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# Regulation of the SOS Response in *Bacillus subtilis*: Evidence for a LexA Repressor Homolog

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**The inducible SOS response for DNA repair and mutagenesis in the bacterium *Bacillus subtilis* resembles the extensively characterized SOS system of *Escherichia coli*. In this report, we demonstrate that the cellular repressor of the *E. coli* SOS system, the LexA protein, is specifically cleaved in *B. subtilis* following exposure of the cells to DNA-damaging treatments that induce the SOS response. The in vivo cleavage of LexA is dependent upon the functions of the *E. coli* RecA protein homolog in *B. subtilis* (*B. subtilis* RecA) and results in the same two cleavage fragments as produced in *E. coli* cells following the induction of the SOS response. We also show that a mutant form of the *E. coli* RecA protein (RecA430) can partially substitute for the nonfunctional cellular RecA protein in the *B. subtilis recA4* mutant, in a manner consistent with its known activities and deficiencies in *E. coli*. RecA430 protein, which has impaired repressor cleaving (LexA, UmuD, and bacteriophage λcI) functions in *E. coli*, partially restores genetic exchange to *B. subtilis recA4* strains but, unlike wild-type *E. coli* RecA protein, is not capable of inducing SOS functions (expression of DNA damage-inducible [*din*::Tn*917-lacZ*] operons or RecA synthesis) in *B. subtilis* in response to DNA-damaging agents or those functions that normally accompany the development of physiological competence. Our results provide support for the existence of a cellular repressor in *B. subtilis* that is functionally homologous to the *E. coli* LexA repressor and suggest that the mechanism by which *B. subtilis* RecA protein (like RecA of *E. coli*) becomes activated to promote the induction of the SOS response is also conserved.**

In the bacterium *Escherichia coli*, the SOS response for DNA repair is regulated by the molecular interaction of the LexA and RecA proteins. The LexA protein participates by singularly repressing the many (ca. 20) unlinked DNA damage-inducible (*din*) chromosomal operons that compose the SOS regulon, including the genes for LexA (*lexA*) and RecA (*recA*) themselves (for review, see reference 54). RecA protein, the most thoroughly characterized enzyme of its type, functions directly in general genetic recombination (42), postreplicational DNA repair, and mutagenesis as well as in induction of the cellular SOS response to DNA damage by its specific interaction with cellular repressors (54). Induction of the SOS response following DNA damage results from the reversible "activation" of RecA protein to a conformation (RecA*) that promotes the specific proteolytic cleavage of the LexA repressor (22), the UmuD protein (3, 39, 50), and the repressors of certain resident bacteriophages (22, 44). Inactivation of the LexA repressor by proteolytic cleavage leads to the increased synthesis of a specific set of proteins which function primarily in DNA repair and mutagenesis (40), resulting in the coordinate expression of the diverse set of cellular phenomena that characterize the SOS response. Among these are an enhanced capacity for DNA repair, chromosome and phage mutagenesis, inhibition of cell division (filamentation), and induction of the RecA protein (54).

The activation of RecA protein is thought to result from the binding of RecA to exposed regions of single-stranded DNA (ssDNA) generated as a result of replication past sites of DNA damage, followed by specific surface interactions with small inducer molecules and target repressor proteins (11, 46). Although the biochemical process by which RecA protein becomes activated following exposure of cells to agents that damage DNA is not well understood, the mechanism of inactivation of the LexA protein is presumed to require the recognition and binding of RecA* to the LexA protein and to involve specific functional groups on the repressor molecule (22).

Recent studies have shown that the SOS response in *Bacillus subtilis* and the SOS response in *E. coli* are remarkably similar from both a phenotypic and regulatory standpoint (24, 25, 28, 30). The SOS response of *B. subtilis* also consists of a set of coordinately induced functions, such as enhanced capacity for DNA repair and mutagenesis, Weigle reactivation, filamentation, and prophage induction (24). As in *E. coli*, the inducible expression of SOS phenomena in *B. subtilis* depends primarily upon the activities of a multifunctional enzyme, designated RecA (formerly called Recbs or Rec protein), the product of the *recA* gene (formerly designated *recE*) (7, 9, 25, 28, 30, 33). *B. subtilis* RecA, like *E. coli* RecA, is a DNA damage-inducible protein and functions in DNA strand exchange (genetic or homologous recombination), postreplication or recombinational repair, and regulation of the SOS response (25, 30). Notwithstanding the significant differences that serve to distinguish the SOS systems in these two bacteria (23, 24, 27), collectively these results demonstrate that the *B. subtilis* RecA protein serves a similar, and perhaps identical, role in catalyzing the processes of DNA recombination and repair and in controlling the many genes that compose the SOS system.

There is also considerable evidence for the existence of a negative regulatory element, similar to the *E. coli* LexA repressor, which serves to control the inducible expression

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of the SOS response in *B. subtilis*. First, that the wild-type RecA protein of *E. coli* can substitute for *B. subtilis* RecA to induce the SOS response in *B. subtilis recA* mutants demonstrates its ability both to become activated by the SOS-inducing signals that are generated in *B. subtilis* following DNA-damaging treatments and to interact with the regulatory components of the SOS system in this bacterium (25, 28). This regulatory interaction presumably is with a cellular LexA-like repressor (or LexA homolog) in *B. subtilis*, since it consequently leads to the specific and coordinate derepression of the SOS response and does so in a manner that is identical to that which occurs in wild-type *B. subtilis* ($recA^+$) cells. Second, purified *B. subtilis* RecA protein is capable of efficiently catalyzing the cleavage of purified *E. coli* LexA protein in an in vitro reaction requiring ssDNA and a nucleotide triphosphate (30). These results suggest that the molecular mechanism by which *B. subtilis* RecA becomes activated to stimulate the proteolytic cleavage of its cognate cellular and prophage repressor(s) in vivo may also be functionally conserved in these phylogenetically divergent eubacteria (56).

In this work, we report the results of experiments in which we have characterized the damage-inducible cleavage of the *E. coli* cellular LexA repressor in *B. subtilis* as a model for understanding the mechanism of SOS induction in *B. subtilis*. We have expressed the *E. coli* LexA protein in *B. subtilis* cells and shown that it is cleaved specifically in vivo in response to DNA damage caused by mitomycin (MMC) and UV radiation. The cleavage of *E. coli* LexA in *B. subtilis* following DNA damage parallels the induction of the SOS response, requires a functional cellular RecA protein, and occurs at the same site in the protein sequence as its inducible RecA*-mediated cleavage in *E. coli* cells. We have also analyzed the ability of similarly expressed *E. coli* RecA430 protein to promote the induction of SOS functions in *B. subtilis recA4* mutants (formerly called *recE4* [9]) that are apparently controlled by a common cellular repressor. That *E. coli* RecA430 catalyzes recombination but not SOS induction in *B. subtilis* indicates that the process of activation of RecA to promote cleavage of the putative LexA homolog is also conserved in these two bacteria.

## MATERIALS AND METHODS

**Bacterial strains and plasmids.** The *B. subtilis* and *E. coli* strains and plasmids used in this study are listed in Table 1. The *B. subtilis dinC22*::Tn*917-lacZ* operon fusion strains (23) produce increased levels of β-galactosidase (*lacZ* gene product) when exposed to a variety of DNA-damaging agents (UV radiation, ethyl methanesulfonate [EMS], and MMC) or when grown to physiological competence independent of any exposure to agents that damage DNA or interfere with DNA replication. The plasmids pPL708-*lexA* and pPL708-*recA430*, constructed as described below, express full-length *E. coli* LexA and RecA430 proteins, respectively, constitutively during the exponential and stationary phases of growth in *B. subtilis* cells under the transcriptional control of the *B. subtilis* bacteriophage SPO2 promoter.

**Materials and media.** Restriction endonucleases and DNA-modifying enzymes were purchased from Bethesda Research Laboratories and New England BioLabs. Affinity-purified goat anti-rabbit immunoglobulin G (IgG)-horseradish peroxidase conjugate, peroxidase color reagent (4-chloro-1-naphthol), and Protein Assay Kit were purchased from Bio-Rad Laboratories. Antibiotics, EMS, MMC, *o*-nitrophenyl-β-D-galactoside, and protein A-Sepharose CL-4B were obtained

TABLE 1. Bacterial strains and plasmids

| Strain or plasmid | Relevant genotype | Source or reference |
|---|---|---|
| *Bacillus subtilis* | | |
| YB886 | *metB5 trpC2 recA*$^+$ *xin-1* SPβ$^-$ | 24 |
| YB1015 | *metB5 trpC2 recA4 xin-1* SPβ$^-$ | 24 |
| WB105[a] | *trpC2 recA*$^+$ | This work |
| WB110[b] | *trpC2 recA4* | This work |
| YB886/*dinC22* | *dinC22*::Tn*917-lacZ recA*$^+$ | 23 |
| YB1015/*dinC22* | *dinC22*::Tn*917-lacZ recA4* | 23 |
| *Escherichia coli* | | |
| MM294 | *recA*$^+$ *lexA*$^+$ | B. Bachmann[c] |
| JL2301 | Δ(*lac-pro*) *sulA211 recA*$^+$ Δ*lexA300*(ΩSpc$^r$) (λ *sulA*::*lacZ* cI *ind att*$^+$) | M. Smith (14) |
| KP301 | *dam-13*::Tn9 *lexA*$^+$ | |
| Plasmids | | |
| pJWL24 | Ap$^r$ *lexA*$^+$ | M. Smith (20) |
| Yrp12-*recA430* | Ap$^r$ *recA430* | D. Ennis (16) |
| pPL608, pPL708 | Cm$^r$ Km$^r$ | P. Lovett (36) |
| pPL608-*recA* | pPL608 derivative carrying *E. coli recA*$^+$ gene | G. Venema (6) |
| pPL708-*lexA* | pPL708 derivative carrying *E. coli lexA*$^+$ gene | This work |
| pPL708-*recA430* | pPL708 derivative carrying *E. coli recA430* gene | This work |

[a] Met$^+$ transformant of YB886.
[b] Constructed by congression (transformation) of *recA4* mutation (9) into WB105.
[c] E. coli Genetic Stock Center, Yale University.

from Sigma Chemical Co. Renografin (RENO-M-60; diatrizoate meglumine) was obtained from Squibb Diagnostics. L-[$^{35}$S]methionine (>800 Ci/mmol) and EnLightning were obtained from Du Pont Biotechnology Systems. Nitrocellulose membranes and Elutip-d columns were purchased from Schleicher & Schuell. Immobilon-P (polyvinylidene difluoride) membranes were from Millipore. T7 DNA polymerase and purified (>99%) *E. coli* RecA protein were purchased from US Biochemicals. Purified *E. coli* LexA protein, in vitro-generated LexA cleavage products, and polyclonal LexA antiserum were the generous gifts of Margaret Smith and John Little, University of Arizona. Polyclonal antiserum to *E. coli* RecA protein was prepared by primary immunization (subcutaneous injection) of New Zealand White rabbits with 100 μg of purified RecA protein suspended in 1 ml of complete Freund's adjuvant, followed by four boosts of 10 to 40 μg each of RecA protein in 0.5 ml of incomplete Freund's adjuvant injected at 3-week intervals. Antisera were collected at 7 and 14 days following the last injection.

Minimal glucose and competence media (GM1 and GM2) were prepared as described previously (57). The *din* operon fusion strains were maintained on LB medium (35) containing erythromycin (0.1 μg/ml) and lincomycin (25 μg/ml). Strains carrying plasmids pPL608, pPL708 (36), and their derivatives, described here, were maintained on LB containing both chloramphenicol and kanamycin at 5 μg/ml.

**Genetic procedures.** Liquid cultures of *B. subtilis* strains were grown to maximize competence, transformed with chromosomal or plasmid DNAs, and fractionated on Renografin block gradients as described before (23, 57). Samples collected from Renografin gradient fractions or from cultures challenged with MMC during exponential

growth in GM1 were assayed for β-galactosidase production by a modification of the Miller procedure (35) as described previously (57). Plasmid DNAs constructed by in vitro ligation were first introduced into *B. subtilis* cells by the transformation of protoplasts, since efficiencies are higher than after transformation of competent cells (4; unpublished results).

**Cloning of the *E. coli recA430* gene in *B. subtilis* plasmid pPL708.** The 3.2-kb *Bam*HI fragment containing the *E. coli recA430* gene was isolated from plasmid Yrp12-*recA430* (16), gel purified (47), ligated to *Bam*HI-digested pPL708 DNA, and transformed into protoplasts of strain YB1015 (*recA4*), with selection on DM3 plates containing kanamycin (100 μg/ml) (4). Putative *recA430*-containing plasmid transformants were then tested for resistance to 0.1% EMS, since strain YB1015 alone will not grow on rich medium containing more than 0.05% EMS (unpublished results). The plasmid structure of several $Cm^r$ $Km^r$ $EMS^r$ transformants was confirmed by restriction enzyme analysis (32), making use of the asymmetric *Pst*I and *Eco*RI sites in the *E. coli recA430* gene to determine its transcriptional orientation (parallel) relative to the SPO2 promoter in plasmid pPL708 (Fig. 1).

**Cloning of the *E. coli lexA* gene in *B. subtilis* plasmid pPL708.** Plasmid pJWL24 (formerly pJL24), which carries the wild-type *E. coli lexA* gene on a 5.7-kb *Bam*HI-*Bgl*II chromosomal DNA fragment (20), was propagated in *E. coli* KP301 (*dam-13*::Tn*9* $lexA^+$) to allow enzyme cleavage at the methylation-sensitive *Cla*I-*Mbo*I site distal to the 3′ end of *lexA*. A 5.2-kb *Cla*I *lexA*-containing fragment was gel purified (47), and the ends of this fragment were filled in with T7 DNA polymerase and deoxyribonucleotide triphosphates (32) and then digested with *Bam*HI. The approximately 1,250-bp *Bam*HI-*Cla*I fragment was ligated to pPL708 DNA previously digested with *Bam*HI and *Sal*I (made blunt-ended by filling in) and used directly to transform protoplasts of *B. subtilis* YB886 ($recA^+$), with selection for $Km^r$ as described above.

To confirm that the fragment isolated from pJWL24 for subcloning into pPL708 contained a functional *E. coli* $lexA^+$ gene, the fragment was ligated to pBR322 and transformed (32) into *E. coli* JL2301, which completely lacks all resident *lexA* sequences and carries a fusion of the LexA-regulatable (repressible) *sulA* promoter to the *lacZ* gene on a λ prophage (14). Ampicillin-resistant transformants of JL2301 harboring plasmids containing the $lexA^+$ fragment formed white (i.e., $Lac^-$) colonies on MacConkey-lactose indicator plates, indicating that the plasmid-encoded LexA protein efficiently repressed the *sulA::lacZ* fusion in this strain. By contrast, strain JL2301 carrying pBR322 alone formed dark red ($Lac^+$) colonies on MacConkey plates, demonstrating that the *sulA::lacZ* fusion was not repressed.

**Electrophoresis and immunoblot procedures.** Sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis was performed essentially as described by Laemmli (19). At each indicated time point, an equivalent sample of cells, corresponding to a volume of 1.0 ml at an $A_{600}$ of 0.6, was collected, chilled on ice for 5 min, centrifuged at 16,000 × *g* at 4°C, and frozen. Cell pellets were resuspended in 10 μl of cold cracking buffer (10 mM Tris-HCl [pH 8.0], 100 mM EDTA, 50 mM NaCl, 2 mM phenylmethylsulfonyl fluoride) containing 1 mg of lysozyme per ml and incubated on ice for 30 min prior to the addition of sample treatment buffer and electrophoresis. Immunoblot transfers to nitrocellulose membranes and antibody detections were performed as described previously (25, 30) unless otherwise indicated.

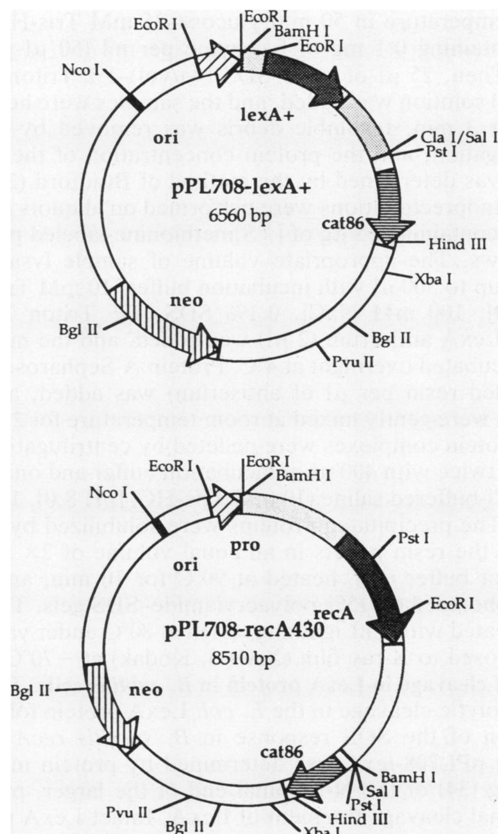


FIG. 1. Structure of *B. subtilis* plasmids pPL708-*lexA* and pPL708-*recA430*. Details of the construction of these plasmids are given in Materials and Methods. *cat-86* is a chloramphenicol acetyltransferase gene (chloramphenicol resistance) derived from *Bacillus pumilis* (36). *neo*, a gene that confers resistance to neomycin and kanamycin, and the replication origin *ori* are derived from the pUB110 moiety of plasmid pPL708, and *pro* is the fragment derived from the *B. subtilis* bacteriophage SPO2, having strong constitutive promoter activity, present in plasmids pPL608 and pPL708 (36). $lexA^+$ and *recA* are the *E. coli* wild-type *lexA* and *recA430* genes, respectively. Arrows indicate direction of transcription for the SPO2 promoter and identified genes.

**Labeling and immunoprecipitation of *E. coli* LexA protein in *B. subtilis*.** The approach we have used to examine the in vivo cleavage of *E. coli* LexA repressor in *B. subtilis* is based upon that of Little (21) and incorporates modifications of procedures for cell lysis and immunoprecipitation similar to those described by Arnosti et al. (1). *B. subtilis* strains WB105 ($recA^+$) and WB110 (*recA4*) carrying plasmid pPL708-*lexA* were grown with aeration at 37°C in minimal glucose medium to a density of about 5 × $10^8$ cells per ml ($A_{600}$ of 0.4 to 0.5) and then radioactively labeled as follows. With cells at the desired density, [$^{35}$S]methionine was added (25 μCi/ml) to a portion of the culture, and the incubation was continued for 5 min to allow time for the uptake and incorporation of labeled methionine into cellular proteins. At this point, the cells were UV irradiated in a glass petri dish at room temperature and then immediately returned to a flask at 37°C and incubated as before. At the indicated time intervals following UV irradiation, 1.0-ml samples were taken, added to 10 μl of nonradioactive 100 mM L-methionine, chilled on ice for 5 min, then centrifuged at 16,000 × *g* for 5 min at 4°C, and rapidly frozen in dry ice. The cell pellets were thawed and lysed by incubation for 15 min at

room temperature in 50 mM glucose–25 mM Tris-HCl [pH 8.0] containing 0.1 mg of lysozyme per ml (50 μl per $10^8$ cells). Then, 25 μl of a 1% SDS (wt/vol)–1% Triton X-100 (vol/vol) solution was added, and the samples were heated at 90°C for 3 min. Insoluble debris was removed by a brief centrifugation, and the protein concentration of the supernatant was determined by the method of Bradford (2).

Immunoprecipitations were performed on aliquots of each sample containing 25 μg of [$^{35}$S]methionine-labeled proteins as follows. The appropriate volume of sample lysate was diluted up to 500 μl with incubation buffer (10 mM Tris-HCl [pH 8.0], 100 mM NaCl, 0.1% SDS, 1% Triton X-100), excess LexA antiserum (2 μl) was added, and the mixtures were incubated overnight at 4°C. Protein A-Sepharose (10 μl of swelled resin per μl of antiserum) was added, and the samples were gently mixed at room temperature for 2 h. The resin-protein complexes were pelleted by centrifugation and washed twice with 400 μl of incubation buffer and once with Tris-HCl-buffered saline (10 mM Tris-HCl [pH 8.0], 100 mM NaCl). The precipitated proteins were solubilized by resuspending the resin pellets in an equal volume of 2× sample treatment buffer (19), heated at 90°C for 10 min, and then electrophoresed on 15% polyacrylamide–SDS gels. The gels were treated with EnLightning, dried at 80°C under vacuum, and exposed to X-ray film (XAR-5, Kodak) at −70°C.

**Site of cleavage in LexA protein in *B. subtilis* cells.** The site of proteolytic cleavage in the *E. coli* LexA protein following induction of the SOS response in *B. subtilis recA*$^+$ cells carrying pPL708-*lexA* was determined by protein microsequencing (34) of the N-terminal end of the larger, putative C-terminal cleavage fragment of LexA. Intact LexA protein and in vivo cleavage products were immunoprecipitated with 10 μl of LexA antiserum from crude extracts of cells from a 500-ml culture of *B. subtilis* WB105 (pPL708-*lexA*) after challenge with MMC (300 ng/ml) for 90 min essentially as described above. The precipitated proteins were electrophoresed on a 15% polyacrylamide–SDS gel and electroblotted onto polyvinylidene difluoride membranes in 25 mM Tris (pH 8.3)–192 mM glycine–15% methanol at 150 mA for 2.5 h at 4°C. Protein bands corresponding in size (13 kDa) to the purified LexA C-terminal fragment were visualized by staining the blot with Coomassie blue, excised, and sequenced directly. The amino acid analysis and sequencing (Edman degradation) were performed on an Applied Biosystems model 477A Protein Sequencer at the Macromolecular Structure Facilities of the Division of Biotechnology, University of Arizona.

## RESULTS

**Synthesis of *E. coli* LexA and RecA proteins in *B. subtilis*.** Chromosomal DNA fragments containing the *E. coli lexA*$^+$ and *recA430* genes were subcloned into the expression vector pPL708 and introduced into *B. subtilis* cells. Since these constructions place the native *E. coli* genes under the transcriptional control of the endogenous *B. subtilis* bacteriophage SPO2 promoter in pPL708 (Fig. 1), they are not regulated (i.e., damage inducible) in their normal way to any observable degree (e.g., Fig. 2; unpublished results) in any of the *B. subtilis* strains following DNA-damaging treatments.

We confirmed the synthesis of the *E. coli* LexA (ca. 22 kDa) and RecA430 (38 kDa) proteins in *B. subtilis* strains carrying the plasmids pPL708-*lexA* and pPL708-*recA430*, respectively, by Western immunoblot analysis with polyclonal antisera to purified *E. coli* LexA and RecA proteins.

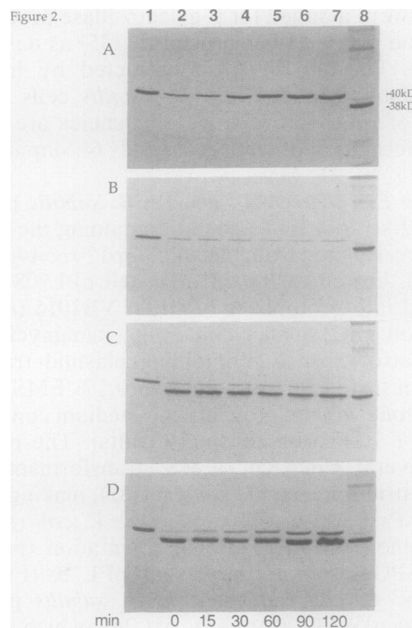


FIG. 2. Induction of *B. subtilis* RecA by *E. coli* RecA430 and RecA$^+$ proteins. Mid-exponential-phase cultures ($A_{600}$ of 0.3 to 0.4) of *din recA*$^+$ and *din recA4* strains of *B. subtilis* were challenged with MMC (0.5 μg/ml), and aliquots were taken at the times indicated for Western immunoblot analysis. Crude extracts from equivalent amounts of cells (corresponding to 1 ml of cells at an $A_{600}$ of 0.6) were electrophoresed in 12.5% polyacrylamide–SDS gels, transferred to nitrocellulose, and incubated with antiserum to purified *E. coli* RecA protein. The positions of the 38-kDa *E. coli* RecA and RecA430 proteins and the 40-kDa *B. subtilis* RecA protein are indicated in panel A. YB886 is *recA*$^+$; YB1015 is *recA4*. (A) YB886/*dinC22*(pPL708); B, YB1015/*dinC22*(pPL708); C, YB1015/*dinC22*(pPL708-*recA430*); D, YB1015/*dinC22*(pPL608-*recA*). Control lanes: Lane 1, YB886/*dinC22*(pPL708) after 60 min of exposure to MMC; lane 8, *E. coli* MM294 (*recA*$^+$), no MMC treatment. Only the portion of each immunoblot having the relevant protein bands is shown.

The immunoreactive 22-kDa and 38-kDa proteins comigrate electrophoretically with *E. coli* chromosomally encoded LexA (data not shown) and RecA proteins (Fig. 2C and D), as well as with purified *E. coli* LexA and RecA, respectively (data not shown), and are not produced in *B. subtilis* strains containing plasmid pPL708 (Fig. 2A and B). The 40-kDa *B. subtilis* RecA protein also cross-reacts with antiserum to *E. coli* RecA and is readily detectable in cell extracts of both *recA*$^+$ and *recA4* strains (Fig. 2). Previous studies (6, 25) have detailed the activities of the 38-kDa wild-type *E. coli* RecA protein in *B. subtilis* strains expressed by the plasmid pPL608-*recA* (Fig. 2D).

**Activities of *E. coli* RecA430 Protein in *B. subtilis* cells.** Following DNA-damaging treatments, *E. coli* RecA protein is capable of effectively substituting for its nonfunctional homolog in *B. subtilis recA4* mutants in restoring both DNA repair and recombination capability and inducing *din* operon expression, Weigle reactivation activity, and the synthesis of *B. subtilis* RecA, but is unable to stimulate prophage induction (6, 25, 28). These results provide considerable evidence that *E. coli* RecA indeed responds to the SOS-inducing signals that are generated in *B. subtilis* cells following DNA damage and can mediate the expression of the genes under the regulatory control of the SOS system in this bacterium.

The most plausible explanation for these properties of *E. coli* RecA is that SOS phenomena in *B. subtilis* are coordinately regulated cellular events whose expression is modulated by a common cellular repressor that is functionally homologous to the LexA protein of *E. coli*. However, it is also conceivable that the *E. coli* RecA protein may play an indirect role in stimulating the expression of SOS functions in *B. subtilis* that either does not involve interaction with a putative LexA homolog or that is mechanistically distinct from its regulatory interaction with the LexA repressor in *E. coli* cells. To address this question, the gene for an *E. coli* RecA protein that is known to be deficient in regulatory activities in *E. coli* was similarly cloned and expressed in the *recA4* mutant of *B. subtilis*. We reasoned that if the SOS-like inducing activity observed previously with the wild-type *E. coli* RecA protein (25) is selectively impaired in *B. subtilis* cells carrying the mutant form of *E. coli* RecA, this would provide additional evidence that (i) the regulatory (repressor-cleaving) function of *E. coli* RecA is required in *B. subtilis*, as it is in *E. coli*, for this activity and (ii) that a LexA repressor homolog likely controls the inducible expression of SOS functions in *B. subtilis*.

The genetic analyses of a large number of *E. coli recA* mutants (11, 52) provide convincing evidence that the recombination and DNA strand exchange activities of the RecA protein are to a certain extent independent of its regulatory activities (i.e., those that promote LexA protein cleavage, λ *c*I repressor cleavage, and UmuD cleavage). This separation of functions of *E. coli* RecA is particularly evident in the so-called split-function phenotype exhibited by the *recA430* mutant (11). The *recA430* mutation generates a RecA protein that is (i) defective in inducible DNA repair and SOS mutagenesis, (ii) unable to promote the cleavage of the λ *c*I repressor and UmuD proteins (3, 43, 50), (iii) capable of only inefficient (20 to 30% of wild-type) cleavage of LexA protein both in vivo and in vitro (11, 31, 46), and (iv) near normal in genetic recombination activity (37, 43). The relatively limited SOS induction in response to DNA damage in *E. coli recA430* cells is consistent with RecA430 being inefficient in promoting the in vivo cleavage of repressor proteins (e.g., LexA and λ *c*I), most likely as a consequence of its marked deficiency in the ATP (and dATP)-dependent binding to ssDNA that is necessary for the formation of the active complex required for cleavage (46).

Because of this specific deficiency in SOS induction in *E. coli*, we examined the extent to which the mutant RecA430 protein can promote the expression of inducible SOS functions in a *B. subtilis recA4* host strain. To determine whether the RecA430 protein (expressed by the plasmid pPL708-*recA430*) was functional in *B. subtilis* cells, we first characterized its ability to catalyze genetic exchange by homologous recombination (i.e., chromosomal DNA-mediated transformation) in a *recA4* mutant. In this context, it is noteworthy that while DNA repair, homologous recombination, and induction of the SOS response, including RecA synthesis and *din* gene expression, are abolished in *B. subtilis* strains carrying the *recA4* mutation (9, 24, 28), the presence of this mutation alone does not diminish the ability of these strains to become competent for DNA uptake and transformation. Strains carrying the *recA4* mutation can undergo transformation with nonhomologous plasmid DNA at wild-type frequencies (23, 24; unpublished results).

As expected, pPL708-*recA430*, like pPL608-*recA* (25), restores chromosomal DNA-mediated transformability to a competent *recA4* mutant (YB1015/*dinC22*), although at a 10-fold lower level than either the *recA*$^+$ strain (YB886/*dinC22*) or the same *recA4* strain carrying pPL608-*recA* (Table 2). The mutant RecA430 protein has no significant effect on either homologous transformation or *din* operon induction in *recA*$^+$ competent cells (Table 2). In addition, pPL708-*recA430* significantly increases the resistance of *recA4* strains to the DNA-damaging agents EMS and MMC (data not shown), although survival is not enhanced to the extent observed with the plasmid pPL608-*recA* (28; unpublished results). This capacity of *E. coli* RecA430 to partially restore genetic transformation and recombinational repair in *B. subtilis* is consistent with its ability to catalyze DNA strand exchange in *E. coli* (42).

TABLE 2. Effect of plasmids pPL708-*recA430* and pPL608-*recA* on chromosomal DNA-mediated transformation frequency and *din* operon expression (β-galactosidase activity) in competent *B. subtilis dinC22*::Tn*917-lacZ* fusion strains[a]

| Fusion strain | Met$^+$ transformation frequency[b] | β-Galactosidase activity[c] ($U/A_{600}$ unit) | |
|---|---|---|---|
| | | T | B |
| YB886/*dinC22*(pPL708) | $7.3 \times 10^{-3}$ | 119.3 | 8.1 |
| YB886/*dinC22*(pPL708-*recA430*) | $5.8 \times 10^{-3}$ | 114.8 | 3.5 |
| YB1015/*dinC22*(pPL708) | $>9.1 \times 10^{-7}$ | 1.2 | 0.7 |
| YB1015/*dinC22*(pPL708-*recA430*) | $2.0 \times 10^{-4}$ | 4.4 | 2.7 |
| YB1015/*dinC22*(pPL608-*recA*) | $2.7 \times 10^{-3}$ | 47.6 | 3.3 |

[a] *dinC22 recA*$^+$ (YB886) and *recA4* (YB1015) fusion strains were grown to maximize competence, transformed with *met*$^+$ DNA, and fractionated in Renografin density gradients as described before (57). β-Galactosidase assays were performed on samples of cells collected from gradient bands after washing and resuspension in GM1 medium. Results shown are representative of duplicate experiments.

[b] Met$^+$ transformation frequencies were determined by dividing the number of CFU of Met$^+$ transformants by the total number of viable cells in an aliquot of unfractionated cells from each culture.

[c] T, top band of Renografin gradients, containing predominantly competent cells; B, bottom band of Renografin gradients, containing predominantly noncompetent cells.

Unlike wild-type *E. coli* RecA, the RecA430 protein was incapable of restoring *din*::Tn*917-lacZ* operon expression (as measured by β-galactosidase activity) in *B. subtilis recA4* mutants following DNA damage. When a representative *din*::Tn*917-lacZ recA4* strain (YB1015/*dinC22*) carrying pPL708-*recA430* was treated with inducing levels of MMC (Fig. 3) or UV irradiation (data not shown), no significant increase in β-galactosidase production was observed compared with the same *din recA4* fusion strain carrying the plasmid pPL608-*recA* or the *din recA*$^+$ strain YB886/*dinC22*(pPL708).

Recent reports (25, 28) have shown that wild-type *B. subtilis* RecA protein regulates its own production and that *E. coli* RecA is capable of inducing the synthesis of the nonfunctional RecA protein in *B. subtilis recA4* cells. When we examined the ability of *E. coli* RecA430 to complement the deficiency in cellular RecA induction in the *recA4* mutant, the results (Fig. 2C) indicated that *E. coli* RecA430 could not restore the synthesis of *B. subtilis* RecA protein in strain YB1015/*dinC22*(pPL708-*recA430*) in response to continuous exposure to MMC. Results with the same strain carrying pPL608-*recA* are shown in Fig. 2D for comparison.

In *B. subtilis*, the development of physiological competence for DNA binding and uptake (8) is accompanied by the induction of the SOS response (23), independent of exposure to DNA-damaging agents. Expression of the various SOS functions during competence in *B. subtilis* is *recA* dependent and presumably occurs as a secondary, albeit direct, conse-

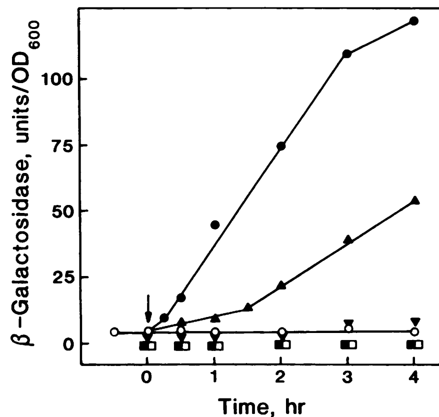


FIG. 3. Effect of plasmids pPL708-*recA430* and pPL608-*recA* on *dinC22*::Tn*917-lacZ* operon induction (β-galactosidase activity) following DNA damage in *din recA*$^+$ and *din recA4* fusion strains of *B. subtilis*. During mid-exponential growth ($A_{600}$ of 0.3) at 37°C in GM1 medium, cultures of YB886/*dinC22* (*recA*$^+$) and YB1015/*dinC22* (*recA4*) were divided (at the time indicated by the arrow) and either challenged with MMC at 0.5 μg/ml (solid symbols) or not treated (open symbols). At the designated time points aliquots (0.5 to 1.0 ml) of each culture were collected and assayed for β-galactosidase activity as described in Materials and Methods. ● and ○, *recA*$^+$ (pPL708); ■ and □, *recA4* (pPL708); ▼, *recA4* (pPL708-*recA430*); ▲, *recA4* (pPL608-*recA*). Data for untreated cultures of the *B. subtilis recA4* (pPL708-*recA430*) and *recA4* (pPL608-*recA*) strains are essentially the same as for the *recA4* (pPL708) strain and are not shown.

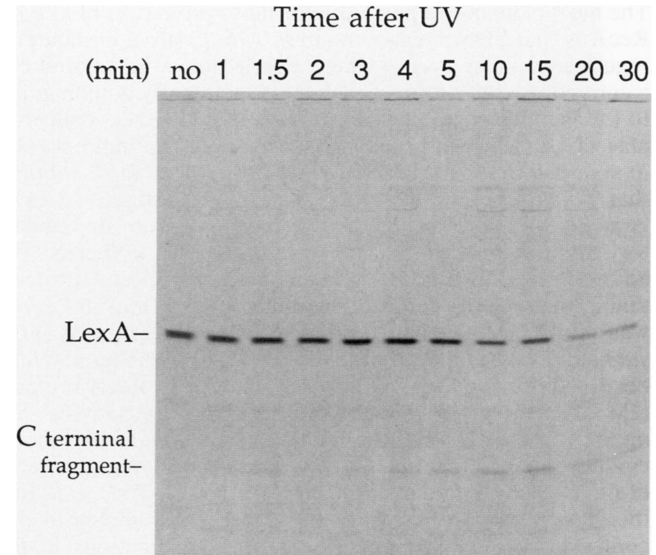


FIG. 4. Onset of induction following UV treatment as shown by cleavage of *E. coli* LexA protein in *B. subtilis recA*$^+$ strain. WB105(pPL708-*lexA*) cells were grown at 37°C and labeled with L-[$^{35}$S]methionine for 5 min, irradiated with UV light (25 J/m$^2$), and sampled at the times indicated. Crude cell extracts were prepared, and radiolabeled LexA protein and cleavage products were immunoprecipitated and subjected to SDS-polyacrylamide gel electrophoresis as described in Materials and Methods. Time is in minutes after UV irradiation. Positions of the intact LexA protein (22 kDa) and its C-terminal fragment (13 kDa) are indicated. The "no" sample in lane 1 was labeled for 5 min and given no UV treatment.

quence of the competence-specific amplification and subsequent "activation" of the cellular RecA protein by metabolic signals (SOS inducing) that are generated during competence development (25, 27). Such inducing signals during competence are believed to originate either from the DNA processing associated with homologous recombination or the generation and presence of single-stranded gapped regions in DNA, both of which occur in competent cells (13). There is now significant evidence that ssDNA, in the presence of RecA and nucleoside triphosphate, is the critical inducing signal leading to the activation of RecA and ensuing induction of the SOS response following DNA damage in *E. coli* (46).

Interestingly, the wild-type *E. coli* RecA protein is activated during competence development in *B. subtilis* cells, as evidenced by the induction of β-galactosidase activity in the competent subpopulation of *din*::Tn*917-lacZ recA4* cells carrying pPL608-*recA* (25) (Table 2). This strongly suggests that the metabolic signals generated during competence are indeed similar (if not identical) to those that result internally from the production and recombinational repair of damaged duplex DNA. Since the concomitant induction of SOS functions during competence is thought to be the result of the cellular RecA*-mediated (or *E. coli* RecA*-mediated) cleavage of the *B. subtilis* LexA homolog (25, 27), we tested the ability of *E. coli* RecA430 to promote *din*::Tn*917-lacZ* operon expression in strain YB1015/*dinC22*(pPL708-*recA430*) that had been grown to competence and fractionated on Renografin gradients to separate competent cells from the noncompetent majority. The results (Table 2) show that while RecA430 can indeed complement the homologous recombination deficiency (Met$^+$ transformation) of *B. subtilis* RecA in competent *recA4* mutants, it is unable to bring about the normal pattern of SOS induction (β-galactosidase production) that is specific to the competent cell fraction (top band of gradients) of the *recA*$^+$ strain (YB886/*dinC22*) or the *recA4* mutant (YB1015/*dinC22*) containing plasmid pPL608-*recA*.

**Cleavage of *E. coli* LexA repressor in *B. subtilis recA*$^+$ and *recA4* strains.** That purified *B. subtilis* RecA protein from a *recA*$^+$ strain catalyzes the proteolytic cleavage of purified *E. coli* LexA repressor protein in an in vitro reaction requiring both ssDNA and nucleoside triphosphates (30) suggests that the LexA repressor homolog in *B. subtilis* is likewise inactivated in vivo by the cleavage-promoting activity of the cellular RecA protein. Furthermore, the ability of *E. coli* RecA protein, but not RecA430, to restore the inducible, coordinate expression of functions presumably repressed by the LexA homolog (*din* gene activation and *B. subtilis* RecA synthesis) to *recA4* mutants provides substantial evidence to support this hypothesis.

To test this model further, we have examined the ability of *B. subtilis* RecA to promote the cleavage of the *E. coli* LexA protein in both *recA*$^+$ and *recA4* mutant cells. In an approach based upon that used by Little (21), exponentially growing liquid cultures of *B. subtilis* strains harboring the plasmid pPL708-*lexA* were pulse labeled with [$^{35}$S]methionine either before or after a low dose of UV radiation sufficient to induce the SOS response. Cell samples were taken at frequent intervals thereafter, crude extracts were prepared, and polyclonal LexA antiserum was used to selectively precipitate radiolabeled LexA protein and its cleavage products. The LexA protein-antibody complexes were subsequently analyzed by SDS-polyacrylamide gel electrophoresis and fluorography.

Following UV irradiation (25 J/m$^2$) of *recA*$^+$ strain WB105(pPL708-*lexA*), LexA protein is clearly cleaved, as

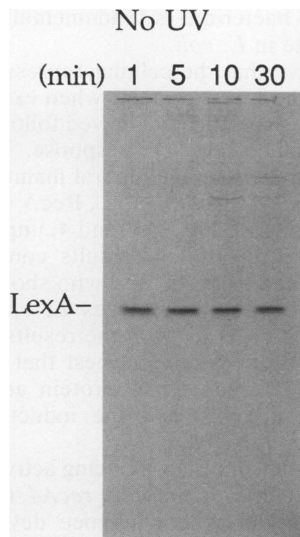


FIG. 5. *E. coli* LexA protein stability in *B. subtilis recA*$^+$ strain WB105(pPL708-*lexA*) without UV treatment. The experiment is similar to that described in the legend to Fig. 4 except that cells were not UV irradiated following the 5-min pulse with radiolabeled methionine.

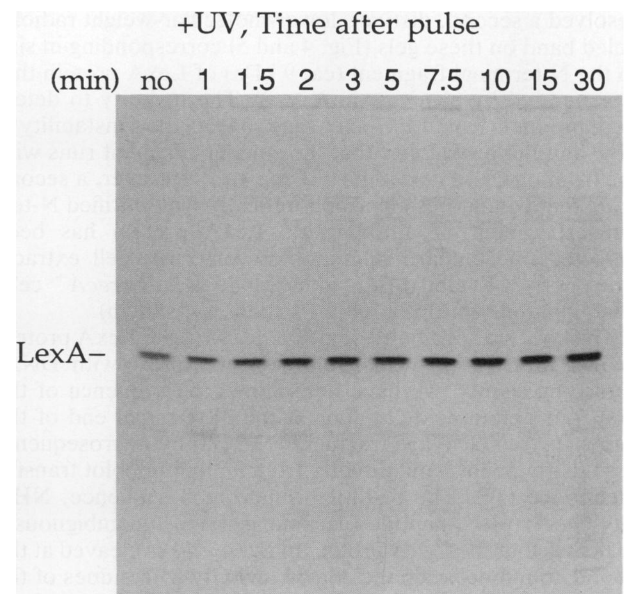


FIG. 6. Fate of *E. coli* LexA protein in *B. subtilis recA4* strain WB110(pPL708-*lexA*). The experiment was similar to that described in procedure B of Little (21), in which cell cultures were UV irradiated (5 J/m$^2$) and incubated at 37°C for 15 min prior to the addition of [$^{35}$S]methionine. Cell samples were treated as described in the legend to Fig. 4. Time is in minutes following radiolabel addition. In lane 1, the "no" sample was labeled for 2 min without prior UV treatment.

shown by the disappearance of the intact protein (22 kDa) and the appearance of a lower-molecular-mass band which corresponds in size (ca. 13 kDa) to the C-terminal fragment produced in *E. coli* (Fig. 4). Little detectable cleavage occurs in the first 2 min after UV irradiation but is very obvious by 5 min and appears to be at a maximum by 10 to 15 min, with approximately 50% of the intact protein having been cleaved by this time. In comparison, the cleavage of LexA in *E. coli* after a similar UV treatment is more rapid, with at least 80% of the protein being cleaved within the first 3 min (21). In uninduced *recA*$^+$ *B. subtilis* cells, LexA protein appears to be quite stable over the period (30 min) of a typical experiment (Fig. 5, lanes 1 through 4).

The fate of newly synthesized LexA protein in induced *B. subtilis recA*$^+$ cells was also analyzed. Cultures were UV irradiated, incubated for 15 min, pulse labeled, and sampled at frequent intervals thereafter (procedure B of reference 21). LexA cleavage was rapid, as evidenced by the presence of radiolabel in both the intact protein and the larger cleavage fragment within 1 to 2 min following label addition (data not shown).

Since the inducible expression of the SOS response in *B. subtilis* requires the functions of the cellular RecA protein and is abolished by the *recA4* mutation (24, 25, 28, 30), we looked for cleavage of LexA protein in the *recA4* strain WB110(pPL708-*lexA*). It was expected that the cellular RecA protein in *recA4* mutants would be severely deficient in its ability to promote the cleavage of *E. coli* LexA protein in vivo following DNA damage, since, as considerable data suggest, it is defective in catalyzing the inactivation of the putative cellular LexA homolog to cause SOS induction in *B. subtilis* (25, 28). In an experiment in which cells were UV irradiated at a low dose (5 J/m$^2$) and then incubated for 15 min prior to the addition of [$^{35}$S]methionine, cleavage of LexA protein following the inducing treatment was not detected in the *recA4* mutant WB110(pPL708-*lexA*) (Fig. 6). In fact, the amount of radiolabeled protein in the full-length LexA protein band from WB110(pPL708-*lexA*) cell extracts increases with time compared with that observed in the *recA*$^+$ strain WB105 with no inducing treatment (Fig. 5) and is an indication of its continued synthesis and overall stability in the *recA4* background. It should be noted that because of the extreme sensitivity of the *recA4* mutant to DNA-damaging agents, the amount of killing resulting from the UV dose (5 J/m$^2$) given to this mutant strain (Fig. 6) is considerably greater than from that administered (25 J/m$^2$) to the *recA*$^+$ strain (Fig. 4). However, a comparison of the levels of LexA protein in cultures of WB110(pPL708-*lexA*) irradiated at both the lower (2 J/m$^2$) and higher (10 and 25 J/m$^2$) UV doses gave results (data not shown) qualitatively identical to those presented in Fig. 6. To confirm that the damage-inducible cleavage of *E. coli* LexA protein in *B. subtilis* is *recA* dependent, we also looked for but could not detect LexA protein cleavage in a strain carrying a chromosomal *recA::cat* gene (Cm$^r$) insertion mutation (null) which abolishes both constitutive and inducible synthesis of any functional cellular RecA protein (data not shown).

We have confirmed that the intact protein and the lower-molecular-weight bands indicated in Fig. 4 through 6 are *E. coli* LexA protein and one of its two presumed cleavage products, respectively, by showing that the immunoprecipitation of both the full-length protein and the 13-kDa fragment from radiolabeled, UV-induced *B. subtilis recA*$^+$ cell extracts was significantly reduced in the presence of an excess of unlabeled purified LexA (data not shown). In addition, the indicated LexA protein and in vivo cleavage fragment band from *B. subtilis* cell extracts had the same electrophoretic mobilities on SDS-polyacrylamide gels as purified LexA protein and C-terminal fragment produce by the autodigestion in vitro of purified LexA protein (22), respectively (data not shown). We have not unambiguously

resolved a second, distinct, lower-molecular-weight radiolabeled band on these gels (Fig. 4 and 5) corresponding in size to the N-terminal fragment (ca. 9 kDa) of LexA protein that is generated by cleavage in *E. coli*. The inability to detect both products from LexA cleavage reflects their instability in vivo and the probability that the smaller fragment runs with the front on these gels (data not shown). However, a second band that comigrates electrophoretically with purified N-terminal fragment of autodigested LexA protein has been detected on standard immunoblots of crude cell extracts from both UV- and MMC-induced *B. subtilis recA*$^+$ cells containing plasmid pPL708-*lexA* (data not shown).

To show the specificity of cleavage of *E. coli* LexA protein in *B. subtilis recA*$^+$ cells following treatment with DNA-damaging agents, we have determined the sequence of the first four amino acid residues at the N-terminal end of the larger in vivo cleavage fragment (13 kDa) by microsequencing this fragment band directly from an immunoblot transfer membrane (34). The resulting amino acid sequence, $NH_2$-Gly-Glu-Pro-Leu-peptide (data not shown), unambiguously indicates that the LexA protein in *B. subtilis* is cleaved at the peptide bond between the Ala-84 and Gly-85 residues of the polypeptide. This is the same Ala-Gly peptide bond of the LexA polypeptide (202 amino acids) at which cleavage occurs in the *E. coli* RecA*-mediated reaction in vivo (15) and in the RecA-independent intramolecular reaction in vitro termed autodigestion (22).

## DISCUSSION

The SOS system for DNA repair and mutagenesis in *B. subtilis* has been shown to parallel the SOS system of *E. coli* in many respects, from the cellular responses of filamentation and prophage induction to the DNA damage-inducible expression of numerous, disparate chromosomal operons and a multifunctional recombination enzyme of the type exemplified by the *E. coli* RecA protein. In view of this remarkable phenotypic similarity, it is reasonable to expect that there is a certain degree of evolutionary conservation in the essential control elements of the SOS systems in these and other bacteria, in both their functional and regulatory properties, if not in their primary gene and protein structures. Indeed, proteins functionally similar to *E. coli* RecA have been detected in many other species of bacteria, including *Erwinia carotovora* (16), *Haemophilus influenzae* (49), *Neisseria gonorrhoeae* (18), *Proteus* spp. (16, 55), *Pseudomonas aeruginosa* (17), a *Synechococcus* sp. (38), and *Vibrio cholerae* (12). The presence of SOS-like damage-inducible phenomena in the yeast *Saccharomyces cerevisiae* (45) and mammalian cells (10, 41) is also an indication of the high degree of evolutionary conservation in both function and the regulation of inducible repair in these organisms. Moreover, that the *E. coli* LexA protein can influence the induction of SOS-like functions in several species of the family *Enterobacteriaceae* (48) demonstrates that the mechanism of negative regulation of SOS-like responses in bacteria may also be highly conserved. Although we find no comparable ability of *E. coli* LexA to influence the expression of SOS-regulated functions or homologous recombination in *B. subtilis* (unpublished results), the results presented here, though indirect, provide additional support for the hypothesis that a cellular SOS repressor which is functionally similar to *E. coli* LexA does exist in *B. subtilis*. Our results also suggest that the molecular protein-protein interaction between the cellular RecA and LexA homologs in *B. subtilis* that ultimately leads to the induction of the SOS response in this bacterium is fundamentally similar to that known to operate in *E. coli*.

We have shown that the cellular repressor of the *E. coli* SOS response, the LexA protein, when expressed in *recA*$^+$ *B. subtilis* cells, is specifically cleaved following exposure to agents which induce the SOS response. This cleavage of LexA in vivo parallels, in a temporal manner, the induction of SOS functions in *B. subtilis*, i.e., RecA synthesis and *din* operon expression (see Fig. 2, 3, and 4; unpublished results) and is RecA dependent. Our results confirm and extend those of Lovett and Roberts (30), who showed that purified *B. subtilis* RecA protein catalyzes *E. coli* LexA protein cleavage in vitro. Together with the results of recent investigations (28, 29), our results suggest that there is a direct correspondence between RecA protein activation (for repressor-cleaving activity) and the induction of the SOS response in *B. subtilis*.

The lack of significant SOS-inducing activity by the *E. coli* RecA430 protein in a *B. subtilis recA4* mutant following DNA damage or during competence development is not surprising, given that RecA430 is impaired in its ability to mediate the induction of comparable SOS functions in *E. coli*. Our present results with the *recA430*-encoded RecA protein, like those with wild-type *E. coli* RecA (Fig. 2 and 3) (25), are therefore consistent with a model for SOS induction in *B. subtilis* that requires the direct involvement of the cellular RecA in promoting the inactivation of a LexA-like repressor element and suggest that the mechanism by which *B. subtilis* RecA becomes activated following DNA damage or during the development of competence is similarly conserved. Particularly noteworthy is our observation that the activities (SOS induction) of the wild-type RecA protein and those of the RecA430 protein, respectively, are identical in response to both DNA damage and the development of competence in *B. subtilis*, suggesting that RecA activation is mediated by binding to regions of ssDNA (46) rather than by a specific interaction with damaged duplex DNA (31) or, alternatively, a model in which DNA damages are obligately required.

The fact that the functional abilities and deficiencies of the mutant *E. coli* RecA430 protein are clearly conserved in *B. subtilis* cells suggests that the SOS-inducing (repressor-cleaving) and recombination activities of the *B. subtilis* RecA protein are also genetically separable functions. The existence of certain *rec* mutations in *B. subtilis*, such as *recA1* (9), provides support for this hypothesis. Like *recA4*, the *recA1* mutation causes a marked deficiency in the induction of the SOS response, both following DNA damage and during competence, and a severe decrease in inducible DNA repair capacity (24, 25, 27, 28, 57). However, compared with *recA4* mutants, *recA1* mutants are only moderately reduced in their capacity for homologous recombination (24, 57) and thus phenotypically resemble the *recA430* mutant of *E. coli*. The *recA4* and *recA1* mutations are known to be closely linked genetically (7), and indeed, recent evidence strongly suggests that both mutations reside in the gene that encodes the *B. subtilis* RecA protein (27, 28, 33).

There is presently no significant biochemical or genetic data on the identity or the nature of the LexA-like repressor in *B. subtilis* regarding its primary structure, specific properties, or chromosomal locus. Presumably, this LexA homolog is a protein which possesses the ability to uniquely recognize and bind to specific DNA sequences in the promoter-operator regions that lie upstream of the genes that compose the SOS regulon in *B. subtilis* and functions to block their transcription in a concerted manner. Numerous

attempts to identify this element in *B. subtilis* by protein-immunological or DNA sequence homology (26, 53; unpublished results) have been unsuccessful. Thus, while the cleavage specificity for LexA protein in *E. coli* and the putative LexA homolog in *B. subtilis* have been retained, there has apparently been considerable divergence in other domains of this protein, such as the DNA-binding domain. A comparison (26) of cloned DNA sequences from the regulatory regions upstream of several *din*::Tn*917* insertions in *B. subtilis* (23) showed no homology to the consensus SOS box, CTGT-$N_x$-CAG, the promoter-binding site for LexA protein that lies upstream of all SOS genes in *E. coli* (54). Cheo et al. (5) have now identified putative DNA-binding sites in the regions upstream of several of these *din*::Tn*917*-*lacZ* insertions, *recA*, and other SOS genes in *B. subtilis* that have the consensus sequence GAAC-$N_4$-GTTC, suggesting that the LexA repressor homolog recognizes and binds to a regulatory DNA sequence structure which is quite different from that recognized by the *E. coli* LexA repressor protein.

Perhaps it is not unexpected to see a considerable degree of structural divergence in the LexA-like repressor from *B. subtilis* (compared with *E. coli* LexA) given that the nucleotide sequence and predicted number of amino acid differences of the *E. coli* and *B. subtilis* RecA proteins is 40% (51) and given the other notable differences between the SOS systems in these two bacteria, such as the *recA*-independent induction of the cellular RecA protein during competence development in *B. subtilis* (27). Thus, while the conservation of function in the SOS systems of *B. subtilis* and *E. coli* is clearly evident at all levels, from the individual genes and their protein products, which catalyze homologous recombination and the repair of stress-induced damage to DNA, it is the specificity of molecular interactions between the RecA homologs and their cognate cellular and phage repressor targets which is ultimately required for the regulatory control of this system for DNA repair and cell survival, a specificity that has apparently been conserved between these two distantly related bacterial species.

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