous proline to supply the auxotrophic defect. If they carry the *pro*P mutation in addition, they require high (10 mM) exogenous proline for growth. Using this strain with three mutations, a Tn10 insertion was selected near *pro*P; this element was then transduced into an Hfr and used to map the site of the *pro*P gene without any complicated strain construction.

(v) *F′ mapping and complementation tests*

Insertion mutations can also be used to augment the F′-mapping scheme described by Low (1973). This chromosome mapping technique involves use of a series of F′ episomes, each carrying a known segment of the chromosome. Each episome is transferred into a strain carrying the mutation to be mapped; selection is made for loss of this mutant phenotype. When an F′ episome is found which complements the unknown mutation, one concludes that the unknown mutation must map in the region of the chromosome homologous to material on the F′ episome. In this scheme, translocatable elements can be used to add a selective marker to each F′ episome.

Forrest Chumley and Rolf Menzel have characterized insertions of Tn10 which seem to be located in F factor material. These drug-resistance determinants can be transduced selectively into any F′ episome, regardless of what bacterial genes are carried by the episome (M. Johnston & C. Beck, unpublished results).

Adding such drug-resistance markers to F′ episomes makes it possible to perform F′ episome transfers by the Low (1973) method without exerting any selective pressure on the mutation to be mapped. The mutation to be characterized can be scored as an unselected marker. At least equally important is the fact that the

diploids generated for the region can be maintained selectively without selecting against the mutant phenotype; this permits long-term growth of the diploids with less danger of losing the mutation of interest. Performing transfers in this way avoids many possible artifacts which can complicate the F′-mapping method; the advantages of treating the gene in question as an unselected marker are particularly great in situations where the mutation to be tested is "leaky", only partially recessive, shows high reversion frequency, or in cases where recombination between episome and chromosome occurs at high frequency.

(vi) *"Cloning" of prokaryotic genes by recombination* in vitro

Recently developed techniques for cloning DNA segments by biochemical construction of recombinant DNA molecules (Lobban & Kaiser, 1973; Jackson *et al.*, 1972; Cohen *et al.*, 1973; Wensink *et al.*, 1974; Cameron *et al.*, 1975) can make good use of drug-resistance insertions when the DNA to be cloned is prokaryotic. For any particular gene, operon, or region to be cloned, the presence of a drug-resistance insertion in or near the relevant locus means that the clone of interest can be selected by virtue of the associated antibiotic-resistance phenotype. This approach makes it possible to clone genes or regions for which no direct selection exists or for which a suitably marked, non-reverting, easily transformable recipient strain is not available. Once the desired clone is obtained, any unwanted insertion mutation can often subsequently be removed by reversion.

(d) *Drug-resistance insertions as portable regions of homology*

(i) *Generation of Hfr strains with specified origin and direction of transfer*

Hfr strains can arise by homologous recombination between bacterial DNA present on an F′ episome and the homologous region on the chromosome. Such Hfr strains have an origin and direction of chromosome transfer determined by the region of homology shared by the F′ episome and the chromosome (Fig. 7). By inserting a drug-resistance element into an episome one can use this principle to make Hfr derivatives anywhere on the bacterial chromosome where one has an homologous drug-resistance element. The drug-resistance element can be inserted in either of two orientations. If there are two episomes carrying insertions oriented in opposite directions, one can produce Hfr derivatives which transfer in either direction from the position of any drug-resistance element on the chromosome.

In order to simplify the election of the Hfr strains, a selective scheme (based on the work of Beckwith *et al.*, 1966) has been used. In the case of *S. typhimurium*, $F'_{ts114}lac^+$ episomes which carry the tetracyline insertion have been used. This episome will not replicate autonomously at 42°C; it carries a region of the *E. coli* chromosome not homologous to any region of the *Salmonella* chromosome. When this episome is present in a strain also harboring a Tn10 insertion in the chromosome, the only region of homology between episome and chromosome is provided by the insertion elements. When such a strain is grown at 42°C selecting Lac$^+$ phenotype, the bulk of the population which grows is composed of cells in which recombination between the two Tn10 elements has served to integrate the $F_{ts}lac$ Tn10 episome into the chromosome. These survivors are Hfr strains whose origin and direction of transfer is determined by the position and orientation of the Tn10 elements involved. An experiment (done by Rolf Menzel) demonstrating this procedure is presented in Table 5.

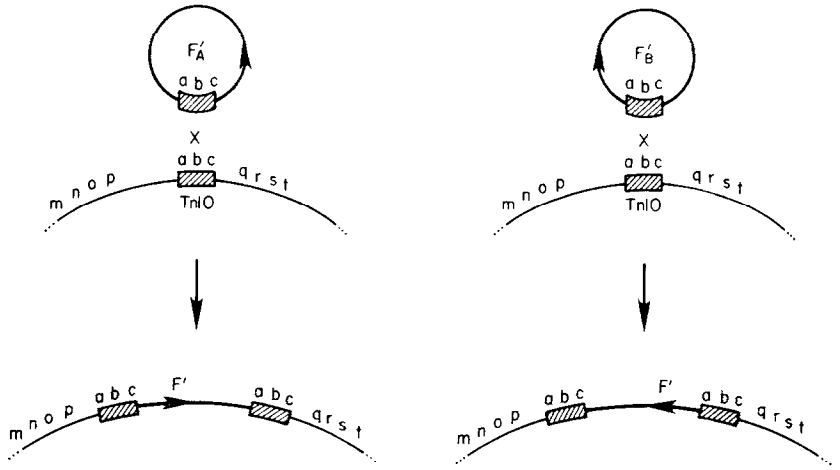


FIG. 7. Hfr strains with an origin and direction of chromosome transfer determined by the region of homology shared by the F′ episome and the chromosome. The hatched boxes represent the region of homology between the episome and chromosome.

In Table 5 it should be noted that the *zzf*-20::Tn10 insertion in the F′*lac* causes clockwise transfer with one chromosomal insertion and counterclockwise transfer with the other two chromosomal insertions tested. The *zzf*-21::Tn10 insertion gives the opposite result. This suggests that the two episomal insertions are oriented in opposite directions with respect to the origin of transfer. By doing similar mating experiments it should be possible to classify any Tn10 insertion as to orientation.

Since chromosome transfer occurs in only one direction in these matings, it appears that the Tn10 insertions do not invert with high frequency; less than 10^{-3} must be inverted. Since the Tn10 element carries an inverted repetition, it was thought possible that recombination between the sequences could cause high-frequency inversion. If this happens at all, the frequency must be less than 10^{-3}, the detection level of this experiment (see Sharp *et al.* (1973) for further discussion).

The method outlined above cannot be used directly to make Hfr derivatives in *E. coli*, since the *lac pro* region on the episome will provide homology and thus produce a high background of Hfr strains whose origin is in that region. Two methods suggest themselves to remedy this problem. First, one might use an episome carrying *Salmonella* material (i.e. F′*his*) with suitable mutants of *E. coli* (*his* deletion). Alternatively, one might use F'_{ts}*lac* episomes in strains carrying chromosomal deletions (such as deletion XIII; Miller, 1972) which remove material homologous to the F′*lac*.

(ii) *Construction of new F′ episomes*

It is possible, by the methods outlined above, to make Hfr strains whose origin and direction of transfer is determined. Therefore, it should also be possible to use these strains to generate new F′ episomes carrying markers adjacent to the site of the insertion element. Since virtually any marker can be adjacent to a tetracycline insertion, any marker can be placed on an episome with the exception of those whose presence on the episome is incompatible with the episome's replication or the viability of the host. Standard methods should accomplish this. One makes an Hfr whose

TABLE 5

Donor genotype			Recombinants	
Insertion carried by F′$_{ts}$*lac* episome	Insertion carried by chromosome	Pre-growth selecting for F′*lac* insertion	*pyr*C$^+$ (clockwise transfer)	*pyr*D$^+$ (counter clockwise transfer)
zzf-20::Tn10	*zcc*-4::Tn10	No	50	4
		Yes	*2000*	6
zzf-20::Tn10	*zcc*-5::Tn10	No	4	50
		Yes	8	*2000*
zzf-20::Tn10	*zcc*-6::Tn10	No	8	30
		Yes	15	*2500*
zzf-21::Tn10	*zcc*-4::Tn10	No	14	20
		Yes	17	*125*
zzf-21::Tn10	*zcc*-5::Tn10	No	25	2
		Yes	*350*	10
zzf-21::Tn10	*zcc*-6::Tn10	No	40	5
		Yes	*500*	7
None	*zcc*-4::Tn10	No	10	1
		Yes	10	6
None	*zcc*-5::Tn10	No	10	1
		Yes	9	2
None	*zcc*-6::Tn10	No	12	5
		Yes	9	7

Crosses presented involve a series of donor strains carrying a F′$_{ts114}$*lac* episome which is temperature-sensitive for replication. Some of these strains have a Tn10 element inserted into the F′$_{ts114}$*lac* episome. Each of the donor strains also carries a Tn10 element inserted into the chromosome between the *pyr*C and *pyr*D genes. These donor strains were crossed with recipients carrying either a *pyr*C$^-$ mutation (located clockwise of the chromosomal Tn10 element) or a *pyr*D$^-$ mutation (located counterclockwise of the Tn10 insertion). Selection was made for transfer of the donor's *pyr*C$^+$ or *pyr*D$^+$ genes. Donors carry a *his* deletion as counter-selective marker. Matings were performed by plating 10^7 donor and 10^7 recipient cells directly onto selective medium. Plates were scored after 2 days; numbers presented are the number of recombinants per plate. No colonies were seen on control plates containing either parent strain alone.

To select for F′$_{ts114}$*lac* integration, donor strains were pregrown at 42°C in minimal lactose medium. When integration was not selected, donors were pregrown in the same medium at 30°C. Pre-growth involved a 10^3 dilution into growth medium and growth to nearly full density.

origin of transfer is near the genes of interest. One then selects for transfer of these genes into a *rec*A$^-$ recipient (Low, 1972). Stable inheritance of donor material by a *rec*$^-$ recipient is frequently due to F′ episome formation.

(iii) *Construction of specialized transducing phages*

Another use of drug-resistance elements in their role as translocatable regions of homology is as substitute attachment sites for phages. A phage which lacks its normal attachment site but which carries a drug-resistance element usually integrates at the position of the drug-resistant element on the chromosome. Aberrant excision of this phage from such positions can result in specialized transducing variants carrying neighboring genes (Campbell, 1962; Gottesman & Beckwith, 1969). Since the drug-resistance elements can be placed almost anywhere, virtually any gene should be moveable onto a phage by this method.

We have used this procedure in *Salmonella* to construct a defective specialized transducing variant of phage P22 which carries the entire histidine operon. A strain which has a tetracycline insertion close to the *his* operon (in the *gnd* gene) was lysogenized with P22 *bpl*, which has a deletion of the attachment site and the *int* gene, but which carries half of the tetracycline element (Chan *et al.*, 1972; Chan & Botstein, 1976). This lysogen was induced and used to transduce a *his*-deletion mutant carrying the *rec*A mutation to histidine independence; the *rec*A mutation prevented generalized transduction. Some of the transductants produced high-frequency transducing lysates capable of transducing the entire *his* operon. The clones appear to harbor the specialized transducing phage in an extrachromosomal "carrier state". Documentation of *his*-specific high-frequency transduction for several "HFT" lysates is given in Table 6. The specialized transducing phages isolated in these particular experiments are defective. It is most likely that the transducing genomes have suffered a loss of phage genes and are associated with a viable non-transducing helper phage. In principle, P22 transducing phages might also be defective if, like P22Tn10, the new composite genome is much too large to fit into a single phage head.

It should be possible to carry out analogous schemes in *E. coli* using phage lambda. In this case, it should not be necessary to transduce into *rec*$^-$ recipients, since generalized transduction is not a problem.

TABLE 6

P22 his$^+$ *high-frequency transducing lysates*

HFT lysate	p.f.u./ml	*his*$^+$/ml	*his*$^+$/p.f.u.	*pyr*A$^+$/ml	*pyr*A$^+$/p.f.u.
1	$5{\cdot}2\times10^{10}$	$1{\cdot}1\times10^{7}$	2×10^{-4}	4×10^{3}	8×10^{-8}
2	5×10^{10}	4×10^{6}	8×10^{-5}	1×10^{4}	2×10^{-7}
3	$1{\cdot}4\times10^{9}$	$1{\cdot}1\times10^{7}$	8×10^{-3}	$<1\times10^{3}$	$<7\times10^{-7}$
8	$1{\cdot}8\times10^{10}$	5×10^{6}	3×10^{-4}	1×10^{3}	6×10^{-8}
10	$7{\cdot}4\times10^{10}$	$3{\cdot}1\times10^{8}$	8×10^{-2}	5×10^{4}	7×10^{-7}
P22$^+$	$4{\cdot}8\times10^{11}$	2×10^{6}	4×10^{-6}	1×10^{5}	2×10^{-7}

The rationale used to generate high-frequency *his*-transducing lysates described in the text. The data here show the relative ability of several of these lysates to transduce the *his* genes and the unlinked *pyr*A gene into *rec*$^+$ recipients. p.f.u., plaque-forming units.

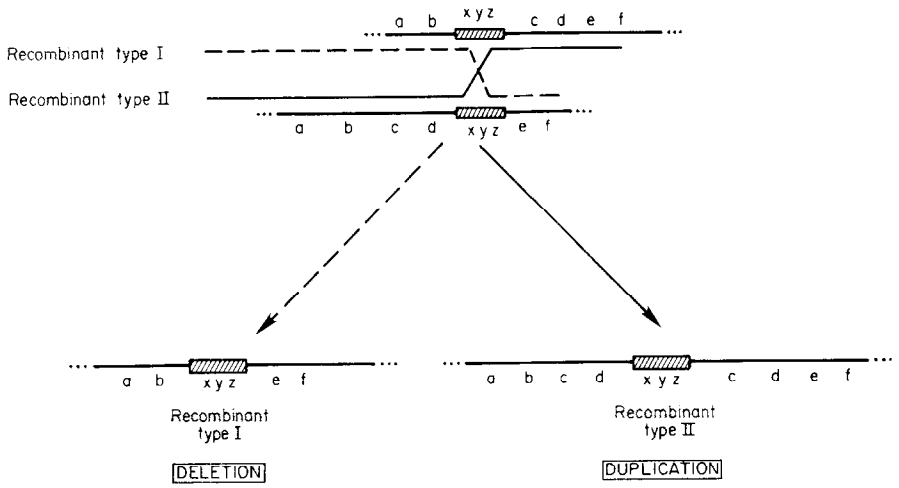
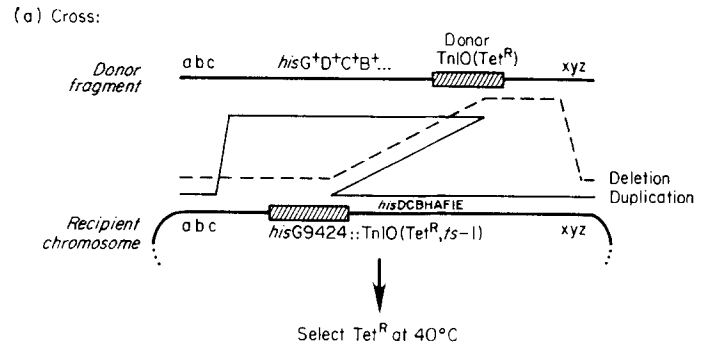


FIG. 8. Homologous recombination between drug-resistance insertions at different positions can produce deletions and duplications with endpoints specified by the positions of the insertions. The box represents the drug-resistance insertion.



(b) Results:

Donor	Total Tet^R recombinants tested	Number of deletions	Number of duplications
1. *his*H8576::TnlO(Tet^R)	22	1	1
2. *his*I9423::TnlO(Tet^R)	12	0	2
3. *zee*-1::TnlO(Tet^R)	14	1	Not tested

FIG. 9. Isolation of duplications and deletions with specified endpoints using a Tn10 (*tet*^{R}-*ts*) recipient.

zee-1::Tn10 carries a Tn10 insertion just outside the *his* operon on the operator-distal side (see Fig. 5).

Recombinants carrying a duplication of material between the insertions in crosses 1 and 2 were identified by their His$^+$Tet^R phenotype. The partial diploid is His$^+$ because the 2 defective *his* regions can complement each other for *his*G and *his*H or 1, respectively.

Recombinants carrying a deletion of material between the insertions were identified by their failure to revert to His$^+$ and because expression of one intervening gene, *his*D, is totally abolished in the deletion. (*his*D function is assayed by utilization of histidinol as a source of histidine. The donor strain expresses *his*D normally; the recipient strain expresses *his*D weakly, due to polar effects of the Tn10 insertion.)

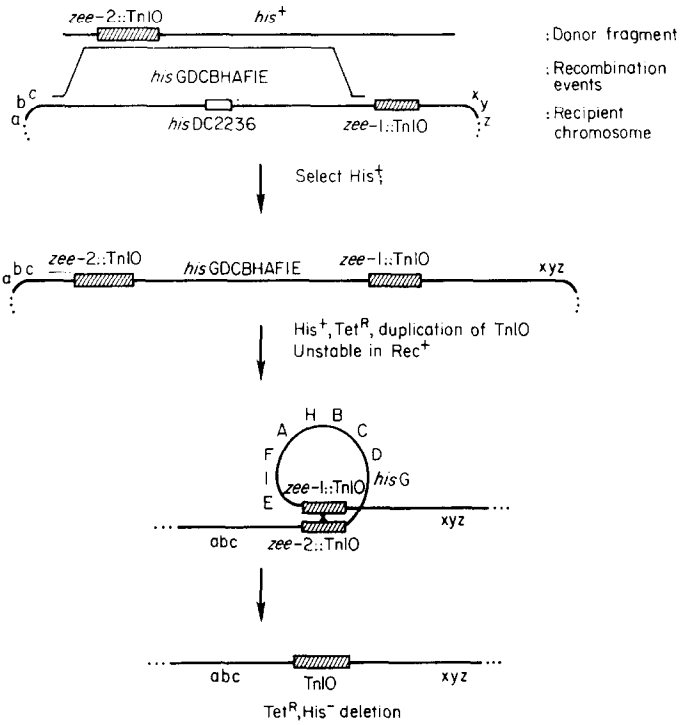


FIG. 10. Isolation of a deletion with endpoints predetermined by the positions of Tn10 insertions. *zee*-1::Tn10 and *zee*-2::Tn10 are Tn10 insertions just outside the *his* operon on the operator distal and operator proximal sides, respectively (Fig. 5).

(iv) *Deletions and duplications with predetermined endpoints specified by positions of insertions*

Genetic exchange between two strains carrying a drug-resistance insertion at different sites can produce recombinants carrying either a deletion or a duplication of the material between the two sites of insertion if the two insertions are in the same orientation with respect to the chromosome as a whole. These recombinants can arise by homologous recombination between insertions at different positions, as illustrated by Figure 8. One complete copy of the resistance element is always retained at the newly created junction. A cross which generates both duplications and deletions in the *his* region is presented in Figure 9. Deletions of this form can also be obtained as occasional segregants from a single strain harboring the insertions at two different positions (see Fig. 10). In Figure 11, a cross is presented which yields duplications in the *his* region.

Construction of these unusual recombinants depends on a mechanism for selecting recombination in the region of interest and for distinguishing deletions and duplications from conventional recombinants. Deletions can often be identified by loss of intervening functions. Duplications are characteristically unstable (Anderson *et al.*, 1976) and can be recognized because they give rise to haploid drug-sensitive segregants (see Fig. 11). Several specific examples of such duplications and deletions that have already been isolated are shown in Figures 9, 10 and 11, which illustrate the types of

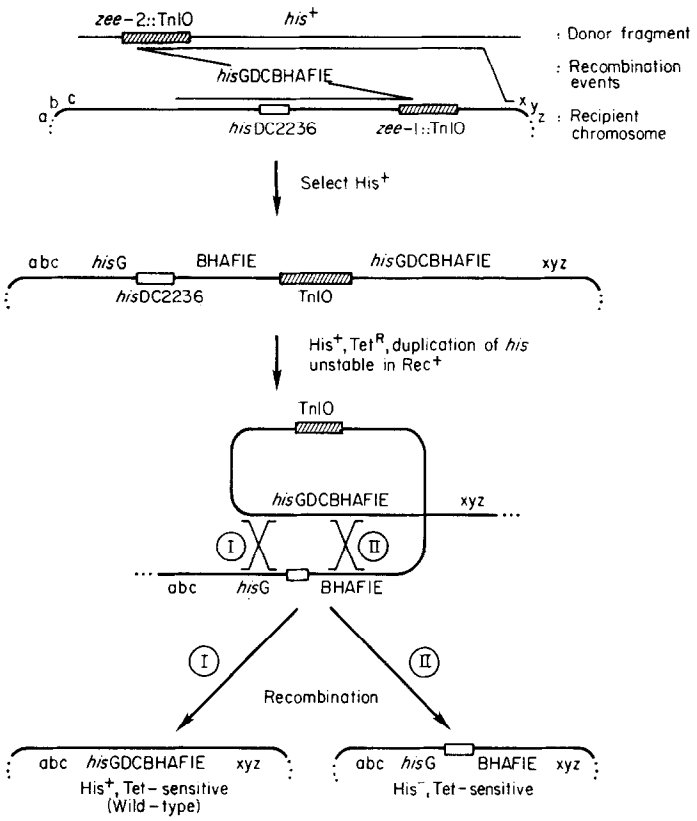


FIG. 11. Isolation and identification of a duplication with endpoints predetermined by the positions of Tn10 insertions.

The cross is the same one used to produce a defined deletion in Fig. 10. Duplications are, in general, characterized by their instability in Rec$^+$ bacteria. In this case the duplications are easily detected, since they will give rise to tetracycline-sensitive segregants (both *his*$^+$ and *his*$^-$) at high frequency.

methods which can be used. The experiments presented in Figures 10 and 11 were done by Forrest Chumley.

A general method for the selection of recombinants in the region of interest involves the use of a recipient strain carrying a mutant Tn10 element, Tn10 (*tet*^{R}-*ts*), in which resistance to tetracycline is thermolabile. A suitable Tn10 (*tet*^{R}-*ts*) mutation can be readily obtained either by mutagenesis of a wild-type Tn10 insertion at the desired locus or by generating an insertion at the desired site using a Tn10 (*tet*^{R}-*ts*) vehicle. In crosses with a *tet*^{R}-*ts* recipient, strains carrying a wild-type Tn10 insertion are used as donor; all of the drug-resistant recombinants obtained (at the non-permissive temperature) will have suffered recombination in the vicinity of the Tn10 element in the donor. As shown by the example in Figure 9, deletions and duplications constitute a significant proportion of such recombinants. Other types of tetracycline-sensitive derivatives of chromosomal Tn10 insertions suitable for use in these experiments can also be obtained without mutagenesis as the products of imprecise excision of Tn10. The isolation and properties of such derivatives are described elsewhere (Botstein & Kleckner, 1977; Kleckner, Reichardt & Botstein, manuscript in preparation).

Other methods for isolating deletions and duplications, and particular ways in which duplications can be used for chromosomal mapping are discussed in sections (e) and (f) below.

(e) *Generation of deletions with one endpoint at an insertion and the other endpoint unspecified*

(i) *Tetracycline-sensitive deletions of the bacterial chromosome*

Tn10 insertions in the *Salmonella his* operon give rise to tetracycline-sensitive variants at high frequencies (10^{-4} in a saturated culture). Extensive genetic analysis has revealed that about 15% of these variants have acquired deletions which remove both the resistance determinant and a set of contiguous chromosomal markers adjacent to the insertion point on one side *or* the other. The deletions shown in Figure 4 were isolated in this way. This approach can be used to isolate deletions in any region of a bacterial chromosome. Tetracycline-sensitive variants can be obtained in large numbers following selection with penicillin in the presence of low levels of tetracycline. Deletions can be identified among these variants by scoring loss of relevant phenotypes and by direct assessment of the ability of variants to donate markers in genetic crosses. For identification of a deletion, it is essential to show that the tetracycline-sensitive mutant fails to act as a donor in crosses to several point mutations mapping near the site of the original Tn10 insertion. Many other non-reverting tetracycline-sensitive variants can occur. This selection for deletions is absolutely general: it does not require direct selection for loss of any chromosomal function in the region of interest.

(ii) *Tetracycline-resistant deletions in the bacterial chromosome*

Tn10 is also capable of generating deletions analogous to those just described, except that the resistance determinant is retained at the genetic position of the deletion. Such deletions are particularly useful because they can be transferred from strain to strain by selecting transfer of drug-resistance. Unlike the tetracycline-sensitive deletions just described, however, these deletions must be selected directly in some way, and thus the ease with which they can be found will depend very much on the particular region of DNA involved. Tetracycline-resistant deletions in a bacterial chromosome can be obtained by selecting loss of a function adjacent to a pre-existing Tn10 insertion. This technique has been used successfully to generate deletions of the *Salmonella* histidine operon. A *Salmonella* strain carrying a Tn10 insertion in the nearby *gnd* gene was subjected to two cycles of penicillin selection in the absence of histidine and the culture plated on medium containing a limiting concentration of histidine where *his*$^-$ bacteria make very small, readily distinguishable colonies. Approximately half of the survivors were *his*$^-$, and among these a substantial proportion were deletions.

(iii) *Deletions in the genome of phage P22Tn10*

Salmonella phage P22 packages its DNA in headfuls and relies on terminal repetition for circularization of the phage genome following injection. A P22 genome which carries the Tn10 insertion is too long to fit in a single phage head. Thus, induction of a P22 prophage carrying a Tn10 insertion produces a burst of particles which carry a headful of DNA; these particles do not have any terminal repetition and are therefore

defective upon single infection. In this way, one selects directly for deletions if one selects particles which are able to circularize following single infection. Following this strategy, Chan *et al.* (1972) selected for tetracycline-resistant lysogens after low multiplicity infection by a P22 Tn10 lysate. The deletions shown in Figure 3, having one endpoint at the Tn10 insertion, were obtained in this way. This strategy should be equally applicable to any temperate phage which packages its DNA by headfuls.

(iv) *Deletions in the genome of phage lambda*

Coliphage lambda packages its DNA by encapsulating all of the DNA between two specific sites on a concatemer. In phages of this type, such as λ, T5, T7, genomes carrying deletions can often be selected because the particles carrying them contain less DNA and are therefore less susceptible to disruption by chelating agents and heat. The additional tool of selecting or screening for loss of an adjacent function can also be used. Both tetracycline-resistant and tetracycline-sensitive deletions can be obtained in this way. A number of λ derivatives carrying tetracycline-sensitive deletions have been isolated by David Barker using this approach.

(f) *Selection of chromosomal duplications with translocatable drug-resistance elements and use of such duplications in chromosomal mapping*

Recent work from several laboratories suggests that tandem duplications are frequent events in bacteria and may include large segments of the chromosome (Straus & Hoffman, 1975; Anderson *et al.*, 1976). Translocatable drug-resistance elements provide a means of selecting duplications and mapping the extent of the duplicated region. This is possible because of the basic nature of mutations resulting from insertion of the drug-resistance element; a chromosomal gene is inactivated and a new selective marker is added (drug-resistance). Because of this, one can select simultaneously for inheritance of both the wild-type copy of the chromosomal gene (i.e. selection for prototrophy) and for the mutant allele (selection for drug-resistance).

For example, one can select for duplications by a transductional cross between a wild-type strain and one carrying a drug-resistance element inserted into a selective gene (i.e. a *his* Tn10 insertion). Selection is made for His^+ and Tet^R. This demands that both the donor (*his*$^+$) allele and the mutant (*his*::Tn10) allele be inherited by the recombinants. This is possible if the recipient carries a duplication of the gene in question; the donor fragment replaces the recipient allele in one of the two copies leaving a merodiploid duplication strain (*his*$^+$/*his*::Tn10). Haploid strains show very few transductants in a cross of this type. However, if a rare individual in the recipient population carries the duplication, it can yield the selected recombinant, thereby "trapping" the spontaneously occurring duplication. Many of the transductants arising in a haploid strain prove to carry this sort of trapped duplication, as shown for the case of *gnd*$^+$/*gnd*::Tn10 duplications in Table 7.

For measuring the extent of a duplication, one crosses a series of characterized auxotrophic insertion mutations caused by a different insertion element into the strain carrying a duplication. If prototrophic, drug-resistant recombinants arise with high frequency, the strain must carry two copies of the locus into which the new element is inserted; presumably the duplication includes that locus. If few such recombinants arise, then the locus in question must lie outside the duplication and be haploid for most cells in the population. The resolution of this method of mapping duplications is limited only by the availability and distribution of drug-resistance

TABLE 7

Using Tn10 to isolate duplications of gnd

Experiment	(A) Total *tet*^{R} transductants	(B) Total *gnd*$^+$ *tet*^{R}	(C) Number of *gnd* duplications
1	2474	3	1
2	2032	1	0
3	904	0	0
4	2707	4	1
5	2636	5	1
6	2074	2	0
7	2637	3	0
8	1202	4	0
9	3404	3	0
10	2402	2	0
Total	22,472	27	3
Relative frequency	1	$1{\cdot}2\times10^{-3}$	$1{\cdot}3\times10^{-4}$

Ten independent clones of an *edd*$^-$, *gnd*$^+$ *Salmonella* strain were transduced to *tet*^{R} with P22 grown on 10 independent clones of a *gnd*::Tn10 donor. The resulting transductants (column (A)) were scored for retention of Gnd$^+$ phenotype by replica-plating onto plates containing gluconate as sole carbon source and tetracycline. Gnd$^+$ Tet^R colonies (column (B)) were purified and tested for instability of both Gnd$^+$ and Tet^R phenotypes. Colonies which segregated Gnd$^+$ Tet^S and Gnd$^-$ Tet^R were assumed to be duplications (column (C)).

element insertions which can be counterselected. This method of determining whether or not a gene is included in a duplication has been used successfully by Phil Anderson for manipulating duplications of the *his* region of *Salmonella*.

4. Genetic Vehicles for Selection of Insertion Mutations

In order to introduce drug-resistance elements into the bacterial chromosome, one needs a vehicle (usually a temperate phage or a plasmid) which carries the desired element. It is also necessary to have some means of selecting against the maintenance of the vehicle in the recipient bacteria, so that the only way the bacteria can become stably drug-resistant is for the drug-resistance element to leave the vehicle and become inserted into the bacterial genome. We have used two different vehicle types: defective transducing phages (i.e. those unable to lysogenize the host) and mutant plasmids (i.e. temperature-sensitive F factors unable to maintain themselves at high temperature).

(a) *Phage vehicles*

The acquisition of drug-resistance elements by phage genomes has been amply described in the literature (Dubnau & Stocker, 1964; Kondo & Mitsuhashi, 1964; Watanabe *et al.*, 1972; Chan *et al.*, 1972; Scott, 1973; Berg *et al.*, 1975; Gottesman & Rosner, 1975). Briefly, phage are grown on strains harboring the desired drug-resistance elements (usually on an R factor) and the lysate is used to transduce a

drug-sensitive strain to drug-resistance. Transductants are then assayed for the production of HFT (high-frequency transducing) lysates. Depending on the physiology of the particular phage used, these HFT lysates may consist of defective particles only, a mixture of defective particles and helper phage, or entirely of non-defective transducing phage particles.

The vehicles which have been found most useful are transducing variants of phage P22 (for insertion mutagenesis of *S. typhimurium*) and phage lambda (for insertion mutagenesis of *E. coli*). Each of these phage vehicles has special properties which influence how it must be used.

(i) *P22 vehicles*

P22Tc-10 is a transducing variant of phage P22 which carries a translocatable tetracycline-resistance element (Tn10) ultimately derived from a drug-resistance plasmid (R222). The insertion of the resistance element makes the composite P22Tc-10 genome too large to fit into a single P22 phage head. Since P22 packages DNA by the headful, a lysate can be produced by induction of a lysogen carrying the oversize P22Tc-10 prophage, but each of the particles in this lysate contains only a fragment of the composite genome. Since these fragments are circularly permuted fragments of the same oversize genome, however, two of the particles in a P22Tc-10 lysate can usually co-operate to reconstruct the complete oversize genome. P22Tc-10 particles are, therefore, semi-defective: although no single particle can productively infect or lysogenize *Salmonella*, high multiplicity infections with a P22Tc-10 lysate result in efficient lytic growth and/or lysogenization (Watanabe *et al.*, 1972; Chan *et al.*, 1972; Tye *et al.*, 1974*a*,*b*; Chan & Botstein, 1976).

Since lysogenization with P22Tc-10 results in stable drug-resistance, additional measures are necessary to make P22Tc-10 into a vehicle usable for insertion mutagenesis. Several alternative schemes have been tried, each with some success. Currently the best P22Tc-10 phage for mutagenizing *Salmonella* is P22Tc10 *int*3 *c2-ts*30 *12*$^-$*am*N11 *13*$^-$*am*H101. An induced lysate of this phage is used to infect *su*$^-$ (no amber or ochre suppressor) *Salmonella*. After adsorption, the infected cells are plated on selective plates (containing tetracycline and 0·01 M-EGTA†) at 41°C. The rationale for these conditions is as follows: the *int*$^-$ mutation prevents integration of the prophage at the normal attachment site; the *c2-ts* mutation prevents maintenance of lysogeny at temperatures above 38°C, the *12*$^-$ mutation causes failure to replicate DNA in any *su*$^-$ strain and thereby reduces killing of infected cells both at the time of initial infection and later on the selection plates; and the *13*$^-$ mutation interferes with lysis of infected cells. In addition, the selective plates contain 0·01 M-EGTA to chelate the Ca^{2+} in the medium. This seems to reduce the background growth of drug-sensitive cells on the selection plates and also prevents injection of DNA from P22 phage heads, inhibiting new cycles of phage growth on the selection plates. The procedure results in about 10^{-6} tetracycline-resistant survivors per infecting phage; about 2% of these survivors are auxotrophs. Not every one of the measures to reduce lysogeny and phage growth is essential for mutagenesis; simply eliminating lysogenization of the phage genome is adequate (but not optimal).

Figure 12 describes the mutagenic properties of the P22Tc10 *int*$^-$ *c2-ts* *12*$^-$*13*$^-$ vehicle. The number of *tet*^{R} transductants is directly proportional to the number of

† Abbreviation used: EGTA, [ethylenebis (oxyethylenenitrilo)] tetraacetic acid, a chelating agent with high affinity for Ca^{2+} ions.

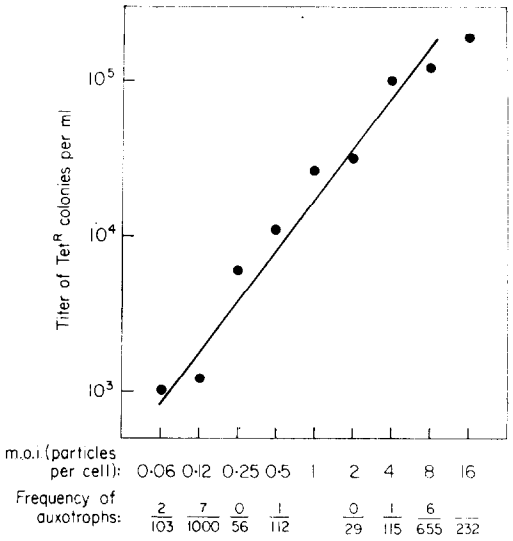


FIG. 12. Translocation of Tn10 from P22 to the *Salmonella* chromosome and generation of chromosomal Tn10 insertion auxotrophs is independent of infection multiplicity.

P22Tn10 particles were obtained by heat induction of a *su*$^+$ (P22Tn10 *int*3 *c2-ts*·29 *12*$^-$*am*N11 *13*$^-$*am*H101) lysogen. Particles were purified in a $CsCl_2$ gradient, and their absolute concentration calculated from the A_{260nm} relative to known P22 standards. Appropriate dilutions of particles were mixed with cells of an *su*$^-$ *Salmonella* strain grown to log phase. After 60 min adsorption at room temperature, the mixture was diluted and spread directly on broth plates containing tetracycline and 0·01 M-EGTA. The plates were incubated at 40°C.

m.o.i., multiplicity of infection.

nput phage particles (5×10^{-6} *tet*^{R} transductants per particle) and independent of multiplicity of infection. The proportion of auxotrophic mutations (about 1%) is likewise independent of multiplicity. Since the infection procedure with this vehicle requires no growth period after infection the mutants obtained in a single infection can safely be assumed to be independent.

(ii) *Lambda vehicles*

Coliphage lambda does not permute its DNA during encapsulation; instead specific sites are cleaved in a particular way so as to produce complementary single-stranded "cohesive" ends. The size of the genome which can be packaged into lambda phage particles is thus limited. Therefore, it is necessary to delete non-essential lambda DNA in order to make room for the insertion of a translocatable drug-resistance element. Two deletion variants of lambda are convenient; phage λ*b*515*b*519 lacks 9·6% of lambda DNA ($4\cdot5\times10^3$ bases) but retains intact the attachment site and functional *int* and *xis* genes; phage λ*b*221 lacks 22% of lambda DNA (10^4 bases) and has lost both the attachment site and the *int* and *xis* genes. Sometimes these vehicles also carry the *nin*5 deletion, which removes another 5·4% of λ DNA ($2\cdot5\times10^3$ bases) (Davidson & Szybalski, 1971).

At present, lambda variants exist which carry the following elements: a tetracycline-resistance element (Tn10) derived from the R-plasmid R222 (Barker, unpublished work), a kanamycin-resistance element (Tn5) derived from the R-plasmid JR67 (Berg *et al.*, 1975), an ampicillin-resistance element (Tn2) derived from the

R-plasmid R-1 (Botstein, unpublished work) and a chloramphenicol-resistance element (Tn9) derived from P1CM (Scott, 1973; Gottesman & Rosner, 1975). Each of these has been used to mutagenize *E. coli* with the production of insertion auxotrophs.

As in the case of P22, it is necessary to prevent lysogenization in order efficiently to select for insertion mutants of the host. In the case of λ*b*221 variants, which are *int*$^-$*att*$^-$ by deletion, this is conveniently done by infection at high multiplicity, outgrowth of the survivors for several generations to segregate away the repressed phage genomes, and plating on medium which contains the appropriate drug. Further cycles of growth and killing on the selective plates can be inhibited by inclusion in the plates of 0·0025 M-sodium pyrophosphate, which kills vegetative lambda particles containing more than a minimum amount of DNA in their heads; like EGTA in the case of P22, sodium pyrophosphate reduces the background in drug-selection plates. Phage λ*b*515*b*519 derivatives containing the *int-am*29 mutation have also been used successfully. In the case of the tetracycline-resistance elements, an alternative scheme appears to work more efficiently; the vehicle in this case contains, in addition to the *b*221 deletion, a temperature-sensitive mutation in the repressor gene (*c*1857) and a mutation in gene *O* (*Oam*29). After infection at high temperature, this phage cannot maintain lysogeny; the *O*$^-$ mutation reduces killing of the infected cells. The latter scheme has the advantages that all mutants are independent, since no outgrowth of infected cells is required.

Using these lambda vehicles, it is now possible to mutagenize *E. coli* with drug-resistance elements so that between 10^{-2} to 10^{-6} (depending on the element) drug-resistant survivors are recovered, of which 1 to 2% are auxotrophs.

(b) *Plasmid vehicles*

Transmissible plasmids (such as F) offer another route by which translocatable drug-resistance elements can be introduced into the bacterial chromosome. As in the case of phage vectors, some method for selecting against the maintenance of the plasmid is required in order to find the relatively rare translocations of the elements from plasmid to chromosome. Three schemes for doing this have been used; first, use of a temperature-sensitive mutant of F which will not replicate at non-permissive temperature; second, introduction of a plasmid into cells already containing a plasmid of the same incompatibility group, and third, introduction of a plasmid into mutant cells unable to maintain this type of plasmid.

(i) *Mutant plasmid vehicles*

The simplest scheme involves an F′ plasmid carrying a *ts* mutation affecting replication (Jacob *et al.*, 1963). For example, a derivative of a F′$_{ts114}$*lac*$^+$ episome was mutagenized with P22Tc-10 in *Salmonella*. Several derivatives carrying the tetracycline-resistant element were isolated. When these are introduced into a *Salmonella* strain at low temperature, tetracycline-resistant derivatives carrying the F′ are easily obtained. When selection is made for tetracycline-resistance at high temperature, two kinds of survivors predominate: Hfr derivatives in which the entire F′ episome has been integrated into the chromosome, and translocations of the tetracycline-resistance element into the chromosome. These two possibilities can be easily distinguished; Hfr strains retain both the Lac$^+$ and Tet^R phenotypes; strains undergoing translocation of Tn10 retain only the Tet^R phenotype and again show the Lac$^-$ phenotype characteristic of *Salmonella*.

Clearly this kind of scheme is generalizable for use in a great variety of enteric bacteria: all those which accept F transfer. Using analogous temperature-sensitive mutants of other plasmids (for example, RP4), the range of bacteria into which these techniques can be applied might be very large indeed. The major technical problem will always be to distinguish plasmid integration from translocation of the drug-resistance element.

(ii) *Use of plasmid incompatibility*

Another scheme is one which has been used successfully by Foster *et al.* (1975) and by Heffron & Falkow (1977). A plasmid carrying a drug-resistance element is introduced into bacteria already carrying a plasmid of the same incompatibility group but not conferring resistance to the drug of interest. Under these circumstances, the incompatibility phenomenon prevents maintenance of the donor plasmid; selection for drug resistance requires translocation of the element, often into the chromosome. This method has general application, since plasmid variants with the same incompatibility spectrum but missing one or another drug-resistance are not rare. Using simultaneous selection for resistance to two drugs (one to maintain the resident plasmid and the other to select the incoming element), it should be possible to obtain insertion auxotrophs without great difficulty.

(iii) *Use of mutant recipients unable to maintain plasmids*

Finally, Heffron (personal communication) used a third scheme for obtaining translocations of an ampicillin-resistance element. This took advantage of the fact that some plasmids depend on non-essential host genes for their maintenance. In the particular example, Heffron used a *pol*A-*ts* host into which he had introduced a *col*E1-derived plasmid carrying the ampicillin-resistance element. The *col*E1 replication apparatus will not function in *pol*A$^-$ cells (Kingsbury & Helinski, 1970) and thus selection at non-permissive temperature for ampicillin-resistance permitted selection of translocations of the ampicillin-resistance element.

(c) *Selection of new insertions of drug-resistance elements using generalized transduction*

Generalized transduction consists of the encapsulation of host DNA by phage particles and the subsequent infection of this DNA into a new host (Ebel-Tsipis *et al.*, 1972; Ikeda & Tomizawa, 1968). When there is homology between the chromosomes of the new host and the piece of DNA transduced, recombination frequently results in substitution of genetic markers from the donor for their alleles in the recipient. If a translocatable element is included as part of a transduced fragment, recombination can serve to integrate the element at a site in the recipient which is homologous to the site at which the element was located in the donor. If recombination is prevented, then the drug-resistant element can only be inherited if it leaves the transduced fragment and inserts (usually at random) into the recipient chromosome. Thus, in the absence of recombination, transduction and selection for drug-resistance allows one to recover the rare cases in which the drug-resistance element has translocated from the original site in the donor DNA to a new site in the recipient. Three ways to prevent homologous recombination in the recipient suggest themselves; first, transduction into a deletion which is larger than a transducing fragment (i.e. a deletion not

ordinarily transducible); second, transduction from a site in the donor which is not homologous to any site in the recipient; third, transduction into a *rec*A$^-$ recipient.

Although not all of these methods have yet been tried successfully, examples of the second (transduction from a strain carrying an R factor into another not containing the plasmid) were found by Watanabe *et al.* (1972).

5. Discussion

The techniques described here provide an extremely powerful supplement to the standard methods of bacterial genetics. For the most part these techniques are familiar ones made easier and more widely applicable to a variety of situations through the use of translocatable genetic material; few of the techniques are new in fundamental concept.

The value of the methods described here is derived from the following characteristics of translocatable drug-resistance elements.

(1) The resistance determinant provides a genetic marker which is subject to positive selection.

(2) The marker can be placed in or near genes of interest. This close linkage makes it possible to move a variety of genes into new genetic backgrounds by positive selection. The drug-resistance determinants can also be applied directly to genetic analysis, since they provide new markers for use in three-point tests and deletion mapping.

(3) The resistance determinant is a movable region of homology which can be used as a sex factor affinity site or a phage attachment site. Two such regions of homology can also recombine with each other to generate duplications, deletions, inversions and translocations.

(4) The translocatable elements cause an increase in the frequency of deletion mutations in the region adjacent to the insertion site. Thus, they can be used to generate deletion mutations in regions of interest.

Since starting to use translocatable drug-resistance elements, we have found them useful in almost every genetic project underway in our laboratories. The techniques described here make a variety of genetic tasks easier. This list of possible uses is certainly not exhaustive; it seems likely that additional applications of translocatable elements will be found.

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