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Transposition of the Kanamycin-Resistance Transposon Tn903

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Summary. The insertion of the kanamycin-resistance transposon, Tn903, into the *Escherichia coli* chromosome was studied. Tn903 is similar in structure to the well known transposons Tn5 and Tn10 in that it has a unique central sequence flanked by inverted repeat sequences extending more than a thousand base pairs. However, the central region of Tn903 has enough single-frame coding capacity only for the drug modifying enzyme, whereas Tn5 and Tn10 carry multigenic unique sequences. In this paper we demonstrate that two different classes of insertion event occur: (1) the first class is a complex event in which all or part of the genome of the bacteriophage lambda vector is co-inserted near the *purE* locus on the *E. coli* chromosome (11.7 min); (2) the second class appears to be a "simple" transposition event in which the transposon alone is inserted at relatively non-specific sites in the chromosome, as has been described for Tn5 and Tn10. Furthermore both classes show dependency on homology-requiring recombination systems. We suggest that Tn903 transposes infrequently because it must utilize a *recA*-controlled host function, whereas Tn5 and Tn10 are *recA*-independent and encode similar but more active functions on the transposon DNA.

Introduction

Insertion sequence (IS) elements are DNA sequences which have been shown to insert into many sites in non-homologous regions of DNA (Shapiro et al., 1977). Transposons share the insertion characteristics of IS elements but, in contrast to IS elements, carry identifiable genes. Most transposons carry genes for polypeptides involved in antibiotic resistance; in fact, many of the resistances carried by the antibiotic resistance plasmids (or "R-factors") have been shown to be encoded by transposons inserted in the plasmids (Shapiro et al., 1977). The well-studied R-factor R6 has been shown to carry the IS elements IS1 and IS2 (Saedler, 1977) and two transposons. One is the tetracycline-resistance transposon Tn10, which is the most intensively studied transposon; it constitutes an extensive inverted duplication in the R6 DNA, with a central region of 6.4 kilobase-pairs (kbp) flanked by 1.4 kbp inverted repeats (Botstein and Kleckner, 1977). The other transposon is a kanamycin resistance element, Tn903 (Nomura et al., 1978; Young et al., 1979) and also constitutes an extensive inverted duplication (Sharp et al., 1973). In Tn903, however, the central unique DNA is only 0.9 kbp, with the flanking repeats each 1.05 kbp. Tn903 is thus of special interest among the drug-resistance transposons because the central DNA has a coding capacity for only about 27,000 daltons of polypeptide, which is the molecular weight of the kanamycin phosphotransferase (Armstrong et al., 1977; Meagher et al., 1977). However, among these four transposable elements, Tn903 has certainly been the least characterized. Indeed, in spite of its transposability, this kanamycin-resistance element has been used as a stable selective marker on many cloning vehicles, such as pSC102, pSC105, pML21, pM131 and pCR1 (Lowett and Helinski, 1976; Cohen, 1977).

To date, Tn903 has been shown to transpose from R factors into a variety of phage chromosomes (Nomura et al., 1978; Young et al., 1979) but has not been observed to transpose into plasmids or the bacterial chromosome (Berg et al., 1978). This narrow range of recipient chromosomes seemed to distinguish Tn903 from other transposable elements. By use of

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an appropriately marked lambda phage as a vector, we demonstrate in this paper that Tn903 does transpose to the *E. coli* chromosome. Furthermore, the transposition events fall into two classes: a "complex" event in which all or part of the vector genome is co-integrated into the bacterial chromosome hear the *purE* locus (11.7 min); and a "simple" event, in which Tn903 alone is inserted into the chromosome, with no evident specificity for a particular chromosomal locus. In addition, we find that both of these classes of transposition events show strong dependence on known homology-recombination systems (i.e., the *recA* system of *E. coli*), in sharp contrast to the characteristic *recA*-independent transposition found for the well-known transposons Tn5 and Tn10 (Berg, 1977; Botstein and Kleckner, 1977).

Materials and Methods

Media and Buffers

Complete medium or broth contained tryptone (1%), yeast extract (0 5%), and NaCl (0.5%). Lambda dilution buffer is 20 mM Tris, pH 8.0 and 20 mM $MgCl_2$. Tryptone/kanamycin (TK) and tryptone/tetracycline (TT) agar is made with either 30 mg kanamycin or 30 mg tetracycline per liter of 1.5% agar, 1% tryptone, 0.5% yeast extract, and 0.5% NaCl.

Isolation of Antibiotic-Resistance Transducing-Phage

All bacterial strains and bacteriophages used in this study are shown in Table 1.

A bacterial strain that hosts a plasmid carrying the antibiotic resistance of interest was lysogenized with *λbbn* by infection at 30° C followed by screening for temperature-sensitivity. Individual colonies were inoculated into a number of separate tube cultures and sub-cultured for several days at 30° C. The cultures were induced in exponential phase in broth by shifting to 42° C for 20 min and then continuing aeration at 37° C for 3 h. The cells were concentrated 30-fold by centrifugation at 4° C and resuspended in lambda dilution buffer. These concentrated, induced cell suspensions were quick-frozen in liquid N_2 and then thawed at 30° C for 30 min. When lysis became apparent by vastly increased viscosity, DNase (Worthington Biochemicals) was added to a final concentration of 10 μg/ml and incubation continued for 10 min. The lysate was then cleared of debris by centrifugation at 4° C. Screening for antibiotic resistance transducing particles was done by infecting 0.2 ml of freshly saturated Ymel overnight culture with 0.1–0.2 ml of the phage suspensions After 30 min preabsorption at 25° C, the infected cells were diluted 30-fold into prewarmed 30° C broth and grown to saturation under aeration in roller tubes. The saturated infected cultures were concentrated 50-fold and spread on either (TK) or (TT) plates. One colony from each infection was picked; all were temperature-sensitive and, after purification on the approximate antibiotic medium, were inoculated into liquid culture, grown at 30° C until mid-exponential phase and thermally induced. Lysis usually occurred within 60 min, and the lysate was treated with a few drops of chloroform before clearing by centrifugation. The resultant lysate contained 100% Kan^R- or Tet^R-transducing particles.

Table 1. Bacteriophages used

Bacterial strains	Genotype	Sources
W3101	*thi⁻ pro⁻ gal⁻*	Collection of M Syvanen
W3101 recA	*thi⁻ pro⁻ gal⁻ recA13*	
Ymel	*pro⁻ su*III	
W3101 pSC102	W3101 (kan^R sul^R)	
W3101 pSC105	W3101 (kan^R tet^R)	
CA8000	HfrH	Cold Spring Harbor Collection
AB1157 (λ)	*thr⁻ leu⁻ thi⁻ lacY⁻ gal⁻ xyl⁻ mtl⁻ proA⁻ his⁻ argE⁻ strA tsx supE* (λ^+)	Constructed by R. Young
PC0135	*thi⁻ purE55 lacY malA xyl mtl gal strA*	B. Bachmann, CGSC 5403

Phage designation	Complete genotype
λ bbn	*λ b519b515cI857nin5 S am7*
λ A⁻B⁻	*λ A am32 B am21 b519b515nin5*
λ pk3	*λ b159cI857*Tn903 (0.923) *S am7*
λ b221 P⁻ k3	*λ* b221*cI857 P am3*Tn903 (0.923) *S am7*
λ b221 red⁻ P⁻ k3	*λ b221 red am270cI857 P am3*Tn903 (0.923) *S am7*

With the exception of *λA⁻ B⁻*, all phages used carry the *S am7* lysis mutation. Also note that the *cI857* and the small deletions *b515, b519,* and *nin5* are not indicated in the normal designation

Physical and Genetic Mapping of the Tn903 Insertion

The preliminary genetic mapping of the Tn903 insertions was done by crossing each Kan^R – transducing phage with lambda phage carrying amber mutations in the *A* and *B* genes, designated *A am B am* An exponential culture of strain Ymel was infected with a multiplicity of 5 each of *λ A am B am* and the λ.:Tn903 *S am 7* isolate. After preabsorption at room temperature for 30 min, the infected cells were diluted 10^3 fold into prewarmed 37° C broth and aerated for 90 min. A few drops of $CHCl_3$ were added, the suspension shaken, and the progeny scored for *am+* recombinants by plating on W3101 at 30° C. Each plaque was tested for kan^R lysogens on TK agar. The fraction of kan^R am^+ plaques was taken as a measure of the distance between the *A am B am* loci (which are at the extreme left end of the lambda vegetative map) and the site of the Tn903 insertion in the phage genome. Physical mapping was performed by heteroduplex analysis as described in Young et al. (1979) Briefly, electron micrographs of heteroduplexes of the λ::Tn903 phage genome with a standard *λ* imm^{434} hybrid phage genome were examined. Standard lengths were the distances between the *b*519 deletion loop and the imm^{434} deletion-substitution "bubble" and between the immunity bubble and the *nin*5 deletion loop. Some transposon orientations were determined by agarose gel electrophoresis of the products of restriction enzyme digestion of various λ::Tn903 insertions. The details are described elsewhere (Young et al., 1979)

Transposition of Tn903

Transposition experiments involved infection of either W3101 (Rec^+) or W3101 *recA* with the appropriate λ·:Tn903 phage. Preabsorption was at 25° C at a multiplicity of ≦1 The infected

cells were then diluted into 30° C or 37° C pre-warmed broth and incubated under aeration for 30 min. The cultures were then concentrated, plated on TK agar, and incubated at 30° C or 42° C until colonies appeared. The Kan^R transductants were purified by streaking on TK plates and tested for the presence of lambda genes by marker rescue spot tests. The spot tests were performed by spotting 5 µl drops of various serially diluted imm^{434} nonsense mutants on lawns of each Kan^R transductant. The presence or absence of a lambda gene could be detected by comparing the plating efficiency of the nonsense mutants on the transductant lawns and on lysogenic and non-lysogenic control lawns. The phages used in these tests are: $\lambda imm^{434} c^- A\,am32$, $\lambda imm^{434} c^{ts} K\,am24$, $\lambda imm^{434} N\,am53$, $\lambda imm^{434} P\,am80$, $\lambda imm^{434} Q\,am7\ Q\,am501$, and $\lambda imm^{434} Ram5$. The W3101 (Rec^+) or W3101 *recA* strains were used as negative controls and W3101 *recA* lysogens of λpk3 as positive controls. The presence of lambda immunity or anti-immunity was tested on the same lawn by streaking *λcI* and *λvir*.

Stability of Kan^R Transcutants

After purification on TK agar, cells from individual clones were transferred to broth, grown to saturation, streaked on both plates and tested by for growth on TK media by either transferring (with a toothpick) or replica plating.

Mapping of Insertion Sites

Preliminary mapping was done by mating isolates of the HfrH strain CA8000, containing insertions of Tn903, with the multiply-marked FB lysogenic strain AB1157 (λ) The λ lysogen of the female strain was used to prevent zygotic induction of those insertions carrying the λ immunity region. Early exponential phase cultures of the male and female bacteria were mixed at a ratio of one Hfr to 10 F^- cells. The mixed cultures were aerated very gently for 2 h, after which Leu^+ Str^R recombinants were selected by plating on the appropriate agar. The recombinants were screened for the acquisition of more distal markers by replica plating, as described by Miller (1972) Estimation of co-transduction frequencies of the kan^R locus with the *purE* marker was done by transducing strain PC0135 (an adenine auxotroph carrying a lesion in *purE*) to prototrophy using a *Plkc* lysate grown on the $purE^+$ strain containing the Tn903 insertion (Miller, 1972).

Results

Transposition of Tn903 to Phage Lambda

Tn5 and Tn10 have been characterized by transposition into phage lambda (Berg et al., 1975; Kleckner et al., 1975) and using lambda vectors for studying transposition into the *E. coli* chromosome (Berg, 1977; Kleckner et al., 1978). We obtained Tn903 insertions in phage lambda by growing lambda on a strain carrying the plasmid R6. Table 2 demonstrates that the frequency of transposition of Tn903 from R6 into phage lambda is about ten-fold less than that of Tn10. Furthermore, Tn903 transposition into phage lambda occurs as frequently from pSC102 and pSC105, which are plasmids constructed in vitro from the pSC101 replicon and restriction fragments from the plasmid R6-5, a derivative of R6 (Cohen et al., 1973).

Table 2

Source plasmid	Frequency of Kan^R transducing particles	Frequency of Tet^R transducing particles
R6	10^{-8} to 10^{-9}	10^{-7} to 10^{-8}
pSC102	10^{-8} to 10^{-9}	–
pSC105	10^{-8} to 10^{-9}	–

Single colonies of W3101 carrying the indicated plasmid and lysogenic for λ *bbn* were picked from TK plates and serially subcultured for three days. 5ml cultures were thermally induced and concentrated phage lysates prepared and screened for drug-resistance transducing particles as described in *Materials and Methods* Frequencies were estimated by reconstruction experiments in which a suspension of λ *bbn* was seeded with known titers of antibiotic-resistance transducing phage and screened as indicated above. Although the absolute frequencies varied within the indicated ranges, the frequency of Tet^R-transducing particles was always about ten-fold greater than the frequency of Kan^R-transducing particles

Initially, 80 independent lambda phage isolates carrying kanamycin resistance were obtained. Most of these phages grow as well as the parental phage, but two absolute defective mutants and five plaque morphology mutants were found. By analyzing these mutants, we discovered a new lysis gene in lambda, R_z which we describe elsewhere (Young et al., 1979). Further genetic and physical analysis of the bulk of the λTn903 isolates was carried out, in order to determine the spectrum of insertion sites in lambda for this transposon.

The approximate location of the Tn903 insertions was determined by crossing each transducing phage with $\lambda\ A\,am\ B\,am$. Since each insertion isolate carried the $S\,am7$ allele, the fraction of λam^+ progeny which retained the kan^R marker represents a measure of the distance between the $A\,am\ B\,am$ site and the Tn903 insertion. Thirty insertions were tested, and Tn903 was found to map at many different sites in the lambda genome (data not shown). Eighteen independent insertions were found between 0.80 and 0.92 and the lambda linear map, so the distribution was not random with respect to the nonessential regions of lambda. A number of these isolates were selected for physical mapping.

The physical location of Tn903 in each of the isolates was determined by measuring heteroduplex molecules in the electron microscope or by analyzing the DNA fragments produced by restriction endonucleases (see Young et al., 1979 for details for this analysis). In Table 3 we list the isolates where the location of Tn903 is accurately known. In some cases, the orientation of the unique sequence of Tn903 with respect to the lambda map was determined. We define

Table 3. Insertion sites of Tn903 in λ

Phage designation	Tn903 Insertion site	Comments
λ pk1	*b2*	
λ pk3	0 923 (II)	
λ pk5	*b2*	
λ dk6	0.951 (I)	lysis-defective
λ pk8	0.705	
λ pk9	*b2*	(between *b*515 and *attP*)
λ pk10	*b2*	(between *b*515 and *attP*)
λ pk21	0 993 (I)	
λ pk22	0 980 (I)	
λ dk23	0.951 (I)	lysis-defective
λ pk24	0.980 (I)	
λ pk25	0 998 (I)	
λ pk26	0 979 (I)	
λ pk34	0.568	
λ pk35	0.80	*cII* insertion
λ pk36	0.76	*rex*⁻

The insertion sites of Tn903 were determined physically for these isolates by heteroduplex analysis. The location is given in fractional lambda lengths. In some cases, inspection of a few heteroduplexes was sufficient to assign the insertion to the *b2* region and the molecules were not measured precisely. The Roman numeral in parenthesis following the insertion indicates the orientation of transposon with respect to the lambda genome, as determined by restriction enzyme analysis using the enzymes *Xho* I and *Sma* I (see Materials and Methods)

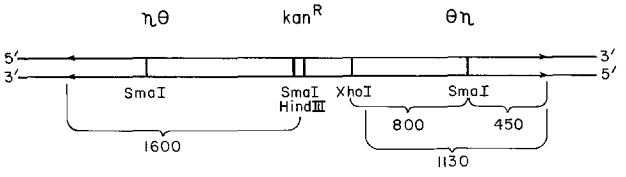

Fig. 1. Physical structure of Tn903. Approximate dimensions in base-pairs (bp) and restriction enzyme cleavage sites are shown. The ηθ designation (Sharp et al., 1973) denotes the flanking repeat sequences

orientation I and II with respect to the order of the *Xho* I site and the *Hin* dIII site in the unique region of Tn903 (see Fig. 1). Orientation I places the *Xho* I site to the right of *Hin* dIII site when gene *S* is at the right of the lambda map as in Fig. 2; in orientation II, the *Hin* dIII site is to the right of the *Xho* I. All seven Tn903 insertions to the right of 0.95 are in orientation I, but a nearby insertion (at 0.923) is in orientation II.

Transposition of Tn903 from Tn903

Bacteriophage λ*cI*857 encodes a thermolabile cI repressor and thus is unable to lysogenize when grown at 42° C. Accordingly when λcI857 carrying a *kan*^{R} gene is infected into sensitive bacteria and grown at 42° C in the presence of kanamycin, the surviving Kan^R bacteria are non-lysogenic. Table 4 lists the frequencies of kanamycin-resistant bacteria that result when a number of different λTn903 derivatives were infected into strain W3101 and Kan^R colonies were selected at 42° C. Table 4 shows that the kanamycin resistance in most of these strains is very unstable. It is shown elsewhere that, in those strains derived from λpk35, λpk3, λdk6, and λpk31, Tn903 is carried on a defective lambda plasmid, which we denote Tn903 λdv (Syvanen, 1979). Of those strains listed in Table 4, only λpk10 yielded isolates in which Tn903 is inserted in the chromosome (i.e., the *kan*^{R} is relatively stable and no plasmid is detected). λpk10 is unable to establish itself as a Tn903 λdv and, as can be seen in Table 4, gives rise to *kan*^{R} clones at a much lower frequency than the other strains. Unexpectedly, even among the stable *kan*^{R} isolates derived from λpk10, many of the clones still carry lambda genes (Table 5).

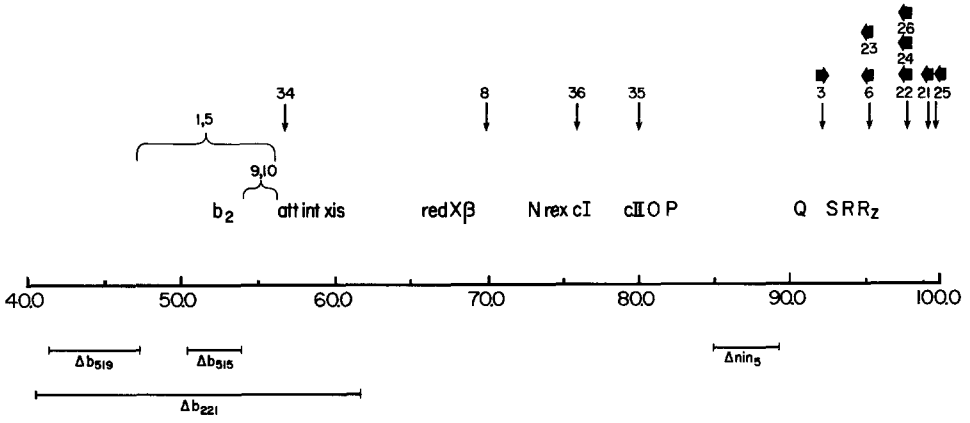

Fig. 2. Insertions of Tn903 in lambda. The right arm of the linear lambda vegetative map is shown with the insertion sites of a number of λTn903 isolates indicated. Where known, the orientation of the transposon is indicated by the thick horizontal arrows. Orientation I is defined as that orientation which placed the *Xho* I cleavage site in Tn903 to the right of the *Hin* dIII site (in the unique central region of the transposon) when the *S* gene is drawn at the right of the lambda map. Thus Tn903 in λpk3 is in orientation II. Only approximate locations are given for isolates λpk1, λpk5, λpk9, and λpk10

Table 4. Isolation of kanamycin-resistant bacteria from λ Tn903 infections

λTn903 used as donor	Location of Tn903 in donor	Frequency Kan^R bacteria	Fraction stable
pk10	*b2* region	1.5×10^{-6}	7/9
pk8	0.705	3×10^{-4}	2/12
pk35	0.797	5×10^{-5}	0/10
pk3	0.923	1.2×10^{-4}	0/11
dk6	0.952	3×10^{-5}	0/12
pk31	0.991	1.5×10^{-4}	0/7
no phage	–	$< 10^{-10}$	–

The frequency of kanamycin resistant (Kan^R) bacteria is the ratio of the number of Kan^R bacteria appearing on a plate per number of bacteria infected with the indicated λTn903 phage (see Materials and Methods) The multiplicity of infection was 0.5. When bacteria were multiply infected. the yield of Kan^R bacteria is reduced (not shown). Stability of the kanamycin-resistance phenotype was tested in selected clones as described in Materials and Methods. All selections for kanamycin resistance were done at 42° C (to prevent lysogenization; see Results)

Table 5. Properties of kanamycin-resistant transductant

Donor λ:Tn903	Frequency of Kan^R bacteria per infected cell	No. of Kan^R isolates tested	Lambda genes				Stability
			P	*Q*	*R*	*A*	
λpk10 at 42° C	1.5×10^{-6}	7	–	–	–	–	< 0.5% (6/7); 50% (1/7)
		1	–	–	–	+	5%
		4	–	+	+	+	40%
		1	+	+	+	+	99%
λpk3 at 42° C	1.2×10^{-4}	13	+	+	–	–	30%
λb221P⁻k3 at 42° C	10^{-6}	13	–	+	+	–	< 0.2% (0 of 500)
λb221P⁻k3 at 30° C	10^{-5}	11	–	+	+	+	< 1% (0 of 10)

All infections were done at m.o.i = 1 into strain W3101 After aerating for 30 min at 30° C to allow expression of the kanamycil-resistance, the infected cultures were plated onto kanamycin medium at the indicated temperature. Frequencies are Kan^R colonies per infected cell after overnight incubation The presence of lambda genes was tested by marker rescue. Stability was measured as the frequency of Kan^S colonies derived from streaking an overnight culture which had been inoculated with a single Kan^R colony

Transposition of Tn903 from Replication-Deficient Phage

In order to eliminate the formation of the Tn903 λdv, we constructed a derivative of λpk3 with a nonsense mutation in the *P* gene. The *P* gene of lambda is required for DNA replication and consequently its inactivation prevents establishment of the λdv plasmid (Berg, 1974). In addition, to prevent integration of some defective fragment through the prophage attachment site, we introduced the *b221* deletion, which removes the *att* site and the *int* and *xis* genes (Davidson and Szybalski, 1971), thus giving *λb221P*⁻k3. When this phage was infected into strain W3101 of *E. coli* at 42° C, kanamycin-resistant clones were obtained and tested for the presence of lambda genes. We found that some of the clones carried the lambda gene *R* and others carried both the *Q* and *R* genes. As we show below, the Tn903 and associated lambda genes are on the bacterial chromosome. The lambda genes found with Tn903 in these cases are those adjacent to Tn903 in the original λTn903 donor (see Table 5, compare Fig. 2).

In similar experiments performed at 30° C (the permissive temperature for the lambda thermolabile repressor), stable lysogens are obtained (Table 5), even though the *att-int* region is deleted in the *λb221* genome. These lysogens are temperature-sensitive, but subsequent growth at 42° C permits selection of temperature-resistant, kanamycin-resistant colonies. All temperature-resistant derivatives isolated in this way carry lambda genes *Q* and *R* but lack other lambda genes.

Influence of Homology-Dependent Recombination Functions on Insertion of Tn903

Given that the insertion of Tn903 into the bacterial chromosome involves integration of all or part of the lambda bacteriophage vector in the chromosome, it was necessary to examine the dependence of this insertion on the known homology-dependent recombination functions – the bacterial *recA* and phage *red* functions (Signer, 1971). A nonsense mutation in the *red* gene was introduced and the number of kanamycin-resistant colonies was scored after 30° C infection into either rec^+ or *recA* bacteria (Table 6). The data show that lysogeny is indeed dependent on functional *rec* and *red* systems. Table 6 (line 1) shows that after λred^+ is infected into $recA^+$ bacteria, kanamycin-resistant lysogens appear at a frequency of 1.2×10^{-5} to 3×10^{-6}. If this infection is repeated with $recA^-$ bacteria, the frequency of lysogeny is reduced (up to 1,000-fold) (Line 3). The most striking reduction occurs with λred^- infection: lysogens can no longer be found, regardless of the bacterial Rec phenotype (lines 2 and 3). The formation of lysogens thus requires both the phage red^+ and bacterial $recA^+$ gene products; the appearance of kanamycin-resistant cells carrying the lambda genes *R* or *Q* and *R*, however, is dependent only on *recA* (Table 6, lines 1–4).

Table 6. Yield of kanamycin resistant bacteria when homology recombination functions are inactivated by mutation

	Genotypes of		Frequency Kan^R at 30° C		Number of Isolates genes	Presence of genes				Immunity
	Donor λTn903	Recipient bacteria	Experiment 1	Experiment 2		N	Q	R	K	
1.	*red*$^+$	*rec*$^+$	3×10^{-6}	1.2×10^{-5}	(15)	+	+	+	+	immune
					(1)	−	−	+	−	sensitive
					(1)	−	−	−	−	sensitive
2.	*red*$^-$	*rec*$^+$	1.5×10^{-7}	5×10^{-7}	(13)	−	+	+	−	sensitive
					(13)	−	−	−	−	sensitive
3.	*red*$^+$	*recA*$^-$	2.7×10^{-8}	5×10^{-9}	(8)	+	+	+	+	immune
					(3)	−	−	−	−	sensitive
	red$^-$	*recA*$^-$	1.2×10^{-8}	4×10^{-9}	(13)	−	−	−	−	sensitive

Each donor phage carries the *b221* deletion and *Pam3* mutation. The phage was infected into the recipient strain (W3101) with the given *recA* allele at an MOI=0.5 and plated onto kanamycin agar at 30° C. The frequency is the number of colonies that appeared divided by the number of infected cells plated. The presence of lambda genes was tested by marker rescue. 'Immune' means the recipient *kan*^{R} strain will not support λ growth; "sensitive" means λ$^+$ makes normal turbid plaques

Table 7. Transposition frequency of Tn903

Genotype of		Frequency of Tn903 transposition (average of 2 experiments)
Donor λTn903	Recipient bacteria	
red$^+$	*rec*$^+$	4×10^{-7}
red$^-$	*rec*$^+$	1.2×10^{-7}
red$^+$	*recA*$^-$	3.5×10^{-9}
red$^-$	*recA*$^-$	6×10^{-9}

The average of the frequency of kanamycin bacteria given in Table 6 for experiment 1 and 2 was determined. This value was then multiplied by the fraction of colonies that appeared to be derived from "simple" transpositions to give the absolute frequency of such events

Table 8A. Mapping by HfrH conjugation

Character	Fraction of recombinants		
	CA8000.:1A	CA8000::2B	CA8000·:7C
Leu$^+$ (*leu*, 1 7 min)	1.00	1.00	1.00
Pro$^+$ (*proAB*, 5.6 min)	0.31	0.40	0.32
Lac$^+$ (*lac*, 7 9 min)	0.26	0.28	0.25
Kan^R	0.10	0.15	0.00
Gal$^+$ (*gal*, 16.7 min)	0.00	0.07	0 01

CA8000 was used as a host for infections by λ*b221 red*$^+$ *cI857 Pam3* KY. The 1A and 2B insertions were Q^+R^+ and lysogenic isolates respectively from *red*$^+$ infections. The 7C insertion is from a *red*$^-$ infection and carries no lambda genes detectable by marker rescue. These HfrH strains were mated with AB1157 (λ) as described in *Materials and Methods*. For each mating 150–200 Leu$^+$ recombinants were selected, and scored for the acquisition of more distal markers by replica-plating

In Table 6 we can now see "simple" transposition of Tn903. We define "simple" transposition as those events which result in kanamycin-resistant colonies which carry no lambda genes and are sensitive to superinfecting lambda; i.e., "simple" Tn903 events correspond to the transposition events described for Tn5 and Tn10 (Berg, 1977; Kleckner et al., 1978). The frequencies of "simple" Tn903 transposition were calculated from the data in Table 6 and are presented in Table 7. There is a 50- to 60-fold reduction in transposition frequency going from a *recA*$^+$ to a *recA*$^-$ recipient; however, the state of the phage *red* recombination system does not affect the frequency of the "simple" events.

Mapping the Insertion Sites

There seem to be two events yielding stable *kan*^{R} clones after infection by a replication-deficient λTn903 vector: insertions of Tn903 accompanied by lambda sequences and insertions without detectable lambda sequences. In view of the absolute dependence on both homology-recombination functions demonstrated by the lysogenic event, and the putative relationship between such lysogens and the "QR" events, it seemed likely that the same site might be involved in these insertions. Preliminary mapping was done by Hfr mating. An Hfr strain was used as the host bacteria in transposition experiments with *red*$^+$ and *red*$^-$ λ*b221P*$^-$k3 phage; a lysogen and two Q^+R^+ isolates, from *red*$^+$ and *red*$^-$ infections respectively,

Table 8B. Co-transduction with purE

Source of P1 lysate	*PurE*$^+$ transductants	*Kan*^{R}	Per cent Co-transduction
Ca8000:.1A	187	148	79
CA8000::2B-1	199	143	72
CA8000 7C	212	0	0

CA8000::2B-1 was obtained by selecting for Kan^R survivors at 42° C and was shown to be Q^+R^+ by marker rescue. P1 lysates were prepared on the indicated strains and used to transduce PC0135 to *purE*$^+$ as described in *Materials and Methods* The PurE$^+$ transductants were scored for Kan^R by replica-plating

were then mated with a female strain carrying multiple markers, including Leu$^-$, which is transferred very early by HfrH (Bachmann et al., 1976). Leu$^+$ recombinants were selected and screened for the acquisition of more distal markers. Table 8A shows representative results from three such matings. The *kan*^{R} locus in the lysogen and the Q^+R^+ strain maps between *lac* and *gal*. More accurate localization was obtained by measuring the co-transduction of *kan*^{R} with the *purE* locus at 11.7 min (Bachmann et al., 1976). The Q$^+$R$^+$ Tn903 insertion co-transduces at ~80% with *purE* (Table 8B); according to the formula of Wu (1966), the *kan*^{R} locus must lie with 0.1 min of *purE*. A similar *purE* linkage was obtained for a Q^+R^+ temperature-resistant derivative of a lysogenic insertion (Table 8B). P1 transduction of the lysogen itself was not attempted, since it is not known what effect the additional lambda DNA present in the lysogen would have on linkage measurements.

The "simple" transposition events do not show the same site specificity, however. Two independent "simple" transposition events from strains represented in Table 7 were mapped using HfrH transfer and *purE* linkage as above. No Kan^R co-transducants or transconjugants were found. We transduced the *kan*^{R} locus from these strains to a variety of other Hfr strains and found that one transfers very early with Hfr KL14 (i.e., 66–76 min) and the other with Hfr KL25 (i.e., 83–93 min), giving two different locations very distant from the lysogen and "QR" locus near *purE*.

Discussion

We have described some of the properties of the kanamycin-resistance transposon, Tn903:

(a) Transposition of Tn903 from the plasmid R6 to phage lambda occurs at about one-tenth the frequency of Tn10. R6 fragments cloned into the plasmid pSC101 also serve as a donor for transposition of Tn903 with same frequency of transposition into phage lambda as does R6.

(b) Insertion of Tn903 occurs at many sites in phage lambda, and in both possible orientations.

(c) Infection of plaque-forming λTn903 phage into *E. coli* (under conditions non-permissive for lysogeny) and subsequent selection for Kan^R results most frequently in the formation of unstable plasmids, which we designate Tn903λdv.

(d) Infection of $\lambda b_{221}P^-$ Tn903 into *E. coli* followed by selection *kan*^{R} results in the insertion of Tn903 into the bacterial chromosome, since Tn903λdv replication is abolished by the mutation in the phage *P* replication gene. These chromosomal insertions occur less frequently than the Tn903λdv formation, per singly infected cell, and fall into two distinct classes:

(1) a class of insertions in which the presence of all of part of the phage lambda donor genome can be detected. At a temperature permissive for lysogeny, stable lysogens are found; at both permissive and non-permissive temperatures, insertions are found which carry only the lambda genes adjacent to the transposon in the vector phage. The *kan*^{R} locus in this class is highly co-transducible (by generalized transduction) with the bacterial *purE* marker, at 11.7 min. We designate this class as "complex" insertions.

(2) a class of insertions in which Tn903 alone is inserted in the bacterial chromosome. Preliminary mapping of two of these insertions has placed them in two different loci, far from the *purE* locus. We designate this class as "simple" insertions.

(e) Both classes of Tn903 insertion events show strong dependence on the homology recombination systems (*rec* and/or *red*) of the infected cell. In particular, the lysogenic insertions are absolutely dependent on the functionality of both recombinational systems. The "simple" insertions occur nearly two orders of magnitude less frequently in *recA*$^-$ infected cells.

Comparison with Tn5 and Tn10

Tn5, Tn10, and Tn903 share the same formal structure (i.e., unique, central DNA flanked by inverted repeats more than one kbp long); Tn10 and Tn903 can be found on the clinically-isolated R factor, R6. All three transposons insert in many different sites in phage lambda (Berg et al., 1975; Berg, 1977; Kleckner et al., 1978; Young et al., 1979). All three transposons, once inserted in a gene, spontaneously excise from the insertion sites at comparable frequencies (Berg, 1977; Kleckner et al., 1978; Young, unpublished results). Both Tn5 and Tn10 transpose from phage lambda to the bacterial chromosome; for these

transposons, the frequency of transposition is high enough and the insertion site specificity so degenerate that the auxotrophic frequency (i.e., inactivation of essential bacterial genes by transposon insertion) is about 10^{-2} per insertion. As a result, Tn5 and Tn10 have become popular tools for mutagenesis, mapping and manipulation of many genomes (Kleckner et al., 1977). Tn903, by virtue of its ability to insert into phage lambda at many sites, has been used for transposition mutagenesis of that genome (Young et al., 1979). However, we find that Tn903 differs radically from Tn5 and Tn10 in transposition to the *E. coli* chromosome.

A useful comparison can be made by considering the frequencies of insertions of the transposon into the bacterial chromosome during single infection with a lambda phage vector carrying the b_{221} deletion and a nonsense mutation in at least one of the phage replication genes. For Tn10 and Tn5, the frequencies are $\sim 10^{-6}$ and $\sim 10^{-4}$, respectively, and are independent of the functionality of the *rec* and *red* recombination systems (Berg, 1977; Kleckner et al., 1978; Young, unpublished results). For Tn903, the frequency of the comparable event (i.e., the "simple" transposition of Tn903) is 10^{-6} to 10^{-7} in $recA^+$ cells and 10^{-8} to 10^{-9} in $recA^-$ cells. Thus, in $recA^+$ conditions, Tn903 transposes from the plasmid R6 to phage lambda and from replicationless lambda to the *E. coli* chromosome at about one-tenth the frequency of Tn10.

Tn903, then, has an intrinsic transposability about one-tenth that of Tn10 and a thousand-fold less than Tn5, in $recA^+$ conditions. However, Tn5 and Tn10 transposition to the *E. coli* chromosome is unaffected by the state of the *recA* and *red* genes, unlike Tn903. We speculate that the lower transposability and the dependence on the host recombination genes both stem from the genetic simplicity of Tn903. The unique central DNA has only coding capacity for the drug modifying enzyme. Furthermore, the *Eco* RI restriction fragment of R6 which includes all of Tn903 (plus about 1.5 kbp of the surrounding R6 DNA) has been cloned into the plasmid ColE1, generating the plasmid pML21 (Hershfield et al., 1976). Only one significant polypeptide is produced from this fragment when the plasmid is introduced into minicells; this polypeptide is the phosphotransferase of Tn903, since it disappears when the kan^R gene is inactivated by insertion of another DNA fragment (Meagher et al., 1977). In comparison, minicell expression experiments with plasmids carrying all or part of Tn5 and Tn10 reveal that many polypeptides are produced from these transposons (Tait and Boyer, 1978; J. Yin, personal communication). We speculate that Tn903 simply lacks one or more genes that are required for (efficient?) transposition and thus depends on a host *recA*-dependent function for its transposability. Studies are now in progress to determine if other *E. coli rec* genes can be implicated in Tn903 transposition.

Co-Integration of the Lambda Genome

The bizarre integration of all or part of the phage vector remains unexplained. An attractive hypothesis would be the existence of limited homology between a sequence near *purE* and either the phage or transposon sequences. Integration of the repressed vector phage by a reciprocal recombination event would result in the lysogenic insertion. However, the lysogenic insertions depend on the functionality of *both recA* and the *red* genes; thus the lysogenic insertions must be more complex that a simple reciprocal recombination. It is just as difficult to rationalize the "Q^+R^+" and "R^+" insertion events. Since these events show dependence on *recA* functionality, it is possible that they represent a step required for the formation of the lysogenic insertions. This reasoning does not explain why the lysogenic events predominate in $recA^+$ red^+ infections, nor does it suggest a plausible mechanism for the formation of "Q^+R^+" insertions. We are attempting to investigate these events physically, using F′ plasmids carrying the *purE* region as targets for Tn903 transposition.

It is possible that similar complex co-integrations occur with λTn5 and λTn10. Although the much higher "simple" transposition frequency of Tn5 would mask these events, it should be possible with Tn10 to detect such integrations. Infections of $\lambda b_{221} O^- P^-$Tn10 do generate a significant fraction of lysogens (Young, unpublished results); however, *recA* and *red* dependence and site-specificity have not yet been determined.

It may be a worthwhile cautionary note, however, that the use of the Kan^R associated with Tn903 as a stable selective marker in cloning plasmids may result in unusual and unpredictable recombination of the cloned DNA into other genomes. Fortunately, many of the most frequently used cloning plasmids carrying Tn903 have been constructed in vitro using steps that include deletion of much of the transposon structure.

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