Excision and Transposition of Tn*5* as an SOS Activity in *Escherichia coli*

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ABSTRACT

Excision and transposition of the Tn*5* element in *Escherichia coli* ordinarily appear to occur by *recA*-independent mechanisms. However, *recA*(Prt^c) genes, which encode RecA proteins that are constitutively activated to the protease state, greatly enhanced excision and transposition; both events appeared to occur concomitantly and without destruction of the donor DNA. The recombinase function of the RecA protein was not required. Transposition was accompanied by partial, and occasionally full, restoration of the functional integrity of the gene vacated by the excised Tn*5*. The stimulation of transposition was inhibited by an uncleavable LexA protein and was strongly enhanced by an additional role of the RecA(Prt^c) protein besides its mediation of LexA cleavage. To account for the enhanced transposition, we suggest that (i) there may be a LexA binding site within the promoter for the IS*50* transposase, (ii) activated RecA may cleave the IS*50* transposition inhibitor, and (iii) the transposase may be formed by RecA cleavage of a precursor molecule.

MOST, if not all, of the recombination events associated with the movement of transposable elements have been considered to ordinarily occur independently of the *recA* gene and the SOS control system because (i) the recombination events involve nonhomologous DNA sequences and (ii) transposition and some transposition related events appear to occur with equal facility in RecA$^+$ and RecA$^-$ cells (BERG 1977; CALOS and MILLER 1980; EGNER and BERG 1981; KLECKNER 1981; BERG 1989). We will demonstrate, however, that both the excision and the transposition of Tn*5* are stimulated by constitutive induction of the SOS regulon and even more by an additional function associated with the RecA protease.

Tn*5* is a 5.8-kilobase (kb) composite transposon that encodes resistance to the antibiotics kanamycin, bleomycin and streptomycin. The resistance genes are flanked by a pair of 1.5-kb insertion sequences (IS*50*) in inverted orientations (ISBERG, LAZAAR and SYVANEN 1982; JOHNSON, YIN and REZNIKOFF 1982; BERG 1989). Only the right repeat, IS*50*R, can function as a transposable element in its own right (BERG *et al.* 1981; MAKRIS, NORDMANN and REZNIKOFF 1988); it encodes both a transposase and an inhibitor of transposition in overlapping sequences in the same reading frame (KREBS and REZNIKOFF 1986; MCCOMMAS and SYVANEN 1988).

The RecA protein of *Escherichia coli* is a multifunctional enzyme that can act both as a recombinase and as a cofactor in the proteolytic cleavage of several proteins (WITKIN 1976; LITTLE and MOUNT 1982; WALKER 1984). For brevity, we refer to the latter property as a protease activity. Under normal conditions, the protease activity of the wild-type RecA protein is very low, though possibly not nil. In the presence of damaged DNA, however, the RecA protein is activated to the protease state (RecA*) in which it mediates the cleavage of the LexA repressor protein; this sequence of events induces the synthesis of the products of numerous unlinked genes that comprise the SOS regulon including, significantly, the *recA* gene (WITKIN 1976; LITTLE and MOUNT 1982; WALKER 1984). A large variety of *recA* alleles with constitutive protease activity (Prt^c) have been isolated and characterized (TESSMAN and PETERSON 1985a,b); cells with such mutations show induction of the SOS regulon in the absence of DNA damaging agents.

The present study was initiated by the serendipitous discovery that cells with the *umuC122*::Tn*5* mutation, which are normally completely defective in Weigle reactivation (BAGG, KENYON and WALKER 1981; ELLEDGE and WALKER 1983), acquired substantial reactivation ability when lysogenized with λ*recA1202*(Prt^c). Among other possibilities, we considered that the protein encoded by the *recA1202*(Prt^c) allele might stimulate excision of Tn*5* from *umuC* in most cells, thereby restoring some *umuC* function. Since the cells remained Kan^r, it seemed likely that practically all the Tn*5* elements had transposed to new chromosomal sites. We therefore addressed the following questions. (i) What proportion of the cells experienced excision of Tn*5*? (ii) How precise was the excision? (iii) Did a transposition event always accompany the excision?

We will show that, in comparison with the *recA*$^+$ allele, *recA*(Prt^c) mutations increased not only the frequency of excision of Tn*5* from the chromosomal *umuC* gene, but also the frequency of transposition.

The increase was at least 100-fold and was LexA-dependent. Stimulation of excision and transposition of Tn*5* in other locations was also observed. Contrary to current models (BERG 1989), the donor DNA was rarely, if ever, destroyed in the *recA*(Prt^c)-promoted events, and excision of Tn*5* was practically always accompanied by transposition to a new chromosomal location.

MATERIALS AND METHODS

Bacteria and bacteriophage: The bacterial strains were isogenic derivatives of *E. coli* K12 (Table 1). The parent strain EST1130 is stable against Mu dl(Ap *lac*) lysis or transposition (TESSMAN and PETERSON 1985a). The UmuC$^-$ phenotype was scored on the basis of increased sensitivity of cells to UV light and the absence of Weigle reactivation (BAGG, KENYON and WALKER 1981). λ*recA1202*(Prt^c Rec$^+$) and λ*recA1211*(Prt^c Rec$^+$) lysogens produce RecA proteins that can be activated by RNA and an enlarged number of nucleotide cofactors (WANG *et al.* 1988; WANG, TESSMAN and TESSMAN 1988); these cells have high levels of constitutive RecA protease activity. The *recA1201*(Prt^c Rec$^-$) allele produces a split phenotype; it encodes a protein with substantial protease activity but highly defective in recombinase activity (TESSMAN and PETERSON 1985b). The *cI857* mutation was crossed into λ*recA1202* c**I** *ind* (λMT75) from λ*recA*$^+$ *cI857 ind* (λ202) to generate λ*recA1202 cI857 ind* (λIT75). In EST1122, which carries a *dinD-lac* fusion promoter, the level of β-galactosidase activity is an indicator of RecA protease activity. Thus, a λ*recA*(Prt^c) lysogen of EST1122 forms dark blue colonies on plates containing 5-bromo-4-chloro-3-indolyl-β-D-galactoside (XGal). Phage S13 was the UV target for Weigle reactivation (TESSMAN 1990).

Media and reagents: M9-CAA medium was M9 salts (MILLER 1972) supplemented (per liter) with 2 g glucose, 5 g vitamin-free casamino acids (Difco), 2 mg thiamine, 10 μM $FeCl_3$, 1 mM $MgSO_4$, and 0.1 mM $CaCl_2$. M9-CAA plates additionally contained 15 g agar per liter; 80 μg XGal per ml was added for M9-CAA-XGal agar. LB broth contained (per liter) 10 g tryptone, 5 g yeast extract and 5 g NaCl; 15 g agar were added for LB agar plates. Antibiotics were used at the following concentrations: kanamycin, 30 μg/ml; rifampin, 25 μg/ml; ampicillin, 50 μg/ml; tetracycline, 25 μg/ml.

DNA manipulations: The preparation and purification of λ DNA were carried out by standard procedures (MANIATIS, FRITSCH and SAMBROOK 1982). DNA was digested with restriction endonucleases obtained from Boehringer Mannheim Biochemicals or Bethesda Research Laboratories according to suppliers' instructions, and electrophoresed in horizontal borate-buffered 0.7% (w/v) agarose gels.

Transposition frequency assay: A *cI857 ind* prophage served as a target for transposition; that event was assayed by thermally inducing the phage and then scoring for the presence of Tn*5* by the ability of the phage to transduce Kan^r (ROTHSTEIN *et al.* 1980; JOHNSON, YIN and REZNIKOFF 1982). Cells lysogenic for λ*recA*(Prt^c) *cI857 ind* and also containing Tn*5* (*e.g.*, *umuC122*::Tn*5*) were grown at 30° in LB broth + 30 μg kanamycin per ml. The prophage in mid-log phase cells was thermally induced by incubation at 45° for 15 min and then at 37° for about 3 h, at which time lysis had occurred. The lysate was treated with pancreatic DNase at 10 μg/ml for 1 hr at 37°. It was then used to lysogenize strain EST1122 [Δ*recA dinD1*::Mu dl(*lac*)]; the ratio of Kan^r colonies to the total number of lysogens constituted the proportion of phage containing a Tn*5* and that was used as the measure of the frequency of transposition into the λ prophage. To lysogenize EST1122, the cells were grown in M9-CAA to 2×10^8/ml at 30° and then infected with the treated λ lysate; the multiplicity of infection (moi) was approximately 1 except for one experiment in which an moi of 0.01 is stated. The infected cells were diluted 10-fold in the same medium and aerated at 32° for 3.5 hr to allow expression of the Kan^r phenotype. The number of Kan^r cells was measured by plating onto M9-CAA-XGal plates containing 30 μg kanamycin per ml; more than 99% of the colonies formed were proven to be lysogenic by streaking the cells against λ*b*$_2$*c* and λ*vir*. The total number of lysogens was determined by plating on M9-CAA agar containing approximately 10^9 λ*b*$_2$*c*.

λ*recA*(Prt^c) lysogens acquire secondary mutations (by proximal mutagenesis) within the *recA* gene with the consequence that lysogens form pale blue rather than dark blue colonies on XGal plates (LIU and TESSMAN 1990). Cells grown in M9-CAA medium gave rise to pale blue colonies at a frequency of about 0.2%; this frequency typically rose to nearly 20% when cells were grown in LB medium where dark blue colonies grew more poorly (LIU and TESSMAN 1990).

Quantitation of Weigle reactivation: The repair sector *W* was used to quantitate Weigle reactivation:

$$W = 1 - \log S_a / \log S_b$$

where *W* is the fraction of lethal damages that are repaired. S_a is the fraction of viruses surviving in a test strain, and S_b is the survival in the reference strain representing $W = 0$. Our procedure of irradiating the S13 virus to the low survival $S_b = 10^{-7}$ made it possible to measure small values of *W* reliably. For example, $W = 0.04$ would correspond to $S_a/S_b = 2$, which was easily measured. $W = 0.03$ would be detected by a 62% increase in survival, $W = 0.02$ by a 38% increase in survival, and $W = 0.01$ by a 25% increase. Fluctuations in repeated measurements indicated that $W = 0.02$ was the practical lower limit of detectable Weigle reactivation.

Rate of spontaneous mutation in the *rpoB* gene (Rif^s → Rif^r): Cultures were grown from single colonies in M9-CAA to approximately 4×10^8 (29 generations) at 32°. The frequency of Rif^r mutants was determined by plating the cells on LB-rifampin agar and incubating at 32° for 24–30 hr.

Heat-pulse curing of lysogens: λ lysogens were grown to early log phase in M9-CAA at 30°, then incubated at 45° in a 5 min pulse to induce the phage. The cultures were then cooled in an ice bath for 5 min to allow the *c*I repressor to renature. Cultures were then grown to saturation at 30° (WEISBERG and GALLANT 1967). The number of viable cells was determined by plating on LB agar at 30°; cured cells were selected by plating on LB agar at 42°.

RESULTS

It is appropriate to describe how the λ*recA1202*(Prt^c) genome was inserted into the Δ*recA umuC*::Tn*5* host strain IT2659. The description is needed because we will demonstrate that Tn*5* excision from *umuC* and transposition to a new location were both so frequent they could possibly have occurred even during the construction of the appropriate starting strains for the experiments. To make lysogens, the phage were first spotted onto a lawn of IT2659

TABLE 1

Bacterial strains

Strain	Relevant genotype and phenotype	Source (reference)
AB1157	*thr-1 leuB6 proA2 his-4 argE3 thi-1 ara-14 lacY1 galK2 xyl-5 mtl-1 rpsL31 tsx-33 supE44*	A. J. CLARK
EST1130	*recA441 sulA11* Δ*lacU169 thr-1 leu-6 his-4 thi-1 argE3 ilv*(Ts) *galK2 rpsL31 dinD1*::Mu dl(Ap *lac* Ts$^+$)	TESSMAN and PETERSON (1985a)
EST945	As AB1157, but Δ*recA306 srl*::Tn*10* λ*recA*$^+$ *att*$^+$ c*I*$^+$	TESSMAN and PETERSON (1985a)
EST1515	As EST1130, but Δ*recA306 srl*::Tn*10*	EST1130 × P1(EST945)
EST1122	As EST1515, but Tet^s	Tet^s from EST1515[a]
IT1819	As EST1130, but S13^s	Our collection
EST2422	As IT1819, but λ*recA*$^+$ *ind* Tet^s	Our collection
IT2006	As EST1122, but λ*recA1201* c*I ind* S13^s	Our collection
IT2228	As EST1122, but λ*recA1202* c*I ind* S13^s	Our collection
GW2100	As AB1157, but *umuC122*::Tn*5*	ELLEDGE and WALKER (1983)
IT1823	As IT1819, but *umuC122*::Tn*5*	IT1819 × P1(GW2100)
IT2659	As IT1823, but Δ*recA306 srl*::Tn*10*	IT1823 × P1(EST945)
IT2675	As IT2659, but λ*recA*$^+$ *cI857 ind*	This work
IT2682	As IT2659, but λ*recA1202 cI857 ind*	This work
IT2683	As IT2659, but λ*recA1211 cI857 ind*	This work
IT2687	As IT2659, but λ*recA1201 cI857 ind*	This work
GW3198	As AB1157, but *umuC36*	ELLEDGE and WALKER (1983)
RS3040	*fadR13*::Tn*10*	B. J. BACHMANN
IT3011	As GW3198, but S13^s	This work
IT3081	As IT3011, but *fadR13*::Tn*10*	IT3011 × P1(RS3040)
IT3098	As IT2228, but *umuC36 fadR13*::Tn*10*	IT2228 × P1(IT3081)
EST1138	As EST1130, but *lexA3*(Ind$^-$)	Our collection
IT2765	As EST1138, but *umuC122*::Tn*5*	EST1138 × P1(IT1823)
IT2857	As IT2765, but Δ*recA306* Tet^s	IT2765 × P1(EST945)
IT2947	As IT2857, but λ*recA*$^+$ *cI857 ind*	This work
IT2953	As IT2857, but λ*recA1202 cI857 ind*	This work
IT2959	As IT2857, but λ*recA1201 cI857 ind*	This work
IT2169	As AB1157, but *lexA51 recA441 sulA211*	Our collection
IT2563	As IT2169, but *umuC122*::Tn*5*	IT2169 × P1(IT1823)
IT2565	As IT2563, but Δ*recA306 srl*::Tn*10*	IT2563 × P1(EST945)
IT3117	As IT2563, but λ*cI857 ind*	This work
IT2689	As IT2565, but λ*recA*$^+$ *cI857 ind*	This work
IT2692	As IT2565, but λ*recA1202 cI857 ind*	This work
IT2696	As IT2565, but λ*recA1201 cI857 ind*	This work
EST1663	As EST1130, but *lexA71*::Tn*5*(Def) Δ*recA306 srl*::Tn*10*	Our collection
IT2873	As EST1663, but λ*recA*$^+$ *cI857 ind*	This work
IT2863	As EST1663, but λ*recA1202 cI857 ind*	This work
IT2869	As EST1663, but λ*recA1201 cI857 ind*	This work
BW9554	*glnA21*::Tn*5*	B. L. WANNER
IT2704	As EST1130, but *glnA21*::Tn*5*	EST1130 × P1(BW9554)
IT2706	As IT2704, but Δ*recA306 srl*::Tn*10*	IT2704 × P1(EST945)
IT2743	As IT2706, but λ*recA*$^+$ *cI857 ind*	This work
IT2747	As IT2706, but λ*recA1202 cI857 ind*	This work
IT2741	As IT2706, but λ*recA1201 cI857 ind*	This work

[a] Procedure of MALOY and NUNN (1981).

cells on M9-CAA agar. After overnight incubation at 32° the center of a turbid spot was picked, streaked on M9-CAA agar, and nine single colonies were chosen. The founder cell for each colony was itself the product of several rounds of cell division (perhaps 5) during which Tn*5* might already have excised and transposed, which would happen on the average for about one of the nine colonies. Each of the nine colonies was inoculated in its entirety into 20 ml of M9-CAA and grown at 32° to 2 × 10^8/ml; a minute remnant of each colony was scored for susceptibility to λb_2c and λ*vir* in order to verify that the original colony contained lysogens. Of the nine cultures, grown for 32 generations from single colonies, six were used directly to determine the spontaneous mutation frequency at the *rpoB* locus by scoring for

Rif^s → Rif^r. The remaining three cultures were used to measure Weigle reactivation. Cultures of λ*recA1201*, λ*recA1211*, and λ*recA*$^+$ lysogens were prepared in the same way.

Excision of Tn*5* from the *umuC* gene: To determine whether the *recA1202*(Prt^c) allele enhanced the excision of Tn*5* from the *umuC* gene, we assayed the reversion of *umuC122*::Tn*5* cells to a UmuC$^+$ phenotype in isogenic *recA*$^+$ and *recA1202* strains, IT2675 and IT2682. The UmuC$^+$ phenotype was scored in two ways (Table 2): (i) frequency of the spontaneous mutation Rif^s → Rif^r and (ii) efficiency of Weigle reactivation of UV-irradiated phage S13. Because spontaneous mutation rates are subject to large fluctuations, the frequency of Rif^s → Rif^r was obtained by averaging the values from six cultures. There was a 37-fold increase in the frequency of Rif^r cells when the λ*recA1202 umuC*::Tn*5* strain (IT2682) was compared with the λ*recA*$^+$ *umuC*::Tn*5* strain (IT2675), indicating that a substantial number of cells had become UmuC$^+$. This increase, however, was less than the 200-fold increase seen in strain IT2228 (λ*recA1202 umuC*$^+$), suggesting that reversion of the *umuC122*::Tn*5* was, on the average, only partial. None of the six independent measurements for IT2682 gave a spontaneous frequency either as low as the average for IT2675 or as high as for IT2228.

The frequency of Rif^r cells did not reveal whether the cultures were homogeneous in terms of *umuC* function. Imprecise excision of Tn*5* might have already occurred in the founder cell of each culture; alternatively, excision might have occurred throughout the growth of the culture, a scenario that would generate a heterogeneous population of *umuC* genes as a consequence of different patterns of excision.

To test these possibilities we isolated 42 single colonies from the three additional cultures of IT2682 described above. For each one we measured the repair sector W for Weigle reactivation of UV irradiated phage S13 (Table 2). From one culture (called #1) we isolated 22 colonies, and from the other two cultures we isolated the remaining 20 colonies, approximately half from each of the two cultures, though we kept no record of which colony came from which of these latter two cultures. The isolates all made dark blue colonies indicating a RecA(Prt^c) phenotype (MATERIALS AND METHODS). For each one we measured the repair sector W for Weigle reactivation of UV irradiated phage S13 (Table 2). Two isolates from different cultures (including #1) produced $W = 0.22$, which is as high as the value in the λ*recA1202 umuC*$^+$ strain IT2228, suggesting that Tn*5* excision had been precise enough to restore all the *umuC*$^+$ function needed for Weigle reactivation. The same two isolates displayed Rif^r mutants at the same high frequency as the control strain that contained the *umuC*$^+$ allele (IT2228). The value $W = 0.16$, from a colony isolated from culture #1, indicates a distinct intermediate class of isolate that showed partial reversion of *umuC122*::Tn*5* to the wild type; most of the other isolates showed W values ≤ 0.05. Thus the original cultures were heterogeneous with regard to precision of excision.

It was conceivable that some of the 42 clones were still heterogeneous, consisting of a mixture of *umuC*::Tn*5* mutants and excisants. Therefore, 11 of the 42 clones were examined for homogeneity by picking four single-colony isolates from each and measuring Weigle reactivation in the 44 resulting cultures. Within experimental error (±0.01) the values of W were the same as for the corresponding original clones, namely $W = 0.22$ (2), 0.16 (1), 0.05 (2), 0.04 (2), 0.03 (2), 0.02 (2), where the number of the original 42 clones studied is in parentheses. Thus each of the 11 clones was homogeneous.

To confirm that Tn*5* had excised from the *umuC* gene, the same 11 of the original 42 clones were selected to be used as donors in P1 transduction experiments designed to test for genetic linkage between the *umuC* gene and Kan^r. The λ*recA*$^+$ strain EST2422 was transduced to Kan^r; the transductants were then scored for their UmuC phenotype by measuring Weigle reactivation after UV activation of the RecA$^+$ protein to the RecA* state. It was expected that the transductants would show the value $W = 0.22$, characteristic of the completely wild-type UmuC$^+$ phenotype of the recipient. This was true in eight cases where the W values of the donors were 0.22 (2), 0.16 (1), 0.05 (2), 0.04 (2), and 0.03 (1), indicating, as expected, that the Tn*5* was no longer located within the *umuC* gene of these donor strains. A total of 17 transductants were studied.

However, for three donors, whose values of W were 0.03 (1) and 0.02 (2), the Kan^r transductants of EST2422 had the value $W \leq 0.01$, indicating that the Tn*5* was still within a nonfunctional *umuC* gene. The discrepancy in the values of W between donor and recipient cells can probably be attributed to the *recA1202* allele in the donor cells, which may produce a small amount of Weigle reactivation ($W = 0.02$–0.03) despite the nonfunctional condition of the *umuC* gene. In *recA*(Prt^c) cells it is likely that $W = 0.03$ is a borderline value that discriminates between excised and nonexcised Tn*5*. Even the *umuC36* mutant (IT3098) exhibited $W = 0.02$ in the *recA1202* strain (Table 2). W was measured for all of nine transductants, three from each donor; the consistently low values suggested that the Tn*5* in the P1 transduction donors may have been stable; if the Tn*5* had behaved as in the parental *umuC*::Tn*5* strain IT2682 these cultures would have contained mixtures of cells, more than half of which would have shown significantly higher

TABLE 2

Reversion of *umuC122*::Tn5

Strain	Relevant genotype[a]	Repair sector[b] W	Rif^r frequency[c]
IT2675	λ*recA*$^+$ *umuC122*::Tn5	≤0.010	$(1.5 \pm 0.8) \times 10^{-8}$
IT2675(UV[d])	λ*recA*$^+$ *umuC122*::Tn5	≤0.010	ND
IT3098	λ*recA1202 umuC36*	0.019 ± 0.002	$(4.2 \pm 1.6) \times 10^{-8}$
IT2682	λ*recA1202 umuC122*::Tn5	0.02 (6/42), 0.03 (10/42) 0.04 (15/42), 0.05 (8/42) 0.16 (1/42), 0.22 (2/42)	$(5.6 \pm 2.6) \times 10^{-7}$[e]
IT2228	λ*recA1202 umuC*$^+$	0.22 ± 0.01	$(3.1 \pm 0.5) \times 10^{-6}$
IT2687	λ*recA1201 umuC122*::Tn5	<0.01 (2/11), 0.02 (2/11) 0.03 (1/11), 0.04 (2/11) 0.05 (1/11), 0.06 (2/11) 0.08 (1/11)	$(9.1 \pm 2.8) \times 10^{-8}$
IT2006	λ*recA1201 umuC*$^+$	0.11 ± 0.01	$(5.8 \pm 1.1) \times 10^{-8}$

[a] All strains shown in Tables 2 to 5 contain the chromosomal Δ*recA306* allele. Phenotypes: *recA1202*, Prt^c Rec$^+$; *recA1201*, Prt^c Rec$^-$; *rec*$^+$(UV), Prt^c Rec$^+$.

[b] The repair sector was measured by Weigle reactivation relative to strain IT2659 (*umuC122*::Tn5 Δ*recA306*) which was the reference strain for $W = 0$. Phage S13 was irradiated to a survival S_b between 6.2×10^{-8} and 2.8×10^{-7}. All plates were incubated at 32°. For IT2682, the repair sector of 42 single-cell isolates was measured; the number of individual isolates represented by the same value of W is given as the numerator in the parentheses. For IT2687, 11 single-cell isolates were studied.

[c] M9-CAA cultures were grown from single colonies to approximately 4×10^8 in 1 ml at 32°. For determination of the Rif^r frequency, the cells were then diluted and plated on LB agar + rifampin at 32° for 24 to 30 hr. Each value was determined from at least six cultures. ND, not determined.

[d] The UV dose was the optimum fluence for SOS induction of 50 J/m^2.

[e] Determined as the average of Rif^r mutant frequencies from six of the original independent lysogenic colonies. From the 42 independent isolates tested for Weigle reactivation, isolates with $W = 0.02$ and 0.22 were further examined for their Rif^r frequencies, and they were $(3.9 \pm 0.2) \times 10^{-8}$ and $(3.4 \pm 0.2) \times 10^{-6}$.

values of W. Thus some of the *recA1202*(Prt^c) *umuC122*::Tn5 strains seemed to harbor a relatively stable Tn5 insertion.

Excision and transposition appear to have been linked; whenever we observed one we also observed the other. One might dispute the existence of linkage by arguing that the same result would have been seen if first transposition had occurred by a replicative mechanism that did not lead to the loss of the Tn5 at the original donor site; subsequently there could have been an independent excision event with partial or full restoration of *umuC* function. This argument appears to fail for two reasons. (i) If an independent excision event is so likely that it would always occur after, but independently of, a transposition event, we would expect it to frequently occur before a transposition event; in that case the cell would no longer be Kan^r. Our observation that the cells always retained their Kan^r is not consistent with that. (ii) In our examination of the 11 of the original 42 clones derived from *umuC122*::Tn5, a multiple number (average = 2.6) of Kan^r P1 transductants were examined in the case of each of those 11 clones. In every case the multiple transductants were identical with regard to whether Kan^r was linked or unlinked to the *umuC* gene. In other words, none of the 11 donor cells showed evidence of having a Tn5 element at more than one distinct place in the cell. That strengthens the conviction that transposition was practically always linked to excision without destruction of the donor DNA.

From the values of W and the cotransduction of Kan^r and UmuC$^-$, we can make a rough estimate of what fraction of the 42 clonal populations had experienced Tn5 excision. If Tn5 excision had occurred in all but 11 cases, the latter being those with $W = 0.02$ (6 cases) and half of those with the borderline value $W = 0.03$ (5 cases), the excision must have occurred in about 70% of the cells in the three cultures from which the 42 clones were isolated. That is a remarkably large proportion of the population. The *umuC* gene was left in a variety of states by the excision events, which may have been perfect in two of the 42 isolates tested, and less than perfect in all the rest.

The protease but not the recombinase activity of the RecA protein was essential for Tn5 excision, as demonstrated by the reversion of *umuC122*::Tn5 in the λ*recA1201*(Prt^c Rec$^-$) lysogen IT2687 (Table 2). This *recA* mutant has by one measure about 30% of the protease activity of *recA1202*, but only 10^{-4} of its recombinational activity (Tessman and Peterson 1985a,b). Yet in a study of the *recA1201* strain that was parallel to the one done on *recA1202*, excision with restoration of some *umuC* function occurred in about nine of 11 single colonies tested. For the *recA1201* allele in IT2006 the maximum value of W was only 0.11 because of the weaker protease activity. This is 50% of the maximum value (0.22) obtained for IT2228 (Table 2). Therefore the values of W for single colonies derived from IT2687 can be compared to the IT2682 values by introducing a factor of 1/2.

Thus, a nonfunctional *umuC* gene derived from IT2687 should only yield the value $W \leq 0.01$, while the value $W = 0.02$ would indicate a partly functional *umuC* gene comparable to what $W = 0.04$ indicated in the case of IT2682. Similarly, the maximum value $W = 0.08$ is probably equivalent to the value 0.16 for IT2682, indicating a restoration of much, but not all, of *umuC* function.

Roughly 80% of the single colonies derived from IT2687 showed some *umuC* function, a frequency comparable to that of strain IT2682. Evidently even the substantially weaker protease strength characteristic of the *recA1201* allele was about as effective in stimulating excision as the stronger *recA1202* mutant, a result that will be seen to be paralleled in the effect on transposition. The increased frequency of Rif^r mutations confirmed that there was some restoration of *umuC* function in IT2687. The mutation frequency of IT2687 was limited by the lower protease activity of the *recA1201* allele (TESSMAN and PETERSON 1985b), but it was intermediate, as expected, between the Rif^r frequencies for IT2675 and IT2006 (Table 2).

We tested whether the UmuC$^+$ cell had a significant growth advantage over the UmuC$^-$ cell when both contained a *recA*(Prt^c) allele. That could provide a trivial explanation for the high frequency of cells in which the Tn*5* had been excised from the *umuC* gene. However, by a comparison of colony sizes, a stable *umuC36* λ*recA1202* mutant (IT3098) was indistinguishable in growth rate from an isogenic *umuC*$^+$ λ*recA1202* (IT2228) cell whether they were grown on M9-CAA or on LB agar.

Transposition accompanied excision: Although there was no selection for Kan^r, all 42 single-cell isolates derived from the λ*recA1202*(Prt^c Rec$^+$) strain IT2682 and all 11 isolates derived from the *recA1201*(Prt^c Rec$^-$) strain IT2687 (Table 2) were scored as Kan^r. This proved that transposition of Tn*5* practically always accompanied its excision in cells with constitutive RecA protease activity; RecA recombinase activity was apparently not needed.

Effect of RecA(Prt^c) on Tn*5* transposition: A quantitative assay of Tn*5* transposition was obtained by determining the fraction of λ prophages containing Tn*5*. This was measured by thermally inducing λ at 45° and then determining the fraction of the released λ particles that could subsequently transduce Kan^r into EST1122, *i.e.*, the number of Kan^r transductants per newly formed lysogen. It should be noted that the λ prophages provided two quite separate functions. One function was to serve as a carrier for introducing different *recA* alleles into *E. coli*. The other function was to serve as a target to measure the frequency of transposition.

The frequency of Tn*5* transposition in a *recA1202*(Prt^c Rec$^+$) mutant increased approximately 100-fold when compared with transposition in a *recA*$^+$ strain (Table 3). The frequency was not significantly reduced in the weaker constitutive protease strains, namely those bearing the *recA1211*(Prt^c Rec$^+$) and *recA1201*(Prt^c Rec$^-$) alleles (TESSMAN and PETERSON 1985a,b). Induction of the activated RecA state (RecA*) by UV irradiation of the λ*recA*$^+$ strain did not markedly increase the transposition frequency above the spontaneous value. Growth of strain IT2675 in mitomycin C (2 μg/ml) increased the transposition frequency by at most 2.5-fold (our unpublished data); this is insignificant compared to the effect of the *recA*(Prt^c) alleles.

Semiquantitative analysis of the transposition frequency: If Tn*5* transposes in all cells and inserts at random into other sites in the chromosome, then the frequency of observing the Tn*5* in a functional λ prophage should be the ratio of the nonessential λ DNA to the rest of the nonessential chromosomal DNA. Only if the Tn*5* inserted into a nonessential region of the λ DNA could the prophage be induced to produce phage that could subsequently transduce Kan^r to the recipient strain (EST1122). The λ prophage region for the *recA* strains is about 46 kbp in length, and approximately one-fourth (11 kbp) is nonessential (COURT and OPPENHEIM 1983), including 1.1 kbp of the *recA* gene. The *E. coli* chromosomal DNA is 4700 kbp long [p. 14 in (NEIDHARDT, INGRAHAM and SCHAECHTER 1990)]. We assume that under our conditions of growth very roughly two-thirds of the *E. coli* DNA is nonessential [p. 465 in (NEIDHARDT, INGRAHAM and SCHAECHTER 1990)]; precision in this number is not needed for our crude calculations. Thus, among the viable transpositions, the fraction that would have landed in a nonessential region of the λ genome should have been about 3×10^{-3} (11/3100), which agrees well with the observed value (Table 3).

We now calculate T, the transposition frequency per generation per cell formed. It can be calculated from f_o, the fraction of a total population of N cells in which no transposition has occurred. We will derive two formulas for T that are mathematically nearly equivalent. (i) Let U_g be the number of cells in which after g generations there has been no transposition. Then $U_g = 2U_{g-1}(1 - T)$. Since $U_o = 1$, it follows that $U_g = 2^g(1 - T)^g$. If the culture is grown to a population of N cells, then $2^g = N$ and $f_o = U_g/N = (1 - T)^g$. Thus, $T = 1 - f_o^{1/g}$, where $g = \log_2 N$. (ii) The average number of transpositions in the lineage of any cell after g generations is gT. From the Poisson distribution approximation, the probability of zero transpositions occurring in any lineage is e^{-gT}. Thus $T = (-\ln f_o)/g$. If $f_o^{1/g}$ is close to 1 then the two formulas for T are approximately equal.

TABLE 3

Effect of *recA*(Prt^c) mutations on transposition of Tn*5* from *umuC122*::Tn*5* into a λ prophage

Donor strain	Relevant genotype and phenotype[a]	Frequency of transposition into λ prophage[b]	Relative frequency
IT2675	λ*recA*$^+$	$(3.9 \pm 0.7) \times 10^{-5}$	1
IT2682	λ*recA1202*(Prt^c Rec$^+$)	$(3.9 \pm 1.6) \times 10^{-3}$	100 ± 44
IT2683	λ*recA1211*(Prt^c Rec$^+$)	$(2.5 \pm 0.9) \times 10^{-3}$	64 ± 26
IT2687	λ*recA1201*(Prt^c Rec$^-$)	$(3.4 \pm 1.1) \times 10^{-3}$	87 ± 32
IT2675(UV[c])	λ*recA*$^+$(RecA*)	$(2.7 \pm 0.4) \times 10^{-5}$	0.69 ± 0.16

[a] Prt^c, constitutive protease activity; Rec$^+$, wild-type recombination frequency in a mating with an Hfr strain; Rec$^-$, recombination frequency 100 times lower than the wild-type frequency; RecA*, RecA protease activated by damaged DNA.

[b] The frequency of Tn*5* transposition into the donor λ prophage was measured by induction of the phage and lysogenization of EST1122. The transposition frequency equals the frequency of Kan^r colonies per λ lysogen.

[c] See footnote *d* in Table 2.

We can only crudely estimate T because we do not know f_o accurately. But for the *recA1202* strain we have already suggested that $f_o \simeq 11/42$. For $g = 32$, both formulas yield $T \simeq 0.042$ as the number of transpositions per generation per cell formed, and $gT \simeq 1.3$ transpositions in the 32 generation lineage of a cell. These values are probably lower limits (see DISCUSSION).

It was necessary to use the special f_o method to calculate T because the frequency of transposition was so high. The much lower frequency in *recA*$^+$ cells allowed a simpler calculation. The frequency of transposition into the λ prophage was only about 4×10^{-5}. We have already calculated the probability of a randomly transposing Tn*5* landing in a nonessential region of the prophage to be about 3×10^{-3}. Therefore the total number of transpositions Tg in the lineage of a cell must be $4 \times 10^{-5}/(3 \times 10^{-3}) = 0.013$; therefore $T = 0.013/32 = 4.0 \times 10^{-4}$, which is 105 times smaller than in the *recA1202* cell.

High frequency of Tn*5* transposition from a λ DNA donor: When the *umuC*::Tn*5* λ*recA1202* strain IT2682 was grown in M9-CAA and plated on M9-CAA-XGal agar, 99.8% of the colonies were dark blue indicating that nearly all the prophages retained the *recA*(Prt^c) allele. The prophage was thermally induced in LB broth and the released phage was used at an moi of 0.01 to lysogenize EST1122 in M9-CAA medium; Kan^r transductants were selected on M9-CAA-XGal agar plates containing the antibiotic. To rule out the unlikely possibility that EST1122 would be transformed with Tn*5* DNA when simultaneously infected with an intact λ particle (KAISER and HOGNESS 1960), particularly in the presence of a RecA* protein (VERICAT, GUERRERO and BARBÉ 1988), the λ lysate was pretreated with pancreatic DNase. The low moi ensured single infection. The frequency of Kan^r cells among lysogens of EST1122 was 1.7×10^{-3}; the frequency was essentially the same (2.0×10^{-3}) when a sample of the lysate that had not been treated with DNase was used.

Once again it is important to note that the λ was serving a dual role; it was not only a vector for the Tn*5* element, but it also carried the *recA*(Prt^c) allele into the recipient, which originally had a Δ*recA* genotype. Because of their newly acquired Prt^c phenotype, EST1122 lysogens that formed dark blue colonies would be expected to show enhanced transposition of the Tn*5* from the infecting phage to the bacterial chromosome. Evidence for that would be the continued presence of the Tn*5* in the cell after the phage was eliminated. Fourteen independently isolated EST1122 lysogens that formed dark blue colonies were cured by the heat-pulse method to see if the Tn*5* had transposed. Twelve of the 14 lysogens remained Kan^r after curing, indicating that transposition to the bacterial chromosome had occurred; in the other two cases the cured cells were Kan^s.

To prove that the *recA1202* allele stimulated the transposition, a control experiment was performed with a λ*recA*$^+$ donor derived from strain IT2675. When this phage was used to lysogenize EST1122, all 18 Kan^r transductants that were examined became Kan^s when subsequently cured of their prophage by the heat-pulse method. This showed, as expected, that there had been no further transposition of the Tn*5* in the *recA*$^+$ cell. Thus, by comparison with the effect of the *recA*$^+$ allele, the *recA1202* allele greatly enhanced transposition. We do not know how many different sites in the λ genome were involved nor do we know at what stage in the infection process the transposition occurred. Since transposition apparently failed to occur in two of the 14 λ*recA1202* Kan^r lysogens, it seems that there may be some relatively stable Tn*5* insertions even in a *recA*(Prt^c) cell.

Location of the transposed Tn*5* in the λ prophage: An aliquot of the λ lysate made by thermal induction of the IT2682 lysogen, but which had not been treated with DNase, was used at an moi of 1 to lysogenize EST1122 in LB broth. Growth in broth was beneficial because, as described in MATERIALS AND METHODS, it provides selective pressure favoring pro-

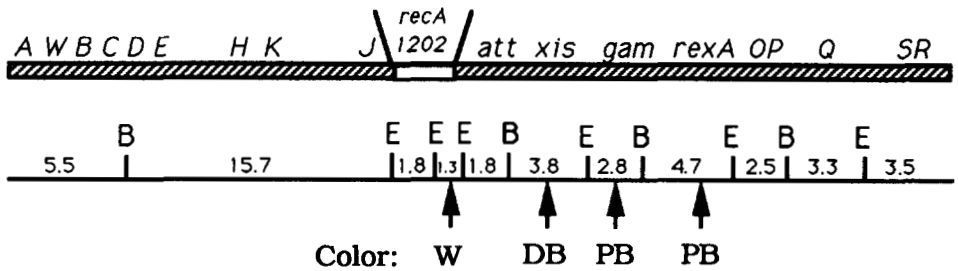

FIGURE 1.—Map of Tn*5* insertion sites in lambda IT75 restriction fragments: E, *Eco*RI; B, *Bam*HI. Color of Kan^r transductants of EST1122: W, white; DB, dark blue; PB, pale blue. ↑, site of Tn*5* insertion. The lengths between restriction sites are designated in kbp.

phages that had previously undergone proximal mutagenesis in the *recA*(Prt^c) gene, which would eliminate the Prt^c phenotype; lysogens containing those prophages produce pale blue or white colonies on the M9-CAA-XGal plates. Those cells would no longer display a high Tn*5* transposition frequency, and consequently the Tn*5* would be frozen at its location at the time the proximal mutagenesis occurred. Kan^r transductants subsequently selected on M9-CAA-XGal plates containing kanamycin displayed a distribution of colony colors: dark blue (66%), pale blue (33%) and white (0.6%). Cells that made pale blue or white colonies were far more sensitive to UV than those that made dark blue colonies (data not shown), indicating that the new colony color was indeed due to a mutation in the *recA1202* gene rather than one in the *lexA* or *dinD1*::Mu d1(*lac*) gene.

Nineteen of these Kan^r EST1122 transductants, 7 dark blue, 11 pale blue and 1 white, were cured of their λ. All formerly pale blue or white transductants became Kan^s after being cured, indicating that the Tn*5* had remained in the phage, as expected. Only one of the seven formerly dark blue transductants became Kan^s, the other six becoming Kan^r, which was consistent with our observation that the intact λ*recA1202* gene did indeed stimulate transposition of the Tn*5* element from its various locations in the λ genome to new sites in the chromosome at some stage after introduction of the phage into EST1122. It will be seen that the white Kan^r transductant contained, as expected, a Tn*5* that had transposed into the *recA* gene, rendering it completely inactive, thus trapping the transposon there.

To determine the location of the Tn*5* more specifically, we performed a restriction endonuclease analysis of λ DNA isolated from six of the 19 Kan^r transductants of EST1122, including three dark blue, two pale blue, and one white colony former (Figure 1). The λ DNA from the two pale blue transductants contained the Tn*5* near the λ*gam* gene and in the λ*rexA* gene, while the λ from the white transductant contained the Tn*5* in the λ*recA* gene. λ DNA from two dark blue transductants showed no sign of the transposon. But one λ isolated from a dark blue transductant was expected to contain the Tn*5* because the transductant subsequently lost its Kan^r when cured of the λ; that λ was found to contain the Tn*5* within the λ*xis* gene.

Dependence of Tn*5* transposition on LexA function and an additional role for RecA(Prt^c) proteins: One role of the *recA*(Prt^c) gene in Tn*5* transposition might well be cleavage of the LexA repressor; that would lead to induction of the genes that by definition make up the SOS regulon. This possibility was tested with two *lexA* mutations, *lexA3*(Ind$^-$) and *lexA51*(Def). A *lexA3*(Ind$^-$) strain makes a noncleavable LexA repressor that prevents the SOS genes from being induced even in the presence of a *recA*(Prt^c) gene. In the *lexA3*(Ind$^-$) *recA*(Prt^c) strains IT2953 and IT2959 the transposition frequencies of Tn*5* did not increase very much above that in the *lexA3 recA*$^+$ strain IT2947 (Table 4), implying that the greatly enhanced transposition of Tn*5* in *recA*(Prt^c) cells is under the control of one or more SOS genes; it is worth emphasizing that one of those SOS genes could be *recA* itself.

The SOS genes become constitutively derepressed in *lexA51*(Def) cells. When those cells contained either the *recA*$^+$ (IT2689) or Δ*recA* (IT3117) allele, the transposition frequency of Tn*5* was higher compared to that in the isogenic *lexA*$^+$ strain IT2675 (Table 4). Those results suggest that the great increase in the transposition frequency of Tn*5* can be attributed to the derepression of LexA-regulated genes. However, although the frequency of Tn*5* transposition was elevated in the *lexA*(Def) λ*recA*$^+$ strain IT2689, it was not as high as that in the *lexA*$^+$ λ*recA1202*(Prt^c) strain IT2682 (Table 3). Furthermore, the frequencies of Tn*5* transposition in *lexA*(Def) strains were raised even higher if they also contained a *recA*(Prt^c) allele rather than either a *recA*$^+$ or Δ*recA* allele (Table 4). Taken together, these results imply that RecA(Prt^c) proteins may play an additional role in increasing the Tn*5* transposition frequency besides its role in cleaving the LexA repressor. We suggest that this additional role may be to mediate the cleavage of the transposition inhibitor protein and possibly also a transposase precursor; the latter cleavage might be needed to make the active form of the transposase.

TABLE 4

Effect of *lexA* mutations on transposition of Tn5 from *umuC122*::Tn5 into prophage λ

Donor strain	Genotype and phenotype	Frequency of transposition into λ prophage[a]	Relative frequency[b]
IT2947	*lexA3*(Ind⁻) λ*recA*⁺	$(2.3 \pm 0.2) \times 10^{-5}$	1
IT2953	*lexA3*(Ind⁻) λ*recA1202*(Prt^c Rec⁺)	$(6.2 \pm 0.3) \times 10^{-5}$	2.7 ± 0.3
IT2959	*lexA3*(Ind⁻) λ*recA1201*(Prt^c Rec⁻)	$(5.8 \pm 0.1) \times 10^{-5}$	2.5 ± 0.2
IT2675	*lexA*⁺ λ*recA*⁺	$(3.9 \pm 0.7) \times 10^{-5}$	1[c]
IT2689	*lexA51*(Def) λ*recA*⁺	$(1.2 \pm 0.3) \times 10^{-4}$	3.1 ± 0.9
IT3117	*lexA51*(Def) λΔ*recA*(Prt⁻ Rec⁻)	$(1.1 \pm 0.2) \times 10^{-4}$	2.8 ± 0.7
IT2692	*lexA51*(Def) λ*recA1202*(Prt^c Rec⁺)	$(4.1 \pm 0.9) \times 10^{-3}$	105 ± 29
IT2696	*lexA51*(Def) λ*recA1201*(Prt^c Rec⁻)	$(3.8 \pm 0.7) \times 10^{-3}$	97 ± 10

[a] Calculated as in Table 3.
[b] Relative to *recA*⁺ allele taken as 1.
[c] IT2675 data from Table 3.

TABLE 5

Effect of *recA*(Prt^c) mutations on frequency of Tn5 transposition from *lexA71*::Tn5 and *glnA21*::Tn5 into λ prophage

Donor strain	Tn5	*recA* genotype	Frequency of transposition into λ prophage[a]	Relative frequency[b]
IT2873	*lexA71*::Tn5	λ*recA*⁺	$(6.3 \pm 1.3) \times 10^{-5}$	1
IT2863	*lexA71*::Tn5	λ*recA1202*	$(1.12 \pm 0.10) \times 10$	18 ± 4
IT2869	*lexA71*::Tn5	λ*recA1201*	$(6.6 \pm 0.4) \times 10^{-4}$	10 ± 2
IT2743	*glnA21*::Tn5	λ*recA*⁺	$(6.4 \pm 0.1) \times 10^{-6}$	1
IT2747	*glnA21*::Tn5	λ*recA1202*	$(1.83 \pm 0.05) \times 10^{-5}$	2.9 ± 0.5
IT2741	*glnA21*::Tn5	λ*recA1201*	$(1.48 \pm 0.33) \times 10^{-5}$	2.3 ± 0.7

[a] Calculated as in Table 3.
[b] Relative to IT2873 and IT2743.

It follows that the derepression of the *recA*(Prt^c) gene that occurs when LexA is cleaved would likely promote transposition of Tn5. But the derepression of yet another gene under LexA control may also be involved. That is because the *lexA*(Def) mutation increased transposition in a λ*recA*⁺ lysogen (IT2689), which has relatively little constitutive protease activity, and even in a Δ*recA* strain (IT3117). We suggest that the Tn5 transposase gene may be the additional SOS gene (see DISCUSSION).

Transposition of Tn5 located in the *lexA* and *glnA* genes: We also measured the transposition of Tn5 elements located in the *lexA* and *glnA* genes (Table 5). In *lexA71*::Tn5 strains, the *recA*(Prt^c) mutations λ*recA1202* and λ*recA1201* enhanced the transposition of Tn5 by approximately 15-fold compared with transposition in an isogenic λ*recA*⁺ strain. Transposition from *glnA21*::Tn5 was enhanced even less, increasing by only threefold in *recA*(Prt^c) strains IT2747 and IT2741 over that that in the *recA*⁺ strain IT2743. The enhanced transposition of Tn5 (and presumably its excision) is apparently dependent on location. The frequency may be sequence-specific or gene-specific. In the λ*recA*⁺ reference strain IT2873, the transposition from the *lexA71* site might have been aided by the LexA⁻ phenotype.

DISCUSSION

The enhanced rate of excision and transposition of Tn5 under conditions of *recA*(Prt^c) constitutive induction of the SOS regulon is the only known case for any bacterial mobile element of a response to the SOS system. The activated RecA protein displayed at least two roles in this response. An obvious one was to turn on the SOS genes by the proteolytic cleavage of LexA. But there was a second role, and it presumably also required the RecA proteolytic function inasmuch as the RecA recombinase function was not required. Most, if not all, of the Tn5 excisions were accompanied by transposition, and most, if not all, of the transpositions were accompanied by excision. By contrast, in either a RecA⁺ or RecA⁻ cell, as well as in most other genetic backgrounds, excision occurs completely independently of transposition (BERG 1977; EGNER and BERG 1981). Significantly, in most, if not all, cases the donor molecule was not destroyed when Tn5 transposition from the *umuC* gene was stimulated by the *recA*(Prt^c) allele; thus we have no reason to suggest the formation of a lethal gap in the donor molecule (BERG 1989).

Activation of the RecA⁺ protein and induction of the SOS regulon by momentary exposure to UV irradiation or continuous exposure to mitomycin C failed to increase either excision or transposition of Tn5 by a substantial amount. This failure could be due to the limited availability of activated RecA proteins when they are induced by DNA damage; most of the activated proteins may be thoroughly engaged in repairing the damages, leaving relatively few molecules to stimulate Tn5 excision and transposition. The situation is different for the RecA(Prt^c) proteins because they are activated in the absence of DNA damage and therefore may be more available to carry

out excision and transposition functions. In contrast to the RecA$^+$ protein, the Prt^c mutants can be activated by an enlarged number of cofactors; they can use other nucleoside triphosphates besides ATP and dATP, and they can use RNA in addition to single-stranded DNA (WANG *et al.* 1988; WANG, TESSMAN and TESSMAN 1988). Therefore, it is conceivable that the activated Prt^c proteins may differ in some significant aspect from RecA$^+$ protein that has been activated by exposure of the cell to DNA damaging agents. A further possibility is that DNA damage may have additional effects that work to inhibit excision and transposition.

An effect of RecA on excision has previously been suggested in relation to mutations in *recB* and *uvrD* that have been found to enhance the excision of Tn*10* and Tn*5* (LUNDBLAD *et al.* 1984; LUNDBLAD and KLECKNER 1985); this enhancement is lost in cells with *recA* mutations. Discussions of this phenomenon have centered on the role of RecA in homologous recombination, a function that appears not to be relevant to our observations.

Constitutively activated RecA(Prt^c) proteins stimulated the excision and transposition of Tn*5* from the *umuC* gene by at least 100 fold compared to RecA$^+$ controls. Excision was detected by two assays for *umuC*$^+$ function: spontaneous mutation to Rif^r and Weigle reactivation of UV damaged phage S13. If we allow for an error of ±0.01, the several values of the repair sector (W) implied that the excision of the Tn*5* left the *umuC* gene in one of at least four distinguishable states, corresponding to W = 0.03, 0.05, 0.16 and 0.22 (Table 2), the latter value suggesting full *umuC*$^+$ function. We conclude that excision can occur with a variable degree of precision.

Some descendants of *recA1202 umuC*::Tn*5* isolates appeared to contain a stabilized Tn*5* at or near the original location. The stable strains showed a small, but finite, repair sector (0.020), which could be due either to the ability of the RecA* protein to function independently of the UmuC protein, or to some slight residual UmuC activity. The Lac$^+$ phenotype of these stable strains indicated that the cells had retained their Prt^c phenotype, which in turn raises the question of how the Tn*5* element was able to evade excision. Since the stability of Tn*5* appears to depend on location, perhaps the Tn*5* had transposed to a new but nearby location conferring greater stability.

Direct evidence for the enhanced transposition of Tn*5* was provided by a comparison of the rate of Tn*5* transposition to a λ DNA target in *recA*$^+$ and *recA*(Prt^c) mutant strains (Table 3). Physical confirmation of the transposition into the phage genome was obtained by restriction endonuclease analysis (Figure 1). Additional evidence of Tn*5* transposition was the fact that all 42 single colonies derived from three cultures of IT2682 (*umuC*::Tn*5* *recA1202*; Table 2) were still Kan^r even though excision had occurred in about 70% of the cells; this also proved that transposition practically always accompanied excision.

When a *umuC122*::Tn*5* strain was lysogenized with any one of three λ*recA*(Prt^c) mutants and then grown for approximately 32 generations, the Tn*5* was found to have transposed into the prophage region of the chromosome at a frequency consistent with random transposition in nearly every cell. In agreement with this is our estimation that 1.3 transpositions occurred during the entire lineage of each cell. It is unlikely that transposition occurred immediately after introduction of the *recA1202* gene; that would be inconsistent with the existence of a variety of excisants with different UmuC$^+$ activities. Excision and transposition may have occurred at a more or less constant rate all through the growth of the culture.

The Tn*5* element was found in the λ prophage about 100 times more frequently in the *recA*(Prt^c) strain than in the *recA*$^+$ control strain. From that value we calculated the transposition frequency, but it was a minimum estimate because the factor of 100 is close to what would be achieved by just one random transposition in every cell. Thereafter, that factor would only rise slowly because most additional transpositions would remove Tn*5* elements as often as they would add them; exceptions would be the occasional transpositions into the λ*recA*(Prt^c) gene, which would terminate the SOS effect and trap the Tn*5* in the prophage. The transposition frequency was also derived from the fraction of clones that did not transpose (f_0 method); a crude calculation showed that there were about 1.3 transpositions in the 32 generation lineage of each cell. This too is a minimum estimate because the failure to transpose may really be due to a separate population of stable *umuC*::Tn*5* alleles that inflate the value of the zero term (f_0) in the Poisson distribution of the number of Tn*5* transpositions in the entire cell lineage. Both minimum estimates yielded high values in the range of 0.03 and 0.04 transpositions per generation per cell formed.

The *recA1202*(Prt^c) allele enhanced transposition of the Tn*5* from unspecified locations in the λ genome at some stage in the infection process. This was demonstrated by thermally inducing λ*recA1202* in strain IT2682 where some of the prophages would contain the Tn*5* as a result of enhanced transposition from the *umuC* gene; the phage were then used to transduce Kan^r into strain EST1122. Most of the transductants made dark blue colonies on XGal plates, indicating that the λ contained an intact *recA1202* gene. Although the Kan^r was introduced into EST1122 by the phage, ten of 12 dark blue transductants that were tested retained their Kan^r when subsequently cured of the prophage, proving that the Tn*5* had moved

from the phage to a bacterial location. The Tn5 did not so readily move from the phage when a λ*recA*$^+$ lysogen (IT2675) was the source of the transducing phage; transduction of Kan^r then occurred at a low frequency (Table 3), and when the cells were subsequently cured of the prophage they lost the Kan^r too, indicating that the Tn5 element had remained in the prophage after infection. It was, therefore, the *recA*(Prt^c) allele, and not simply the infection process, that was responsible, at least in part, for the high frequency of Tn5 transposition from the infecting λ particle.

The extent to which a *recA*(Prt^c) gene stimulates transposition depends on the location of the Tn5; evidence of that was the lower frequency of transposition from the *lexA71*::Tn5 site and an even lower frequency from the *glnA21*::Tn5 site (Table 5), compared to the frequency from *umuC122*::Tn5 or from various λ::Tn5 sites. We observed additionally that relatively stable λ::Tn5 insertions may also exist. Since transcription of the *glnA* gene was repressed under our experimental conditions, it is worth considering that the frequency of transposition may be directly related to the level of transcription.

The enhanced rate of excision and transposition in the RecA(Prt^c Rec$^-$) strain IT2687 (Table 2 and 3) showed that the protease and not the recombinase activity of RecA was responsible. The high rate of transposition was apparently due in part to the inactivation of the LexA repressor; in a *lexA3*(Ind$^-$) strain, which makes a noncleavable repressor, the stimulatory effect of the *recA*(Prt^c) mutation was blocked (Table 4). This confirms that one role of the RecA(Prt^c) protein in excision and transposition of Tn5 is derepression of the SOS regulon.

What activity regulated by the LexA repressor might control excision and transposition? We will see that RecA(Prt^c) activity itself may play a direct role. That may not be the entire story, however, because transposition was elevated in a *lexA51*(Def) strain even when it contained the *recA*$^+$ or Δ*recA* allele (Table 4); this suggests that derepression of some SOS gene other than *recA* might also be involved.

The RecA(Prt^c) protein, for its part, appears to have another role to play in enhancing transposition besides its role in mediating the cleavage of the LexA repressor. That became evident when the *recA*(Prt^c) allele was seen to increase transposition even in the *lexA*(Def) strain, where the SOS regulon is constitutively derepressed without any need for RecA cleavage of LexA (Table 4). The additional role of the activated RecA protein in enhancing transposition could quite possibly be to mediate the cleavage of another repressor or an inhibitor.

Candidates for another SOS gene and for another RecA* target readily come to mind. An argument can be made that the LexA protein regulates the transcription of the Tn5 transposase gene and that the RecA* protease activity regulates the cleavage of the transposition inhibitor. Tn5 transposition is largely controlled by two IS*50*R encoded proteins, p1 and p2 (ISBERG, LAZAAR and SYVANEN 1982; JOHNSON, YIN and REZNIKOFF 1982). p1 is the transposase, encoded by the *tnp* gene, which acts preferentially in *cis* to promote Tn5 transposition; p2, encoded by the *inh* gene, can act in *trans* to inhibit transposition. The relative amounts of p1 and p2 should determine in part the Tn5 transposition frequency (JOHNSON and REZNIKOFF 1984; YIN and REZNIKOFF 1988).

We have found a potential LexA binding site that resembles a consensus sequence (WALKER 1984); the site extends suggestively from base −29 to −14 in the promoter region of the *tnp* gene:

tnp sequence: CTTTcccgttttcCAG

Consensus sequence: CTGTatata-a-aCAG

We found no similar site in the promoter region of the *inh* gene. If it is indeed repressed by the LexA protein, the *tnp* gene would be, by definition, a member of the SOS regulon.

If the RecA* activity mediated cleavage of the transposition inhibitor, then the combination of the RecA* protein and the cleaved LexA protein would result in less p2 and more p1, which would be doubly effective in increasing the transposition frequency and might explain our observations. This theory contains an interesting complication arising from the fact that p2 is encoded by a DNA sequence nested within the coding region for p1 and in the same reading frame. If p2 is cleaved, would p1 also be cleaved, and would that eliminate transposase activity? One possible solution might be that its N-terminal extension protects p1 against proteolytic cleavage; more intriguing however, is the possibility that p1 is only the precursor of the active transposase, the latter being produced by cleavage of p1. That would be analogous to the case of the UmuD protein, which is cleaved by RecA* to form the active UmuD′ fragment (BURCKHARDT *et al.* 1988; NOHMI *et al.* 1988; SHINAGAWA *et al.* 1988). If the C-terminal fragment obtained from the postulated cleavage of p1 is the active transposase, then cleavage of p2 might also produce the active transposase inasmuch as the C-terminal product should be identical with that produced from p1.

The close proximity of the *recA*(Prt^c) gene to the Tn5 element when both are in the λ genome could contribute to the high rate of transposition that we observed when a λ*recA*(Prt^c) particle was used to transduce the Tn5 element; that proximity would help to bring the activated RecA protein into contact with p1 and p2. There is a relevant precedent for proximal effects of *recA*(Prt^c) genes (LIU and TESSMAN 1990).

Transposition of Tn*5* even in *recA*$^+$ cells is, in part, under SOS control. This was established by the observation that transposition was threefold higher in the *lexA51*(Def) strain IT2689 (Table 4) relative to its value in the *lexA*$^+$ strain IT2675 (Table 3). We can draw an important conclusion from the fact that the *recA*(Prt^c) strains IT2692 and IT2696 (Table 4) contributed approximately a 30-fold further increase in the transposition frequency: the additional role of RecA mentioned above is even more important than its role in effecting the cleavage of LexA. More specifically, RecA-mediated cleavage of the transposition inhibitor and cleavage of a transposase precursor may provide the main control of transposition; LexA might not be a strong repressor of the transposase gene, possibly in keeping with the failure of the putative LexA binding site to match any of the seven bases comprising the secondary part of the consensus sequence. The same line of reasoning suggests that inactivating the LexA repressor enhances transposition of Tn*5* primarily through amplification of the RecA protein.

It is believed that the SOS response is momentarily triggered spontaneously in a small proportion of cells by chance activation of the RecA protein. Such activation is thought to produce spontaneous induction of the λ prophage (LITTLE 1990); it could play a similar role in Tn*5* transposition. However, to explain the observations that spontaneous transposition is *recA*-independent (BERG 1977; CALOS and MILLER 1980; KLECKNER 1981), we would have to assume that there also exists a *recA*-independent mechanism that normally masks the effect of the rare SOS response.

It has been suggested that the donor molecule is destroyed after being vacated by the Tn*5* element in the process of transposition (BERG 1989). Experimental support for that view is lacking, however. In contrast, we have shown here that when Tn*5* transposed via an SOS pathway from its location in the *umuC* gene, the donor molecule appeared to survive in most, if not all, cases; moreover, the survival did not depend on RecA$^+$-mediated homologous recombination.

Under our constitutive SOS conditions, most of the excision events restored some function to the vacated gene; those events were always linked to transposition. But when the SOS system is repressed, that linkage is usually absent. We call attention, however, to previous indications that some linkage does still exist: when *lacZ*::Tn*5* cells revert to LacZ$^+$, for example, 1% of those events seem to be accompanied by transposition (BERG 1977). That rare linkage of perfect or quasi-perfect excision with transposition in a *recA*$^+$ cell might be explainable in terms of chance induction of the SOS response.

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