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The *Salmonella typhimurium* LT2 *uvrD* Gene Is Regulated by the *lexA* Gene Product

PATTY P. PANG AND GRAHAM C. WALKER*

Biology Department, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139

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The *uvrD* gene product apparently plays a role in the repair of UV damage, in mismatch repair, and in genetic recombination. A lower level of expression of the *Salmonella typhimurium* LT2 *uvrD* gene was observed in maxicells prepared from an *Escherichia coli* strain that contained a *lexA*$^+$ plasmid than in maxicells prepared from an *E. coli* strain that lacked functional LexA protein. These results suggest that the *uvrD*$^+$ gene is repressed by the LexA protein and is thus a member of the set of genes whose expression is increased by "SOS"-inducing treatments.

Treatment of *Escherichia coli* with agents that damage DNA or block DNA replication causes the induction of a set of physiological phenomena, collectively termed the "SOS" responses (9, 26). Studies with *lac* fusions have identified a set of genes induced by these treatments, including *uvrA* (8, 8a), *uvrB* (4, 18), *umuDC* (1), *mucAB* (S. J. Elledge and G. C. Walker, submitted for publication), *sfiA* (7), and *himA* (11). These genes are all controlled by the RecA and LexA proteins (9).

SOS responses have also been reported in *Salmonella typhimurium*; in particular, the induction of prophages and Weigle reactivation have been shown to be *recA*$^+$ dependent (3, 22). In addition, if *lac* fusions to the *umuC* and *mucB* genes (3a, 15) are introduced into *S. typhimurium* on a plasmid, they can be induced by UV irradiation (P. P. Pang, S. J. Elledge, and G. C. Walker, unpublished data). These observations strongly suggest that regulation of SOS functions in *S. typhimurium* is similar to that in *E. coli* and that *S. typhimurium* LT2 codes for a LexA analog that is able to efficiently repress the expression of at least the *umuDC* and *mucAB* operons and probably other *din* (damage-inducible) genes from *E. coli*. Furthermore *tif*-like mutations mapping at the *recA* locus of *S. typhimurium* have also been isolated (J. Roth, personal communication).

An important process associated with the maintenance of genetic fidelity at the time of DNA replication is mismatch repair. In *E. coli* and *S. typhimurium*, mismatch repair has been postulated to use methylation at the N6 position of adenine in GATC sequences to discriminate daughter strands from parental strands at replication forks (5, 12, 16, 17, 23–25). Recent studies suggest that the *uvrD* gene product, together with the products of *mutH*, *mutL*, and *mutS* loci, may play a role in this methyl-directed mismatch repair system. Mutations at these loci cause a high spontaneous mutation frequency (5, 6, 16, 17). In addition, mutations at the *uvrD* locus have been identified that affect genetic recombination and sensitivity to UV light, indicating that its gene product also plays a role in recombination and in the repair of UV damage.

Insertion mutations at the *mutH*, *mutL*, *mutS*, and *uvrD* loci in *S. typhimurium* LT2 also cause a high frequency of spontaneous mutations (20, 21). We have recently cloned one of these genes, *uvrD*, and have shown that the gene product has the same apparent molecular weight as the *uvrD* product from *E. coli*. It also appears to complement an *E. coli uvrD* insertion mutation completely (14). These observations suggest that the *S. typhimurium uvrD* gene product is very similar to that of *E. coli*.

Since the *uvrD* gene product plays a role in the repair of UV damage, we were interested in determining whether the *uvrD* gene is regulated by the *lexA* gene product. Since neither class of *lexA* mutation, Ind$^-$ or Def (9), has yet been isolated in *S. typhimurium*, we decided to examine the regulation of the *S. typhimurium uvrD* product in *E. coli* by looking at the levels of UvrD protein synthesized in *E. coli* maxicells in the presence or absence of the *E. coli* LexA protein. We had previously used this approach to verify the LexA regulation of the *umuDC* operon (3a).

pGW1801, a plasmid carrying the *uvrD* gene from *S. typhimurium* (14), was transformed into

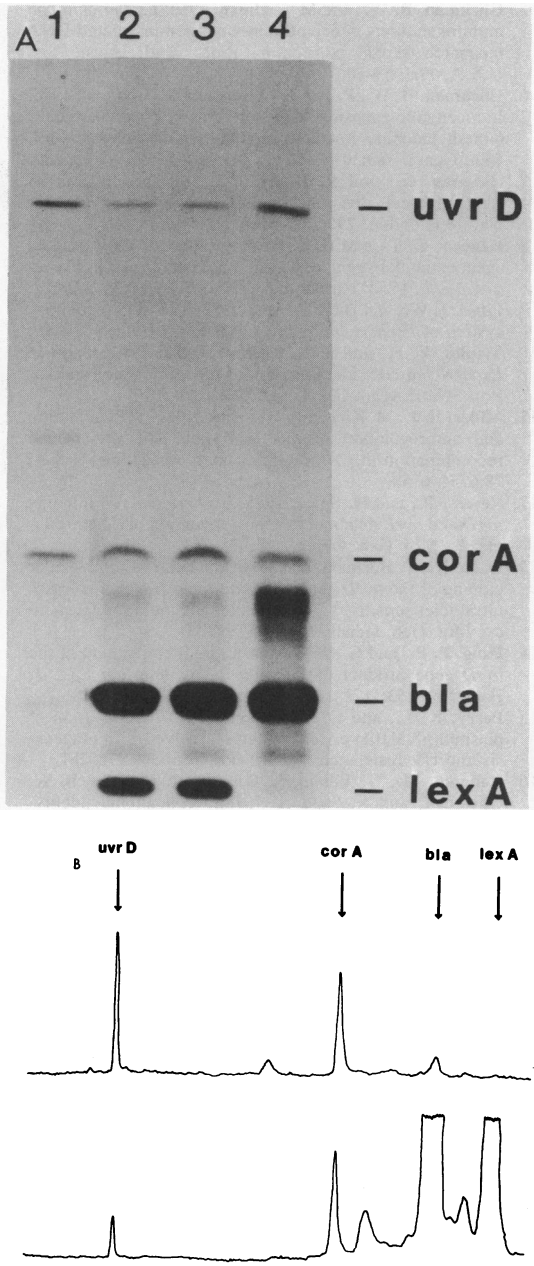

FIG. (A) Analysis of plasmid-encoded proteins by the maxicell technique. The plasmids studied, pGW1801 (*uvrD*$^+$), pRB160 (*lexA*$^+$), and pBR322, were introduced into strain RB901 (*spr-51 ΔrecA21*). Maxicells (14) were prepared, and plasmid-encoded proteins were labeled with [^{35}S]methionine and analyzed on 10% sodium dodecyl sulfate-polyacrylamide gels. Lane 1, RB901(pGW1801); lanes 2 and 3, RB901(pGW1801)(pRB160); lane 4, RB901(pGW1801)(pBR322). Proteins used as molecular weight standards were phosphorylase *b*, bovine serum albumin, ovalbumin, carbonic anhydrase, soybean trypsin inhibitor, and lysozyme. (B) Densitometer scans of autoradiograph. Upper tracing: lane 1, RB901(pGW1801). Lower tracing: lane 2, RB901(pGW1801)(pRB160).

TABLE 1. Bacterial strains

Strain	Genotype	Source or reference
E. coli K12		
RB901	F$^-$ *thr leu lacY? rpsL31 Δ(recA-srl)21 spr-51 (lexA3) sfiA11*	R. Brent (2)
GW3715	RB901(pGW1801)	P. Pang (14)
GW3727	RB901(pGW1801)(pRB160)	This paper
GW3729	RB901(pGW1801)(pBR322)	This paper

E. coli Δ*recA* *lexA*(Def) strains that either contained or did not contain pRB160, a derivative of pBR322 carrying the *E. coli lexA*$^+$ gene (2) (Table 1). As a control, pGW1801 was also transformed into an *E. coli* Δ*recA* *lexA*(Def) strain carrying pBR322. The pGW1801 plasmid is compatible with pBR322 and its derivatives. Plasmid-encoded proteins were then labeled with [^{35}S]methionine by the maxicell procedure described previously (14, 19), and the proteins were separated by electrophoresis on 10% sodium dodecyl sulfate-polyacrylamide gels. Several individual maxicell preparations were made from each of the above strains.

Figure 1A shows an autoradiograph of a few such preparations. A lower level of UvrD protein was observed when the cells from which the maxicells were prepared also carried the *lexA*$^+$ gene on pBR322. pBR322 alone had no effect on the level of UvrD protein. To quantitate more accurately the level of UvrD protein observed under the various conditions, the autoradiographs were scanned with a densitometer; representative tracings are shown in Fig. 1B.

The 38-kilodalton protein whose gene maps close to the *uvrD* gene on pGW1801 appears to be the *S. typhimurium* equivalent of the *E. coli* CorA product since it is virtually the same size as the *E. coli* CorA protein (10, 13) and its gene is required for the complementation of an *E. coli corA* mutant (data not shown). The *corA* gene does not appear to be in the same operon as the *uvrD* gene (14). In quantitating the levels of *uvrD* gene product made under the various conditions employed in this study, we used the level of the 38-kilodalton CorA protein as a standard, making the assumption that its level is not affected by the presence or absence of LexA protein. Ratios of the intensity of the UvrD protein band to that of the CorA protein under the various conditions are shown in Table 2. The level of UvrD protein in the absence of the *E. coli* LexA protein is about three times higher than in the presence of the LexA protein. The absolute

TABLE 2. Ratios of UvrD protein to CorA protein in the presence and absence of LexA protein[a]

Plasmids present in maxicells	UvrD/CorA[b]
pGW1801 (*uvrD*$^+$) and pBR160 (*lexA*$^+$)	0.24 ± 0.07 (2.08)
pGW1801 (*uvrD*$^+$)	0.88 ± 0.15 (0.9)

[a] Ratios were obtained by comparing the intensities of the protein bands of autoradiographs. Six independent maxicell preparations of each strain were analyzed.

[b] Data are expressed as mean ± standard deviation (median).

amount of CorA protein labeled in each maxicell preparation was very similar, regardless of the strain used to prepare the maxicells.

We thus conclude that the *uvrD* gene of *S. typhimurium* is repressed by the *E. coli* LexA protein and therefore probably by the *S. typhimurium* LexA equivalent. Our results are consistent with the findings of E. Siegel (XIII International Congress of Microbiology, 1982, p28:1, p. 100), who used a *uvrD*::Mu *d* (Ap *lac*) fusion in *E. coli* to show that the *uvrD* gene is induced about three- to fourfold by UV irradiation and mitomycin C. Also, a putative LexA-binding site has been found near the promoter region of the *E. coli uvrD* gene (S. R. Kushner, personal communication). Thus, it seems likely that *uvrD* is another member of the set of *din* genes whose expression is increased, in a *recA*$^+$ *lexA*$^+$-dependent fashion, by SOS-inducing treatments.

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