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Purified *lexA* protein is a repressor of the *recA* and *lexA* genes

(SOS response/*Escherichia coli*/*recA* protease/*lexA* target genes/SOS boxes)

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ABSTRACT **_Escherichia coli_ shows a pleiotropic response (the SOS response) to treatments that damage DNA or inhibit DNA replication. Previous evidence has suggested that the product of the *lexA* gene is involved in regulating the SOS response, perhaps as a repressor, and that it is sensitive to the *recA* protease. We show here that *lexA* protein is a repressor of at least two genes, *recA* and *lexA*. Purified protein bound specifically to the regulatory regions of the two genes, as judged by DNase I protection experiments, and it specifically inhibited *in vitro* transcription of both genes. The binding sites in *recA* and *lexA* were found to be about 20 base pairs (bp) and 40 bp long, respectively. The 40-bp sequence in *lexA* was composed of two adjacent 20-bp sequences, which had considerable homology to one another and to the corresponding *recA* sequence. These 20-bp sequences, which we term "SOS boxes," show considerable inverted repeat structure as well. These features suggest that each box represents a single repressor binding site. Finally, we found that purified *lexA* protein was a substrate for the *recA* protease in a reaction requiring ATP or an analogue, adenosine 5′-[γ-thio]triphosphate, and denatured DNA.**

Escherichia coli displays a pleiotropic response to conditions that damage DNA or inhibit DNA replication (1). This response, often termed the "SOS response," includes phenomena such as prophage induction, enhanced DNA repair capacity, and induced mutagenesis. Except for the well-studied case of prophage induction (2, 3), the molecular mechanisms underlying particular SOS functions are not known, nor are the relationships among these diverse processes. It has recently become clear, however, that a considerable part of the SOS response is controlled by a complex system of gene regulation—a system involving the products of at least two genes, *lexA* and *recA*.

Current models for this system (see *Discussion* and refs. 2–12) suggest that the *lexA* protein is a repressor that directly regulates the expression of a group of unlinked genes. Early evidence for this model came from studies on the regulation of the *recA* gene (4, 5, 13, 14). In wild-type cells, *recA* is expressed at a low rate during exponential growth, and at a rate roughly 10-fold higher after inducing treatments. This pattern is altered in cells with two types of *lexA* alleles: *lexA*$^-$ strains (15) are noninducible, whereas *spr* strains (14) are constitutive. More recent evidence indicates that another gene repressed by *lexA* protein is *lexA* itself (8, 9). Finally, recent genetic studies (10, 16, 17) suggest that *lexA* protein also controls at least six other genes, among them the *uvrA* and *uvrB* genes, which are involved in excision repair. The function of some of the other gene products regulated by *lexA* is not yet certain, nor is it clear whether all *lexA*-controlled genes have been identified.

We report here that purified *lexA* protein can protect specific regions near the start of the *recA* and *lexA* genes against nuclease attack and that it can inhibit specifically the *in vitro* transcription of these genes. These data support a repressor model for its function. A parallel study by Brent and Ptashne (18) reaches the same conclusion.

In order to derepress the SOS system, the function of this repressor must be destroyed. We show here that, as expected from studies in a crude system (11), purified *lexA* protein is a substrate for the *recA* protease. The fact that this reaction requires single-stranded DNA argues for activation of *recA* protein by a cofactor signalling DNA damage, as proposed (3).

MATERIALS AND METHODS

Bacterial and Plasmid Strains. Bacterial strains used, with only relevant markers listed, were AB1157 (*recA*$^+$ *lexA*$^+$), the "maxicell" strain CSR603 (*recA1 uvrA6 phr-1*), and DM511 (*lexA3 tsl-1*), all previously described (8, 11, 19, 20); derivatives carrying F′ factors were made by conjugation (21), and those carrying plasmids derived from pBR322 were made by transformation (20). Plasmids used were F′ *lacI*^{q}, which overproduces *lac* repressor about 10-fold (22), from M. Calos; pLJ3, carrying the *lac*UV5 promoter, (23), from T. Roberts; pBR322 (24) and five of its derivatives: pJL3, which carries the *recA* regulatory region and most of *recA* (25); pJL5, carrying the *recA* regulatory region on a 145-base pair (bp) *Msp* I endonuclease fragment extending from −126 to +19 in the *recA* sequence (26, 27) in the *Cla* I site of pBR322 (this work); pJL21, carrying *lexA*$^+$ (20); pJL42, a *lexA*$^+$ subclone of pJL21 containing an 1150-bp *Eco*RI/*Cla* I fragment (this work); and pJL45, a derivative of pJL21 in which the *lexA* regulatory region (Fig. 3) was replaced with a 95-bp *Eco*RI/*Pvu* II fragment (R. Brent, personal communication) from pLJ3 carrying the *lac*UV5 promoter. Its construction will be described elsewhere.

DNase I Protection Experiments. These experiments were modified from procedures given in ref. 28. Reaction mixtures contained 20 mM Tris·HCl at pH 7.4, 10% sucrose, 1 mM dithiothreitol, 0.1 mM EDTA, 1.5 mM $CaCl_2$, 2.5 mM $MgCl_2$, 0.1% bovine serum albumin, 50 mM NaCl, and labeled DNA at about 10 nM. To aliquots at 22°C was added *lexA* protein, as indicated; after 10 min, pancreatic DNase I was added to a concentration of 10 ng/ml. After 10 min more at 22°C, samples were treated as described (28) and analyzed by electrophoresis in an 8% polyacrylamide/7 M urea gel (0.5 mm × 16 cm × 33 cm) as described (ref. 29, procedures 17 and 18), followed by autoradiography. DNAs were the following. The *recA* regulatory region: pJL5 was digested with *Eco*RI or *Hin*dIII, labeled with ^{32}P at its 5′ ends (ref. 29, procedure 5b), and cut with *Rsa* I; the operator-bearing fragment was isolated (ref. 29, procedure 9, 8% gel). The *lexA* regulatory region was on a 148-bp *Eco*RI/*Bcl* I fragment from pJL42; DNA was cut with one enzyme, labeled, cut with the other enzyme, and isolated as described above.

***In Vitro* Transcription.** Transcription was performed as described (30), except that [α-^{32}P]CTP (10 Ci/mmol; 1 Ci = 3.7

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Abbreviations: bp, base pairs; ATP[S], adenosine 5′-[γ-thio]triphosphate.

$\times 10^{10}$ becquerels) was 5 μM, GTP and UTP were 10 μM, templates were generally at 5 nM, and reactions were stopped 10 min after addition of nucleoside triphosphates (NTPs). In most experiments, incubations were in three stages: 1, *lexA* protein was added, or omitted in controls; 2, after 10 min at 37°C to allow *lexA* protein binding, RNA polymerase was added; 3, after 10 min to allow polymerase binding, NTPs, including [α-^{32}P]CTP, and heparin were added; 4, after 10 min, reactions were terminated by adding an equal volume of formamide. Templates for transcription were isolated as above. The *recA* regulatory region was a *Sac* II/*Cla* I fragment 475 bp long from pJL3, from −145 to +330 relative to the start-point of *recA* mRNA. Transcription from this template was repressed by *lexA* protein, as seen in Fig. 6 (not shown), but for the experiment in Fig. 6 this fragment was further digested with *Hha* I, treated with phenol, precipitated with ethanol, and redissolved. The *lexA* and *amp* regulatory regions were from pJL42; a 740-bp

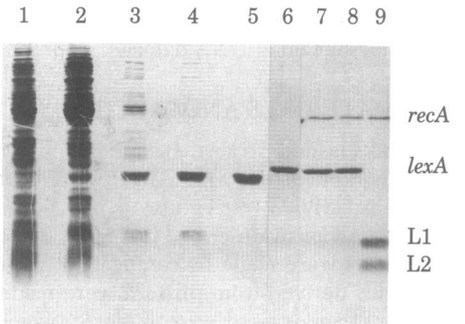

FIG. 1. Purification of *lexA* protein and cleavage of the purified material. Lanes 1–5: Samples of fractions I–V, respectively (see below), were analyzed by electrophoresis in polyacrylamide gels (14% gels) containing sodium dodecyl sulfate as described (8) and stained with Coomassie blue (32). Lanes 6–9: Cleavage of purified material by treatment with purified *recA* protease. The complete reaction mixture contained 10 mM Tris·HCl at pH 7.4, 5% sucrose, 0.05 mM EDTA, 0.5 mM dithiothreitol, 4 mM $MgCl_2$, heat-denatured calf thymus DNA at 0.5 μg/ml, 1 mM adenosine 5′-[γ-thio]triphosphate (ATP[S]), *tif* protein at 10 μg/ml, and *lexA* protein at 80 μg/ml. Reaction mixtures were incubated 90 min at 37°C, then 20-μl aliquots were mixed with 10 μl of 4× sample buffer (11), heated 3 min at 100°C, and analyzed as above (13% gel). Lane 6, *lexA* protein alone, with no treatment; lane 7, ATP[S] omitted; lane 8, denatured DNA omitted; lane 9, complete reaction. *recA*, *lexA*, L1, and L2 indicate positions of *tif* protein, *lexA* protein, and *lexA* protein cleavage products (11). In a separate experiment, *lexA* protein incubated without protease showed the same pattern as untreated material (not shown). Comparison of lanes 5 and 6 shows that a small amount of material the size of the cleavage products accumulated in the preparation during the 5 weeks between the time the two gels were run.

lexA protein was purified, by a procedure to be detailed elsewhere, from a mixture of two cultures: 10 ml of the maxicell strain JL472 (CSR603/F′ *lacI*^{q}/pJL45) was labeled with 0.5 mCi of [^{35}S]methionine as described (11) except that 1 mM isopropyl thiogalactoside was present after irradiation; cells were mixed with cells from 4 liters of strain JL475 (AB1157/F′ *lacI*^{q}/pJL45) grown in 0.5% yeast extract/1% Bactotryptone/0.5% NaCl/thiamin at 1 μg/ml/0.25 mM isopropyl thiogalactoside. Sonication and low-speed centrifugation yielded fraction I; after precipitation with Polymin P, salt elution, and ammonium sulfate precipitation, the protein was dialyzed against buffer A (20 mM Tris·HCl, pH 7.4/10% sucrose/1 mM dithiothreitol/0.1 mM EDTA) plus 40 mM NaCl (fraction II). Protein was successively fractionated on DEAE-cellulose (fraction III), phosphocellulose (fraction IV), and hydroxyapatite, then dialyzed into buffer A + 50 mM NaCl (fraction V). Yields in each column step were roughly 50%, and the final yield was about 250 μg as judged by the Bradford assay (33) with bovine serum albumin as standard. Molarities given were calculated on the basis of this assay and a monomer molecular weight of 25,000 for *lexA* protein (8, 9, 11); we do not know, however, if all the protein molecules were active, or whether the active form is multimeric.

*Msp*I fragment was isolated that contained both promoters. The fragment was treated with *Eco*RI; for identification of individual transcripts the products were separated, but for the experiment in Fig. 6 the *Eco*RI digest was treated as above.

Materials. Restriction enzymes *Alu* I, *Bcl* I, *Bst*NI, *Eco*RI, *Msp* I, *Rsa* I, and *Sac* II, polynucleotide kinase, and phage T4 DNA ligase were from New England BioLabs; *Hha* I was from Bethesda Research Labs (Rockville, MD); and *Cla* I and *Hin*dIII were from Boehringer Mannheim. Enzymes were used according to the manufacturer's specifications, except for *Eco*RI (20). Pancreatic DNase I (type D) and bacterial alkaline phosphatase (BAPF) were from Worthington; RNA polymerase was from Enzo Biochemicals (New York); chicken blood DNA and calf thymus DNA were from Calbiochem; and isopropyl β-D-thiogalactoside was from Sigma. The *tif*-1 form of the *recA* protease was made in phage-infected cells as described (11) and purified as described (31) through the glycerol gradient step. [α-^{32}P]CTP (480 Ci/mmol), [γ-^{32}P]ATP (2600 Ci/mmol), and [^{35}S]methionine (1100 Ci/mmol) were from New England Nuclear.

RESULTS

Purification of *lexA* Protein. In order to provide a plentiful source of *lexA* protein, we made a multicopy plasmid that carries

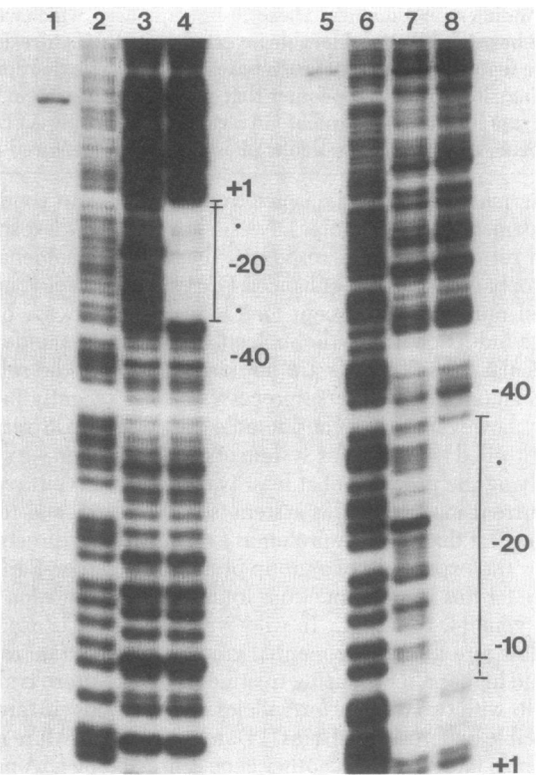

FIG. 2. Binding of *lexA* protein to the *recA* operator. Fragments were 5′-end-labeled to the left (lanes 1–4) or the right (lanes 5–8) of the operator region shown in Fig. 3*A*. Samples were treated with DNase I in the absence (lanes 3 and 7) or presence (lanes 4 and 8) of 200 nM *lexA* protein. Control lanes contained the same DNA samples cleaved chemically at purines (lanes 2 and 6; ref. 29) or partially digested with *Alu* I (lanes 1 and 5). Because chemical cleavage generates 3′-phosphate groups, fragments migrate roughly one-half bp faster, both in this gel and that of Fig. 4, than do the identical fragments bearing the 3′-OH groups given by restriction enzymes and DNase I. The positions are numbered relative to the start-point of *recA* mRNA given in ref. 26 (the start-point is given in ref. 27 as position +2 of this sequence). Results identical to those in lanes 5–8 were obtained with a fragment from pJL3 labeled at position +56 and extending beyond the operator (not shown).

a fusion between the *lexA* gene and the *lac* promoter. In strains carrying this plasmid, pJL45, roughly 1% of the total cell protein was *lexA* protein after induction with isopropyl thiogalactoside (not shown). To eliminate any possible selective disadvantage of high levels of *lexA* protein, this plasmid was maintained in a host containing excess *lac* repressor; cultures were derepressed with isopropyl thiogalactoside to serve as a source of *lexA* protein. From extracts of such cultures, we purified *lexA* protein to at least 95% of physical purity (Fig. 1, lanes 1–5). As judged by the semiquantitative functional assays described below, this purified material retained activity after storage at 5°C for several months.

Cleavage of *lexA* Protein. As expected from studies in crude extracts (11), purified *lexA* protein was a substrate for the *recA* protease, in a reaction closely resembling cleavage of purified λ repressor (3). Cleavage in this purified system was dependent upon the presence of both ATP[S] and denatured DNA (Fig. 1, lanes 7–9). ATP was able to substitute for ATP[S] (not shown). The *lexA* protein was attacked at a rate at least 10-fold greater than was λ repressor when both substrates were present in the same reaction mixture at about 40 and 20 μg/ml, respectively (data not shown).

Specific Binding to *recA* and *lexA* Operator Regions. Using the DNase I protection technique for visualizing specific DNA-protein interactions (34, 35), we found that a segment of DNA adjacent to the start of the *recA* gene (26, 27) was protected in both strands from limited DNase I attack by the presence of purified *lexA* protein (Fig. 2). This protected segment is about 20 bp in length (Fig. 3A). It lies 10 to 30 bp upstream from the start of *recA* mRNA synthesis, and it overlies a 22-bp segment that is a perfect inverted repeat except for the innermost 2 bp. From these studies and other evidence given below, we conclude that this protected segment is an operator for *recA* and shall term it such.

Similar experiments (Fig. 4) with the *lexA* regulatory region (9, 36, 37) revealed that a longer region, about 40 bp in length, was protected from attack by *lexA* protein. This effect was less dramatic than with the *recA* operator, because the unprotected operators were favored sites for DNase attack (lanes 3 and 7), and protected DNA still suffered some attack (lanes 4 and 8). The DNA sequence of this region was determined (29) and is shown in Fig. 3*B*. The region protected by *lexA* protein straddles the start-point of the *lexA* mRNA (36, 37). Analysis of this sequence and comparison with the operator in *recA* suggests that the protected region in *lexA* is actually composed of two adjacent binding sites, which we shall term operators 1 and 2. Both are 20 bp in length, and they show homology with each other and with the *recA* operator (Fig. 5). All three operators share the sequence -C-T-G-T-A-T- at positions 3–8; their right halves have less homology.

Several other considerations also argue that the protected region in *lexA* consists of two adjacent and similar binding sites. Each of the two operators in *lexA* shows some inverted repeat structure, more pronounced in operator 1 than in operator 2 (Fig. 3*B*). In both operators, a site at or very near position 14 showed enhanced susceptibility to DNase attack in the presence of *lexA* protein, suggesting that the *lexA* protein interacted in the same way with both operators. Finally, the two operators in *lexA* both contain the same A+T-rich palindrome, G-T-A-T-A-T-A-C, in the same position of the sequence. Over a range of *lexA* protein concentrations giving partial to almost complete protection, we saw no evidence for preferential binding to one operator over the other (not shown), a finding compatible with cooperative binding of repressor to the two sites (35), with equal binding affinity of repressor for the two sites, or with a single 40-bp binding site.

A final experiment (not shown) suggested that the right end of at least one operator is not crucial to *lexA* protein binding. The DNA used in Fig. 4 (lanes 1–4) was further cut by *Bst*NI near the end of *lexA* operator 2 (Fig. 3); on this DNA, *lexA* protein was still able to protect operator 1 and the left half of operator 2 to about the same extent as on uncut DNA over a range of protein concentrations which gave partial to almost complete protection. It was unclear whether the remaining portion of

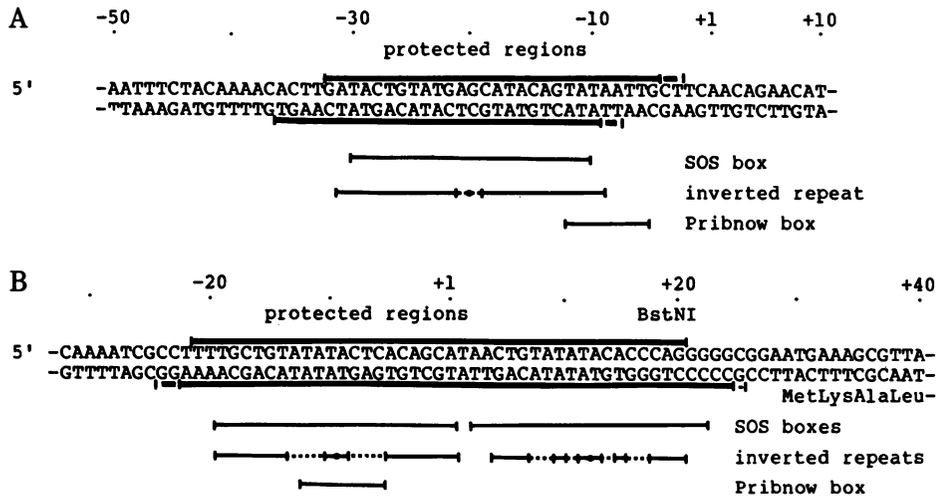

FIG. 3. Sequences in the *recA* and *lexA* regulatory regions protected by *lexA* protein. (*A*) *recA*. The sequence and mRNA start-point are from refs. 26 and 27. The regions protected from DNase I attack by *lexA* protein are indicated for the top and bottom strands by heavy bars. Because not every position is attacked by DNase I, the ends of the protected regions are not precisely defined; the uncertainty is indicated by broken bars at the segment ends. Moreover, because the regions protected on the two strands are not exactly the same, the ends of the operator are not precisely defined by this type of analysis. (*B*) *lexA*. The DNA sequence of the segment between the *Eco*RI and *Bcl* I sites (at positions −72 and +77) was determined (ref. 29, procedures 10–13), but only a portion is shown; this sequence was determined by using this fragment labeled at either end, and the overlap extended from position −54 to +58, relative to the mRNA start-point (refs. 18, 36, and 37; R. Brent, personal communication). This sequence agrees with those given in refs. 18, 36, and 37. Plasmid pJL45 is presumed to have been fused at position +19; a possible Shine–Dalgarno sequence for *lexA*, -A-G-G-G-G-G-, immediately follows this point and may function in pJL45 at the indicated ATG codon. Not shown are direct and inverted repeats between the two operators based on the -G-T-A-T-A-T-A-C- palindromes in each SOS box. Starting at position 29, an open reading frame extends to the right for 606 bases (ref. 36 and unpublished data).

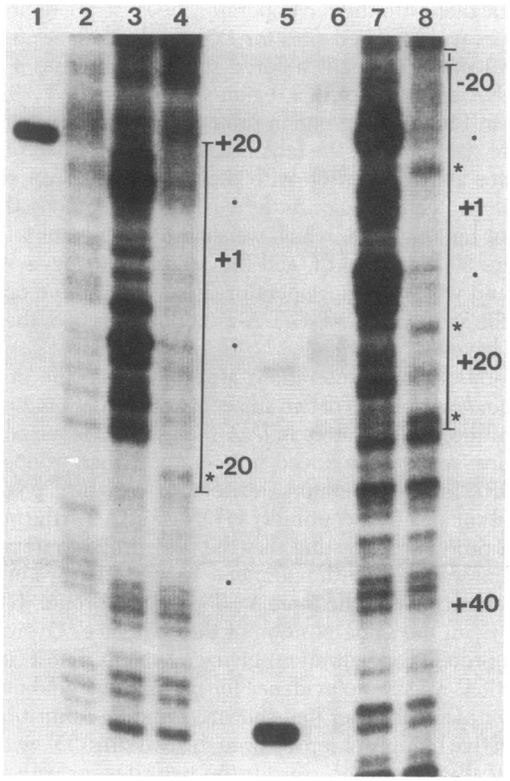

FIG. 4. Binding of *lexA* protein to the *lexA* operators. The experiment was identical to that of Fig. 2, except that fragments bearing the *lexA* regulatory region were used and control samples (lanes 1 and 5) were partially digested with *Bst*NI. See Fig. 3 for the sequence of this region. The purine ladders (lanes 2 and 6) were visualized more clearly by longer exposure (not shown). Asterisks indicate sites at which binding of *lexA* protein appeared to enhance DNase attack.

operator 2 was protected or not. Because the degree of specificity required for apparent protection by this assay is not great, however, a difference in binding constants might be detectable by a more quantitative assay.

Specific Inhibition of *in Vitro* Transcription. To test whether the specific binding interaction described above could prevent *in vitro* transcription of the *recA* and *lexA* genes, we used a purified system containing RNA polymerase, a restriction fragment containing a particular regulatory region, and NTPs, including [α-^{32}P]CTP. Labeled transcripts were analyzed by gel electrophoresis and autoradiography. A which forms a band in the autoradiogram.

We examined transcription from three promoters—the *recA* and *lexA* promoters, and a control promoter, from the *amp* or

TACTGTATGAGCATACAGTA	recA operator
TGCTGTATATACTCACAGCA	lexA operator 1
AACTGTATATACACCCAGGG	lexA operator 2
taCTGTATataCacaCAG-a	consensus

FIG. 5. Homologies among sequences in *lexA* binding sites. The sequences are from Fig. 3. Our data do not prove that the binding sites end precisely at the ends of the boxes, but we assume this to be so for the sake of discussion. In the consensus sequence, homologies shared by all three boxes are given by capital letters, those present in two of the three by lower-case letters. Shortly before we first observed protection of these boxes, R. Brent (personal communication) pointed out to us the homology between the *recA* box and three boxes at *lexA*, including one at −47 to −28 that is not protected.

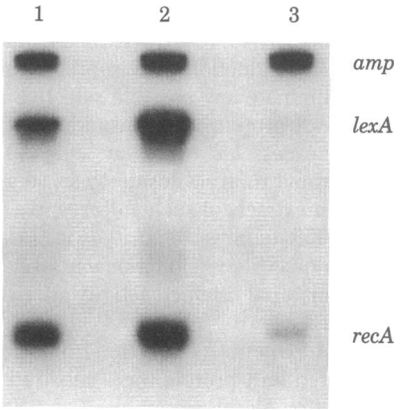

FIG. 6. Inhibition of *recA* and *lexA* transcription by *lexA* protein. The protocol is described in *Materials and Methods*, except that in lane 1 RNA polymerase was added first, *lexA* protein (400 nM) after 10 min, and heparin and NTPs after 10 min more. In lane 2, no *lexA* protein was added; in lane 3, *lexA* protein was added first at 400 nM. Transcripts were identified in reactions containing only one promoter fragment (not shown). In separate comparisons with each promoter alone, each showed the same response to prior addition of *lexA* protein as here (not shown). In another experiment (not shown) we found no effect of *lexA* protein on transcription from the *lac*UV5 promoter.

β-lactamase gene of pBR322. When we added *lexA* protein prior to RNA polymerase, the amounts of both the *recA* and *lexA* transcripts were greatly reduced, while that of the *amp* transcript was unaffected (Fig. 6, lane 3), in comparison with a reaction mixture lacking *lexA* protein (lane 2). Over a range of *lexA* protein concentrations between 50 and 800 nM, we saw no evidence for strongly preferential repression of either *lexA* or *recA* relative to the other (not shown). We conclude that purified *lexA* protein specifically inhibits transcription of the *recA* and *lexA* genes, and that it is therefore a repressor of these two genes.

When RNA polymerase was added prior to *lexA* protein, much less inhibition of transcription was observed (Fig. 6, lane 1). This finding suggests that, as in the case of λ repressor (38), *lexA* protein acts by binding and blocking access of RNA polymerase to the promoter. The roughly 2-fold repression observed with the *lexA* transcript suggests that *lexA* protein might be able to displace polymerase, for example by binding to operator 2, or alternatively that RNA polymerase dissociates from the *lexA* promoter more rapidly than from *recA*.

DISCUSSION

The interaction of the *lexA* repressor with its operators resembles better-characterized repressor–operator interactions. In various DNase protection experiments we have looked at about 1800 bp of DNA sequences lying adjacent to the *recA* or *lexA* operators or in pBR322, and no other segments 20 bp or more in size appeared to be protected by *lexA* protein. These data suggest that binding to the operators is highly sequence specific. Complexes between the *recA* operator and *lexA* protein were stable for a period of hours as judged by their cosedimentation in sucrose gradients (unpublished data); those between the *lexA* regulatory region and the repressor were less stable and appeared to dissociate during sedimentation. This difference in stability of complexes is consistent with measurements of the relative binding constants (18). The operators overlap the promoter regions of the two genes, and repressor appears to work by excluding RNA polymerase from the promoter. The operators have 2-fold rotational symmetry (Fig. 3). Interestingly, the location of the operators with respect to the promoter is different in *recA* and *lexA*; the significance of this structural feature is not yet understood. Finally, the presence of two bind-

ing sites at *lexA* instead of one suggests the possibility of cooperative binding to *lexA*, as in the case of λ repressor (35).

Genetic evidence suggests that *lexA* protein also regulates the expression of many other genes in addition to *recA* and *lexA* (see Introduction). It is probable that this regulation will operate by repression as well, and that these other genes will contain binding sites for *lexA* protein similar to those described here. We propose to term these binding sites "SOS boxes" to emphasize their commonality.

Regulation of *lexA* target genes contrasts with other systems in which several functions are controlled by a single repressor. In systems such as *lac* and *trp*, one regulatory region controls a set of linked genes with related functions. In the *arg* regulon (39), the genes are unlinked, but still they act in the same metabolic pathway. Finally, in the SOS system, a set of unlinked genes is controlled by the *lexA* repressor; these genes probably do not all act on the same pathway, yet their coordinated expression is thought to aid cell survival.

Our findings lend further biochemical support to the current model (2–12) for regulation of *recA* and other genes. The essential postulates of this model are four in number: (*i*) In exponentially growing cells, *lexA* protein represses *recA*, *lexA*, and other genes involved in the SOS response; our data show that *recA* and *lexA* are repressed *in vitro*. (*ii*) In induced cells, *lexA* protein is specifically cleaved by the *recA* protease; this reaction takes place in a purified *in vitro* system (Fig. 1). (*iii*) *recA* protease is inactive in exponentially growing cells, but is reversibly activated by one or more signal molecules, as yet unknown, that are generated by impairments to normal DNA replication and symbolize that state. We have found that the activity against purified *lexA* protein requires single-stranded DNA as a cofactor (Fig. 1), as in the case of λ repressor (3); perhaps, as suggested (3), this cofactor is a signal molecule. (*iv*) Cleavage inactivates *lexA* protein, leading to derepression of target genes for as long as functional *lexA* protein cannot accumulate. We have found that the ability of *lexA* protein to protect the *recA* operator from DNase I is reduced by at least 90% by cleavage (unpublished data), suggesting that the cleavage products have little or no residual activity. We conclude that this model, originally based largely on genetic evidence, is also completely consistent with the known biochemical properties

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