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Analysis of the SOS Inducing Signal in *Bacillus subtilis* Using *Escherichia coli* LexA as a Probe

CHARLES M. LOVETT, JR.,* THOMAS M. O'GARA, AND JAMES N. WOODRUFF

Department of Chemistry, Williams College, Williamstown, Massachusetts 01267

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We analyzed the *Bacillus subtilis* SOS response using *Escherichia coli* LexA protein as a probe to measure the kinetics of SOS activation and DNA repair in wild-type and DNA repair-deficient strains. By examining the effects of DNA-damaging agents that produce the SOS inducing signal in *E. coli* by three distinct pathways, we obtained evidence that the nature of the SOS inducing signal has been conserved in *B. subtilis*. In particular, we used the *B. subtilis* DNA polymerase III inhibitor, 6-(*p*-hydroxyphenylazo)-uracil, to show that DNA replication is required to generate the SOS inducing signal following UV irradiation. We also present evidence that single-stranded gaps, generated by excision repair, serve as part of the UV inducing signal. By assaying the SOS response in *B. subtilis dinA*, *dinB*, and *dinC* mutants, we identified distinct deficiencies in SOS activation and DNA repair that suggest roles for the corresponding gene products in the SOS response.

The *Escherichia coli* SOS system comprises about 20 unlinked genes that code for proteins involved in DNA repair processes including excision repair, recombinational repair, and error-prone repair (SOS mutagenesis) (for reviews, see references 23 and 42). The SOS genes are coordinately induced when *E. coli* is exposed to physical or chemical agents that damage DNA; their increased expression results in characteristic SOS phenomena such as enhanced DNA repair capacity, enhanced mutagenesis, and inhibition of cell division. SOS gene expression is controlled by the action of two proteins (which are themselves products of SOS genes): LexA protein represses the transcription of SOS genes, and RecA protein, when activated by an inducing signal, promotes the proteolytic inactivation of LexA repressor. Thus, induction of the SOS system is the immediate consequence of LexA cleavage. Although RecA is required for efficient LexA cleavage in vivo and at physiological pH in vitro, the proteolytic mechanism involves LexA functional groups in a reaction that is accelerated by activated RecA (21, 39).

The events leading to the activation of RecA and the exact nature of the inducing signal are not completely understood, although they must depend, in part, on the type of DNA lesion. *E. coli* RecA is activated for repressor (LexA and certain phage repressors) cleavage in vitro when it binds single-stranded DNA and nucleoside triphosphate (7, 22, 35). Consistent with the single-stranded DNA requirement, there is strong evidence that the SOS inducing signal is single-stranded DNA exposed by the metabolic processing of damaged DNA; however, the origin and precise nature of the signal depend on the inducing treatment (38). In any case, the repair of damaged DNA would, according to the model, reduce the level of inducing signal (and therefore the level of activated RecA). In turn, LexA repressor would accumulate and again repress SOS gene transcription.

Exposure of the bacterium *Bacillus subtilis* to DNA-damaging agents results in an SOS response that includes enhanced DNA repair capacity, enhanced mutagenesis, and inhibition of cell division (26, 34, 46). Genetic and biochemical studies have identified several components of the *B. subtilis* SOS system including the RecA protein (31), the SOS repressor (28), and at least three distinct damage-inducible (*din*) genes, designated *dinA*, *dinB*, and *dinC* (17, 25). Like its counterpart in *E. coli*, *B. subtilis* RecA protein (formerly referred to as the *recE* gene product) is induced by DNA-damaging treatments and required for general genetic recombination, recombinational repair, and induction of *B. subtilis* SOS functions (10, 11, 26, 30). In vitro, *B. subtilis* RecA catalyzes DNA strand exchange and promotes the cleavage of *E. coli* LexA in a reaction that requires single-stranded DNA (31). Several lines of evidence indicate that *recA*, *dinA*, *dinB*, and *dinC* share a regulatory mechanism involving the RecA-dependent inactivation of an SOS repressor (6, 25, 28, 30, 31). Moreover, the ability of *B. subtilis* RecA to promote cleavage of *E. coli* LexA (31) and the ability of *E. coli* RecA to induce SOS functions in *B. subtilis* (9, 27) indicate that the mechanism of SOS regulation has been conserved between *E. coli* and distantly related *B. subtilis*.

We report here a quantitative characterization of the *B. subtilis* SOS response using *E. coli* LexA protein as a probe to follow both SOS activation and cellular recovery from DNA damage (i.e., the repair of damaged DNA) in wild-type and DNA repair-deficient strains. Our results indicate that LexA cleavage in *B. subtilis* reflects the activation of *B. subtilis* RecA and that the subsequent accumulation of LexA is due to removal of the SOS inducing signal; thus, measuring LexA levels following DNA damage provides an assay for the *B. subtilis* SOS response. Using this assay, we specifically addressed two questions. (i) Has the nature of the SOS inducing signal been conserved in *B. subtilis*? In particular, we used the *B. subtilis* DNA polymerase III inhibitor 6-(*p*-hydroxyphenylazo)-uracil (HPUra) in a unique way, not possible in *E. coli*, to assess the role of DNA replication in generating the SOS inducing signal. (ii) What role do the *dinA*, *dinB*, and *dinC* gene products play in the *B. subtilis* SOS response? Our results provide clues to the involvement of these *din* gene products in SOS activation and DNA repair.

MATERIALS AND METHODS

Bacterial strains and plasmids. The bacterial strains used in this study are listed in Table 1. Each of the strains (which are derivatives of strain YB886) has been cured of the bacteriophage SPβ and rendered noninducible for the endogenous

* Corresponding author. Phone: (413) 597-2124. Fax: (413) 597-4116. Electronic mail address: clovett@williams.edu.

TABLE 1. Bacterial strains and plasmids

Strain or plasmid	Relevant genotype	Source or reference
B. subtilis strains		
YB886	*metB5 trpC2 xin-1* SPβ⁻	47
YB965	*metB5 hisB leuA8 polA5 xin-1* SPβ⁻	26
YB1005	YB886 *uvrA42*	15
YB1015	YB886 *recA4*	15[a]
YB1017	YB886 *recM13*	15[b]
YB1260	YB886 *recA1*	26
YB886-*dinA77*	YB886 (*dinA77*::Tn*917-lacZ*)	25
YB886-*dinB7*	YB886 (*dinB7*::Tn*917-lacZ*)	25
YB886-*dinC17*	YB886 (*dinC17*::Tn*917-lacZ*)	25
Plasmids		
pRB480	Ap^r *lexA*$^+$	R. Brent
pPL608	Cm^r Km^r	44
pPL708-*lexA*	Cm^r Km^r *lexA*$^+$	45
pPL608-*lexA*	Km^r *lexA*$^+$	This study

[a] Formerly YB886 *recE4*.
[b] Formerly YB886 *recG13*.

prophage PBSX (47). Plasmid pRB480, containing a *Hin*dIII-ended *lexA* gene, was obtained from R. Brent. Plasmid pPL708-*lexA*, containing the entire *lexA* gene, was obtained from M. Wojciechowski. Plasmid pPL608, containing the *B. subtilis* SPO2 promoter and a promoterless *cat86* gene, confers resistance to chloramphenicol (5 μg/ml) and kanamycin (5 μg/ml) and has been described elsewhere (44). Plasmid pPL608-*lexA* was prepared by ligation of *Hpa*I-*Hin*dIII-digested pRB480 with *Hpa*I-*Hin*dIII-digested pPL608. YB886 cells were made competent and transformed with the ligation mixture by the procedure of Bott and Wilson (2). Kanamycin-resistant, chloramphenicol-sensitive transformants were tested for the expression of LexA by immunoblot analysis as described below. Cesium chloride-purified pPL608-*lexA* was similarly introduced into the strains listed in Table 1 by transformation of competent cells.

Materials. The *B. subtilis* RecA and *E. coli* LexA proteins used as standards were purified as described previously (24, 31). Polyclonal *B. subtilis* RecA antiserum was prepared by the subcutaneous injection of New Zealand White rabbits with RecA protein of greater than 95% purity. Initially, 100 μg of RecA protein suspended in 1 ml of complete Freund's adjuvant was injected, and two boosters of 100 μg of RecA suspended in 1 ml of incomplete Freund's adjuvant were injected at 3-week intervals. The antiserum was collected 10 days after the third injection. Polyclonal *E. coli* LexA antiserum was provided by M. Sassanfar. HPUra was provided by G. Wright. Restriction enzymes and DNA-modifying enzymes were purchased from Bethesda Research Laboratories and U.S. Biochemicals. Affinity-purified goat anti-rabbit horseradish peroxidase conjugate was purchased from Bio-Rad Laboratories. Mitomycin, nalidixic acid, and 4-chloro-1-napthol were purchased from Sigma Chemical Co. Nitrocellulose filters were purchased from Schleicher and Schuell. Stock solutions of HPUra, mitomycin, and nalidixic acid were made in 50 mM NaOH (200 μM), ethanol (1 mg/ml), and 100 mM NaOH (5 mg/ml), respectively.

UV irradiation. Liquid cultures of *B. subtilis* were grown in Luria-Bertani (LB) broth at 37°C to an optical density at 600 nm (OD_{600}) of 0.5 and immediately put on ice. Cells from 15-ml aliquots were pelleted by centrifugation at 6,000 × *g* for 15 min, and the supernatant was discarded. Pelleted cells were resuspended in 15 ml of ice-cold minimal salts and irradiated with an 8-W germicidal lamp in a large petri dish on ice. Cells were immediately pelleted by centrifugation at 6,000 × *g* for 15 min, and the supernatant was discarded. Cells were resuspended in 1 ml of ice-cold LB broth and added to 30 ml of LB broth prewarmed to 37°C, and aliquots were removed at the indicated time intervals and put on ice. For each time point, an equal volume of cells (corresponding to 1 ml at an OD_{600} of 0.6) was pelleted by centrifugation. Cell pellets were resuspended in 20 μl of ice-cold 20 mM Tris-HCl (pH 7.5)–10% (wt/vol) sucrose containing 0.5 mg of lysozyme per ml and subjected to five cycles of freezing and thawing. An equal volume of 2× sample buffer (0.16 M Tris-HCl [pH 6.8], 20% [vol/vol] glycerol, 0.28 M 2-mercaptoethanol, 0.4% sodium dodecyl sulfate [SDS], 0.001% [wt/vol] bromphenol blue) was added to each sample. Samples were then subjected to SDS-polyacrylamide gel electrophoresis (SDS-PAGE) followed by immunoblot transfer as described elsewhere (31).

Treatment with mitomycin, nalidixic acid, and HPUra. Cells were grown in LB broth at 37°C, and chemicals were added to give the indicated concentrations during exponential growth (OD_{600} = 0.3). HPUra was added immediately prior to inducing treatment where indicated. For each time point, an equal volume of cells (corresponding to 1 ml at an OD_{600} of 0.6) was removed and treated as described for UV irradiation. HPUra and nalidixic acid were removed from the media by chilling on ice, pelleting cells by centrifugation at 6,000 × *g* for 15 min, and washing pellets with ice-cold minimal salts. Cells were resuspended in 1 ml of ice-cold LB broth and added to 30 ml of LB broth prewarmed to 37°C; aliquots were removed at the appropriate time intervals and treated as described for UV irradiation.

Quantitation of *B. subtilis* RecA and *E. coli* LexA proteins. *B. subtilis* strains were treated with the indicated doses of UV, mitomycin, nalidixic acid, or HPUra, and cell samples were analyzed by immunoblot transfer as described previously (30). Nitrocellulose membranes containing electrophoretically transferred proteins were incubated with *B. subtilis* RecA antiserum (diluted 1:5,000) and *E. coli* LexA antiserum (diluted 1:3,000) followed by peroxidase-conjugated anti-rabbit antibody (diluted 1:3,000) essentially as described previously (31). Samples of purified *B. subtilis* RecA (30 to 500 ng) and *E. coli* LexA (1 to 100 ng) proteins were electrophoresed alongside cell samples. Immunoblots were scanned with an LKB laser-enhanced densitometer, and the amounts of RecA and LexA were interpolated from purified protein standard curves as described previously (30). All datum points are average values from at least two trials.

RESULTS

Expression and cleavage of *E. coli* LexA in *B. subtilis*. We constructed a derivative of plasmid pPL608 (44) in which the *E. coli lexA* gene is under the transcriptional control of the *B. subtilis* phage SPO2 promoter and the translational control of the *Bacillus pumilus cat86* gene (see Materials and Methods and Fig. 1A). To obviate the possibility that the cloned *lexA* gene would itself be induced by DNA damage and therefore not give an accurate measure of the LexA cleavage rate, we eliminated the *lexA* promoter-operator region. Plasmid pPL608-*lexA* contains an *Hpa*I-*Hin*dIII fragment containing all but the first nine nucleotides of the *lexA* gene inserted into *Hpa*I-*Hin*dIII-digested pPL608, devoid of most of the *cat86* structural gene. As shown in Fig. 1B, *Hpa*I cuts after nucleotide 62 of the *cat86* structural gene and after nucleotide 11 of the *lexA* structural gene. Thus, pPL608-*lexA* codes for a

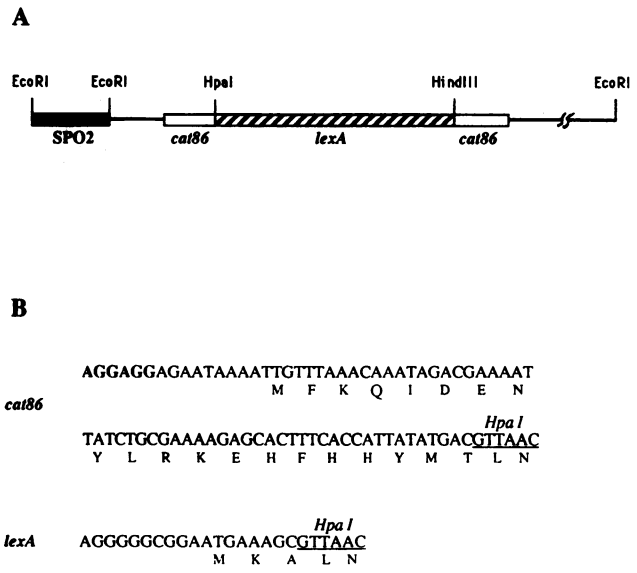

FIG. 1. Construction of plasmid pPL608-*lexA*. Plasmid pPL608-*lexA* was constructed by relacing the *Hpa*I-*Hin*dIII fragment of the *cat86* gene in plasmid pPL608 with an *Hpa*I-*Hin*dIII fragment of *E. coli lexA*. (A) Schematic diagram of pPL608-*lexA*. (B) Comparison of the 5′ sequences of the *cat86* and *lexA* genes reveals that translation of pPL608-*lexA* would produce a Cat86-LexA fusion protein in which the first 3 amino acids of LexA are replaced by the first 20 amino acids of Cat86. Putative ribosome-binding site is indicated by boldface type.

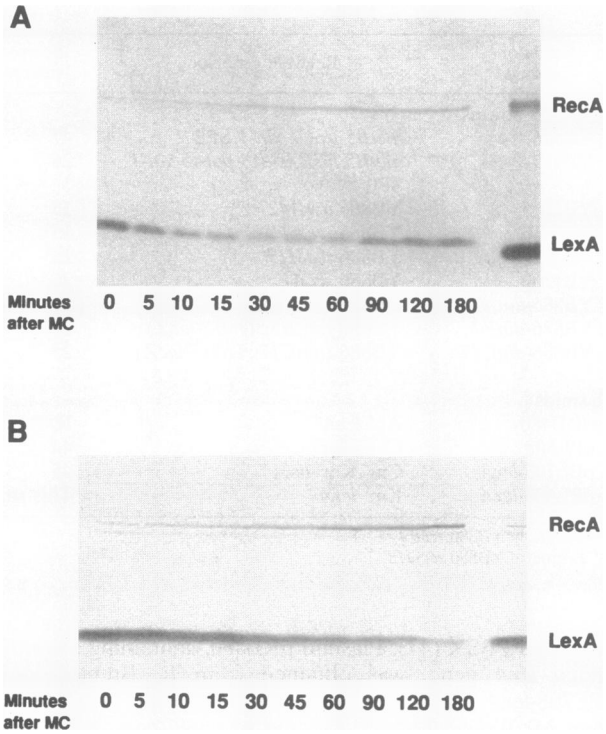

FIG. 2. Immunoblots of wild-type *B. subtilis* cells following exposure to mitomycin. Log-phase *B. subtilis* cells (YB886) containing plasmid pPL608-*lexA* (A) or pPL708-*lexA* (B) were treated with mitomycin at 0.5 μg/ml and harvested at the designated times after mitomycin addition. Equivalent amounts of cells (corresponding to 1 ml at an OD_{600} of 0.6) were lysed and subjected to SDS-PAGE followed by immunoblot transfer using *B. subtilis* RecA and *E. coli* LexA antisera as described in Materials and Methods. Purified RecA and LexA proteins were loaded as markers.

24.7-kDa LexA hybrid protein in which the first three amino acids of LexA are replaced by the first twenty amino acids of the Cat86 protein. The migration of the LexA hybrid relative to wild-type LexA (molecular mass = 22.4 kDa) through an SDS-polyacrylamide gel is consistent with a protein of the expected size (Fig. 2A). Native LexA, expressed in *B. subtilis* on plasmid pPL708-*lexA* (45), is electrophoretically indistinguishable from the purified protein (Fig. 2B). For simplicity, the hybrid protein will be referred to as LexA in the remainder of this report.

We quantified the amounts of *E. coli* LexA and *B. subtilis* RecA in pPL608-*lexA*-containing strains by densitometric scanning of immunoblots (see Materials and Methods). The amounts of protein were interpolated from standard curves that were approximately linear for LexA levels of 1 to 50 ng and RecA levels of 30 to 400 ng by use of our antisera and immunoblot assay conditions. Our assay conditions permitted the simultaneous quantification of both LexA and RecA over the ranges of values that occur in induced cells. On the basis of a value of 3×10^8 cells per ml at an OD_{600} of 1.0, we determined that uninduced wild-type cells contained about 4,000 molecules of LexA per cell and 4,500 molecules of RecA per cell. The amount of LexA expressed in uninduced cells depended on the genetic background and ranged from about 3,400 molecules per cell in the *uvrA42* strain to about 5,000 molecules per cell in the *recA4* strain.

Figure 2A shows a typical stained immunoblot of wild-type cells containing pPL608-*lexA*. The level of the LexA hybrid protein decreases over a period of 30 min (in this case following mitomycin treatment) and subsequently increases. The constant level of LexA following DNA damage in mutants that either produce defective RecA protein or are otherwise deficient in SOS induction indicates that this decrease is due to cleavage mediated by activated *B. subtilis* RecA (see below).

To assess the intrinsic stability of the LexA hybrid protein, we added chloramphenicol (50 μg/ml) to block protein synthesis. When chloramphenicol was added to uninduced wild-type cells, the level of LexA decreased slowly with a half-life of about 65 min; moreover, the addition of chloramphenicol prior to induction of treatment did not affect the initial rate of LexA disappearance; however, there was no subsequent resynthesis (data not shown). The accumulation of LexA after cleavage should reflect the decrease in activated RecA that occurs upon the repair of damaged DNA; this is supported by our results with the *uvrA42* mutant described below. We did not detect LexA cleavage products in our assay; however, Wojciechowski et al. (45) showed that the native LexA protein is cleaved in *B. subtilis* to yield cleavage products identical to those produced by *E. coli* RecA. Moreover, purified *B. subtilis* RecA produces cleavage products that are electrophoretically indistinguishable from those produced by *E. coli* RecA (31).

We found that the LexA hybrid was a better probe of the *B. subtilis* SOS response than native LexA protein expressed on pPL708-*lexA* (45). Figure 2B shows an immunoblot of wild-type cells containing pPL708-*lexA* treated under conditions identical to those in Figure 2A. Comparison of the two immunoblots reveals that the basal level of LexA in cells containing pPL708-*lexA* is more than twofold higher and the rate of cleavage is at least 50% lower than in cells containing our plasmid; moreover, LexA produced from pPL708-*lexA* did not return to its original level 2 h after mitomycin treatment.

The lower basal level of LexA observed in cells containing our construct could be due to attenuated translation of the *cat86* regulatory region (12); alternatively, either the plasmid copy number is lower or the hybrid protein is less stable. The apparently lower cleavage rate of LexA in cells containing pPL708-*lexA* may reflect the presence of the *lexA* operator. If LexA represses its own expression in uninduced cells, the observed cleavage rate would be the net effect of both cleavage and increased expression. Alternatively, the higher level of the native LexA may saturate the inactivation system. Although it is also possible that the additional amino acids in the LexA hybrid protein may impart structural changes that increase the catalytic rate constant, the cleavage rate for the LexA hybrid is comparable to the rate of LexA cleavage in vitro (see below). Notwithstanding the differences between the two proteins, it is clear that the *lexA* plasmid used in this study provides a suitable probe for both the activation of the SOS response and the repair of damaged DNA in *B. subtilis*.

Although the interaction of RecA with LexA has been highly conserved between *E. coli* and *B. subtilis*, there is no evidence for a conserved repressor-operator interaction between these species. In fact, the two SOS operators differ in sequence and separation of half-sites (28, 42). Moreover, purified *E. coli* LexA does not bind to any of the *B. subtilis din* promoter regions in mobility shift assays nor does it inhibit the binding of the *B. subtilis* SOS repressor to *din* promoter regions (28). Consistent with the inability of LexA to bind *B. subtilis* operators in vitro, we found that LexA does not affect the basal levels of RecA in any of the strains tested and it does not significantly reduce the induction of RecA and *din* operons (data not shown).

SOS response to different inducing agents in wild-type cells. The SOS response in both *E. coli* and *B. subtilis* is activated by physical and chemical agents that produce, either directly or indirectly, a variety of DNA lesions. We chose to investigate the effects of UV, mitomycin, and nalidixic acid—agents that presumably generate the *E. coli* SOS inducing signal in three distinct ways. Efficient induction by UV requires an active replication fork (38), supporting the model in which RecA is activated by single-stranded gaps exposed when replication is interrupted by pyrimidine dimers (36). Mitomycin-induced cross-links should block replication and therefore must generate the signal differently. According to the model, UvrABC endonuclease makes single-strand cuts, on both sides of the DNA cross-link, producing a substrate for RecA-mediated recombinational repair (37); the hypothesis that this action results in an SOS inducing signal is supported by evidence showing that efficient induction by mitomycin requires excision repair (38). Nalidixic acid is a DNA gyrase inhibitor that blocks replication and causes double-strand breaks in DNA (16, 40, 41). Induction by nalidixic acid requires the unwinding activity of RecBCD nuclease (5).

Figure 3A and C shows the levels of RecA and LexA in wild-type cells at the indicated times following exposure to UV, mitomycin, or nalidixic acid. For each treatment, LexA cleavage precedes RecA induction, and there is a correlation between the kinetics of RecA induction and the kinetics of LexA cleavage. For optimum doses of inducing agent (30), the induction and cleavage kinetics for UV- or mitomycin-treated cells are about the same. On the basis of the amount of LexA cleaved in the first 5 min after UV irradiation, the initial cleavage rate is at least 2.8 ng of LexA per min per 60 ng of RecA or 70 mmol of LexA per min per mol of RecA (Table 2). This rate compares well with in vitro cleavage rates (discussed below). Previous studies have shown that nalidixic acid induction of RecA is significantly lower than induction by mitomycin or UV (30). Corresponding to the reduced rate of RecA induction, the LexA cleavage rate after nalidixic acid treatment is less than 20% of the rates with UV or mitomycin.

TABLE 2. LexA cleavage rates

Strain	Cleavage rate[a] (mmol of LexA/min/mol of RecA) with indicated treatment			
	UV	Mitomycin	Nalidixic acid	No DNA-damaging treatment
YB886 (wild type)	70	56	10	0
YB886 + HPUra	33	76	20	13
YB1005 (*uvrA42*)	51	9	7	0
YB1005 + HPUra	23			
YB965 (*polA5*) + HPUra	38			
YB1015 (*recA4*)	0	0	0	
YB1260 (*recA1*)	0	0	0	
YB1017 (*recM13*)	0	0	0	
YB886-*dinA77*	61	13	12	0
YB886-*dinB7*	43	51	9	0
YB886-*dinC17*	0	61	3	0

[a] Cleavage rates were determined from the maximum rate over a 5-min period; in most cases, this corresponds to initial rates, however, some strains exhibited lag periods before the maximum cleavage rate was observed. The amount of LexA cleaved was divided by the amount of RecA present in the cell (which was strain dependent). On the basis of repeated trials, the average precision in these values is about 7%.

Figure 3C shows that LexA accumulation occurs between 20 and 30 min after UV irradiation whereas its accumulation following mitomycin induction does not begin until nearly 60 min after treatment. The delay with mitomycin is probably due to slower removal of the inducing signal (i.e., repair of DNA damage), because the time course for LexA accumulation is similar when the drug is removed from the medium. We found that at a twofold higher dose of mitomycin there is no detectable accumulation of LexA 2 h after inducing treatment (although the kinetics of RecA induction and LexA cleavage are the same). In wild-type *E. coli* cells, LexA begins to accumulate about 20 min after UV irradiation, although the dose that promotes maximum RecA induction in *E. coli* is 20-fold lower than that for *B. subtilis* (38). The level of LexA continues to decrease for 2 h after addition of nalidixic acid; however, LexA rapidly returns to its initial level when the drug is removed from the medium.

SOS response in *rec* mutants. We examined LexA cleavage and RecA induction in three *rec* mutants that are deficient in all SOS functions as well as RecA and *din* operon induction following DNA damage (26, 30). Several lines of evidence indicate that the *recA1* and *recA4* mutants contain mutations in the *recA* gene that render the protein highly deficient in SOS induction and recombination (28–30, 33). These strains are completely deficient in RecA induction, as shown in Fig. 3B. Consistent with the lack of RecA induction, these strains show no detectable cleavage of LexA following any of the three DNA-damaging treatments (Fig. 3D and data not shown). Moreover, the basal level of LexA in these strains is slightly higher than that in wild-type cells, suggesting that there might be a low level of activated RecA in uninduced $RecA^+$ cells. Cells containing the *recM13* mutation are also deficient in SOS induction, but in this case, the RecA protein is probably normal (1, 26, 30). We found that there is no detectable LexA cleavage in *recM13* strains treated with any inducing agent (Fig. 3D and data not shown), suggesting that the mutation blocks the activation of RecA. Because RecA activation is inhibited regardless of the inducing treatment, the *recM* gene

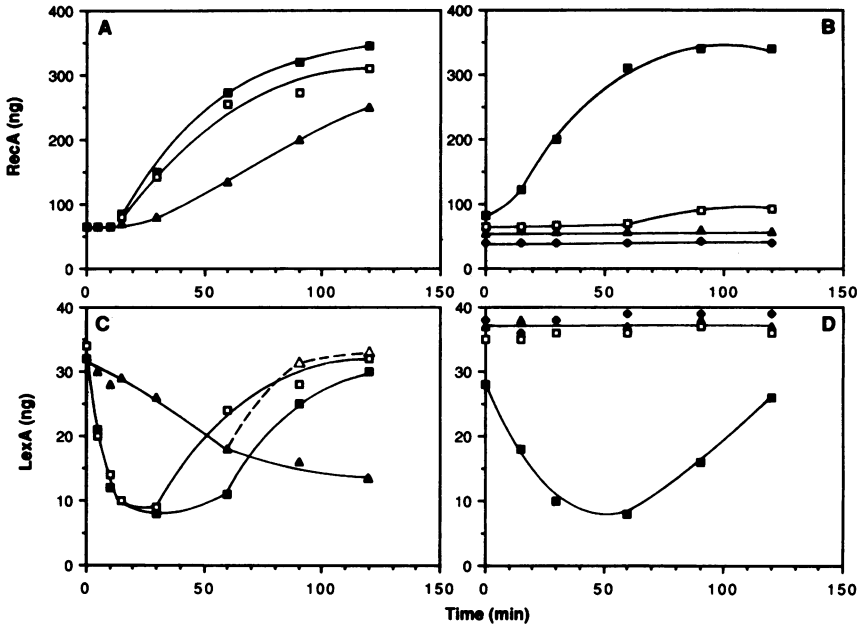

FIG. 3. Kinetics of *B. subtilis* RecA induction (A, B) and *E. coli* LexA disappearance and accumulation (C, D) in Rec$^+$ and *rec* mutant cells following exposure to UV radiation, mitomycin, or nalidixic acid. Log-phase *B. subtilis* cells were exposed to the indicated doses of inducing agent, harvested at the designated times following inducing treatment, and subjected to immunoblot transfers as described in the legend to Fig. 2. Immunoblots were quantified by scanning densitometry, and the amounts of RecA and LexA were determined from standard curves obtained by use of purified proteins as described. (A, C) YB886 (wild type) treated with mitomycin at 0.5 μg/ml (■), UV radiation at 50 J/m^2 (□), or nalidixic acid at 50 μg/ml (▲). The dashed line in panel C shows LexA levels when nalidixic acid was removed from the medium after 60 min of incubation. (B, D) ▲, YB1260 (*recA1*); ◆, YB1015 (*recA4*); □, YB1017 (*recM13*); ■, YB1016 (*polA5*) treated with mitomycin at 0.5 μg/ml.

product may act in a general way to effect the productive interaction of RecA with single-stranded DNA. Characterization of the *recM* gene revealed putative DNA-binding and ATP-binding motifs that may be involved in this function (1). Although there is no detectable cleavage, there is a low level of RecA induction by mitomycin (Fig. 3B), suggesting a low level of *B. subtilis* repressor inactivation. Our inability to detect LexA cleavage may be due to a lower activity of RecA towards the foreign repressor than to the *B. subtilis* repressor.

SOS response in *din* mutants. The *dinA*, *dinB*, and *dinC* genes were identified as DNA damage-inducible operons by transpositional mutagenesis using Tn*917-lacZ*; *din*::Tn*917-lacZ* fusion strains exhibit increased β-galactosidase activity following treatment with mitomycin or UV (25). Several lines of evidence indicate that all three *din* genes are part of the *B. subtilis* SOS regulon: (i) β-galactosidase induction in *din* fusion strains requires a functional RecA protein (25), (ii) mutations that block SOS functions also inhibit the induction of *din* operons (30), and (iii) there is a consensus sequence overlapping each *din* gene promoter (including *recA*) (6) that is the binding site for the *B. subtilis* repressor (28).

Most of the *E. coli* SOS genes are involved in either SOS induction or some form of DNA repair mechanism—recombinational repair, excision repair, or translesion DNA synthesis (error-prone repair). It therefore seemed likely that *B. subtilis din* fusion strains would have deficiencies that could be detected by our assay. We chose to examine LexA cleavage and accumulation in the *dinA77*, *dinB7*, and *dinC17* fusion strains because, with Tn*917* inserted close to the 5′ end of the corresponding genes, they are probably null mutants (6).

Because the *dinA* gene maps near the *B. subtilis uvrA* locus (17) and has sequence homology with the *E. coli uvrB* gene (6), we compared the effects of the *dinA77* and *uvrA42* mutations; the latter is deficient in the incision step of excision repair (18). Figure 4 shows the kinetics of RecA induction and LexA cleavage in *dinA77*::Tn*917-lacZ* and *uvrA42* strains following treatment with UV, mitomycin, or nalidixic acid. Following UV irradiation, the kinetics of LexA cleavage are approximately the same in both strains as in wild-type cells. However, over extended periods of time there is no accumulation of LexA protein in *dinA77* and *uvrA42* cells. This is the expected result if these cells are unable to excise pyrimidine dimers. It is also consistent with the decreased survival rate in these strains following UV irradiation (15, 25). Although the kinetics of cleavage are apparently normal, the induction of RecA is reduced in these strains; this may be a consequence of decreased gene expression due to the presence of pyrimidine dimers. Following exposure to mitomycin, both strains exhibit a marked reduction, relative to the wild type, in the rate of LexA cleavage. The recovery from mitomycin damage in these cells is apparently normal because LexA begins to accumulate at about 1 h after treatment; this is somewhat surprising because, according to the *E. coli* model, mitomycin cross-links are ultimately repaired by UvrABC (37). In contrast with UV and mitomycin treatments, the response to nalidixic acid in *dinA77* and *uvrA42* strains is similar to that in wild-type cells.

Figure 5 shows the induction and cleavage kinetics for *dinB* and *dinC* fusion strains. Mutations in either *dinB* or *dinC* affect the SOS response to UV irradiation, albeit in different ways. The *dinB* mutation reduces slightly the rates of LexA cleavage and RecA induction following UV irradiation and delays by nearly 60 min the recovery from UV-induced damage (Fig. 5A and C). By contrast, SOS induction and recovery following mitomycin-induced damage in the *dinB7*::Tn*917-lacZ* strain are apparently normal (compare Fig. 3C and 5C); nalidixic acid induction in this strain is also similar to that in wild-type cells

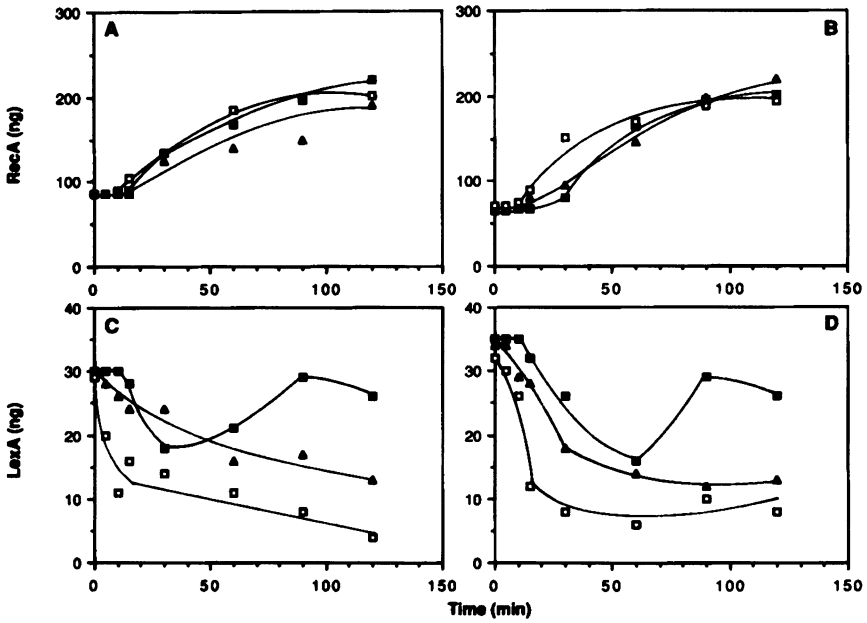

FIG. 4. Kinetics of *B. subtilis* RecA induction and *E. coli* LexA disappearance and accumulation in excision repair-deficient strains following exposure to UV radiation, mitomycin, or nalidixic acid. Log-phase YB1005 (*uvrA42*) (A, C) or YB886-*dinA77* (B, D) cells were exposed to mitomycin (■), UV (□), or nalidixic acid (▲) at the dose indicated in the legend to Fig. 3, harvested at the designated times following inducing treatment, and analyzed as described in the legend to Fig. 3.

(data not shown). Thus, the effect of this mutation seems to be specific for UV-induced lesions. In *dinC17*::Tn*917-lacZ* cells, there is no significant cleavage of LexA over a period of 3 h after UV irradiation (Fig. 5D). The *dinC* operon is itself induced by UV in this strain (25), and Fig. 5B shows that RecA is also induced; however, the *dinC17* mutation reduces the rate and extent of RecA induction. It is not clear how a low level of SOS induction occurs while LexA cleavage is not detected; however, it is not unlikely that the native repressor is inactivated to some extent because inactivation of the native repressor is probably more efficient than cleavage of the foreign repressor. Surprisingly, *dinC17*::Tn*917-lacZ* cells have normal

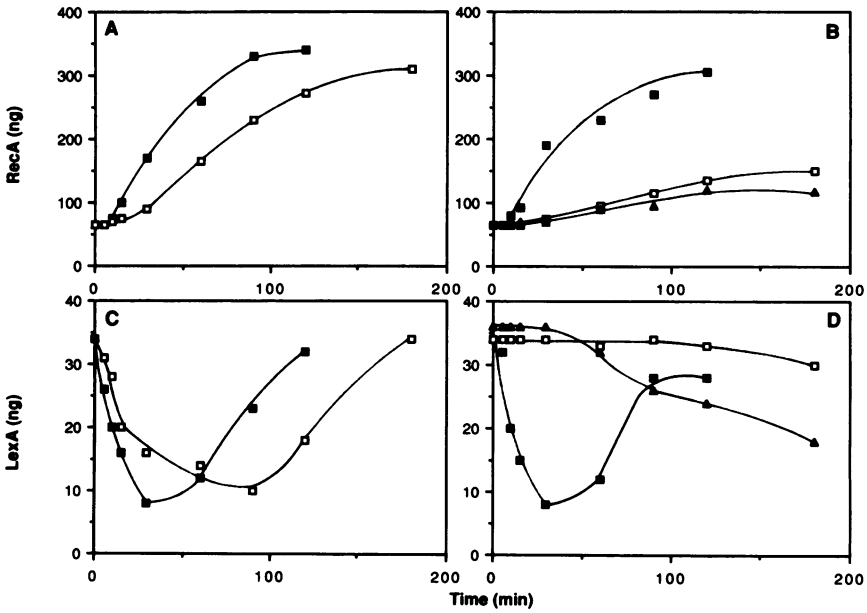

FIG. 5. Kinetics of *B. subtilis* RecA induction and *E. coli* LexA disappearance and accumulation in *dinB* and *dinC* mutants following exposure to UV radiation, mitomycin, or nalidixic acid. Log-phase YB886-*dinB7* (A, C) or YB886-*dinC17* (B, D) cells were exposed to mitomycin (■), UV (□), or nalidixic acid (▲) at the dose indicated in the legend to Fig. 3, harvested at the designated times following inducing treatment, and analyzed as described in the legend to Fig. 3.

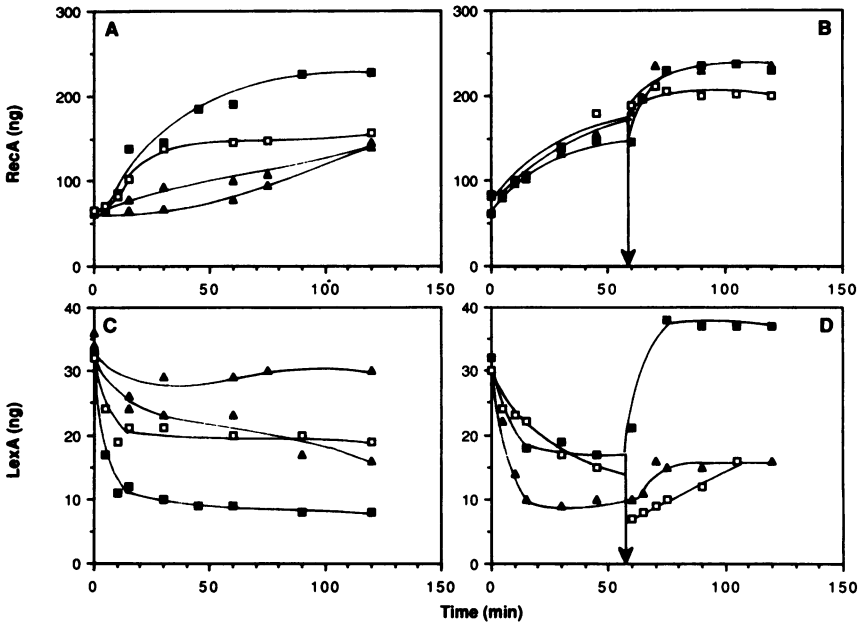

FIG. 6. Effects of HPUra on *B. subtilis* RecA induction and *E. coli* LexA disappearance and accumulation in wild-type and excision repair-deficient strains following exposure to UV radiation, mitomycin, or nalidixic acid. Log-phase *B. subtilis* cells were treated with HPUra at 200 μM, exposed to the doses of inducing agent indicated in the legend to Fig. 3, harvested at the designated times following inducing treatment, and analyzed as described in the legend to Fig. 3. (A, C) YB886 (Rec$^+$) treated with mitomycin at 0.5 μg/ml (■), UV radiation at 50 J/m^2 (□), nalidixic acid at 50 μg/ml (▲), or no DNA-damaging agent (△). (B, D) Rec$^+$ (■), *uvrA42* (□), or *polA5* (▲) cells treated with HPUra (200 μM) and irradiated with UV at 50 J/m^2. HPUra was removed from the media after 60 min, as indicated by the arrows.

survival curves following UV irradiation, despite reduced induction of the SOS response (data not shown). Both LexA cleavage and RecA induction are also significantly reduced in *dinC17* cells following nalidixic acid treatment; this is consistent with a previous report of no detectable nalidixic acid induction of the *dinC* operon in a *dinC22*::Tn*917-lacZ* fusion strain (30). By contrast, comparison of Fig. 3C and 5D indicates that induction and recovery following mitomycin treatment are not affected by the *dinC17* mutation. Thus, it appears that the *dinC* gene product is required for efficient generation of the inducing signal following UV irradiation and nalidixic acid treatment and is not critically required for other aspects of the SOS response.

The effect of replicative arrest on the *B. subtilis* SOS response. The role of DNA replication in the SOS response has been the subject of a variety of investigations in both *B. subtilis* and *E. coli*. In either bacterium, blocking DNA replication with drugs (e.g., nalidixic acid) or by temperature shifts with DNA replication mutants induces the SOS response (30, 38). On the other hand, active DNA replication is evidently the primary requirement in *E. coli* for efficient SOS induction following UV irradiation (38). As discussed above, this apparent paradox is explained by the existence of several distinct pathways for generating the SOS inducing signal.

The reduced form of HPUra is a potent and specific inhibitor of *B. subtilis* DNA polymerase III; it does not inhibit other *B. subtilis* DNA polymerases or any of the DNA polymerases of *E. coli* (4). This drug therefore provides a means for studying the role of replication in *B. subtilis* DNA repair that is not possible in *E. coli*. The effects of HPUra on DNA repair in *B. subtilis* following UV irradiation have been examined. Brown (4) showed that replicative repair (presumably by DNA polymerase I) occurs in UV-irradiated *B. subtilis* cells treated with doses of HPUra that completely block DNA replication. Fields and Yasbin (13) showed that HPUra inhibits Weigle reactivation, which is the increased survival of UV-irradiated bacteriophage in a UV-irradiated host. There is evidence that, in *E. coli*, this SOS phenomenon is primarily due to error-prone translesion DNA synthesis (8, 43). The HPUra inhibition of Weigle reactivation is consistent with evidence supporting a direct functional role of *E. coli* DNA polymerase III in error-prone repair (3, 19).

We used our assay to examine the effects of inhibiting DNA replication on the activation of RecA and the subsequent repair of damaged DNA. In the absence of DNA damage, a dose of HPUra that inhibits DNA replication by more than 95% causes a low level of LexA cleavage in wild-type cells and RecA is induced about twofold (Fig. 6A and C; Table 2). In wild-type cells treated with either mitomycin or nalidixic acid, HPUra increases the LexA cleavage rate by an amount roughly equal to the rate with HPUra alone; however, LexA does not return to its original level in mitomycin-treated cells as it does in the absence of the drug (Fig. 6A and C). In contrast, HPUra reduces the rate and extent of LexA cleavage following UV irradiation (Fig. 6C). Consistent with the results for *E. coli* (38), the reduced cleavage rate after UV treatment should correspond to less activated RecA in the absence of DNA replication. The decrease in SOS induction by UV in the absence of DNA replication could account, in part, for HPUra inhibition of Weigle reactivation (13).

Because the cleavage rate following UV irradiation is greater than that with HPUra alone, RecA is probably activated to some extent either by binding gaps generated during excision repair or by binding thymine dimers directly as suggested by Lu and Echols (32). To distinguish between these possibilities, we quantified LexA in *uvrA42* and *polA5* strains following UV irradiation and HPUra treatment. The *uvrA42* strain, deficient in the incision step of excision repair (18),

should have more dimers than the wild type at comparable doses of UV; thus, if thymine dimers serve as part of the inducing signal, the LexA cleavage rate should be greater than that of the wild type. The *polA5* strain produces a defective DNA polymerase I and presumably is deficient in filling gaps left after dimer excision (20); thus, if gaps generated by excision repair serve as part of the inducing signal, the LexA cleavage rate should be greater than that of the wild type. Figure 6D shows that the LexA cleavage rate is increased relative to that of the wild type in *polA5* cells and decreased in *uvrA42*, thus supporting the latter model.

Because the inhibitory effect of HPUra is reversible (4), we reasoned that removal of HPUra from the media should activate RecA in *uvrA42* mutants. Figure 6D shows that when the drug is removed 60 min after irradiation, there is a rapid decrease in the amount of LexA in these cells. By contrast, removal of the drug from wild-type cells results in a rapid increase in the level of LexA. In this case, the DNA should be repaired by the time the drug is removed, and LexA accumulates when replication resumes.

DISCUSSION

We have cloned the *E. coli lexA* gene in *B. subtilis* such that LexA is expressed as a fusion protein in which the first 3 amino acids of LexA have been replaced by the first 20 amino acids of the Cat86 protein. Using a quantitative immunoblot assay with antisera raised against *B. subtilis* RecA and *E. coli* LexA, we have simultaneously measured the levels of these proteins in crude extracts following DNA-damaging treatments. In every case, with a variety of DNA repair mutants and three distinct DNA-damaging agents, there is a close correlation between the rates of LexA cleavage and RecA induction. LexA is cleaved following SOS inducing treatments in *B. subtilis* $RecA^+$ cells at a rate comparable with that promoted by purified *B. subtilis* RecA in the presence of single-stranded DNA (31). Cleavage of LexA requires a functional RecA protein and does not occur in strains that are deficient in SOS induction. Taken together, these results indicate that LexA cleavage in *B. subtilis* following DNA-damaging treatments reflects the activation of RecA by an inducing signal (presumably single-stranded DNA).

The rate of LexA cleavage in vivo compares well with the rate of *B. subtilis* RecA-mediated LexA cleavage in vitro (31). On the basis of the amount of LexA cleaved in $RecA^+$ cells in the first 5 min after UV irradiation, the initial rate of cleavage is at least 70 mmol of LexA per min per mol of RecA. In vitro *B. subtilis* RecA-mediated LexA cleavage requires both single-stranded DNA and nucleoside triphosphate, and the rate depends on the nucleoside triphosphate used; the cleavage rate is 320 mmol of LexA per min per mol of RecA with dATP and 30 mmol of LexA per min per mol of RecA with ATP. RecA would have access to all nucleoside triphosphates in vivo, and the rate is likely to be intermediate between these values.

The basal level of LexA in uninduced cells depends on the genetic background, ranging between 3,400 and 5,000 molecules per cell. This range probably reflects differences in the amount of activated RecA in uninduced cells because the basal level of LexA is highest in *recA4* and *recA1* strains; these strains produce defective RecA proteins that do not promote any detectable LexA cleavage even following inducing treatments. Thus, it is unlikely that any cleavage occurs in uninduced *recA4* and *recA1* cells. The lack of cleavage activity in these mutants may be due either to a deficiency in effectively interacting with the inducing signal or to an inability to interact with LexA. The basal level of LexA is lowest in *polA5* and *uvrA42* mutants, both of which are defective in different stages of excision repair. We attribute this to a background level of DNA lesions in uninduced cells that are normally repaired by excision repair. *uvrA42* strains are unable to initiate excision repair, so that lesions otherwise repaired by excision repair would be encountered by an active replication fork and result in discontinuous replication leaving a gap that could activate RecA. Our results are consistent with the reduced level of LexA reported for an uninduced *E. coli uvrA* mutant (38).

Our results indicate that the nature of the SOS inducing signal, as characterized in *E. coli*, has been conserved in *B. subtilis*; these results also provide additional support for the *E. coli* models. Efficient induction of the *E. coli* SOS response by UV is dependent on active DNA replication (38); this is consistent with the model in which the replication fork stops at pyrimidine dimers and reinitiates beyond the lesion leaving a single-stranded gap to activate RecA (36). Using the potent and specific DNA polymerase III inhibitor HPUra, we have provided strong evidence that generation of the inducing signal in *B. subtilis* following UV irradiation primarily requires DNA replication. HPUra reduces the rate of LexA cleavage in UV-irradiated wild-type cells; subtracting the cleavage due to HPUra, we estimate that the cleavage rate is reduced by about 70%. Our results with UV-irradiated *uvrA42* cells provide additional evidence that the progress of the replication fork is necessary and sufficient for generation of the inducing signal. There is a rapid decrease in the level of LexA in irradiated *uvrA42* cells (which should contain unrepaired pyrimidine dimers) when HPUra is removed from the medium.

Discontinuous DNA replication is not the only source of inducing signal following UV exposure, because UV irradiation causes a nearly threefold increase in the rate of LexA cleavage in wild-type cells treated with HPUra. Using the *polA5* mutant, we have provided evidence that part of the inducing signal following UV irradiation is derived from single-stranded gaps generated during excision repair. The rate and extent of LexA cleavage in UV-irradiated *polA5* cells treated with HPUra (which should be unable to fill in gaps generated during excision repair with either DNA polymerase I or DNA polymerase III) are greater than in similarly treated wild-type cells. On the basis of in vitro studies with *E. coli* RecA, it has been suggested that damaged DNA can serve as an alternative inducing signal (32). Our results with HPUra-treated *uvrA42* cells clearly demonstrate that this is not the case in *B. subtilis*. In the absence of DNA replication, the rate of LexA cleavage is lower in UV-irradiated *uvrA42* cells than in irradiated wild-type cells. If pyrimidine dimers served to activate RecA in vivo, there would be more activated RecA in the excision repair mutants due to a greater number of unrepaired dimers.

The SOS inducing signal generated following mitomycin exposure must be derived in a way that does not require DNA replication, because mitomycin cross-links would stop the progress of the replication fork. Indeed, our results indicate that mitomycin induction of the SOS response is not reduced when replication is blocked. *E. coli* SOS induction by mitomycin is reduced in a *uvrA* mutant (38), supporting the model in which mitomycin lesions are processed by UvrABC-mediated incisions, on both sides of the cross-link, to produce a single-stranded substrate for recombinational repair (and thereby activate RecA) (37). Our results with the *uvrA42* and *dinA77* mutants are consistent with this model. There is a 10-minute lag before cleavage occurs in mitomycin-treated excision repair mutants and a corresponding lag in RecA induction. LexA is then cleaved at a rate that is about 20% of the wild-type rate. Thus, a low level of inducing signal is generated in these

mutants, perhaps at a blocked replication fork, by a mechanism that does not involve excision repair. This may be similar to the mechanism that activates RecA when replication is blocked by HPUra.

As expected, there is no accumulation of LexA in excision repair mutants following UV irradiation. However, it is curious that recovery from mitomycin damage in *uvrA42* and *dinA77* mutants appears normal; although the rate and extent of LexA cleavage are reduced in these strains, the time course for LexA accumulation is similar to that in the wild type. Because these strains are sensitive to mitomycin at the doses used in this study, it seems unlikely that the damage is accurately repaired (reference 15 and data not shown). However, it is not clear how the inducing signal could be removed without some sort of repair mechanism, although it is difficult to reconcile these results with the *E. coli* repair models.

Our results with the *dinB7* mutant indicate a deficiency in a repair pathway that is specific for UV photoproducts, whereas the recovery from mitomycin damage is apparently normal. This is also difficult to reconcile with the *E. coli* models, in which the complete repair of either pyrimidine dimers or mitomycin lesions is accounted for by a combination of excision repair and recombinational repair (37). The existence of a distinct repair pathway that repairs mitomycin adducts (or at least removes the inducing signal) but not UV photoproducts is suggested by our results with the *uvrA42* and *dinB7* mutants. In that case, the *dinB* gene product may be involved in either excision repair or recombinational repair. There is no significant homology between the *B. subtilis dinB* gene and any of the known *E. coli* SOS genes.

The *dinC* gene product is apparently required for SOS induction by UV and, to a lesser degree, nalidixic acid, but not mitomycin. The striking deficiency in LexA cleavage activity following UV irradiation is surprising, because *dinC* mutants are not UV sensitive (data not shown) and the *dinC* operon is itself induced by UV in *dinC* fusion strains (25). We have demonstrated that DNA replication is required for the activation of RecA following UV damage; however, the viability of *dinC* mutants argues that the *dinC* gene product is not required for DNA replication. It is possible that the *dinC* gene product either facilitates the interaction between RecA and the single-stranded gap or promotes the dissociation of polymerase from the dimer site. In any case, the characterization of the *dinC* gene product promises to reveal additional information about the mechanism of RecA activation by UV damage. There is no significant homology between the *dinC* gene and any of the known *E. coli* SOS genes.

We have presented evidence that the activation of RecA by the SOS inducing signal has been conserved between *E. coli* and the distantly related *B. subtilis*. Other conserved features of the SOS systems in these two species include the inducible SOS functions themselves (i.e., enhanced DNA repair capacity, enhanced mutagenesis, and filamentation), RecA structure and activities (31, 40a), and the interactions between RecA and the SOS repressor (27, 31). On the other hand, our results with *B. subtilis din* mutants suggest that either certain aspects of SOS activation and DNA repair have not been conserved or the *E. coli* models are not complete. Several features of the *B. subtilis* SOS response apparently have not been conserved. For example, there is evidence that the repair pathway that contributes primarily to Weigle reactivation in *B. subtilis* differs from that in *E. coli*; the repair mechanism in *B. subtilis* is apparently specific for pyrimidine dimers whereas in *E. coli* a variety of lesions are repaired (14).

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