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Induction of the SOS Response in *Escherichia coli* Inhibits Tn*5* and IS*50* Transposition

MICHAEL D. WEINREICH, JOHN C. MAKRIS,† AND WILLIAM S. REZNIKOFF*

Department of Biochemistry, University of Wisconsin, 420 Henry Mall, Madison, Wisconsin 53706

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In response to DNA damage or the inhibition of normal DNA replication in *Escherichia coli*, a set of some 20 unlinked operons is induced through the RecA-mediated cleavage of the LexA repressor. We examined the effect of this SOS response on the transposition of Tn*5* and determined that the frequency of transposition is reduced 5- to 10-fold in cells that constitutively express SOS functions, e.g., *lexA*(Def) strains. Furthermore, this inhibition is independent of *recA* function, is fully reversed by a wild-type copy of *lexA*, and is not caused by an alteration in the levels of the Tn*5* transposase or inhibitor proteins. We isolated insertion mutations in a *lexA*(Def) background that reverse this transposition defect; all of these mapped to a new locus near 23 min on the *E. coli* chromosome.

Tn*5* is a composite transposon consisting of two 1.5-kb IS*50* elements (termed IS*50*R and IS*50*L) oriented as inverted repeats flanking a unique central region that encodes resistance to kanamycin, bleomycin, and streptomycin (Fig. 1) (for a review, see reference 1). IS*50*R encodes two proteins: P1, the transposase that is absolutely required for transposition, and P2, which acts to inhibit transposition by an unknown mechanism (14, 18, 57). P2 is translated in the same reading frame as P1 but lacks the N-terminal 55 amino acids of P1 (23). The analogous proteins of IS*50*L, P3 and P4, are prematurely truncated in the carboxy-terminal region by an ochre (UAA) codon and have no known role in transposition (41). The minimum sequence required at the ends for full transposition proficiency is 19 bp (15, 42) for both Tn*5* and IS*50*. Tn*5* transposition utilizes two outside ends (OE), and IS*50* transposition utilizes one OE and one inside end (IE) (Fig. 1). The base sequences at the OE and IE are not entirely identical, and this results in different frequencies of transposition for IS*50* and Tn*5* (29, 42).

A variety of host proteins are known that influence the frequency of Tn*5* transposition. These include Dam (55), DnaA (56), integration host factor (IHF) (30), HU (52), DNA polymerase I (43, 48), SulA (44), and gyrase (13). These proteins are proposed to fall into two classes: (i) those that regulate transposition (e.g., Dam) and (ii) those that act mechanistically at some step in the transposition process (probably DnaA). It is important to investigate host involvement in this process to gain an understanding of how transposition is regulated and also to identify key proteins that would be required for a detailed in vitro analysis of transposition. For instance, IHF was determined through genetic and biochemical analyses to be involved in λ integration (32); it is also involved in a number of transposition reactions (9). Similarly, HU was determined to be involved in Mu transposition (7, 12), and it has been recently shown by immunoelectron microscopy (26) to participate with MuA protein in transpososome assembly at the Mu ends before nicking and strand exchange. There certainly may be other, undefined host functions that influence transposition; since SOS induction generates such a distinct state of DNA metabolism and repair, we wanted to determine whether this altered physiology had any effect on Tn*5* transposition.

Induction of the SOS system in *Escherichia coli* occurs in response to agents that damage DNA or that interfere with normal DNA replication (for a review, see reference 50). In addition to the induction of enzymes that effect DNA repair and the increased expression of LexA itself, this response also results in the induction of lambdoid prophages (40), a transient inhibition of cell division (11), a phenomenon termed SOS mutagenesis (51), and the increased expression of *himA* (33), which encodes a subunit of IHF. This complex response results from cleavage of the global SOS repressor, LexA, facilitated by an activated form of RecA (27). LexA is normally bound to specific DNA sequences termed SOS boxes and coordinates the transcriptional repression of the SOS genes (2, 28, 53). Slight variations in each binding site determine the strength of the LexA interaction and, hence, the degree of repression (53). The SOS-inducing signal is most probably made up of extended regions of single-stranded DNA. Induction also requires ATP or dATP (6). When RecA-ATP binds to single-stranded DNA, it presumably undergoes a conformational change that activates it for cleavage of LexA (27). Recently, Sassanfar and Roberts (45) showed that, in fact, DNA replication is required for the generation of the SOS signal after exposure to agents that cause lesions in the DNA. This has been interpreted to mean that the replication machinery bypasses the lesion and reinitiates replication downstream, generating an interval of single-stranded DNA.

We investigated whether any aspect of the SOS response would influence the transposition frequency of a resident Tn*5* element. Using different genetic means to induce SOS and several independent transposition assays, we found that induction of the SOS response causes a significant decrease in the frequency of Tn*5* and IS*50* transposition. We then show that this defect in transposition can be reversed by any one of several mini-Mu insertions near 23 min on the *E. coli* map.

MATERIALS AND METHODS

Bacterial strains. All strains are listed in Table 1. The *dam-13*::Tn*9* mutation was transferred via P1*vir*-mediated

* Corresponding author.
† Present address: Genentech Inc., South San Francisco, CA 94080.

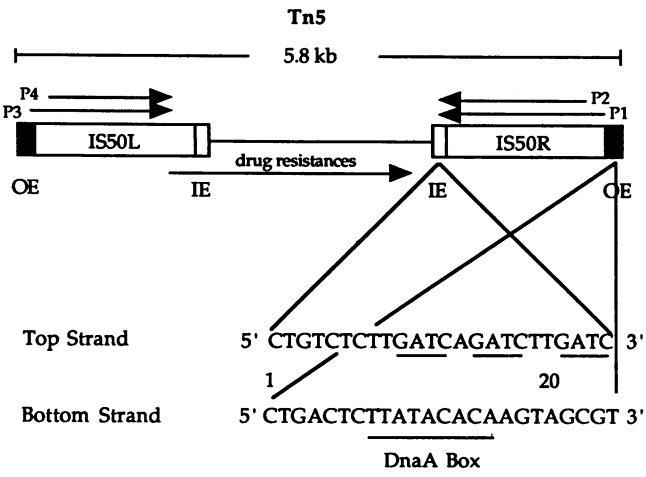

FIG. 1. Functional organization of Tn5. The arrows represent the coding regions for the proteins of Tn5. Shown in detail are the 24 bases at the OE and IE in the 5'-to-3' direction. Dam methylation sites are underlined at the IE, as is the DnaA site at the OE.

transduction into DM2550 and DE190 to generate JCM200 and JCM201. The *lexA3*(Ind$^-$) mutation was similarly transduced from DE1238 into DM2550 and DE190 by selecting for the linked *malB*::Tn*9* (Cm^r) and screening for colonies that were sensitive to 0.5 μg of mitomycin C per ml, generating MDW26 and MDW27, respectively. The chromosomal Tn5 insertions were P1 transduced into DM2550 and DE190 from D38.21 (*ilvD*::Tn5) (55), CBK105 (W3110 *thy trpD*::Tn5), or CBK130 (W3110 *thy proC*::Tn5). CBK105 and CBK130 were

TABLE 1. Bacterial strains

Strain	Relevant genotype	Source
DM2550	Δ(*lac-gpt*)*6 rpsL supD43 thi-1 ilu*(Ts)	D. Mount
DE190	DM2550 *lexA51*(Def) *sulA211*	D. Mount
DE1238	DM2550 *recA730 sulA211 lexA3*(Ind$^-$)-*malB*::Tn*9*	D. Mount
DM2568	DE190 *recA*$^+$ *srl*::Tn*10*	D. Mount
DM2572	DE190 *recA430 srl*::Tn*10*	D. Mount
GW1000	Δ(*lacZ169*) F$^-$ *thr-1 leu-6 his-4 arg-33 galK2 strA31 ilu*(Ts) *recA441 sulA211*	G. Walker
GW1001	GW1000 *lexA71*(Def)::Tn*5*	G. Walker
RZ 47.01	Δ(*lac-pro*) *ara rpsL thi-1*/pOX*gen*38	Laboratory collection
RZ224	Δ(*lac-pro*) *ara polA thi-1* Spc^r *rpsL* Nal^r λ^r	Laboratory collection
JCM200	DM2550 *dam-13*::Tn*9*	This study
JCM201	DE190 *dam-13*::Tn*9*	This study
MDW26	DM2550 *lexA3*(Ind$^-$)-*malB*::Tn*9*	This study
MDW27	DM2550 *lexA3*(Ind$^-$)-*malB*::Tn*9* *sulA211*	This study
MDW266	DM2550 *ilvD*::Tn*5*	This study
MDW268	DE190 *ilvD*::Tn*5*	This study
MDW321	DM2550 *trpD*::Tn*5*	This study
MDW322	DE190 *trpD*::Tn*5*	This study
MDW323	DM2550 *proC*::Tn*5*	This study
MDW324	DE190 *proC*::Tn*5*	This study
MM13	DE190 *zcc-13*::MudII4041	This study
MM23	DE190 *zcc-23*::MudII4041	This study
MM34	DE190 *zcc-34*::MudII4041	This study
MM64	DE190 *zcc-64*::MudII4041	This study
λ psulA	*sulA*::*lacZ* c*Iind att*$^+$	J. Little

obtained from Claire Berg. Transduction with Mu lysates was essentially as described previously (4).

To ensure that strains had obtained the *dam-13*::Tn*9* allele, plasmid DNA was prepared and checked for sensitivity to *Mbo*I cleavage and resistance to *Dpn*I cleavage.

Plasmids. Plasmids pRZ1495 and pRZ1496 were described previously (29). They are Tn5 derivatives that contain a promoterless *lacZ* gene (replacing IS*50*L) fused in frame to an OE. This structure, along with a wild-type IS*50*R, brackets a tetracycline resistance cassette to form the OE-OE construct pRZ1495. pRZ1496 differs by having an IE cassette replacing the OE of IS*50*R; this allows one to monitor IS*50*-type transposition events, that is, events utilizing one OE and one IE. Transposition of these constructs is determined by the papillation assay (see below). pRZ1495 was ultimately derived from pRZ102, which has a Tn5 insertion in the colicin E1 gene (which is SOS regulated) and is *cea imm*$^+$ *kil* (19). pRZ1495 has 500 bp of heterologous DNA toward the 5' end of the colicin E1 gene from the Tn5 insertion point, and transcription from the *lexA*-regulated promoter may not impinge on the Tn5 element. However, since this was a concern, we also measured papillation from a Tn*5lac* insertion in pOX*gen*38 (17) to verify that the papillation effect we describe was not dependent on context.

pRZ102 contains a wild-type Tn5 insertion in ColE1 (19). pRZ901 and pRZ910 contain carboxy-terminal protein fusions of *lacZ* to the P1 and P2 proteins (55). pRZ910 has a methionine-to-isoleucine change at the N-terminal amino acid of P2 and produces only the P1 β-galactosidase fusion protein. pRZ4716 is pRZ1495 with a deletion between the *Not*I and *Nhe*I sites of IS*50*R, creating an Tn*5lac* element that does not encode functional transposase but can be complemented in *trans* to transpose. pOX385 contains the Tn*5lac* transposon from pRZ1495 inserted into pOX*gen*38 by a transposition event. It confers resistance to tetracycline, gentamicin, and spectinomycin and is phenotypically Lac$^-$. pATT20 contains the *Eco*RI-*Hin*dIII fragment of pJWL184 inserted into pGB2 (5, 48a). This construct has wild-type *lexA* under the control of the *lacUV5* promoter. Since it contains a pSC101 origin, pATT20 exists at about five copies per cell.

β-Galactosidase assays. All β-galactosidase assays were performed essentially as described by Miller (34), except that chloroform and 0.1% sodium dodecyl sulfate were used to permeabilize the cells.

Transposition assays. Transposition was measured by two independent means: a papillation assay (24) or a mating-out assay (18).

The papillation assay was performed as described by Krebs and Reznikoff (24). In this method, the appearance of Lac$^+$ papillae on an otherwise Lac$^-$ colony represents transposition events of the Tn5 or IS*50* element into an actively transcribed gene in the proper frame and orientation to produce hybrid β-galactosidase fusion proteins. Briefly, strains containing either pRZ1495 or pRZ1496 were grown overnight in Luria broth containing 15 μg of tetracycline per ml. Dilutions were plated onto lactose-MacConkey agar also containing tetracycline to give 20 to 40 colonies per plate. For each strain, at least five colonies on each of three plates were monitored for the accumulation of papillae over several days, and the average number of papillae per colony was then plotted as a function of time. The standard deviation was determined to be within 10 to 15%. Quantitative comparisons between strains in any particular experiment gave roughly the same differential rate over the time course of the assay. However, we routinely chose time points between 72

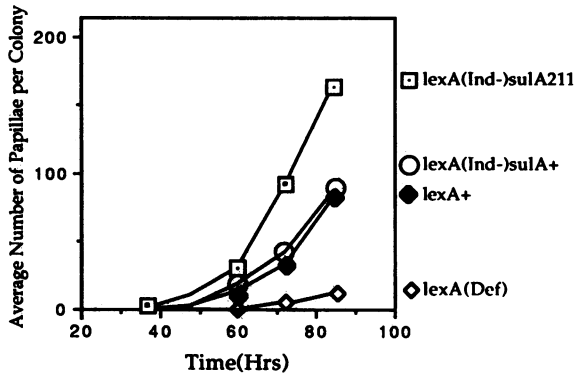

FIG. 2. Effect of SOS expression on Tn*5* transposition: papillation of the Tn*5* (OE-OE) construct pRZ1495 on lactose-MacConkey plates. The *lexA*(Def) strain is DE190, the *lexA* wild-type strain is DM2550, the *lexA3*(Ind$^-$) *sulA*$^+$ strain is MDW26, and the isogenic *sulA211* strain is MDW27. DE190 also differs from DM2550 by containing the *sulA211* allele; however, this allele is not responsible for the decrease in transposition (Fig. 3A; see the text). The top curve represents papillation in DE190, which has been transduced to *lexA3*(Ind$^-$), thus, repression of the SOS system reverses the transposition defect exhibited in SOS-induced cells.

and 96 h for the comparative differences cited in the text. Each experiment was repeated at least two times with similar results.

The doubling time of each strain containing these plasmids was approximately the same for each isogenic series grown in LB containing 15 μg of tetracycline per ml, so differences in the rate of papillae accumulation between strains are not due to different growth rates of the strains being compared.

Since plating of Mu transduction cultures of DE190 directly onto lactose MacConkey agar gave poor yields, transduction mixtures were diluted and plated onto glucose-minimal Miller medium (34) containing 0.3% Casamino Acids, 40 μg of 5-bromo 4-chloro-3-indoyl-β-D-galactoside per ml, and 0.05% phenyl-β-D-galactoside (PG plates) also containing 20 μg of kanamycin and 15 μg of tetracycline per ml. Papillation then was measured as above.

The mating-out assay was performed by the method of Johnson et al. (18).

RESULTS

Tn*5* and IS*50* transposition is decreased in *lexA*(Def) strains. To investigate the effect of SOS expression on transposition, we utilized a *lexA*(Def) strain that constitutively expresses SOS functions. The papillation assay was used to quantitate this effect. In this method, the accumulation of Lac$^+$ papillae on a Lac$^-$ colony over time is used as a measure of the transposition frequency (24). The papillation constructs replace IS*50*L with a promoterless *lacZ* gene, which also lacks translational signals, fused in frame with either an IE or an OE. When this element transposes into an actively transcribed gene in the correct frame and orientation, hybrid β-galactosidase proteins are produced, giving rise to red papillae on lactose-MacConkey agar. With this assay, the level of Tn*5lac* transposition from pRZ1495 shows a 5- to 10-fold decrease in a strain that expresses SOS functions constitutively versus the wild-type parent [Fig. 2, *lexA*(Def) versus *lexA*$^+$]. Identical results were also obtained when the Tn*5lac* construct was present on pOX*gen*38 (17), a single-copy F derivative, and so this reduction in transposition is independent of both Tn*5* copy number and the context of the insertion. Furthermore, this decrease in transposition is also seen for IS*50* (see Fig. 5B). The data included in Fig. 2 also suggest that wild-type SulA is inhibitory to Tn*5* transposition (see below).

In an independent assay, a wild-type Tn*5* insertion in ColE1 also shows the same effect as that determined with the papillation assay. In this method, the pOX*gen*38 episome serves as the target for transposition, and the number of exconjugants that have Tn*5* insertions divided by the total number of exconjugants obtained represents the transposition frequency. A fivefold decrease in transposition occurs in the *lexA*(Def) strain compared with that in the wild type (Table 2). A trivial explanation for this would be that the ColE1 plasmid is unstable in the *lexA*(Def) strain and is lost at a high frequency during the mating. However this is ruled out because the plasmid used shows identical stability in the two strains in the absence of selection (52).

Tn*5* insertions examined in additional contexts show that this phenomenon is a general one. The transposition frequencies of three different chromosomal Tn*5* insertions were determined by the mating-out assay in the *lexA*$^+$ and *lexA*-(Def) backgrounds. Table 2 shows that insertions in *trpD* and *proC* exhibit three- and fivefold reduced frequencies, respectively, in the SOS-induced strain compared with the wild type. However, the Tn*5* insertion in the *ilvD* locus showed identical transposition rates in both genetic backgrounds. We cannot explain this difference at present; however, since this is the only exception we have found to the general behavior of Tn*5* and IS*50*, we believe that this represents some sequence-specific or positional effect of the insertion (see Discussion). Taken together, these results suggest that LexA itself plays a positive role in Tn*5* and IS*50* transposition, that the induction of an SOS gene product acts to inhibit transposition, or that an SOS-independent function acts to inhibit transposition in the presence of SOS proteins.

TABLE 2. Mating-out frequencies showing effect of SOS on Tn*5*

Strain	Donor of Tn*5*	Transposition frequency[a]	Relative frequency
DM2550 (LexA$^+$)	pRZ102	$(2.5 \pm 0.7) \times 10^{-5}$	1.0
DE190 [LexA(Def)]	pRZ102	$(5.5 \pm 2.5) \times 10^{-6}$	0.2
MDW321 (LexA$^+$)	*trpD*::Tn*5*	$(1.5 \pm 0.3) \times 10^{-4}$	1.0
MDW322 [LexA(Def)]	*trpD*::Tn*5*	$(5.0 \pm 2.0) \times 10^{-5}$	0.3
MDW323 (LexA$^+$)	*proC*::Tn*5*	$(3.0 \pm 1.1) \times 10^{-4}$	1.0
MDW324 [LexA(Def)]	*proC*::Tn*5*	$(5.4 \pm 2.9) \times 10^{-5}$	0.2
MDW266 (LexA$^+$)	*ilvD*::Tn*5*	$(4.1 \pm 0.8) \times 10^{-5}$	1.0
MDW268 [LexA(Def)]	*ilvD*::Tn*5*	$(3.5 \pm 1.5) \times 10^{-5}$	0.9

[a] Transposition frequencies represent the average of four measurements and have been confirmed on different days with similar results.

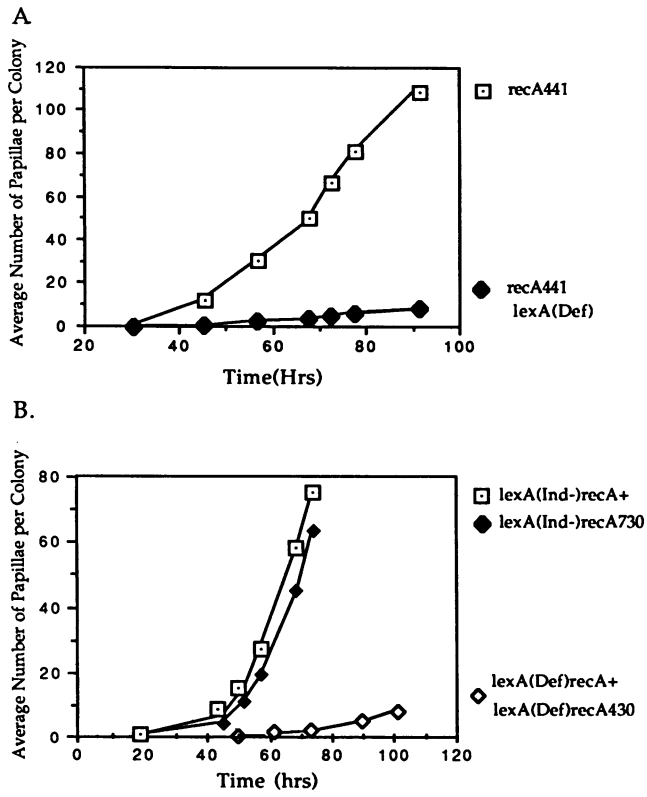

FIG. 3. Effect of RecA proteolysis on Tn5 transposition. (A) Papillation of the Tn5 construct pRZ1495 in strains GW1000 *recA441* and GW1001 *recA441 lexA71*(Def) at 32°C. Both strains contain the *sulA211* allele, showing that it is the absence of LexA that causes the transposition defect. (B) Tn5 papillation in DE1238 *recA730 lexA3*(Ind$^-$) and MDW27 *recA*$^+$ *lexA3*(Ind$^-$) (□, ◆), showing that constitutive expression of RecA proteolysis without SOS induction does not alter Tn5 transposition. Similarly, the curve showing Tn5 papillation in DM2568 *recA*$^+$ *lexA*(Def) and DM2572 *recA430 lexA*-(Def) (◇) indicates that *recA* proteolysis is not required for the SOS-dependent inhibition of transposition.

RecA protease activity does not directly influence Tn5 transposition. To examine the role of RecA in this phenomenon, we investigated the effect of several *recA* alleles that alter the proteolytic function of RecA. At elevated temperatures (>40°C) the RecA441 protein (3) is constitutively activated for LexA cleavage; however, its proteolytic activity is elevated over that of the wild type even at 30°C (54). A comparison of a *recA441 lexA*$^+$ strain with the isogenic *lexA71*(Def) strain at 32°C shows the same characteristic decrease in transposition due to constitutive expression of the SOS system (Fig. 3A). This confirms the previous observation in an independent genetic background. This proteolytic activity is not required for the transposition decrease, however, as shown by the following experiment. The RecA430 protein is severely impaired in its proteolytic activity (8). A comparison of Tn5 papillation in a *lexA*(Def) background containing wild-type *recA* versus the *recA430* mutant reveals no difference in Tn5 transposition rates; i.e., they are transposing at the same low rate characteristic for SOS-induced cells (Fig. 3B). This strongly suggests that an activated form of RecA is not required for the decrease in Tn5 transposition other that to induce SOS by cleavage of LexA.

Further evidence that the proteolytic activity of *recA* does not influence Tn5 transposition is shown by examining another *recA* allele, *recA730*. The RecA730 protein is constitutively activated for its protease activity regardless of the temperature, yet it has normal recombination activity. A comparison of DE1238 (*recA730*) versus MDW27 (*recA*$^+$) reveals no significant differences in transposition (Fig. 3B). In these experiments, the *lexA3*(Ind$^-$) allele is present to ensure that the SOS response is not induced by RecA. Thus, the proteolytic activity of RecA is being examined independently of SOS induction, and we detect no influence of this activity on transposition.

Wild-type *lexA* is able to reverse the transposition defect in a *lexA*(Def) strain. Since strains that constitutively express SOS functions are somewhat mutagenic (8, 47, 50), it was necessary to show that the effect on Tn5 transposition involved LexA itself and was not due to not some unknown mutation elsewhere on the chromosome. This was studied by introducing a functional copy of *lexA* into DE190 and then measuring transposition frequencies to see whether the transposition phenotype of the *lexA*(Def) strain could be reversed. DE190 transduced to *lexA3*(Ind$^-$) no longer is defective in transposition (Fig. 2); thus, repression of the SOS system reverses the transposition defect. We also transformed plasmid pATT20 into wild-type and *lexA*(Def) strains. This plasmid has wild-type *lexA* cloned into the low-copy-number, pSC101-derived plasmid pGB2. The transposition frequencies in these strains are now identical (52). Thus, the reduction in transposition seen in the *lexA*-(Def) strain is due to the absence of LexA itself.

Effect of *lexA3*(Ind$^-$) and *sulA211* alleles on Tn5 transposition. We also determined whether the noninducible *lexA3*(Ind$^-$) mutation had any effect on Tn5 transposition, because perhaps a transient induction of SOS is required after transposition events. The LexA3 protein has a change from Gly-Ala to Gly-Asp at the peptide bond that is subject to proteolysis and is resistant to cleavage (31). Isogenic strains that are either *lexA*$^+$ or *lexA3*(Ind$^-$) show no changes in transposition frequencies [Fig. 2; *lexA*$^+$ versus *lexA*(Ind$^-$) *sulA*$^+$]. Thus, even if Tn5 transposition events cause damage to the DNA (e.g., double-stranded breaks), SOS induction is not required for cell survival after transposition.

Induction of *sulA* is not responsible for the transposition decrease seen in our *lexA*(Def) strains, since they are also SulA$^-$ (see below). SulA is an SOS protein that inhibits cell division during SOS induction (10, 11). Constitutive expression of *sulA* results in lethal filamentation, and *lexA*(Def) strains are not viable in combination with this wild-type SulA activity (35). This is relevant because an earlier report by Sasakawa et al. (44) showed that SulA was inhibitory for Tn5 transposition and that overproduction of SulA caused a further reduction in the transposition frequency. This was presumed to be due to its inhibition of cell division, since the addition of DL-pantoyl lactone to the medium, thus bypassing this cell division block by inducing septation directly, reversed the SulA-dependent inhibition of transposition. A comparison of MDW26 and MDW27, which differ only in *sulA* alleles, indeed reveals a difference in transposition. The *sulA211* strain shows a modest twofold increase (determined by papillation and mating out) over the isogenic *sulA*$^+$ strain (Fig. 2). This suggests that even the low basal level of SulA in wild-type cells is inhibitory to Tn5 transposition.

The *lexA*(Def) mutation does not significantly influence IS*50* protein expression. We investigated whether the transposition decreases seen in the *lexA*(Def) strain could be explained by alterations in the expression of the transposase (P1) or the inhibitor (P2). Figure 4 shows the activities of

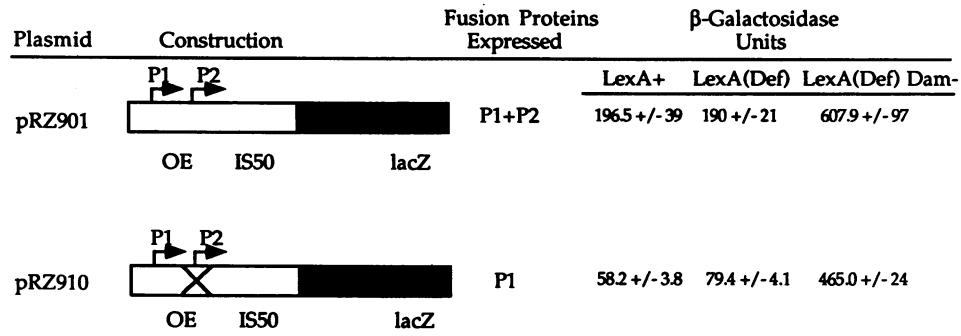

FIG. 4. Effect of *lexA*(Def) on IS*50* gene expression. β-Galactosidase was assayed as described by Miller (34); the data represent averages of quadruplicate measurements. Plasmid pRZ901 expresses the β-galactosidase fusion proteins for both P1 and P2, whereas pRZ910 only expresses the P1 fusion protein. The *lexA*$^+$ strain is DM2550, the *lexA*(Def) strain is DE190, and the *lexA*(Def) *dam-13* strain is JCM201.

β-galactosidase fusions to the carboxy termini of P1 and P2. There are only small changes in P1 and P2 protein expression in the *lex*(Def) strain compared with those in the wild type. Furthermore, since the ratio of transposase to the inhibitor (P1/P2 ratio) is the crucial quantity for determining transposition frequency (16) and since this ratio is slightly increased in the *lexA*(Def) strain (Fig. 4), it can be concluded that LexA is not exerting its effect by altering the expression of the transposition proteins.

The SOS induction effect is dominant to *dam*. Methylation of a GATC site in the P1 promoter inhibits its activity (55). Under Dam$^-$ conditions, the activity of this promoter is enhanced and transposase levels rise relative to those of the inhibitor, causing an increase in transposition. We wanted to test whether increased transposase levels could overcome the SOS-dependent inhibition of transposition. We confirmed that the transposase levels rise approximately fivefold relative to the inhibitor in the *lexA*(Def) *dam-13* strain compared with the *lexA*(Def) strain (Fig. 4). Figure 5A shows Tn*5* papillation data for strains that contain *lexA*(Def) and *dam-13* mutations. The *dam lexA*$^+$ strain shows the increase in transposition that is characteristic of increased transposase expression. However, inactivation of Dam in a *lexA*(Def) background does not alter the frequency of transposition, and thus the SOS effect is dominant to the increases seen under *dam* conditions.

This dominance is also seen for IS*50* transposition (Fig. 5B). Inactivation of Dam in a *lexA*(Def) strain shows only a small increase in papillation compared with the large (100- to 1,000-fold) increase known to occur for IS*50* in *dam* strains. This large *dam*-mediated increase in transposition is due to elevated transposase expression and also to increased use of an unmethylated IE, which contains two GATC Dam methylation sites (29, 55). Thus, IS*50* behaves analogously to Tn*5*, both in the transposition decrease in SOS-induced cells and in the dominance of this effect to *dam* conditions (although the dominance is not complete for IS*50*).

Isolation of mutations that restore the wild-type transposition phenotype. We assumed that either an SOS gene product or some other *E. coli* protein was acting to inhibit Tn*5* transposition under conditions of SOS induction and that by knocking out this gene we could reverse the transposition defect in a *lexA*(Def) host, so we randomly mutagenized DE190 containing pRZ1495 by using mini-MudII4041 (4) and screened insertion mutants (kanamycin-resistant colonies) for increased papillation. We obtained six such mutants in an initial screen of ~1,200 colonies and verified that the increase was host associated. These insertions were P1 transduced back into the original *lexA*(Def) background in case multiple insertions had occurred and retested for the increased transposition phenotype. One of the mutations was not associated with the Mu element and was not studied further. That these mutants were still *lexA*(Def) was determined by lysogenizing them with a λp*sulA*::*lacZ* operon fusion and measuring the β-galactosidase levels. The SulA gene is derepressed approximately 50- to 100-fold in a *lexA*(Def) background (11), and this transcriptional fusion is therefore a sensitive measure of the level of LexA in the cell. Four of the mutants were still *lexA*(Def) by this criterion (Table 3), and the papillation profiles for these (Fig. 6)

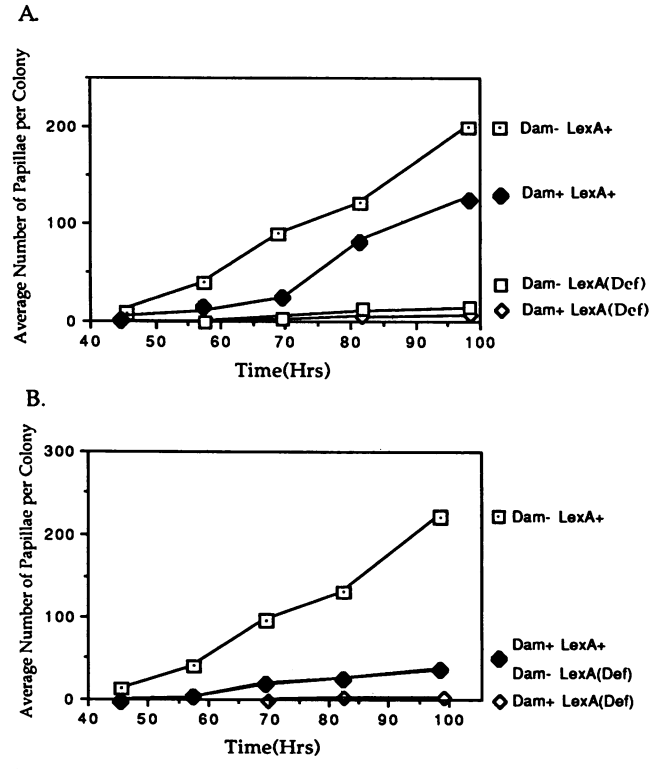

FIG. 5. Dominance of *lexA*(Def) to *dam* for Tn*5* and IS*50* transposition. (A) Tn*5* papillation in the following strains: JCM200, Dam$^-$ LexA$^+$; DM2550, Dam$^+$ LexA$^+$; DE190, Dam$^+$ LexA(Def); and JCM201, Dam$^-$ LexA(Def). (B) IS*50* transposition in the same strains. The IE-OE papillation construct used was pRZ1496.

TABLE 3. Map positions of pseudorevertants

Insertion	% Linkage to Tn*10* (22.25 min)	β-Galactosidase activity[a] of λp*sulA*::*lacZ*
MM13	54 (188/350)[b]	2,152
MM23	52 (213/409)	2,167
MM34	68 (204/300)	2,062
MM51	0 (0/100)	42.5
MM64	64 (269/420)	2,020
DE190 [LexA(Def)]	Not applicable	2,173
DM2550 (LexA$^+$)	Not applicable	31.6

[a] β-Galactosidase activities are in Miller units (34) and represent averages of three measurements. Standard deviations were 1 to 5%. All of the insertions are in *lexA*(Def) backgrounds except for MM51, which shows the wild-type repressed value for the *sulA* fusion phage.

[b] The numbers in parenthesis indicate Kan^s/Tet^r colonies used to calculate the P1 linkages.

indicate that transposition is increased 10- to 20-fold relative to the *lexA*(Def) parent strain. That this increase represents a transposase-dependent phenomenon and not an unusual recombination process is shown by the fact that when these strains are transformed with pRZ4716, a derivative of pRZ1495 that contains a 300-bp deletion in the transposase coding region but contains the end sequences intact, no papillation occurs over the same time course.

These four insertions (three of them independently isolated) were mapped by Hfr crosses and then by P1-mediated transduction (46) to near 22 min on the *E. coli* map (Table 3). The only known SOS gene in this region is *sulA*. However, these insertions are not in *sulA* for the following reasons: (i) a Tn*5* insertion in *sulA* is 25% linked with *zcc-282*::Tn*10*, whereas these pseudorevertants are 50 or 70% linked to this Tn*10*; (ii) a Tn*5* insertion in *sulA* does not alter the transposition phenotype in a *lexA*(Def) background (52), whereas these Mu insertions do; and (iii) if the Mu insertion were in *sulA* we would have been unable to obtain Kan^s (*sulA*$^+$) recombinants at a high frequency since these would be lethal in the *lexA*(Def) background.

We cloned DNA adjacent to one of these insertions (MM13) by selection for Kan^r clones of *Hin*dIII-digested chromosomal DNA ligated into pBR322. These clones all contained approximately 7.5 kb of chromosomal DNA. This

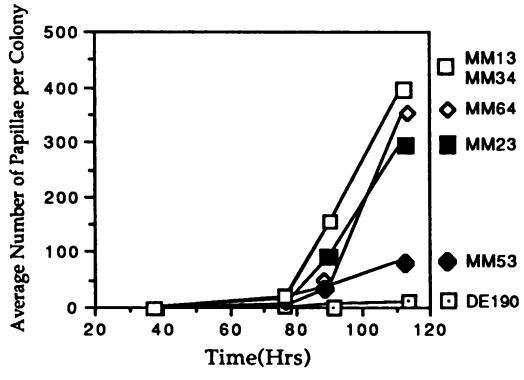

FIG. 6. Tn*5* papillation of mini-MudII4041 pseudorevertants. Each of the Mu insertions is in the DE190 *lexA*(Def) background and was assayed as in Fig. 2, except the plates were PG indicator plates instead of lactose-MacConkey plates (see Materials and Methods). (The same results are obtained with lactose-MacConkey medium.) MM53 is actually *lexA*$^+$ and probably resulted from a Mu insertion linked to *lexA*$^+$. It is shown for comparison.

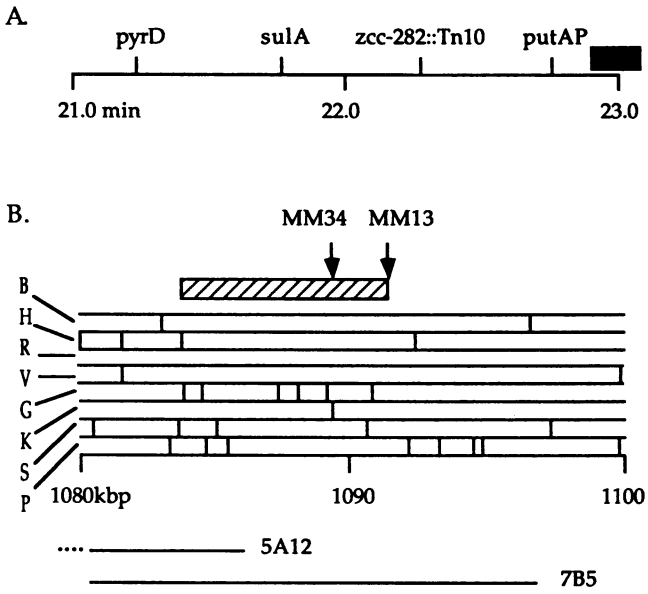

FIG. 7. Position of two Mu insertions on the genetic and physical maps. (A) Genetic map of the 21- to 23-min region of the *E. coli* chromosome showing selected markers. The black box represents the approximate region of the Kohara map shown below. (B) Kohara map of the 1,080- to 1,100-kbp region of the *E. coli* chromosome. The hatched box represents the DNA cloned adjacent to insertion MM13 to the nearest *Hin*dIII site, which also overlaps the DNA cloned from the MM34 insertion. Arrows indicate the approximate positions of the Mu insertions. Restriction enzymes: B, *Bam*HI; H, *Hin*dIII; R, *Eco*RI; V, *Eco*RV; G, *Bgl*I; K, *Kpn*I; S, *Pst*I; P, *Pvu*II. Lines below the figure indicate the DNA from this region present in the Kohara phages 5A12 and 7B5. We observed two *Eco*RV sites within the 10-kb *Hin*dIII fragment covered by our clones that do not appear on the published map, this was the only discrepancy.

DNA was sequenced beginning in the Mu R end, through the junction point, and included 100 bp of the adjacent chromosomal sequence. This 100-bp sequence was used to search the bacterial GenBank library with the FASTA algorithm (36) to determine whether this gene had been previously sequenced; no matches were found. This indicates that this gene has not been previously defined, at least not at the sequence level.

This cloned chromosomal DNA was used as a probe to localize this mutation on the *E. coli* physical map. DNAs from nine λ phages of the Kohara mini-set library spanning min 21 to 23 (21) were probed with this cloned DNA by dot blot hybridization. In addition, we similarly cloned a 5.4-kb chromosomal fragment adjacent to the insertion in MM34, which showed a slightly different linkage to the Tn*10* at min 22.3 and used this DNA to probe these same nine Kohara phages. The same two phages, 5A12 and 7B5, hybridized with both chromosomal probes. This information, together with a restriction analysis of the cloned chromosomal DNA (data not shown), allows us to localize these two Mu insertions to ca. 1,090 kbp (approximately 23 min) on the Kohara map. These data are presented in Fig. 7 along with a genetic map of the 21- to 23-min region.

DISCUSSION

We have shown that full induction of the SOS response in *E. coli* leads to a 5- to 10-fold decrease in the frequency of

Tn*5* transposition. This same general phenomenon holds true for IS*50*. This effect does not depend on the proteolytic activity of RecA, other than to cleave LexA and thereby induce the SOS response, since the decrease is seen in a *lexA*(Def) strain carrying the *recA430* allele, which is severely deficient in this proteolytic activity. Also, since the P1/P2 ratio is relatively unchanged in the SOS-induced background, inactivation of LexA or induction of some other SOS protein does not alter transposition by decreasing transposase expression the inhibitor (P2). This phenomenon is independent of the Tn*5* copy number and the sequence context of the Tn*5* insertion. The one exception to this is the chromosomal *ilvD*::Tn*5* insertion. Although we do not understand this anomaly, one difference is that the position of the insertion is quite close to the *E. coli* chromosomal origin of replication, whereas the other chromosomal insertions are much farther removed from *oriC*. Perhaps there is also some local sequence or conformational effect that is different for this insertion and that influences transposition.

We postulate that the decrease in transposition is due to the presence of an *E. coli* gene product that acts negatively at some step in the transposition pathway, as opposed to a direct involvement of LexA in Tn*5* and IS*50* transposition. This is a reasonable hypothesis for two reasons. First, a computer search revealed that there are no LexA binding sequences present in IS*50*, allowing up to four mismatches from the published consensus (53). If a looser consensus is used for the computer search, several possible LexA sites appear within IS*50*; however, these all have mismatches at highly conserved base pairs in the recognition sequence. One such weak site in the transposase promoter, for example, has (in addition to numerous mismatches at lesser conserved base pairs) a mismatch at position three, which is invariant in all LexA binding sites examined so far. Furthermore, we have shown that transposase levels are relatively unchanged in a *lexA*(Def) strain, which is inconsistent with an interaction of LexA at this site. Although these considerations do not rule out the possibility that LexA interacts directly with IS*50* DNA at some less conserved site, it seems unlikely. A direct interaction also seems unlikely because LexA is not known to participate in the higher-order DNA-protein structures necessary for transposition or other recombination events.

Second, mini-Mu insertion mutations in a *lexA*(Def) background have been isolated that increase transposition to levels above those of the wild type. These mutations were mapped to 23 min on the chromosome and most probably represent a newly identified gene(s). Since these insertions fall into two groups, one 54% linked and one 68% linked to a Tn*10* insertion at min 22.25, it is possible that these insertions fall within an operon. Perhaps a single downstream gene product is acting to inhibit transposition and the upstream Mu insertions are polar onto expression of this gene. Two of the Mu insertions have been shown to lie within about 2 kb of each other; however, this does not allow us to determine whether a single gene or than more than one gene is involved. These Mu insertions do not influence Tn*5* transposition in a wild-type background (52), which is consistent with this locus being under SOS control. Conclusive proof regarding LexA regulation will have to await cloning and characterization of the entire locus.

There are two host proteins induced in response to DNA damage that are already known to influence Tn*5* transposition. The *himA* gene, encoding one subunit of IHF, shows a small two- to fourfold SOS induction that is mediated through the LexA repressor (33). IHF plays a positive role in Tn*5* and IS*50* transposition but only under *dam* conditions; in a wild-type background, IHF has no influence on Tn*5* or IS*50* transposition (30). It seems unlikely, therefore, that the two- to fourfold increase in IHF levels after SOS induction is responsible for the decreases we detect in transposition. Recently, it has been suggested that DnaA levels also rise about fourfold upon exposure to DNA-damaging agents; however, the *dnaA* gene is not directly regulated by LexA since no increases in DnaA over wild-type levels were observed in a *lexA*(Def) background (37). DnaA is the initiator protein required for cellular DNA replication from *oriC*, and it also stimulates Tn*5* transposition 10- to 100-fold (56). Since we used *lexA*(Def) strains to study SOS derepression and these conditions were not reported to alter DnaA levels, it is unlikely that the phenomenon we describe is caused by altered DnaA expression. We would predict, however, that small increases in DnaA might in fact stimulate transposition if DnaA availability were a rate-limiting factor for Tn*5* transposition.

The dominance of SOS induction to that of the methylation effects on Tn*5* DNA is interesting. Since increasing the amount of transposase in a *lexA*(Def) background does not result in Tn*5* transposition increases, this suggests that the putative SOS inhibitor is acting at a step in transposition that comes after binding of the transposase to the ends or that it interferes with this initial binding. Transposase seems to have a much higher affinity for the unmethylated IE than for the methylated IE (22), and this results in at least a 100-fold increase in IS*50* transposition in *dam* cells. Since IS*50* does show a small increase in the *lexA*(Def) *dam* strain (reflecting increased IE activity?) and Tn*5* does not, one might argue that transposase still binds normally to the end sequences but is defective at a subsequent step in transposition. These explanations are highly speculative and can be investigated only when the biochemical identity of the inhibitor is known.

A recent report (25) claims that the proteolytic activity of RecA greatly increases the transposition frequency of Tn*5* and that the full effect also requires SOS induction. This is directly contrasted with our observations, because we detect no influence of RecA protease constitutive mutants on transposition and find that SOS induction causes a decrease in Tn*5* transposition. Kuan et al. observe that excision and transposition of Tn*5* from a *umuC*::Tn*5* insertion is greatly increased when these strains are lysogenized with a lambda phage containing *recA1202*, a protease constitutive mutant, compared with that in the *recA*$^+$ control. We believe that this represents a specialized phenomenon and is not generally true for Tn*5*. First, Kuan et al. note a tremendous variation in the stimulation of these excision events depending on the particular Tn*5* context: e.g., from 3-fold to 100-fold. Second, their strain background always contains a MudI(Ap *lac*) fusion in *dinD*, an SOS locus of unknown function (and therefore, the full complement of SOS functions is not induced in their strains); also, the most dramatic effect they report (from *umuC*::Tn*5*) represents an insertion in another SOS locus that might itself affect the response. Although we believe that Kuan et al. report an intriguing phenomenon, we believe that our observations represent the more generalized behavior for Tn*5* since we have observed the same phenomenon with five separate Tn*5* insertions in two independent genetic backgrounds. It is possible, however, that the context surrounding a particular Tn*5* insertion profoundly affects its transposition behavior under certain circumstances.

It is interesting to speculate about the utility the phenomenon we describe. The SOS system is normally induced in

response to challenges from the environment that would disrupt the integrity of the DNA of the organism. If the DNA damage is extensive enough, certain resident prophages are excised and undergo lytic development (40), as if to escape the impending death of the cell. However, a transposon cannot survive in the extracellular environment like, for instance, a mature phage particle can. So, for the case of Tn*5*, it seems that the bacterium has evolved a mechanism to even further decrease the likelihood of transposition, which under conditions of stress would most likely be undesirable. This is not a trivial point, because SOS induction also results in prolonged stable DNA replication that does not require coupling to the cell cycle and that can greatly increase the DNA content of the cell (20). This is relevant because IS*50* transposition events are probably coupled to the passage of a replication fork, during which the IE becomes transiently hemimethylated in a similar manner to that of IS*10* (38). When the IE of IS*50* is unmethylated the transposition frequency increases 100-fold, so the increased SOS replication activity might otherwise (i.e., in the absence of the SOS Tn*5* or IS*50* inhibitor) increase the movement of the IS*50* element.

In conclusion, it is a logical extension of our results to state that Tn*5* and IS*50* transposition is sensitive to adverse environmental stimuli via the coupling of the cellular SOS response. It is interesting to note that Roberts and Kleckner (39) found that Tn*10* transposition events caused a transient induction of the SOS response, presumably through the degradation of the gapped donor molecule (a product of conservative transposition), although SOS induction was not required for Tn*10* transposition. Furthermore, they reported that partial induction of the SOS response with mitomycin C did not significantly alter the frequency of transposition. It will be interesting to see whether other transposable elements in nature respond in a manner similar to (or opposite) that of Tn*5*.

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REFERENCES

1. **Berg, D. E.** 1989. Transposon Tn*5*, p. 185–210. *In* D. E. Berg and M. M. Howe (ed.), Mobile DNA. American Society for Microbiology, Washington, D.C.
2. **Brent, R., and M. Ptashne.** 1981. Mechanism of action of the *lexA* gene product. Proc. Natl. Acad. Sci. USA **78**:4204–4208.
3. **Castelaazi, M., J. George, and G. Buttin.** 1972. Prophage induction and cell division in *E. coli*. I. Further characterization of the thermosensitive mutation *tif-1* whose expression mimics the effects of UV irradiation. Mol. Gen. Genet. **119**:139–152.
4. **Castilho, B. A., P. Olfson, and M. J. Casadaban.** 1984. Plasmid insertion mutagenesis and *lac* gene fusion with mini-Mu bacteriophage transposons. J. Bacteriol. **158**:488–495.
5. **Churchward, G., D. Belin, and Y. Nagamine.** 1984. A pSC101-derived plasmid which shows no sequence homology to other commonly used cloning vectors. Gene **31**:165–171.
6. **Craig, N. L., and J. W. Roberts.** 1980. *E. coli recA* protein-directed cleavage of phage λ repressor requires polynucleotide. Nature (London) **283**:26–30.
7. **Craigie, R., D. Arndnt-Jovin, and K. Mizuuchi.** 1985. A defined system for the DNA strand-transfer reaction at the initiation of bacteriophage Mu transposition: protein and DNA substrate requirements. Proc. Natl. Acad. Sci. USA **82**:7570–7574.
8. **Ennis, D. G., B. Fisher, S. Edmiston, and D. W. Mount.** 1985. Dual role for *Escherichia coli* RecA protein in SOS mutagenesis. Proc. Natl. Acad. Sci. USA **82**:3325–3329.
9. **Friedman, D. I.** 1988. Integration host factor: a protein for all reasons. Cell **55**:545–554.
10. **George, J., M. Castellazzi, and G. Buttin.** 1975. Prophage induction and cell division in *E. coli*. III. Mutations *sfiA* and *sfiB* restore division in *tif* and *lon* strains and permit the expression of mutator properties of *tif*. Mol. Gen. Genet. **140**:309–332.
11. **Huisman, O., and R. D'Ari.** 1981. An inducible DNA replication-cell division coupling mechanism in *E. coli*. Nature (London) **290**:797–799.
12. **Huisman, O., M. Faelen, D. Girard, A. Jaffe, A. Toussaint, and J. Rouviere-Yaniv.** 1989. Multiple defects in *Escherichia coli* mutants lacking HU protein. J. Bacteriol. **171**:3704–3712.
13. **Isberg, R. I., and M. Syvanen.** 1982. DNA Gyrase is a host factor required for transposition of Tn5. Cell **30**:9–18.
14. **Isberg, R. I., A. L. Lazaar, and M. Syvanen.** 1982. Regulation of Tn5 by the right repeat proteins: control at the level of the transposition reactions? Cell **30**:883–892.
15. **Johnson, R. C., and W. S. Reznikoff.** 1983. DNA sequences at the ends of transposon Tn5 required for transposition. Nature (London) **204**:280–282.
16. **Johnson, R. C., and W. S. Reznikoff.** 1984. Role of the IS50R proteins in the promotion and control of Tn5 transposition. J. Mol. Biol. **177**:645–661.
17. **Johnson, R. C., and W. S. Reznikoff.** 1984. Copy number control of Tn5 transposition. Genetics **107**:9–18.
18. **Johnson, R. C., J. C. P. Yin, and W. S. Reznikoff.** 1982. Control of Tn5 transposition in *Escherichia coli* is mediated by protein from the right repeat. Cell **30**:873–882.
19. **Jorgenson, R. A., S. J. Rothstein, and W. S. Reznikoff.** 1979. A restriction enzyme cleavage map of Tn5 and location of a region encoding neomycin resistance. Mol. Gen. Genet. **177**:6047–6051.
20. **Kogoma, T., T. A. Torrey, and M. J. Connaughton.** 1979. Induction of UV-resistant DNA replication in *Escherichia coli*: induced stable DNA replication as an SOS function. Mol. Gen. Genet. **176**:1–9.
21. **Kohara, Y., K. Akiyama, and K. Isono.** 1987. The physical map of the whole *E. coli* chromosome: application of a new strategy for rapid analysis and sorting of a large genomic library. Cell **50**:494–508.
22. **Krebs, M. P.** 1986. Ph.D. thesis. University of Wisconsin, Madison.
23. **Krebs, M. P., and W. S. Reznikoff.** 1986. Transcriptional and translational initiation sites of IS50. J. Mol. Biol. **192**:781–791.
24. **Krebs, M. P., and W. S. Reznikoff.** 1988. Use of a Tn5 derivative that creates *lacZ* translational fusions to obtain a transposition mutant. Gene **63**:277–285.
25. **Kuan, C.-T., S.-K. Liu, and I. Tessman.** 1991. Excision and transposition of Tn5 as an SOS activity in *Escherichia coli*. Genetics **128**:45–57.
26. **Lavoie, B. D., and G. Chaconas.** 1990. Immunoelectron microscopic analysis of the A, B, and HU protein content of bacteriophage Mu transpososomes. J. Biol. Chem. **265**:1623–1627.
27. **Little, J. W., S. H. Edmiston, L. Z. Pacelli, and D. W. Mount.** 1980. Cleavage of the *Escherichia coli lexA* protein by the *recA* protease. Proc. Natl. Acad. Sci. USA **77**:3225–3229.
28. **Little, J. W., D. W. Mount, and C. R. Yanisch-Perron.** 1981. Purified *lexA* protein is a repressor of the *recA* and *lexA* genes. Proc. Natl. Acad. Sci. USA **78**:4199–4203.
29. **Makris, J. C., P. L. Nordmann, and W. S. Reznikoff.** 1988. Mutational analysis of insertion sequence 50 (IS50) and transposon 5 (Tn5) ends. Proc. Natl. Acad. Sci. USA **85**:2224–2228.
30. **Makris, J. C., P. L. Nordmann, and W. S. Reznikoff.** 1990. Integration host factor plays a role in IS*50* and Tn*5* transposition. J. Bacteriol. **172**:1368–1373.
31. **Markham, B. E., J. W. Little, and D. W. Mount.** 1981. Nucleotide sequence of the *lexA* gene of *Escherichia coli* K-12.

Nucleic Acids Res. **9**:4149–4161.
32. **Miller, H. I., and D. I. Friedman.** 1977. Isolation of *Escherichia coli* mutants unable to support lambda integrative recombination, p. 349–356. *In* A. Bukhari, J. Shapiro, and S. Adhya (ed.), DNA insertion elements, plasmids and episomes. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
33. **Miller, H. I., M. Kirk, and H. Echols.** 1981. SOS induction and autoregulation of the *himA* gene for site specific recombination in *E. coli*. Proc. Natl. Acad. Sci. USA **78**:6754–6758.
34. **Miller, J.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
35. **Mount, D. W.** 1977. A mutant of *E. coli* showing constitutive expression of the lysogenic induction and error prone DNA repair pathways. Proc. Natl. Acad. Sci. USA **74**:300–304.
36. **Pearson, W. R., and D. J. Lipman.** 1988. Improved tools for biological sequence comparison. Proc. Natl. Acad. Sci. USA **85**:2444–2448.
37. **Quinones, A., W.-R. Juterbock, and W. Messer.** 1991. Expression of the *dnaA* gene of *Escherichia coli* is inducible by DNA damage. Mol. Gen. Genet. **227**:9–16.
38. **Roberts, D., B. C. Hoopes, W. R. McClure, and N. Kleckner.** 1985. IS10 transposition is regulated by DNA adenine methylation. Cell **43**:117–130.
39. **Roberts, D., and N. Kleckner.** 1988. Tn10 transposition promotes RecA-dependent induction of a λ prophage. Proc. Natl. Acad. Sci. USA **85**:6037–6041.
40. **Roberts, J. W., and C. W. Roberts.** 1975. Proteolytic cleavage of bacteriophage λ repressor in induction. Proc. Natl. Acad. Sci. USA **72**:147–151.
41. **Rothstein, S. J., and W. S. Reznikoff.** 1981. The functional differences in the inverted repeats of Tn5 are caused by a single base pair nonhomology. Cell **23**:191–199.
42. **Sasakawa, C., G. F. Carle, and D. E. Berg.** 1983. Sequences essential for transposition at the termini of IS50. Proc. Natl. Acad. Sci. USA **80**:7293–7297.
43. **Sasakawa, C., Y. Uno, and M. Yoshikawa.** 1981. The requirement of both DNA polymerase and 5′ to 3′ exonuclease activities of DNA polymerase I during Tn5 transposition. Mol. Gen. Genet. **182**:19–24.
44. **Sasakawa, C., Y. Uno, and M. Yoshikawa.** 1987. Lon-SulA regulatory function affects the efficiency of transposition of Tn5 from λ b221 cI857 Pam Oam to the chromosome. Biochem. Biophys. Res. Commun. **142**:879–884.
45. **Sassanfar, M., and J. W. Roberts.** 1990. Nature of the SOS-inducing signal in *Escherichia coli*. J. Mol. Biol. **212**:79–96.
46. **Singer, M., T. A. Baker, G. Schnitzler, S. M. Deischel, M. Goel, W. Dove, K. J. Jaaks, 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.
47. **Sweasy, J. B., E. M. Witkin, N. Sinha, and V. Roegner-Maniscalco.** 1990. RecA protein of *Escherichia coli* has a third essential role in SOS mutator activity. J. Bacteriol. **172**:3030–3036.
48. **Syvanen, M., J. D. Hopkins, and M. Clements.** 1982. A new class of mutants in DNA polymerase I that affects gene transposition. J. Mol. Biol. **158**:203–212.
48a. **Thliveras, A., and D. Mount.** Unpublished data.
49. **Uhlin, B. E., and A. J. Clark.** 1981. Overproduction of the *Escherichia coli recA* protein without stimulation of its proteolytic activity. J. Bacteriol. **148**:386–390.
50. **Walker, G. C.** 1984. Mutagenesis and inducible responses to deoxyribonucleic acid damage in *Escherichia coli*. Microbiol. Rev. **48**:60–93.
51. **Weigle, J. J.** 1953. Induction of mutation in a bacterial virus. Proc. Natl. Acad. Sci. USA **39**:628–636.
52. **Weinreich, M. D., and W. S. Reznikoff.** Unpublished data.
53. **Wertman, K. F., and D. W. Mount.** 1985. Nucleotide sequence binding specificity of the LexA repressor of *Escherichia coli* K-12. J. Bacteriol. **163**:376–384.
54. **Witkin, E. M., and T. Kogoma.** 1984. Involvement of the activated form of RecA protein in SOS mutagenesis and stable DNA replication in *Escherichia coli*. Proc. Natl. Acad. Sci. USA **81**:7539–7543.
55. **Yin, J. C. P., M. P. Krebs, and W. S. Reznikoff.** 1988. The effect of *dam* methylation on Tn5 transposition. J. Mol. Biol. **199**:35–45.
56. **Yin, J. C. P., and W. S. Reznikoff.** 1987. *dnaA*, an essential host gene, and Tn5 transposition. J. Bacteriol. **169**:4637–4645.
57. **Yin, J. C. P., and W. S. Reznikoff.** 1988. p2 and inhibition of Tn5 transposition. J. Bacteriol. **170**:3008–3015.