RECOMBINATION BETWEEN IS5 ELEMENTS: REQUIREMENT FOR HOMOLOGY AND RECOMBINATION FUNCTIONS

MICHAEL S. TIMMONS,* M. LIEB† and RICHARD C. DEONIER*

** Molecular Biology, Department of Biological Sciences, University of Southern California, Los Angeles, California 90089-1481, and † Department of Microbiology, University of Southern California School of Medicine, Los Angeles, California 90033*

Manuscript received November 20, 1985
Revised copy accepted April 7, 1986

ABSTRACT

Intermolecular recombination between two IS5 elements was measured, using bacteriophage lambda recombination vectors, and was compared to recombination between two copies of an SV40 segment cloned into the same vectors. Experiments were conducted in the presence and in the absence of RecA and Red functions, and with the recombining inserts in the same or in reversed orientation. Under all conditions, IS5 elements recombined in a manner similar to the SV40 inserts, indicating that IS-encoded functions did not confer measurable additional intermolecular recombination ability to IS5 in *E. coli* K-12. Bacteriophages containing reversed IS5 inserts, for which the 16 base pair (bp) termini are identical in 15 positions and which display 12 bp of uninterrupted homology, recombined at approximately the same low frequency under Rec^+ and Rec^- conditions, indicating that these short homologies were not good substrates for the Rec system. Bacteriophages having reversed inserts recombined better under Red^+ than under Red^- conditions, but the crossovers were located in nonhomologous regions flanking the element termini. This suggests that 12-bp homologies are not good substrates for the Red system.

IS elements are normal components of the chromosomes of *E. coli* K-12 and of other *E. coli* strains (HU and DEONIER 1981; GREEN *et al.* 1984). These elements can promote genetic change by transposition. Moreover, recombination between pairs of identical elements can produce inversions (SAVIĆ, ROMAC and EHRLICH 1983; LOUARN *et al.* 1985), additions (DAVIDSON *et al.* 1975) or deletions of large DNA segments (TIMMONS, BOGARDUS and DEONIER 1983). In recombination, do IS elements in *E. coli* K-12 behave identically to DNA segments of approximately the same length that are not capable of transposition?

Any IS × IS recombination that depends on element-encoded information might indicate the nature of biochemical activities associated with transposases. While transposition of IS elements is a rare event, the frequency of transposition intermediates might be significantly higher. For Tn*10* and IS5 there is accumulating evidence for double strand breaks at the element termini (MOR-

ISATO and KLECKNER 1984; ISBERG and SYVANEN 1985), which would allow transposition without a cointegrate intermediate (BERG 1977, 1983). Double strand breaks might initiate strand exchanges (SZOSTAK *et al.* 1983) leading to IS × IS recombination. For those elements that transpose by a replicative mechanism (for review, see GRINDLEY and REED 1985), replication forks at the termini of abortive or productive transposition intermediates might stimulate recombination. Finally, some elements that transpose by the replicative mode encode resolvases, which catalyze site-specific recombination.

Most studies of IS × IS recombination have employed assays of intramolecular events. In a *recA*$^+$ host, amplification of drug resistance genes on an R plasmid proceeds as readily by recombination within directly repeated nontransposable gene segments as by recombination between directly repeated IS*1* elements (PETERSON and ROWND 1985). In the absence of *recA* function, intramolecular recombination between a pair of directly repeated IS*50* elements on a plasmid proceeds with a frequency only slightly higher than that seen with direct repetitions of a nontransposable segment of lambda DNA (ISBERG and SYVANEN 1985). These findings suggest that intramolecular recombination between IS elements is not dependent on IS-encoded information; instead, slipped mispairing during DNA replication is a likely mechanism for forming the observed products (ALBERTINI *et al.* 1982).

However, there are studies suggesting that intermolecular as well as intramolecular IS × IS recombination may be influenced by element-encoded functions. For example, IS*2* × IS*2* recombination is more frequent than IS*3* × IS*3* recombination in F integration or excision (DEONIER and MIRELS 1977; DEONIER and HADLEY 1980). Also, there is evidence for *recA*-independent recombination following transformation of bacteria with plasmid DNA containing directly repeated IS*50* elements (ZUPANCIC *et al.* 1983) or IS*1* elements (BRAEDT 1985). In both of the latter two studies, recombination products were so abundant after transformation and subsequent growth that they could readily be detected by fluorescence after gel electrophoresis. Yet, in another study, directly repeated IS*1* elements on a small plasmid produced deletions with a frequency of only 10^{-6} during growth in a *recA*$^-$ strain (IIDA, SCHRICKEL and ARBER 1982). Whether the apparent elevation in IS × IS recombination is entirely attributable to selection after transformation (PHADNIS and BERG 1985) or depends, at least in part, on element-encoded information remains to be determined. It is not yet clear whether IS elements are active or passive participants in recombination.

We have chosen to study IS × IS recombination using an assay system that reveals intermolecular recombination. IS*5* was chosen for the present study of IS × IS recombination because of its abundance in *E. coli* K-12 (SCHONER and SCHONER 1981), because of its known transposition proficiency (LIEB 1981) and because of its role in chromosome rearrangements. The experiments were designed to meet the following criteria: (1) recombinants should be detected early to reduce the effects of selection during growth; (2) both recombinant and parental genomes should be recovered quantitatively; (3) intermolecular, rather than intramolecular, recombination should be measured to reduce the

TABLE 1

Bacterial and bacteriophage strains

Strain	Relevant genotype	Source (reference)
Bacteria (all are *Escherichia coli* K-12 derivatives)		
C600	λ^-	H. SHIZUYA (SIGNER, BECKWITH and BRENNER 1965)
C600 (λ^+)		M. LIEB
JC5495	λ^- *recA13 recB21*	H. ECHOLS (WILLETTS and CLARK 1969)
RD17	λ^- *recA56*	This laboratory (DEONIER and MIRELS 1977)
S683	(P2) (λ) derived from 594 (CAMPBELL 1965)	G. SMITH (personal communication)
MST100	λ^- *recA*$^-$ (pMST100); pMST100 = pBR322::8.3-kb, IS*5A*-containing *Bam*HI fragment	This laboratory (TIMMONS, SPEAR and DEONIER 1984)
Bacteriophages		
λKH100	*cI ind*$^-$::IS*5*	W. SZYBALSKI (BLATTNER et al. 1974)
λrva[a]	*spi84*	D. CARROLL (CARROLL, AJIOKA and GEORGOPOULOS 1980)
λrvb[a]	*red*$^+$ *gam*$^+$ *imm*434	D. CARROLL (CARROLL, AJIOKA and GEORGOPOULOS 1980)
λrvc[a]	*red*$^-$ *gam*$^+$ *imm*434	D. CARROLL (CARROLL, AJIOKA and GEORGOPOULOS 1980)

[a] These phages also contain several deletions resulting in retention of only one *Hin*dIII site, which lies within the immunity region (*imm*λ or *imm*434). they also contain *nin*5, a mutation that allows λ to reproduce in the absence of the product of gene *N* (deleted in λrva).

effects of "slipped-mispairing" mechanisms; and (4) control experiments should measure recombination between nontransposing DNA segments that are approximately the same size as IS*5*.

MATERIALS AND METHODS

Bacterial and bacteriophage strains: Descriptions and sources of bacterial and phage strains employed in this research are presented in Table 1. λrva-, λrvb- and λrvc-derived phage constructs contained either the 1.77-kb *Hin*dIII fragment of SV40, the 1.76-kb *Hin*dIII fragment of λKH100 that contains IS*5*, or the 3.4-kb *Hin*dIII/*Bam*HI fragment of pRH112 that contains IS*5A*. IS*5A* refers to a naturally occurring IS5 element located between *proB* and *argF* on the *E. coli* K-12 chromosome (TIMMONS, BOGARDUS and DEONIER 1983). IS*5A* may or may not be identical with IS5 in λKH100. For both the SV40 and IS*5A* inserts, orientation I means that the *Eco*RI site is to the right relative to the λ vegetative map, and orientation II means the opposite orientation. Bacteriophage constructs were named by adding to the name of the parent phage an abbreviation for the source of the insert and its orientation (*e.g.*, λrvaSV(I) is a λrva derivative containing the 1.77-kb *Hin*dIII fragment of SV40 inserted in orientation I, *i.e.*, with the *Eco*RI site to the right). Stocks of λrva, λrvb and λrvc contained variants that were able to grow on the selective host, S683. The frequencies of such variants in the stocks used for the crosses are λrvaSV(I), 2.4×10^{-7}; λrvaIS5(I), 2.8×10^{-7};

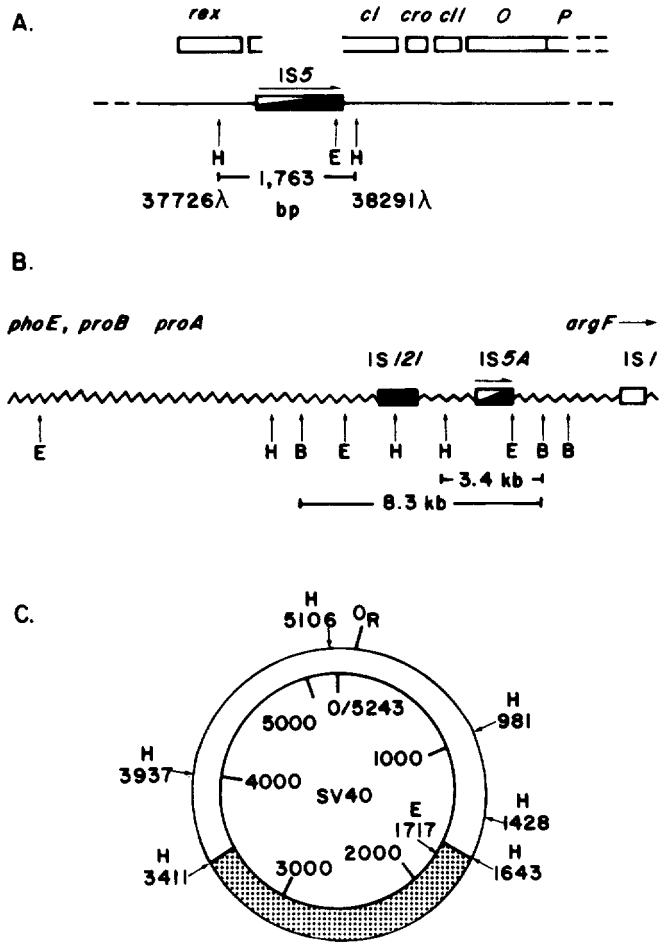

FIGURE 1.—Sources of inserts cloned into λrva, λrvb and λrvc (see Table 1). *Eco*RI, *Bam*HI and *Hin*dIII sites are represented by E., B and H, respectively. A, Genetic and restriction maps of the immunity region of IS*5* insertion mutant λKH100. The 1763-bp *Hin*dIII fragment was cloned into λrva giving phages λrvaIS*5*(I) and λrvaIS*5*(II). Orientation I is indicated by a rightward arrow. B, Relevant restriction sites in the *proAB* region of the Δ*traFproA*$^+$ plasmid pRH112 (HADLEY and DEONIER 1980). The 8.3-kb *Bam*HI fragment was cloned in the *Bam*HI site of pBR322 to make pMST100 (TIMMONS, SPEAR and DEONIER 1984). The 3.4-kb *Hin*dIII/*Bam*HI fragment was subcloned from pMST100 to construct phages λrvbIS*5A*(I), λrvbIS*5A*(II), λrvcIS*5A*(I) and λrvcIS*5A*(II). C, *Hin*dIII restriction map of SV40 DNA (FIERS *et al.* 1978; VAN HEUVERSWYN and FIERS 1979). The 1768-bp restriction fragment from bp 1643 to bp 3411 (shaded) was used to construct phages λrvaSV(I), λrvbSV(I), λrvbSV(II), λrvcSV(I) and λrvcSV(II). The single *Eco*RI site in this fragment was used to orient the insert relative to the phage map.

λrvaIS*5*(II), 1.1×10^{-7}; λrvbSV(I), 1.3×10^{-6}; λrvbSV(II), $< 10^{-6}$; λrvbIS*5A*(II), 4.9×10^{-6}; λrvbIS*5A*(I), 1.1×10^{-4}; λrvcSV(I), 2.8×10^{-6}; λrvcSV(II), 3.7×10^{-6}; and λrvcIS*5A*(II), 4.2×10^{-6}.

Construction of λrva, λrvb and λrvc derivatives: Sources of restriction fragments cloned into λrva, λrvb and λrvc are shown in Figure 1. pMST100 contains the chromosomal IS*5A* element (TIMMONS, SPEAR and DEONIER 1984). The phages used in this study were constructed by ligation of *Hin*dIII-digested λrva, λrvb or λrvc DNA with

the SV40 or IS5-containing *Hin*dIII or *Hin*dIII/*Bam*HI fragments described in Figure 1. For construction of λrvbIS5*A* and λrvcIS5*A* phages, the ends of the *Hin*dIII/*Bam*HI fragment of pMST100 containing IS5*A* were filled in using the Klenow fragment of DNA polymerase I, and were ligated to phosphorylated *Hin*dIII linkers before insertion into λrvb or λrvc. Ligation products were used to transfect JC5495, which was plated together with C600 indicator bacteria. λrvb- and λrvc-derived constructs were recognized by their clear-plaque phenotypes (CARROLL, AJIOKA and GEORGOPOULOS 1980), and λrva derivatives were identified by physical analysis. The presence of the desired inserts in all phages was confirmed by restriction analysis, and the presence of IS5 elements in the λrvaIS5, λrvbIS5*A* and λrvcIS5*A* phages were verified by hybridization to pGM1 (KAMP *et al.* 1979).

Growth of phage stocks and cross procedures: To reduce the background of bacterial DNA in phage stocks used for physical analysis, the procedures of DAVIS *et al.* (1980) and of MANIATIS, FRITSCH and SAMBROOK (1982) were combined and modified. Phage eluted from single plaques were used to make plate stocks on C600 lawns, using 1% T-agarose plates. Phage were eluted into SM buffer (MANIATIS, FRITSCH and SAMBROOK 1982); cell debris was removed by centrifugation; and the supernatants were treated with RNase A and DNase I (50 μg/ml of each for 30 min at 37°). The phage were precipitated by adjusting lysates to 10% (w/v) polyethylene glycol and 1 M NaCl. The phage were resuspended in SM buffer, and the protocol of DAVIS *et al.* (1980) was then followed, omitting a second treatment with RNase A. The above procedure compensated for low titers of phage constructs and for high bacterial DNA backgrounds, both of which impaired visualization of phage DNA digests when conventional rapid screening procedures were employed.

For use in crosses, phage stocks of λrva and λrvb derivatives were grown by infection of C600 on Trypticase plates (1% BBL Trypticase peptone, 1% agar, 0.5% NaCl) supplemented with 0.06% glucose, 5 μM $FeCl_3$, 75 μM $CaCl_2$, 2 mM $MgSO_4$ and 10 μg/ml thiamine. To reduce the frequency of spontaneous *gam*$^-$ mutants, phage stocks of λrvc and of its derivatives were grown as liquid lysates on the *recA*$^-$ strain RD17 in NZY broth medium (BLATTNER *et al.*, 1977) supplemented with 10 mM $CaCl_2$.

Phage crosses were conducted with modification of published procedures (CARROLL, AJIOKA and GEORGOPOULOS 1980: D. CARROLL, personal communication). A mixture of 1.5×10^9 of each parental phage was preabsorbed for 20 min at room temperature with 3×10^8 cells from overnight cultures grown in NZ broth (1% N-Z-Amine, Type A, 0.5% NaCl), in a total volume of 0.3 ml. The adsorption mixture was diluted with 3 ml of NZY broth supplemented with 0.2% glucose and 20 mM $MgCl_2$, transferred to a 50-ml Erlenmeyer flask and shaken vigorously for 90 min at 37°. Lysates were treated with chloroform and then were titered on C600, C600(λ$^+$) and the λ,P2 double lysogen S683, using NZ plates (1% agar). The toip agar contained NZ broth and 10 mM $MgCl_2$.

The frequencies of Spi$^-$ Imm434 recombinants (*i.e.*, the products of recombination within the inserts) were calculated by dividing the phage titer on the double lysogen by the titer on C600. Titers of recombinants were corrected for the number of variants in the parental stock that were able to grow on S683 (see above). These would produce, during the cross, progeny that would incorrectly be scored as recombinants in the lysate. The titer of each parent phage in the lysate was multiplied by the frequency of variants in the parental stock, and this number was subtracted from the observed titer on S683 to estimate the frequency of true recombinants. Control experiments indicated that corrections for unadsorbed phage would not be significant (data not shown), and no such corrections were applied.

RESULTS

Recombination assay system: The recombination assay system is shown in Figure 2, using λrvaIS5(II) and λrvcIS5*A*(II) as an example. Wild-type λ (and *red*$^-$ *gam*$^+$ λ) is Spi$^+$, which means sensitive to P2 interference (*i.e.*, these phages

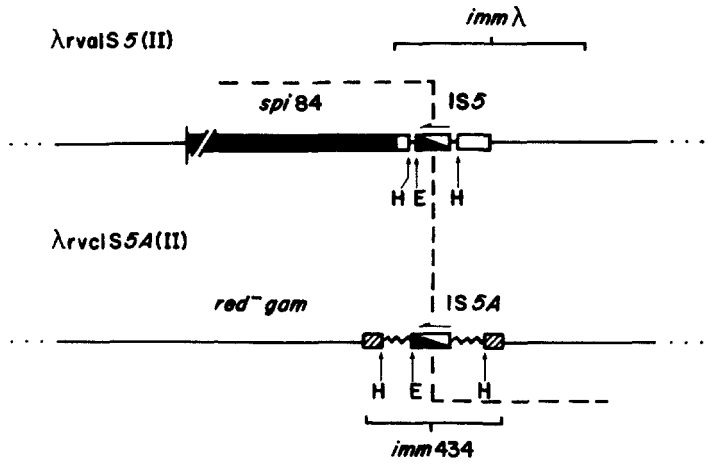

FIGURE 2.—Production of a recombinant by a crossover within homology between selected markers (*spi*84 and *imm*434) of λrva and λrvc. The homology is provided by IS5 in a cross between λrvaIS5(II) and λrvcIS5A(II). The dashed line indicates the selected recombinant. Restriction sites are labeled as in Figure 1. Sawtooth lines represent *E. coli* K-12 DNA flanking IS5A, and the shaded region is the Spi⁻ substitution. Open boxes indicate the *imm*λ region in λrvaIS5(II), and hatched boxes indicate the *imm*434 region in λrvcIS5A(II). the IS5 polarities were determined with the aid of the asymmetrically located *Eco*RI site.

will not grow on P2 lysogens). Red⁻ Gam⁻ phages are Spi⁻: such phages will grow on P2 lysogens (LINDAHL *et al.* 1970). *Spi*84 is a mutation in which the *bio* region of *E. coli* K-12 is substituted for the phage region containing the *red* and *gam* genes. The λrva derivatives are Spi⁻ *imm*λ; therefore, they will grow on P2 lysogens, but not on λ lysogens. Because λrvb and λrvc derivatives are *gam*⁺ (Spi⁺) *imm*434, they will grow on λ lysogens, but not on P2 lysogens. The indicated crossover produces Spi⁻ *imm*434 recombinants, which will grow on a λ,P2 double lysogen, whereas neither of the two parents will do so. The reciprocal recombinants also will not grow on λ,P2 double lysogens. Recombination frequency is defined as the fraction of the bacteriophage progeny from a cross that will plate on S683, a λ,P2 double lysogen. λrva and λrvb derivatives are employed for crosses under Red⁺ conditions (λrvb carries *red*⁺), and λrva and λrvc are employed for Red⁻ crosses. Because λrvb and λrvc are *gam*⁺, the *recBC* enzyme is antagonized in both types of crosses, resulting in suboptimal functioning of the bacterial Rec system (UNGER and CLARK 1972). The two different IS5 elements inserted within the *Hin*dIII sites of the λrva and λrvb (λrvc) genomes differ with respect to the flanking DNA sequence, but they have the same orientation and, thus are the only homologous segments between the selectable markers. Consequently, progeny recombinant for the outside sequences are expected to have experienced crossovers within these inserts [see CARROLL, AJIOKA and GEORGOPOULOS (1980) for references and discussion].

Recombination between homologous inserts crossed in various genetic backgrounds: Recombination between homologous IS5 elements was measured by crossing λrva derivatives (*red*⁻) with λrvb derivatives (*red*⁺) or with λrvc

derivatives (*red*$^-$) in *recA*$^+$ (C600) or *recA*$^-$ (RD17) bacteria, as indicated in Table 2. Since we select for recombination between outside markers, we detect only recombinants resulting from an odd number of exchanges in the insert. For comparison, recombination between phage derivatives containing a 1.77-kb segment of SV40 was measured under the same conditions. From crosses A, B and C (Table 2) it is seen that, under Red$^+$ conditions (in *recA*$^+$ or *recA*$^-$ bacteria), the frequency of recombination between IS5 elements in the same orientation (both elements in orientation I or both elements in orientation II) is lower than the frequency of recombination between SV40 inserts. The ratio of the recombination frequencies in crosses B or C to recombination frequencies for SV40 inserts is approximately equal to the ratio of the IS5 length to the length of the SV40 insert (0.68).

Under RecA$^+$ Red$^-$ conditions, recombination frequencies are lower than under RecA$^+$ Red$^+$ conditions, and under RecA$^-$ Red$^-$ conditions, recombination is further diminished, but significant amounts of recombination persist (compare lines A and B to line I, which gives results for crosses between phages with no inserts at all). Recombination between IS5 elements is not more frequent than recombination between SV40 fragments. We conclude that for homologous crosses under RecA$^+$ Red$^+$, RecA$^+$ Red$^-$, RecA$^-$ Red$^+$ or RecA$^-$ Red$^-$ conditions, the transposable character of the IS5 elements does not confer any recombinational properties to these elements beyond those attributable to homology alone.

Recombination when homology is limited to the inverted repeats of IS5: Even under RecA$^-$ Red$^-$ conditions, homologous inserts recombined at frequencies 3- to 5-fold higher than the detection limits. We reasoned that, if IS5 elements were to encode information that catalyzed reciprocal recombination the inverted repetitions at the IS5 termini, which are homologous in 15 of 16 positions (SCHONER and KAHN 1981; ENGLER and VAN BREE 1981), might be the target sites for this activity. Since the homology between the inverted repeats at the termini remains when one of the IS5 elements is reversed relative to the other, recombination attributable to IS functions might still proceed. In the reversed orientation, the limited amount of homology might preclude generalized homologous recombination between the inverted repeats. We therefore conducted crosses using parents containing IS5 inserts having reversed orientation. Under RecA$^-$ Red$^-$ conditions, the IS5 inserts (line D) did not recombine with each other significantly more frequently than reversed SV40 inserts (line G) or IS5 inserts crossed with SV40 inserts (lines F and H). Under Red$^+$ conditions, however, recombination between the reversed IS5 inserts (lines D and E) was significantly higher than recombination frequencies seen with nonhomologous inserts.

Physical analysis of 18 recombinants produced in cross D (Red$^+$ RecA$^+$) revealed that crossovers had not occurred within the 16-bp termini of IS5 (see below). Because the 16-bp termini are flanked by heterologous regions on both sides, we consider it unlikely that many of the illegitimate recombinants are a result of exchange initiated within the 16-bp region. We have no explanation for the higher recombination frequencies, under Red$^+$ conditions, between

TABLE 2

Recommendation between IS5 or SV40 insertions

Inserts in cross[a] and orientation	Homology (bp)	Recombination frequency in[b,c] RecA+ Red+	RecA+ Red−	RecA− Red+	RecA− Red−
Crosses with homologous inserts					
A. SV[d](I) × SV(I)	1768	1.4×10^{-3}	3×10^{-4}	1.8×10^{-3}	$3.0(\pm 2) \times 10^{-5}$
B. IS*5*(II) × IS*5A*(II)	1195	9.3×10^{-4}	1×10^{-4}	1.2×10^{-3}	$2.0(\pm 0.2) \times 10^{-5}$
C. IS*5*(I) × IS*5A*(I)	1195	9.4×10^{-4}	NT[e]	1.0×10^{-3}	NT
D. IS*5*(I) × IS*5A*(II)	2 × 12[f]	$2.1(\pm 0.4) \times 10^{-5}$	2×10^{-6}	$1.2(\pm 0.3) \times 10^{-5}$	$7(\pm 3) \times 10^{-6}$
E. IS*5*(II) × IS*5A*(I)	2 × 12[f]	$7.6(\pm 5.0) \times 10^{-5}$	NT	$5.2(\pm 2.4) \times 10^{-5}$	NT
Crosses with nonhomologous inserts					
F. SV(I) × IS*5A*(II)	0[g]	NT	4×10^{-6}	NT	$6(\pm 2) \times 10^{-6}$
G. SV(I) × SV(II)	0[g]	$<1 \times 10^{-7}$	4×10^{-7}	$<1 \times 10^{-7}$	$1(\pm 0.5) \times 10^{-6}$
H. IS*5*(II) × SV(I)	0[g]	NT	4×10^{-7}	NT	$2(\pm 1) \times 10^{-6}$
I. λrva × λrvb or λrvc	0	5×10^{-6}	2×10^{-6}	3×10^{-6}	7×10^{-6}

[a] The first insert listed in each cross is cloned into λrva, and the second insert listed is cloned into λrvb or λrvc.

[b] RecA+ crosses were conducted in C600, and RecA− crosses were conducted in RD17; Red+ crosses were performed with λrvb, and Red− crosses were performed with λrvc. Crosses done in X7026 (a *recA*+ strain otherwise isogenic with RD17) and in C600 yielded the same crossover frequencies (data not shown).

[c] Frequencies reported are the fraction of bacteriophage progeny from the cross that are Spi− Imm434. Each cross was performed at least twice. Experiments for which standard deviations are repeated were performed at least four times.

[d] SV designates the bp 1961 to bp 3459 *hin*dIII fragment from SV40 (*cf.* Figure 1).

[e] NT, not tested.

[f] The two 12-bp homologies for IS*5* elements with reversed polarity are within separated inverted repeat sequences, which are homologous in 15 of 16 positions.

[g] Computer-assisted scan indicates no homologies larger than 11 bp between SV40 inserts in reversed orientation or between IS*5A* and the SV40 sequence in the indicated orientation.

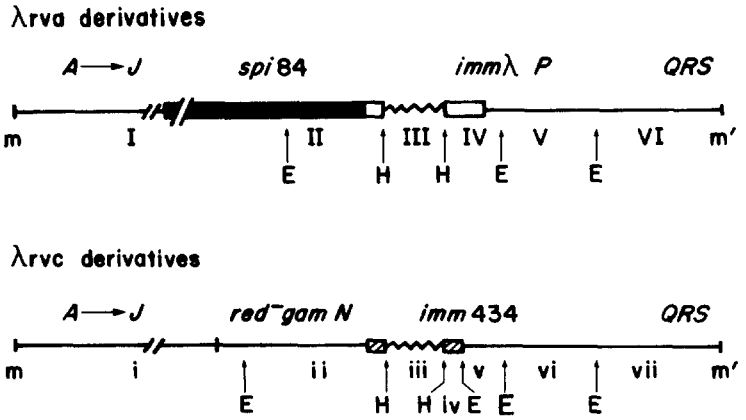

FIGURE 3.—Restriction sites defining intervals in which crossovers might occur in λ*rva* and λ*rvc* derivatives (*cf.* Table 3). Inserts are denoted by sawtooth lines. Boxed regions are designated as in Figure 2. The intervals are drawn approximately to scale. Restriction sites are designated as in Figure 1.

inverted IS*5* elements than between nonhomologous inserts. However, there is no evidence for aberrantly frequent recombination at the IS*5* termini.

Structure of recombinants produced under RecA⁻ Red⁻ conditions: To determine whether the Spi⁻ *imm*434 progeny obtained under RecA⁻ Red⁻ conditions were a result of illegitimate recombination, homologous recombination or mutation of the parents, the physical structure of the DNA from presumptive recombinants was analyzed. Because *Hin*dIII and *Eco*RI digests of the parental phage DNAs each produce characteristic fragments (Figure 3), electrophoresis of restriction digests of recombinant progeny will reveal the interval in which recombination occurred.

Phages from ten to 12 plaques from each of crosses A, B, D, F and G done under RecA⁻ Red⁻ conditions were repurified on the selective strain S683; bacteriophage DNA was isolated as described in MATERIALS AND METHODS, and it was digested with *Eco*RI or *Hin*dIII. The parental intervals in which recombination actually occurred are indicated in Table 3. DNA from all 12 recombinant progeny from the cross with similarly oriented SV40 inserts (Table 3, cross A) yielded the *Eco*RI and *Hin*dIII restriction fragments expected for homologous recombination within the inserts. Nine of the 12 progeny from crosses with similarly oriented IS*5* elements gave the restriction fragments expected if recombination occurred within IS*5* elements. In one recombinant, breaks had occurred in intervals III and ii, and two DNAs had the parental λrvcIS*5A*(II) structure (Table 3, cross B).

Of the 12 recombinant progeny examined from an IS*5*(I) × IS*5A*(II) cross (Table 3, cross D), six had the λrvcIS*5A*(II) parental structure, and restriction analysis of the remainder indicated crossovers in a variety of intervals. Two of the recombinants arose from crossovers within the inserted fragments, but the crossovers did not occur between pairs of IS*5* terminal inverted repeats. The assay system evidently is not sufficiently sensitive to detect any rare recombinations between such short regions under *recA⁻ red⁻* conditions. The structures

TABLE 3

Physical alterations in DNA of recombinant bacteriophage isolated under RecA⁻ Red⁻ conditions

	Cross (no. of plaques screened)[a]	Intervals recombined[b]	No. of examples of each mutant or recombinant class
A.	λrvaSV(I) × λrvcSV(I) (12)	III × iii[c]	12
B.	λrvaIS*5*(II) × λrvcIS*5A*(II) (12)	None	2 (~λrvcIS*5A*(II))[d]
		III × ii	1
		III × iii[e]	9
D.	λrvaIS*5*(I) × λrvcIS*5A*(II) (12)	None	6 (~λrvcIS*5A*(II))[d]
		I × ii	1
		I × iii	1
		II × ii	1
		II × iii	1
		III × iii[f]	2
F.	λrvaSV(I) × λrvcIS*5A*(II) (10)	None	4 (~λrvcIS*5A*(II))[d]
		I × ii	2
		II × ii	2
		IV × ii	1
		? × ?[g]	1
G.	λrvaSV(I) × λrvcSV(II) (11)	None	3 (~λrvaSV(I))[d]
		None	1 (~λrvcSV(II))[d]
		I × ii	1
		II × ii	4
		III × iii	1
		IV × iv	1

[a] Crosses *cf.* Table 2 were done in RD17 (*recA*) and plaques were purified from the λ, P2 lysogen S683. In all cases, there was a continuum of plaque sizes. For each cross, six small- to medium-size plaques and six medium- to large-size plaques were picked so that the screening would include the range of plaque types.

[b] Compare Figure 3.

[c] *Eco*RI and *Hin*dIII fragments were consistent with a phage genome produced by homologous recombination within the SV40 sequences.

[d] Restriction patterns were identical to one of the parents. λrvc-derived mutants are presumably *gam*⁻ variants of the parents. No strict correlation between plaque size and phage structure was seen.

[e] *Hin*dIII fragments were those expected from a phage genome produced by homologous recombination within the IS*5* elements.

[f] Crossovers were within the inserts, but not between pairs of IS*5* inverted repeat sequences.

[g] Structure not interpretable at this level of analysis.

of some recombinants suggested that they might have been formed by IS*5*-mediated replicon fusions, but the few examples observed were not examined in detail. A variety of intervals paricipated in the production of recombinant progeny from SV(I) × SV(II) crosses or from SV(I) × IS*5*(II) crosses (Table 3, crosses F and G). These progeny probably arose by illegitimate recombination or recombination in small homologies between the λ and 434 immunity regions (WESTMORELAND, SZYBALSKI and RIS 1969; WILGUS, MURAL and FRIEDMAN

1973). All of the recombinants produced by crossovers outside one or both inserts differed in structure.

Except in cross A, a considerable fraction of the selected progeny produced restriction patterns identical to those of a parental phage. This was anticipated, because at these low recombination frequencies, parental mutants represented a substantial fraction of the phages plating on the selective host. The fraction of presumptive recombinants having parental restriction patterns were < 0.1, 0.17, 0.5, 0.4 and 0.4 for crosses A, B, D, F and G, respectively. These values are in reasonable agreement with the calculated fraction of phage progeny in these particular crosses that are parental variants that grew on the selective host (0.04, 0.09, 0.2, 0.2 and 0.4, respectively).

DISCUSSION

No element-specific recombination was detected in a system allowing selection of IS*5* × IS*5* recombinants. The frequency of illegitimate recombination ($\sim 4 \times 10^{-6}$; *cf.* Table 2, crosses F, G and H, last column) measured in the *recA*$^-$*red*$^-$ background under conditions of limited homology was much larger than the measured transposition frequency for IS*5* into the cI gene of bacteriophage lambda ($\sim 10^{-8}$; M. LIEB, unpublished results). The Red system was more active than the Rec system in the present experiments. This was anticipated because the crosses were conducted under Gam$^+$ conditions, and the *gam* gene product antagonizes the *recBC* nuclease, an important component of the Rec system.

Vectors having reversed copies of IS*5* did not undergo recombination *via* the 15 of 16-bp homologies in the inverted repeats at the ends of the elements. Under *recA*$^+$*red*$^-$ conditions, the recombination frequency was approximately the same as if no inserts were present at all, a result that agrees with the finding that 17-bp homologies were not good substrates for the Rec system (WATT *et al.* 1985). This also agrees with the results of SINGER *et al.* (1982), who found that at least 50 bp of homology ware required for efficient recombination in T4, which codes for its own recombination system. In contrast to the observations of WATT *et al.* (1985), we find a background level of homology-independent recombination ($10^{-6} - 10^{-5}$; *cf.* Table 2, crosses F-H and last column of cross I) that is 100- to 1000-fold higher than the levels that they report for λ *red*$^-$ *gam*$^-$ recombining with plasmids. We presume that these low levels of nonhomologous (illegitimate) recombination may differ depending on the nature of recombination vectors employed. For example, their modes or sites of replication in the cell may influence the probability of recombination between them. We are not certain that the elevated frequencies seen for reversed IS*5* (but not for reversed SV) inserts when the Red system was operating are significant. If they are, our data would suggest that illegitimate recombination was somewhat enhanced by the Red system.

The present results indicate that intermolecular IS*5* × IS*5* recombination in *E. coli* K-12 is mediated largely by the Red or RecA recombination pathways. The λrva/λrvb system could be employed for IS*1* and for IS*50*, to test whether IS × IS intermolecular recombination for these elements is as frequent as it is

in intramolecular recombination. Assays that detect intermolecular recombinants should be less sensitive to slipped mispairing than assays that measure intramolecular recombination. Our studies suggest, however, that the presence of *gam*$^-$ mutants in λ stocks plus a relatively high frequency of illegitimate recombination may limit the usefulness of the λrva, b, c system for studies of recombination when recombination frequencies are low.

We thank DANA CARROLL and SCOTT WRIGHT for advice on the use of the λrva/λrvb recombination test system, and GERALD SMITH for providing strain S683. This work was supported by National Science Foundation grant PCM8110604 (to M.L.), National Science Foundation grant PCM8110605 (to R.C.D.) and United States Public Health Service grant GM24589 from the National Institutes of Health (to R.C.D.).

LITERATURE CITED

ALBERTINI, A. M., M. HOFER, M. P. CALOS and J. H. MILLER, 1982 On the formation of spontaneous deletions: the importance of short sequence homologies in the generation of large deletions. Cell **29:** 319–328.

BERG, D. E., 1977 Insertion and excision of the transposable kanamycin resistance determinant Tn*5*. p. 205. In: *DNA Insertion Elements, Plasmids, and Episomes*, Edited by A. I. BUKHARI, J. A. SHAPIRO and S. L. ADHYA. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.

BERG, D. E., 1983 Structural requirement for IS*50*-mediated gene transposition. Proc. Natl. Acad. Sci. USA **80:** 792–796.

BLATTNER, F. R., M. FIANDT, K. K. HASS, P. A. TWOSE and W. SZYBALSKI, 1974 Deletions and insertions in the immunity region of coliphage lambda: revised measurement of the promoter-startpoint distance. Virology **62:** 458–471.

BLATTNER, F. R., B. G. WILLIAMS, A. E. BLECHL, K. DENNISTON-THOMPSON, H. E. FABER, L.-A. FURLONG, D. J. GRUNWALD, D. O. KIEFER, D. D. MOORE, J. W. SCHUMM, E. L. SHELDON and O. SMITHIES, 1977 Charon phages: safer derivatives of bacteriophage lambda for DNA cloning. Science **196:** 161–169.

BRAEDT, G., 1985 Recombination in *recA* cells between direct repeats of insertion element IS*1*. J. Bacteriol. **162:** 529–534.

CAMPBELL, A., 1965 The steric effect in lysogenization by bacteriophage lambda I. Lysogenization of a partially diploid strain of *Escherichia coli* K12. Virology **27:** 329–339.

CARROLL, D., R. S. AJIOKA and C. GEORGOPOULOS, 1980 Bacteriophage lambda cloning vehicles for studies of genetic recombination. Gene **10:** 261–271.

DAVIDSON, N., R. C. DEONIER, S. HU and E. OHTSUBO, 1975 Electron microscope heteroduplex studies of sequence relations among plasmids of *Escherichia coli* X. Deoxyribonucleic acid sequence organization of F and of F-primes, and the sequences involved in Hfr formation. p. 56. In: *Microbiology 1974*. Edited by D. SCHLESSINGER. American Society for Microbiology, Washington, D.C.

DAVIS, R. W., M. THOMAS, J. CAMERON, T. P. ST. JOHN, S. SCHERER and R. A. PADGET, 1980 Rapid DNA isolations for enzymatic and hybridization analysis. p. 404. In: *Methods in Enzymology*, Vol. 65. Academic Press, New York.

DEONIER, R. C. and R. G. HADLEY, 1980 IS*2*-IS*2* and IS*3*-IS*3* relative recombination frequencies in F integration. Plasmid **3:** 48–64.

DEONIER, R. C. and L. MIRELS, 1977 Excision of F plasmid sequences by recombination at

directly repeated insertion sequence 2 elements: involvement of *recA*. Proc. Natl. Acad. Sci. USA **74:** 3965–3969.

ENGLER, J. A. and M. P. VAN BREE, 1981 The nucleotide sequence and protein coding capability of the transposable element IS*5*. Gene **14:** 155–163.

FIERS, W., R. CONTRERAS, G. HAEGEMAN, R. ROGIERS, A. VAN DE VOORDE, H. VAN HEUVERSWYN, J. VAN HERREWEGHE, G. VOLCKAERT and M. YSEBAERT, 1978 Complete nucleotide sequence of SV40 DNA. Nature **273:** 113–120.

GREEN, L., R. D. MILLER, D. E. DYKHUIZEN and D. L. HARTL, 1984 Distribution of DNA insertion element IS*5* in natural isolates of *Escherichia coli*. Proc. Natl. Acad. Sci. USA **81:** 4500–4504.

GRINDLEY, N. D. F. and R. R. REED, 1985 Transpositional recombination in prokaryotes. Annu. Rev. Biochem. **54:** 863–896.

HADLEY, R. G. and R. C. DEONIER, 1980 Specificity in the formation of Δ*tra* F-prime plasmids. J. Bacteriol. **143:** 680–692.

HU, M. and R. C. DEONIER, 1981 Comparison of IS*1*, IS*2* and IS*3* copy number in *Escherichia coli* strains K-12, B, and C. Gene **16:** 161–170.

IIDA, S., S. SCHRICKEL and W. ARBER, 1982 On the segregation of IS*1*-mediated cointegrates between bacteriophage P1 DNA and plasmid pBR322 derivatives. FEMS Microbiol. Lett. **15:** 269–273.

ISBERG, R. R. and M. SYVANEN, 1985 Tn*5* transposes independently of cointegrate resolution: evidence for an alternative model for transposition. J. Mol. Biol. **182:** 69–78.

KAMP, D., L. T. CHOW, T. R. BROKER, D. KOWK, D. ZIPSER and R. KAHMANN R, 1979 Site-specific recombination in phage Mu. Cold Spring Harbor Symp. Quant. Biol. **43:** 1159–1167.

LIEB, M., 1981 A fine structure map of spontaneous and induced mutations in the lambda repressor gene, including insertions of IS elements. Mol. Gen. Genet. **184:** 364–371.

LINDAHL, G., G. SIRONI, H. BAILY and R. CALENDAR, 1970 Bacteriophage lambda: abortive infection of bacteria lysogenic for phage P2. Proc. Natl. Acad. Sci. USA **66:** 587–594.

LOUARN, J. M., J. P. BOUCHÉ, F., LEGENDRE, J. LOUARN and J. PATTE,, 1985 Characterization and properties of very large inversions of the *E. coli* chromosome along its origin-to-terminus axis. Mol. Gen. Genet. **201:** 467–476.

MANIATIS, T., E. F. FRITSCH and J. SAMBROOK, 1982*Molecular Coating, A Laboratory Manual*. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.

MORISATO, D. and N. KLECKNER, 1984 Transposase promotes double strand breaks and single strand joints at Tn*10* termini *in vivo*. Cell **39:** 181–190.

PETERSON, B. C. and R. H. ROWND, 1985 Recombination sites in plasmid drug resistance gene amplification. J. Bacteriol. **164:** 1359–1361.

PHADNIS, S. H. and D. E. BERG, 1985 *recA*-independent recombination between repeated IS*50* elements is not caused by an IS*50*-encoded function. J. Bacteriol. **161:** 928–932.

SAVIĆ, D. J., S. P. ROMAC and S. D. EHRLICH, 1983 Inversion in the lacrose region of *Escherichia coli* K-12: inversion termini map within IS*3* elements $\alpha_3\beta_3$ and $\beta_5\alpha_5$. J. Bacteriol. **155:** 943–946.

SCHONER, B. and M. KAHN, 1981 The nucleotide sequence of IS*5* from *Escherichia coli*. Gene **14:** 165–174.

SCHONER, B. and R. G. SCHONER, 1981 Distribution of IS*5* in bacteria. Gene **16:** 347–352.

SIGNER, E. R., J. R. BETHWITH and S. BRENNER, 1965 Mapping of suppressor loci in *Escherichia coli*. J. Mol. Biol. **14:** 153–166.

SINGER, B. S., L. GOLD, P. GAUSS and D. H. DOHERTY, 1982 Determination of the amount of homology required for recombination in bacteriophage T4. Cell **31:** 25–33.

SZOSTAK, J. W., T. L. ORR-WEAVER, R. J. ROTHSTEIN and F. W. STAHL, 1983 The double strand-break repair model for recombination. Cell **33:** 25–35.

TIMMONS, M. S., A. M. BOGARDUS and R. C. DEONIER, 1983 Mapping of chromosomal IS*5* elements that mediate type II F-prime plasmid excision in *Escherichia coli* K-12. J. Bacteriol. **153:** 395–407.

TIMMONS, M. S., K. SPEAR and R. C. DEONIER, 1984 Insertion element IS*121* is near *proA* in the chromosomes of *Escherichia coli* K-12 strains. *J. Bacteriol.* **160:** 1175–1177.

UNGER, R. C. and A. J. CLARK, 1972 Interaction of the recombination pathways of bacteriophage λ and its host *Escherichia coli* K-12: effects on exonuclease V activity. J. Mol. Biol. **70:** 539–548.

VAN HEUVERSWYN, H. and W. FIERS, 1979 Nucleotide Sequence of the *Hin*d-C fragment of simian virus 40 DNA. Eur. J. Biochem. **100:** 51–60.

WATT, V. M., C. J. INGLES, M. S. URDEA and W. J. RUTTER. 1985 Homology requirements for recombination in *Escherichia coli.* Proc. Natl. Acad. Sci. USA **82:** 4768–4772.

WESTMORELAND, B. C., W. SZYBALSKI and H. RIS, 1969 Mapping of deletions and substitutions in heteroduplex DNA molecules of bacteriophage lambda by electron microscopy. Science **163:** 1343–1348.

WILGUS, G. S., R. J. MURAL and D. J. FRIEDMAN. 1973 λ*imm*λ·434: a phage with a hybrid immunity region. Virology **56:** 46–53.

WILLETTS, N. S. and A. J. CLARK, 1969 Characteristics of some multiply recombination-deficient strains of *Escherichia coli.* J. Bacteriol. **100:** 231–239.

ZUPANCIC, T. J., S. I., MARVO, J. H. CHUNG, E. G. PERALTA AND S. R. JASKUNAS, 1983 *RecA*$^-$ independent recombination between direct repeats of IS50. Cell **33:** 629–637.

Communicating editor: G. MOSIG