Tn7 Transposition Creates a Hotspot for Homologous Recombination at the Transposon Donor Site

Ann T. Hagemann[1] and Nancy L. Craig[2]

Department of Microbiology and Immunology and Department of Biochemistry and Biophysics, G. W. Hooper Foundation, University of California, San Francisco, San Francisco, California 94143

Manuscript received June 17, 1992
Accepted for publication September 11, 1992

ABSTRACT

Homologous recombination at the bacterial transposon Tn7 donor site is stimulated 10-fold when Tn7 is activated to transpose at high frequency in RecD$^-$ *Escherichia coli*, where recombination is focused near the ends of double-chain breaks. This is observed as an increase in recombination between two *lacZ* heteroalleles when one copy of *lacZ* carries within it a Tn7 that is transposing at high frequency. This stimulation of recombination is dependent upon the presence of homology with the donor site, is independent of SOS induction, and is not due to a global stimulation of recombination. When stimulated by Tn7 transposition, the conversion events giving rise to Lac$^+$ recombinants occur preferentially at the site of Tn7, suggesting that transposition is stimulating gene conversion at the donor site. These results support the model that Tn7 transposition occurs by a "cut and paste" mechanism, leaving a double-chain break at the donor site that is repaired by the host homologous recombination machinery; normally, repair would use homology in a sister chromosome to regenerate a copy of the transposon. This proposed series of events allows transposition that is nonreplicative, *per se*, to be effectively replicative.

THE bacterial transposon Tn7 (BARTH *et al.* 1976) (reviewed by CRAIG 1991) is observed to transpose both *in vitro* and *in vivo* by a nonreplicative or "cut and paste" mechanism. *In vitro*, transposition occurs without DNA synthesis, producing a broken donor molecule lacking the transposon and a simple insertion of the transposon into the target molecule (BAINTON, GAMAS and CRAIG 1991). There is evidence from transposition *in vivo* that both chains of transposon DNA present in the substrate are also present in the transposition product (M. LOPATA, K. ORLE, R. GALLAGHER and N. L. CRAIG, unpublished), consistent with a nonreplicative mechanism for transposition. The fate of the donor *in vivo* is unknown, but the observation that Tn7 transposition induces the SOS response of *Escherichia coli*, a normal response to DNA damage including double-chain breaks, suggests that a broken donor is a product of transposition *in vivo* as well as *in vitro* (A. STELLWAGEN and N. L. CRAIG, unpublished).

Several events could follow breakage of the transposon donor *in vivo* [see BERG, BERG and SASAKAWA (1984), BENDER and KLECKNER (1986), and BENDER, KUO and KLECKNER (1991) for discussions of donor fate]. One result of donor breakage could simply be loss of the replicon carrying the break. Replicon loss need not result in cell death since exponentially growing *E. coli* contains more than one copy of the chromosome (COOPER and HELMSTETTER 1968; KUBITSCHEK and FREEDMAN 1971).

Another response to donor breakage could be ligation of the break to rescue the replicon. Ligation of the break without any DNA degradation would leave some extra bases at the original site of Tn7. Insertion of Tn7 involves duplication of 5 base pairs (bp) at the site of insertion, but transposition of Tn7 *in vitro* removes only part of that duplication (BAINTON, GAMAS and CRAIG 1991). Therefore, ligation of the break created by Tn7 transposition would leave a remnant of the duplication in the donor site. Alternatively, the break could be processed into a "precise excision" product by annealing complementary chains at each end of the donor break, within the duplicated sequence. The extra bases could be removed by exonucleolytic degradation of the duplicated sequence on one strand at each end of the donor break, before or after annealing. When Tn7 transposes from a disrupted gene, a resection/ligation mechanism would be expected to restore function of the gene, whereas simple ligation of the break might not. Donor sealing by either of these two mechanisms could be facilitated by transposon-encoded functions.

Another possible fate of a break left in the donor DNA following Tn7 transposition is double-chain-break repair by homologous recombination (SZOSTAK *et al.* 1983). In a bacterium, the only source of DNA sequence homology normally available for repair of a break would be in a sister chromosome. Repair of a

[1] Present address: Institute of Molecular Biology, University of Oregon, Eugene, Oregon 97403-1229.
[2] Present address: Howard Hughes Medical Institute, Department of Molecular Biology and Genetics, Johns Hopkins University School of Medicine, 725 N. Wolfe Street, 615 PCTB, Baltimore, Maryland 21205.

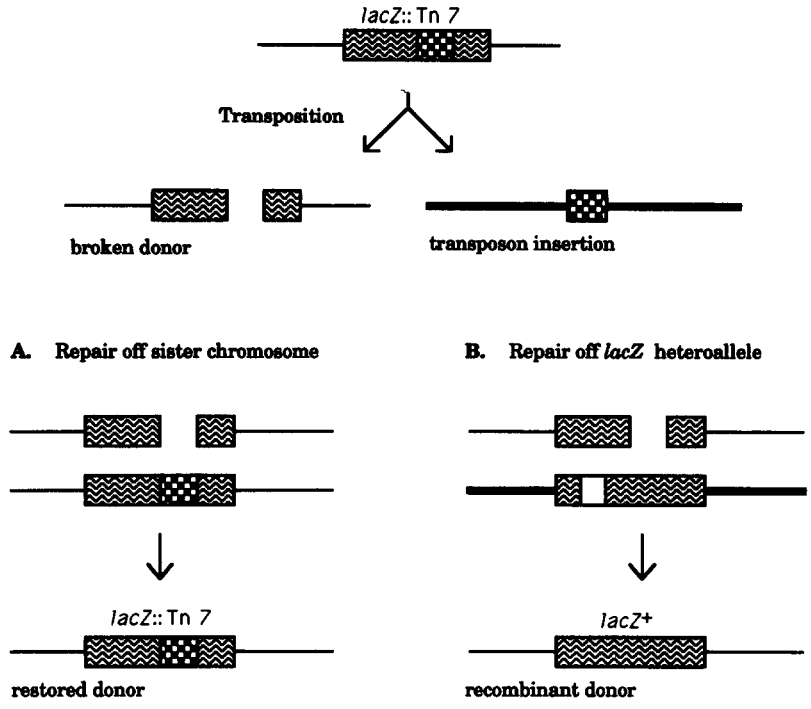

FIGURE 1.—A model for recombinational donor repair (after SZOSTAK *et al.* 1983) following nonreplicative transposition. (**A**) A broken donor is normally repaired by homologous recombination with the sister chromosome, regenerating a copy of the transposon. (**B**) If an alternative source of homology is available, the donor break may be repaired such that the transposon is lost and gene function is restored.

broken transposon donor using homology in the sister would restore a copy of the transposon at the donor site (see Figure 1). The repaired donor would be genetically identical to the original donor substrate and to the product of a replicative transposition event. This genetically invisible repair event at the donor site may be detectable if an alternative source of homology is provided for repair of the break. If a second, ectopic copy of the gene disrupted by the transposon is present, repair of the donor break using the ectopic copy could restore gene function at the donor site. Such an association between homologous recombination and transposition has been reported for the Drosophila *P* element (ENGELS *et al.* 1990) and the Tc1 transposon of *Caenorhabditis elegans* (PLASTERK and GROENEN 1992).

We report that Tn7 transposition stimulates recombination between two Lac$^-$ heteroalleles of *lacZ* when Tn7 resides within one of the copies of *lacZ*. We observe this Tn7 transposition-dependent stimulation of homologous recombination in RecD$^-$ *E. coli*, where recombination is focused at the sites of double-chain breaks (THALER *et al.* 1989). A recombinational model for repair of the donor DNA predicts that the recombination events stimulated by transposition will result in conversion at the copy of *lacZ* acting as the transposon donor, *i.e.*, the putative site of the double-chain break, resulting in a replacement of the transposon with Lac$^+$ information. We observe that conversion to *lacZ*$^+$ stimulated by Tn7 transposition occurs preferentially at the copy of *lacZ* carrying the transposon, supporting the recombinational repair model.

MATERIALS AND METHODS

Media, bacterial strains and growth conditions: Media used were LB (rich) and, for recombination assays, M9 (minimal) containing 0.2% lactose as the sole carbon source [see MILLER (1972) for recipes]. For mapping sites of conversion, transductants were plated on E medium (minimal with citrate) (VOGEL and BONNER 1956) with 0.2% glucose. All minimal media were supplemented with arginine and histidine, each at 20 μg/ml. Selection for trimethoprim resistance was done on Isosensitest agar (Oxoid). Drug concentrations were 100 μg/ml trimethoprim (Tp), 20 μg/ml nalidixic acid (Nal), 100 μg/ml kanamycin (Kan) and 10 μg/ml gentamycin (Gen).

Bacterial strains were constructed by conjugation and phage P1-mediated transduction using standard procedures, and are described in Table 1. All ATH strains were derived from SK296 (ZIEG and KUSHNER 1977), which is a derivative of AB1157. The *recD1903*::mini*tet* allele was transduced from DPB271 (BIEK and COHEN 1986). The *lexA3* allele (MOUNT, LOW and EDMISTON 1972) was moved from DE405 (D. ENNIS) by cotransduction with the linked *mal*::Tn*9*, selecting for chloramphenicol resistant transductants and screening for UV sensitivity. The *trpB83*::Tn*10* allele was transduced from NK5151 (N. KLECKNER), and *proC* and *zaj-3099*::Tn*10kan* were cotransduced from CAG18594 (SINGER *et al.* 1989).

The conjugable plasmids used were pOX38-Gen, which lacks *attTn7*, and pOX-*attTn7-2*, which is a derivative of pOX38-Gen carrying *attTn7* embedded in a transposition-defective miniTn*10*; Tn7 transposes at low frequency to pOX38-Gen and at high frequency to pOX-*attTn7-2* (WADDELL and CRAIG 1988; ARCISZEWSKA, DRAKE and CRAIG 1989). In order to avoid accumulating transposons in the episomes, all experiments were carried out by introducing the appropriate F factors immediately before each use. F factor donor strains OH124 and LA223-2 were mated with the experimental strains and transconjugants were selected by plating on Gen+Kan to select for both the F factor and for miniTn*7-kan* in the *attTn7* site of the recipient chromosome. In the case of ATH160, which carries whole Tn7 in *attTn7*, mating mixtures were plated on Gen and single colonies tested for Tp^R (encoded by Tn7), since growth was poor on plates containing Tp+Gen. Young colonies were picked into LB broth and grown at 30°. For transposition

TABLE 1

Bacterial strains

Name	Genotype	Source or reference
ATH152	*thi-1 his-4 argE3 rpsL31 lacMS286 φ*80dII*lacBK1 trpB83*::Tn*10*	This paper
ATH154	*thi-1 his-4 argE3 rpsL31 lacZ4524*::Tn*7φ*80dII*lacBK1 recD1903*::mini*et attTn7*::miniTn*7-kan*	This paper
ATH160	*thi-1 his-4 argE3 rpsL31 lacMS286φ*80dII*lacBK1 recD1903*::mini*et attTn7*::Tn*7*	This paper
ATH161	*thi-1 his-4 argE3 rpsL31 lacZ4524*::Tn*7 recD1903*::mini*et attTn7*::miniTn*7-kan*	This paper
ATH162	*thi-1 his-4 argE3 rpsL31 lacMS286φ*80dII*lacBK1 recD1903*::mini*et proC zaj-3099*::Tn*10kan*	This paper
ATH176	*thi-1 his-4 argE3 rpsL31 lacBK1 φ*80dII*lacZ4524*::Tn*7 recD1903*::mini*et attTn7*::miniTn*7-kan*	This paper
ATH178	*thi-1 his-4 argE3 rpsL31 lacZ4524*::Tn*7 φ*80dII*lacBK1 recD 1903*::mini*et attTn7*::miniTn*7-kan lexA3 mal*::Tn*9*	This paper
ATH179	*thi-1 his-4 argE3 rpsL31 lacBK1φ*80dII*lacZ4524*::Tn*7 recD1903*::mini*et attTn7*::miniTn*7-kan lexA3 mal*::Tn*9*	This paper
CW51	*recA56 Δlac-proXIII ara arg* Nal^R Rif^R	WADDELL and CRAIG (1988)
OH124	CW51 pOX38-Gen	O. HUGHES as in ARCISZEWSKA, DRAKE and CRAIG (1989)
LA223-2	CW51 pOX-*attTn7-2*	ARCISZEWSKA, DRAKE and CRAIG (1989)

assays, aliquots were removed during exponential phase and transferred to 37° for mating. Recombination assays were done on saturated cultures.

Transposition assay: Frequencies of transposition into F factors were determined by a mating-out assay in which the F^+ strain in question was mated with CW51. Cultures of both partners were grown to 2–5 × 10^8 cells/ml and mixed in equal volumes. Mating mixtures were plated on Nal+Gen to select for transconjugants (selecting against both the donor and the recipient, respectively), and also on Nal+Tp to determine the frequency with which transconjugants had acquired Tp^R by gaining the transposon. In order to determine efficiencies of plating on the above drug combinations, $Nal^RTp^RGen^R$ transconjugants grown in the absence of drugs were plated on LB, LB+Nal+Gen and Isosensitest agar+Nal+Tp, and all were within a factor of two.

Recombination assay: Frequencies of recombination to Lac^+ were determined by plating dilutions of cultures on rich medium (2 × 10^{-7} ml) and on minimal medium containing lactose (2 × 10^{-3} ml), with incubation at 30° for 2 days. Recombination frequency is defined as the number of colony-forming units on lactose divided by the colony-forming units on LB. Lac^+ colonies were purified by streaking on LB with 60 μg/ml X-gal, and blue colonies picked for further analysis.

Mapping the site of conversion: Lac^+ recombinants were mapped by P1 transduction to determine the site of conversion. Phage P1 was grown on individual Lac^+ clones and used to transduce both *proC* and *trpB* versions of the Lac^- starting strain (ATH162 and ATH152) to Pro^+ and Trp^+, respectively, and transductants were replica-printed onto M9 lactose plates to determine with which marker lac^+ was linked.

RESULTS

Experimental design: The chromosomal duplication of the *lac* operon used to assay homologous recombination in the presence and absence of high frequency Tn7 transposition is diagrammed in Figure 2. One copy of the *lac* operon is in the normal location at minute 8 of the *E. coli* genetic map. The second copy has been integrated at minute 27, in the φ80 phage attachment site (φ80dII*lac*; KONRAD 1977), and is in inverted orientation relative to the normal *lac*. The *lacZ* gene in one of these copies is disrupted by a Tn7 insertion and the other is inactivated by a small deletion not overlapping the Tn7 site. Recombination between these two *lacZ* alleles can generate a functional *lacZ* gene. The copy of *lac* at minute 8 is closely linked with *proC*, and the copy at minute 27 is closely linked with *trpB*, facilitating linkage mapping of the site of conversion in Lac^+ recombinants. Two previous analyses of Lac^+ recombinants generated between *lac* and φ80dII*lac* in two different strain backgrounds (including this one) failed to find any that had inverted the segment of the chromosome between the *lac* regions, suggesting that this inversion is nonpermissible (KONRAD 1977; ZIEG and KUSHNER 1977).

Recombination between the heteroalleles of *lacZ* was assayed by plating on minimal lactose medium, upon which colonies arose in numbers not linearly related to the number of cells plated. For this reason, all genotypes assayed for homologous recombination were plated at the same dilution to allow direct comparison. Growth rate is likely to affect the frequency of recombinants, since recombinant colonies were of heterogenous size and continued to arise on plates following the 2 days of incubation, suggesting that they arose on the minimal lactose plates during slow growth of Lac^- cells. Although recombination frequencies varied depending on incubation time and temperature, the ratios of recombinants of different genotypes remained relatively constant. This can be seen in the data of Figure 3, where pairs of points

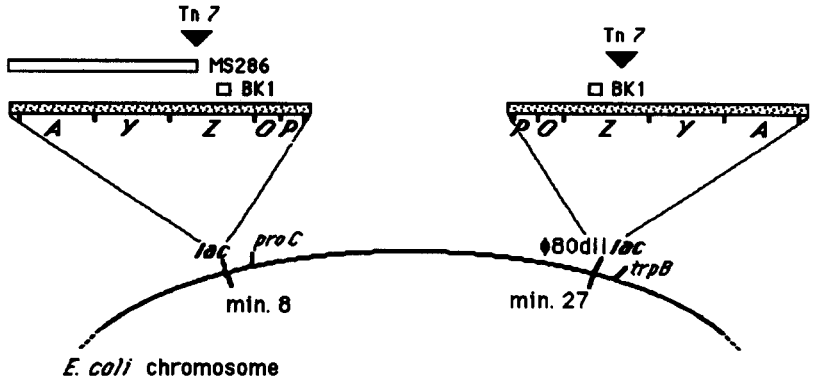

FIGURE 2.—Configuration of the *lac* duplication used to assay recombination. The *lac* sequences are stippled boxes and deletions are open boxes (ZIEG and KUSHNER 1977). Tn7 is in *lacZ*, either within or very close to the region deleted by *MS286*. The *BK1* deletion does not overlap the Tn7 insertion or the *MS286* deletion.

collected concurrently fall within a wide range of recombination frequencies but maintain comparable ratios of stimulation by Tn7 transposition.

Tn7 transposes at a much higher frequency in the presence of *attTn7*, its preferred chromosomal insertion site (LICHTENSTEIN and BRENNER 1981), than in the absence of *attTn7* (ROGERS *et al.* 1986; WADDELL and CRAIG 1988; KUBO and CRAIG 1990). The presence of *attTn7* coordinately stimulates both excision of the transposon from the donor and its insertion into the target *in vitro* (BAINTON, GAMAS and CRAIG 1991); *in vivo*, SOS induction by Tn7 requires *attTn7*, suggesting that *attTn7* also stimulates excision and insertion coordinately *in vivo* (A. STELLWAGEN and N. L. CRAIG, unpublished). However, the normal chromosomal *attTn7* site in the *lac* duplication strains does not activate transposition because it is already occupied by miniTn7-*kan*. This element consists of 109 bp of the left end of Tn7 and 199 bp of the right end, flanking a gene encoding Kan^R. MiniTn7-*kan* is missing some sequences required in *cis* for transposition but carries enough of the Tn7 ends to render this *attTn7* site immune to subsequent insertions (ARCISZEWSKA, DRAKE and CRAIG 1989). The presence of Tn7 in a target DNA greatly reduces the frequency of transposition into that target, *i.e.*, provides *cis*-acting immunity (ARCISZEWSKA, DRAKE and CRAIG 1989; HAUER and SHAPIRO 1984). An immune *attTn7* site also fails to activate transposon excision *in vitro* (BAINTON, GAMAS and CRAIG 1991), and fails to provoke induction of SOS *in vivo* (A. STELLWAGEN and N. L. CRAIG, unpublished). Thus, in the *lac* duplication strains, the stimulation to high frequency of transposition, both excision and insertion, requires the introduction of another, unoccupied *attTn7* site. In experiments to determine the effect of high frequency Tn7 transposition on recombination, either an F factor carrying an *attTn7* site (pOX-*attTn7-2*), or the same F factor without *attTn7* (pOX38-Gen), is introduced by conjugation and the resulting transconjugants are assayed for recombination and transposition.

The RecBC(D$^-$) pathway of homologous recombination in *E. coli* exhibits high levels of recombination near double-chain breaks (THALER *et al.* 1989). For this reason, we looked for a stimulation of recombination by Tn7 transposition in *recD* mutants, in which most recombination follows the RecBC(D$^-$) pathway.

Tn7 transposition stimulates recombination to Lac$^+$: Frequencies of recombination to Lac$^+$ for *recD* strains carrying two heteroallelic mutant copies of *lacZ*, one containing Tn7, are plotted in Figure 3A. The strains in each pair differ in the presence or absence of an *attTn7* site in the F factor. A tenfold increase in the frequency of recombination is observed when transposition is activated by *attTn7*. Recombination to Lac$^+$ is stimulated by transposition when *lac*::Tn7 resides at minute 8 or minute 27. Transposition frequencies in these strains increase about four orders of magnitude in the presence of *attTn7*, as shown in Table 2.

Does Tn7 stimulate recombination globally? Since SOS induction, an *E. coli* response to high levels of Tn7 transposition (A. STELLWAGEN and N. L. CRAIG, unpublished) causes an increase in expression of some recombination genes, including *recA* and several RecF pathway genes [see WALKER (1984) for review], it is possible that SOS induction could cause a global stimulation of recombination following Tn7 transposition. For this reason, recombination frequencies were determined for heteroalleles of *lacZ* in a *lac* duplication strain carrying Tn7 at a chromosomal location distant from *lac*. These constructs showed no stimulation of recombination when Tn7 was activated to transpose at high frequency (Figure 3C).

Recombination was also assayed in *lexA3* mutant versions of the *lac* duplication strains carrying Tn7 in *lac*. The SOS response is not inducible in a *lexA3* mutant (MOUNT, LOW and EDMISTON 1972), so any increase in recombination would be independent of the SOS response. In the *lexA3* strains, recombination was stimulated by Tn7 to a greater extent than in *lexA*$^+$ (Figure 3B). The greater stimulation of recombination in these strains may be due to a decrease in the background frequency of recombination. The frequency of transposition was unaffected by the *lexA3* mutation (Table 2).

The inability of Tn7 transposition to stimulate recombination assayed at markers distant from the

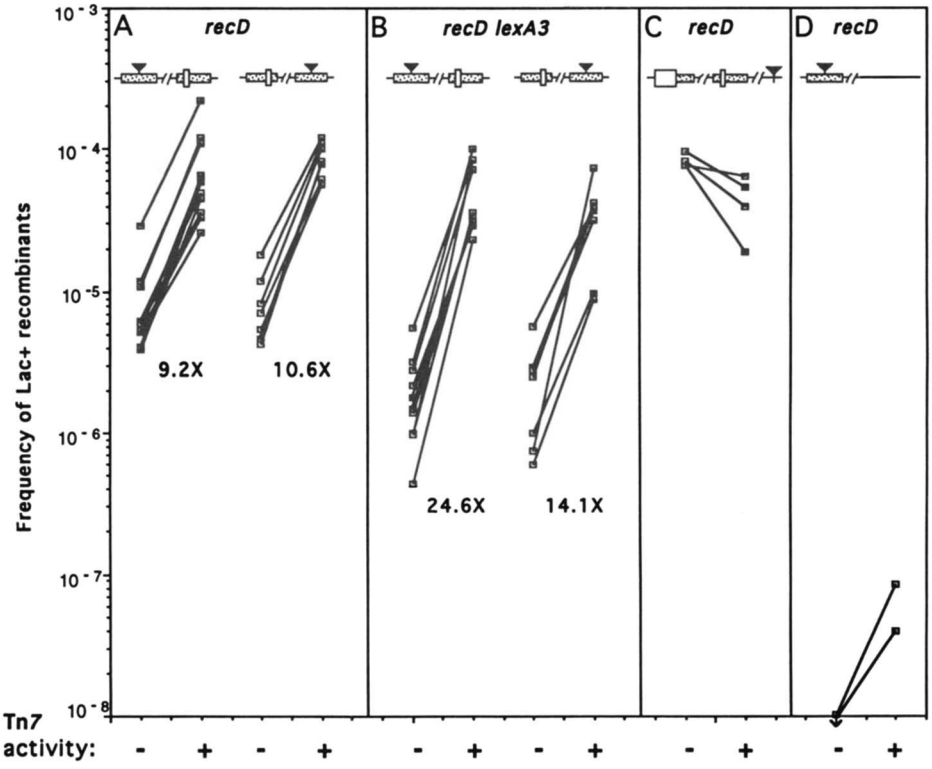

FIGURE 3.—Recombination to Lac$^+$ is stimulated by Tn7 transposition. Each point represents a culture grown from a single colony. Points plotted at any single position along the horizontal are repetitions of the same genotype, and lines connect points collected concurrently from cultures that differ in the presence/absence of the *attTn7* site in the target episome. Tn7 activity is represented for simplicity as "+" (high frequency transposition) or "−" (low frequency transposition); actual frequencies of transposition are given in Table 2. The fold stimulation was calculated for each pair of points, and the median value is given beneath each set of lines, where applicable. The configuration of markers is diagrammed above each data set; the copy of *lac* (stippled box) at the left is at minute 8, the other copy of *lac* is at minute 27 of the *E. coli* map. Tn7 is the black triangle and deletions are open boxes. Relevant aspects of the genotypes are given. Strains names are, from left to right: A, ATH154, ATH176; B, ATH178, ATH179; C, ATH160; D, ATH161.

TABLE 2

Activity of Tn7 and its effect on site of conversion to lac$^+$

Strain	*lac* allele at min. 8	*lac* allele at min. 27	Episome	Transposition frequency	Percent conversion to *lac*$^+$ at: min. 8	min. 27
ATH154	*lacZ4524*::Tn7	*lacBK1*	pOX	1.5×10^{-6}	35	65
			pOX-*att*	1.1×10^{-1}	75	25
ATH176	*lacBK1*	*lacZ4524*::Tn7	pOX	1.0×10^{-6}	74	26
			pOX-*att*	6.8×10^{-2}	24	76
ATH178	*lacZ4524*::Tn7	*lacBK1*	pOX	3.8×10^{-6}	34	66
			pOX-*att*	7.5×10^{-2}	90	10
ATH179	*lacBK1*	*lacZ4524*::Tn7	pOX	5.7×10^{-7}	57	43
			pOX-*att*	5.8×10^{-2}	8	92
ATH160	*lacMS286*	*lacBK1*	pOX	6.5×10^{-7}	NA	NA
			pOX-*att*	9.6×10^{-2}	NA	NA
ATH161	*lacZ4524*::Tn7	None	pOX	4.0×10^{-7}	NA	NA
			pOX-*att*	9.2×10^{-2}	NA	NA

To determine percent of conversion at each *lac* site, a minimum of 50 Lac$^+$ recombinants were analyzed, from at least five independent cultures. Transposition frequencies are means, and were measured for the same cultures (≥5) from which convertants were selected. pOX is pOX38-Gen, and pOX-*att* is pOX-*attTn7-2*. NA is not applicable.

transposon donor site and the stimulation of recombination by Tn7 transposition in *lexA3* mutants demonstrate that Tn7 transposition stimlulates recombination at the donor site independently of SOS.

Is donor site homology required to generate Lac$^+$? In order to address the possibility that simple sealing of a donor break following transposition can cause reversion of *lacZ*::Tn7, a strain was made that lacks the second copy of *lac*. In this construct there is no homology to the donor site except in a sister chromosome, so homologous recombination cannot gen-

erate a wild-type *lacZ* gene. In order to detect Lac$^+$ revertants it was necessary to plate these cells at densities higher than those for measuring homologous recombination, so frequencies should not be compared quantitatively (see comment under *Experimental design*). No revertants arose from the strain lacking *attTn7* (Figure 3D), at a limit to detection of reversion of 6×10^{-9}. In the presence of *attTn7*, this strain displayed some reversion to Lac$^+$, but at a frequency several orders of magnitude lower than the frequency with which Lac$^+$ colonies arose in the presence of the second copy of *lac*. Therefore, precise resealing cannot account for the stimulation by Tn*7* in the proportion of Lac$^+$ colonies that arise in the presence of homology with the donor site, but resealing may occur at a low frequency following transposition. Since the stimulation of formation of Lac$^+$ colonies by Tn*7* transposition requires a source of homology to the donor site in *lac*, we can conclude that the Lac$^+$ colonies are arising by homologous recombination.

Does the Tn*7* donor site give or take information in homologous recombination? If the stimulation of homologous recombination by Tn*7* transposition is due to creation of a recombinationally active double-chain break, repair of this break should replace the information that was originally present (Tn*7*) with the information from the homologous sequence (see SZOSTAK *et al.* 1983 for a possible mechanism). Most of the recombinants stimulated by Tn*7* are therefore predicted to carry the *lac*$^+$ information at the original location of Tn*7*, rather than the original location of the deletion. An analysis of positions of conversion to *lac*$^+$ in both spontaneous and Tn*7*-stimulated recombinants revealed a bias in conversion, as shown in Table 2. Among the spontaneous convertants, those arising in the absence of *attTn7*, a majority had converted the deletion-containing *lacZ* allele to *lac*$^+$, whereas among the Tn*7*-stimulated convertants most had replaced Tn*7* with *lac*$^+$ information. Among transposition-stimulated recombinants, the bias toward conversion at the Tn*7*-containing allele is strongest in *lexA3* mutants. This would be expected if the level of spontaneous recombination is reduced in *lexA3* mutants, as proposed above, allowing the events stimulated by Tn*7* and converting *lacZ*::Tn*7* to *lacZ*$^+$ to comprise a larger fraction of the total than in *lexA*$^+$.

DISCUSSION

Stimulation of recombination by transposition: We report that Tn*7* transposition stimulates homologous recombination between the Tn*7* donor site and a homologous sequence. This was seen between duplicate, heteroallelic copies of *lacZ*, one carrying Tn*7* and the other carrying a small deletion, and was not due to a global stimulation of recombination. Conversion preferentially replaced the transposon in Tn*7*-stimulated recombinants, but not in the spontaneous recombinants, indicating that the recombinagenic activity of Tn*7* involves loss of the transposon.

The recombinational repair model diagrammed in Figure 1 predicts that when given a source of homology alternative to the sister chromosome, a break induced by a nonreplicative transposition event can be repaired such that the transposon is replaced with *lac*$^+$ information. The results presented here are consistent with this model. Under most circumstances, the same type of recombinational repair would replace the gap left by transposition with a copy of the transposon obtained from a sister chromosome. This would be an invisible event because the repaired donor would be genetically identical to the original donor. It is only when a duplication of the DNA in which Tn*7* resides is introduced that there is an opportunity to lose the transposon by recombination with the homologous sequence.

Ectopic *versus* sister repair: Tn*7* stimulates homologous recombination at the donor site by about 10-fold, producing Lac$^+$ recombinants at a frequency of 10^{-4} to 10^{-5}, while a much larger fraction of the cells, roughly 10^{-1}, have experienced a transposition event in their history. Failure to see Lac$^+$ recombinants at a frequency closer to that of transposition suggests that the broken donor replicon is not always repaired by homologous recombination with the ectopic copy of *lac*. This could be due to a preference for repair by recombination with a sister chromosome. Alternative fates, such as imprecise sealing of the break or loss of the donor replicon, seem unlikely in light of evidence that in *E. coli* repair of double-strand breaks occurs efficiently when homologous recombination is possible, but occurs infrequently (if at all) when homologous recombination is not possible. X-ray induced double-strand breaks are repaired efficiently by the RecBC pathway when cells contain several copies of the chromosome. However, when only one copy of the chromosome is present (due to growth in minimal medium or growth to stationary phase) little or no repair is seen (KRASIN and HUTCHINSON 1977; SARGENTINI, DIVER and SMITH 1983; SARGENTINI and SMITH 1986). Recombination between sister chromosomes at allelic positions has no genetic consequence, therefore is not detectable except as repair of DNA damage. Perhaps a double chain break finds homology in its sister more efficiently than it finds ectopic homology. This could be due to constraints imposed by nucleoid structure or simply to proximity of the sister, especially if the break occurs in a recently replicated region of the chromosome. An association of Tn*7* transposition with replication seems possible by analogy with Tn*10* which transposes preferentially from hemimethylated and therefore newly replicated DNA, possibly ensuring that a sister is available for repair of the broken donor (ROBERTS *et al.* 1985). In support of this analogy, Tn*7*, like Tn*10*, enjoys a

greater than 10-fold increase in transposition frequency in *E. coli* mutants (*dam*$^-$) lacking DNA adenine methylase (O. HUGHES and N. L. CRAIG, unpublished; ROBERTS *et al.* 1985).

Another explanation for sister preference is that the homology available for repair from the sister is more extensive than from the ectopic copy of *lac* (4.7 megabases *vs.* ~10 kilobases), and therefore there may be a greater probability of interaction with the sister. If degradation of DNA from a Tn*7*-induced break occurs past the position of the deletion in the second copy of *lacZ*, then a Lac$^+$ recombinant cannot arise. If degradation from the break removes the entire *lac* region, recombination with the ectopic copy of *lac* is prohibited, but an interaction with the sister chromosome is still possible. This was the motivation for using nuclease-deficient *recD* mutants; an attempt to repeat these experiments in Rec$^+$ strains revealed that recombination to Lac$^+$ is stimulated only twofold by Tn*7* transposition (data not given). This Rec$^+$ result is consistent with the view that limited *lac* homology is more prohibitive for recombination in the wild-type background, where ExoV nuclease is present, than in *recD* mutants, lacking ExoV. The results presented here for *recD* strains are useful in illustrating the possibility for recombinational repair, and if the homology available for recombination to Lac$^+$ were more extensive, the *recD* mutation might not be necessary. In the natural condition, with Tn*7* hopping from a unique sequence in the chromosome of wild-type *E. coli*, repair off the sister chromosome to regenerate the transposon will have no homology limitation, therefore there is no obvious barrier to this event.

Excision of Tn*7*: We observed a low level of transposition-dependent reversion of *lacZ*::Tn*7* in the absence of *lac* homology (Figure 3D). This precise excision occurred at a low frequency compared to homologous recombination promoted by Tn*7* transposition. Transposons Tn*5* and Tn*10*, both of which have long (>1.3 kilobases) terminal inverted repeats and move nonreplicatively, are precisely excised at low frequencies independently of transposition [see BERG (1989) and KLECKNER (1989) for reviews]. It has been suggested that excision of these elements may be facilitated by pairing of the long terminal repeats of the transposons through a mechanism involving slippage of base pairing in replication of repeated sequences (EGNER and BERG 1981; FOSTER *et al.* 1981; originally proposed for formation of frameshift mutations by STREISINGER *et al.* 1966). Tn*7*, having only 30 bp inverted repeats separated by 14 kilobases (LICHTENSTEIN and BRENNER 1982), is probably not excised by the putative pairing slippage mechanism proposed for Tn*5* and Tn*10*.

Phage Mu transposes mostly replicatively and lacks long inverted repeats [see PATO (1989) for review]. Mu is precisely excised in a transposase-dependent reaction that is thought to be facilitated by transposase-mediated alignment of the ends (NAG and BERG 1987). Tn*7* excision, which is dependent upon transposition, could occur by a mechanism similar to that proposed for Mu, or by low efficiency sealing of the broken donor product following transposition. The level of Tn*7* precise excision seen in a RecD$^+$ strain is 10–20-fold lower than in RecD$^-$ (data not shown), suggesting that a double-chain break species is being degraded by ExoV in the RecD$^+$ strain. The absence of ExoV in RecD$^-$ could allow more of the donor breaks to be joined precisely by a resection/ligation mechanism, as described in the introduction. The combination of high levels of nonreplicative transposition characteristic of Tn*7* and the absence of ExoV in these RecD$^-$ mutants allows detection of events that occur at a frequency 10^{-6} that of transposition. RecD$^-$ mutants were examined for precise excision of Tn*10* and no increase was seen over wild-type (AMUNDSEN *et al.* 1990), but the frequency of Tn*10* transposition in that system was probably about 2×10^{-5} (FOSTER *et al.* 1981), compared to 10^{-1} for Tn*7* here.

Is Tn*7* transposition replicative in two steps? Double-strand breaks are repaired efficiently in both wild-type and RecD$^-$ *E. coli* (KRASIN and HUTCHINSON 1977; SARGENTINI, DIVER and SMITH 1983; MURIALDO 1988). The repair of a Tn*7*-induced break to produce a Lac$^+$ recombinant need only insert a short region, either by gap repair involving DNA synthesis, as in the double-strand-break repair model of SZOSTAK *et al.* 1983, or by two conservative splice events, one on each side of the break. Repair that restores a copy of the transposon requires insertion of at least 14 kilobases, the length of Tn*7*. Like recombination to Lac$^+$, this could be accomplished by splicing together parts of two chromosomes, with little or no DNA synthesis, or by copying the entire transposon using repair synthesis. Following transposition, if gap-filling replaces Tn*7* by copying the transposon from a sister, transposition will be occurring by a mechanism that results in its net replication even though Tn*7* does not instruct the replication machinery. Tn*7* could ensure its replication by leaving behind a break that the cell would fill with a new copy of the transposon. The observation of homologous recombination (gene conversion) associated with Tn*7* transposition in *E. coli* and with movement of transposons in organisms as distantly related to *E. coli* as *C. elegans* (PLASTERK and GROENEN 1992) and Drosophila (ENGELS *et al.* 1990) suggests that allowing the host machinery to replicate the transposon is an effective evolutionary strategy for transposable elements.

We wish to thank RIK MYERS, OWEN HUGHES, FRANK STAHL, ANNE STELLWAGEN and DAVID THALER for interesting discussions and helpful suggestions. A.T.H. gratefully acknowledges support from the American Cancer Society, grant PF-3557, and the University of California Cancer Research Coordinating Committee.

This work was supported by National Institutes of Health grant AI21533 to N.L.C.

LITERATURE CITED

AMUNDSEN, S. K., A. M. NEIMAN, S. M. THIBODEAUX and G. R. SMITH, 1990 Genetic dissection of the biochemical activities of RecBCD enzyme. Genetics **126:** 25–40.

ARCISZEWSKA, L. K., D. DRAKE and N. L. CRAIG, 1989 Transposon Tn*7* *cis*-acting sequences in transposition and transposition immunity. J. Mol. Biol. **207:** 35–52.

BAINTON, R., P. GAMAS and N. L. CRAIG, 1991 Tn7 transposition *in vitro* proceeds through an excised transposon intermediate generated by staggered breaks in DNA. Cell **65:** 805–816.

BARTH, P. T., N. DATTA, R. W. HEDGES and N. J. GRINTER, 1976 Transposition of a deoxyribonucleic acid sequence encoding trimethoprim and streptomycin resistances from R483 to other replicons. J. Bacteriol. **125:** 800–825.

BENDER, J., and N. KLECKNER, 1986 Genetic evidence that Tn10 transposes by a nonreplicative mechanism. Cell **45:** 801–815.

BENDER, J., J. KUO and N. KLECKNER, 1991 Genetic evidence against intramolecular rejoining of the donor DNA molecule following IS*10* transposition. Genetics **128:** 687–694.

BERG, D. E., 1989 Transposon Tn*5*, pp. 185–210 in *Mobile DNA*, edited by D. E. BERG and M. M. HOWE. American Society for Microbiology, Washington, D.C.

BERG, D. E., C. M. BERG and C. SASAKAWA, 1984 Bacterial transposon Tn*5*: evolutionary inferences. Mol. Biol. Evol. **1:** 411–422.

BIEK, D. P., and S. N. COHEN, 1986 Identification and characterization of *recD*, a gene affecting plasmid maintenance and recombination in *Escherichia coli*. J. Bacteriol. **167:** 594–603.

COOPER, S., and C. E. HELMSTETTER, 1968 Chromosome replication and the division cycle of *Escherichia coli* B/r. J. Mol. Biol. **31:** 519–540.

CRAIG, N. L., 1991 Tn*7*: a target site-specific transposon. Mol. Microbiol. **5:** 2569–2573.

EGNER, C., and D. E. BERG, 1981 Excision of transposon Tn*5* requires the inverted repeats but not the transposase functions of Tn*5*. Proc Natl. Acad. Sci. USA **78:** 459–463.

ENGELS, W. R., D. M. JOHNSON-SCHLITZ, W. B. EGGLESTON and J. SVED, 1990 High-frequency P element loss in Drosophila is homolog dependent. Cell **62:** 515–525.

FOSTER, T. J., V. LUNDBLAD, S. HANLEY-WAY, S. M. HALLING and N. KLECKNER, 1981 Three Tn10-associated excision events: relationship to transposition and role of direct and inverted repeats. Cell **23:** 215–227.

HAUER, B., and J. SHAPIRO, 1984 Control of Tn7 transposition. Mol. Gen. Genet. **194**:149158.

KLECKNER, N., 1989 Transposon Tn*10*, pp. 227–268 in *Mobile DNA*, edited by D. E. BERG and M. M. HOWE. American Society for Microbiology, Washington, D.C.

KONRAD, E. B., 1977 Method for the isolation of *Escherichia coli* mutants with enhanced recombination between chromosomal duplications. J. Bacteriol. **130:** 167–172.

KUBITSCHEK, H. E., and M. L. FREEDMAN, 1971 Chromosome replication and the division cycle of *Escherichia coli* B/r. J. Bacteriol. **107:** 95–99.

KUBO, K. M., and N. L. CRAIG, 1990 Bacterial transposon Tn7 utilizes two different classes of target sites. J. Bacteriol. **172:** 2774–2778.

KRASIN, F., and F. HUTCHINSON, 1977 Repair of DNA double-strand breaks in *Escherichia coli*, which requires *recA* function and the presence of a duplicate genome. J. Mol. Biol. **116:** 81–98.

LICHTENSTEIN, C., and S. BRENNER, 1981 Site-specific properties of Tn7 transposition into the *E. coli* chromosome. Mol. Gen. Genet. **183:** 380–387.

LICHTENSTEIN, C., and S. BRENNER, 1982 Unique insertion site of Tn7 in the *E. coli* chromosome. Nature **297:** 601–603.

MILLER, J. H., 1972 *Experiments in Molecular Genetics*. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

MOUNT, D. W., K. B. LOW and S. EDMISTON, 1972 Dominant mutations (*lex*) in *Escherichia coli* K-12 which affect radiation sensitivity and frequency of ultraviolet-induced mutations. J. Bacteriol. **112:** 886–893.

MURIALDO, H., 1988 Lethal effect of λ DNA terminase in recombination-deficient *Escherichia coli*. Mol. Gen. Genet. **213:** 42–49.

NAG, D. K., and D. E. BERG, 1987 Specificity of bacteriophage Mu excision. Mol. Gen. Genet. **207:** 395–401.

PATO, M. L., 1989 Bacteriophage Mu, pp. 23–52 in *Mobile DNA*, edited by D. E. BERG and M. M. HOWE. American Society for Microbiology, Washington, D.C.

PLASTERK, R. H. A., and J. T. M. GROENEN, 1992 Targeted alterations of the *Caenorhabditis elegans* genome by transgene instructed DNA double strand break repair following Tc1 excision. EMBO J. **11:** 287–290.

ROBERTS, D., B. C. HOOPES, W. R. MCCLURE and N. KLECKNER, 1985 IS10 transposition is regulated by DNA adenine methylation. Cell **43:** 117–130.

ROGERS, M., N. EKATERINAKI, E. NIMMO and D. SHERRATT, 1986 Analysis of Tn7 transposition. Mol. Gen. Genet. **205:** 550–556.

SARGENTINI, N. J., W. P. DIVER and K. C. SMITH, 1983 The effect of growth conditions on inducible, *recA*-dependent resistance to X rays in *Escherichia coli*. Radiat. Res. **93:** 364–380

SARGENTINI, N. J., and K. C. SMITH, 1986 Quantitation of the involvement of *recA*, *recB*, *recC*, *recF*, *recJ*, *recN*, *lexA*, *radA*, *radB*, *uvrD*, and *umuC* genes in the repair of X-ray-induced DNA double strand breaks in *Escherichia coli*. Radiat. Res. **107:** 58–72.

SINGER, M., T. A. BAKER, G. SCHNITZLER, S. M. DEISCHEL, M. GOEL, W. DOVE, K. J. JAACKS, A. D. GROSSMAN, J. W. ERICKSON and C. A. GROSS, 1989 A collection of strains containing genetically linked alternating antibiotic resistance elements for genetic mapping of *Escherichia coli*. Microbiol. Rev. **53:** 1–24.

STREISINGER, G., Y. OKADA, J. EMRICH, J. NEWTON, A. TSUGITA, E. TERZAGHI and M. INOUYE, 1966 Frameshift mutations and the genetic code. Cold Spring Harbor Symp. Quant. Biol. **31:** 77–84.

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.

THALER, D. S., E. SAMPSON, I. SIDDIQI, S. M. ROSENBERG, L. C. THOMASON, F. W. STAHL and M. M. STAHL, 1989 Recombination of bacteriophage λ in *recD* mutants of *Escherichia coli*. Genome **31:** 53–67.

VOGEL, H., and D. BONNER, 1956 Acetylornithinase of *Escherichia coli*. Partial purification and some properties. J. Biol. Chem. **218:** 97–106.

WADDELL, C. S., and N. L. CRAIG, 1988 Tn7 transposition: two transposition pathways directed by five Tn7-encoded genes. Genes Dev. **2:** 137–149.

WALKER, G. C., 1984 Mutagenesis and inducible responses to deoxyribonucleic acid damage in *Escherichia coli*. Microbiol. Rev. **48:** 60–93.

ZIEG, J., and S. R. KUSHNER, 1977 Analysis of genetic recombination between two partially deleted lactose operons of *Escherichia coli* K-12. J. Bacteriol. **131:** 123–132.

Communicating editor: D. E. BERG