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Interaction of *Escherichia coli* RecA Protein with LexA Repressor

II. INHIBITION OF DNA STRAND EXCHANGE BY THE UNCLEAVABLE LexA S119A REPRESSOR ARGUES THAT RECOMBINATION AND SOS INDUCTION ARE COMPETITIVE PROCESSES*

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The *Escherichia coli* RecA protein is involved in SOS induction, DNA repair, and homologous recombination. *In vitro*, RecA protein serves as a co-protease to cleave LexA repressor, the repressor of the SOS regulon; in addition, RecA protein promotes homologous pairing and DNA strand exchange, steps important to homologous recombination and DNA repair. To determine if these two functions of RecA protein are competing or parallel, the effect of uncleavable LexA S119A repressor on RecA protein-dependent activities was examined. LexA S119A repressor inhibits both the single-stranded DNA (ssDNA)-dependent ATP hydrolysis and DNA strand exchange activities of RecA protein. As for wild-type LexA repressor (Rehrauer, W. M., Lavery, P. E., Palmer, E. L., Singh, R. N., and Kowalczykowski, S. C. (1996) *J. Biol. Chem.* 271, 23865–23873), inhibition of ATP hydrolysis is dependent upon the presence of *E. coli* single-stranded DNA binding (SSB) protein, arguing that LexA repressor affects the competition between RecA protein and SSB protein for ssDNA binding sites. In contrast, inhibition of DNA strand exchange activity is SSB protein-independent, suggesting that LexA S119A repressor blocks a site required for DNA strand exchange. These results imply that there is a common site on the RecA protein filament for secondary DNA and LexA repressor binding and raise the possibility that the recombination and co-protease activities of the RecA protein filament are competitive.

RecA protein is essential to homologous genetic recombination in *Escherichia coli* (1–3). The homologous pairing and DNA strand exchange activities of RecA protein have been well defined (for recent reviews see Refs. 4–6). In association with ATP, RecA protein binds to ssDNA[1] to form a contiguous filament, called the presynaptic complex or filament (7). Upon recognizing homologous duplex DNA, the presynaptic complex promotes both pairing and DNA strand exchange in the 5′ to 3′ direction relative to the displaced ssDNA (8).

The presynaptic complex functions not only in recombination; it is also a key component in the signal transduction pathway for the DNA damage-inducible SOS regulon (for reviews see Refs. 9, 10). LexA repressor is the repressor that regulates expression of the genes in the SOS regulon. Once DNA damage is incurred, LexA repressor is inactivated by self-cleavage in a RecA protein-dependent manner (11, 12). The signal for SOS induction is ssDNA formed as a result of DNA damage (13). This ssDNA can be produced either by DNA polymerase arresting at sites of DNA damage (14) or by RecBCD protein unwinding at dsDNA breaks (15). Presumably, RecA protein forms presynaptic filaments on these regions of ssDNA, resulting in repressor cleavage (16). Thus, both the recombinational and co-protease activities of RecA protein share a minimal requirement for the presence of ssDNA and ATP.

Despite the many individual studies on the DNA strand exchange and co-protease activities, it is not clear whether these represent totally independent functions. These two activities of RecA protein may involve common sites on the presynaptic complex; in support, electron micrographic reconstruction studies suggest that the binding site for LexA repressor lies within the deep helical groove of the RecA protein filament (17). This is a site also believed to harbor the secondary DNA binding site essential for DNA strand exchange (18). Therefore, it is possible that binding of a second DNA molecule and LexA repressor to the RecA protein filament are mutually exclusive events. Since LexA repressor and DNA potentially compete for binding to the RecA protein filament, the recombinational and SOS induction roles of RecA protein may be competitive.

In support of the competitive nature of the binding to RecA protein between LexA repressor and a second DNA molecule, the presence of either ss- or dsDNA inhibits the co-protease activity of RecA protein (13, 19–22). Additionally, the co-protease activity of RecA protein is inhibited by SSB protein when the RecA protein concentration is sub-saturating relative to the ssDNA concentration (22). Finally, wild-type LexA repressor in excess of RecA protein decreases ssDNA-dependent ATP hydrolysis by RecA protein in an SSB protein-dependent manner.[2] To further investigate this phenomenon, quantitative analysis was undertaken using an uncleavable form of LexA repressor, LexA S119A repressor. Both the active site and substrate in the LexA repressor self-cleavage reaction exist on each individual LexA repressor molecule; the active site is composed of both the serine at position 119 and the lysine at position 156, whereas the substrate is the peptide bond between residues 85 and 86 (23, 24). The LexA S119A repressor is rendered uncleavable due to the substitution of the serine at position 119 with an alanine (24). The LexA S119A repressor

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[1] The abbreviations used are: ssDNA, single-stranded DNA; SSB, single-stranded DNA binding; PEP, phosphoenolpyruvate; PVA, polyvinyl alcohol; ϵM13, etheno-modified M13 ssDNA containing 1,N^6-ethenoadenosine and 3,N^4-ethenocytidine residues; DTT, dithiothreitol; dsDNA, double-stranded DNA; RFI, relative fluorescence increase; STMP, salt titration-midpoint.

[2] E. Palmer and S.C. Kowalczykowski, unpublished observations.

used here demonstrated competitive inhibition of RecA protein co-protease activity, indicating that the mutant protein is wild-type in its interaction with RecA protein. We also found that LexA S119A repressor inhibits both RecA protein ssDNA-dependent ATP hydrolysis and DNA strand exchange. Inhibition of ATP hydrolysis was observed only in the presence of *E. coli* SSB protein. Consistent with this, LexA S119A repressor slowed RecA protein displacement of SSB protein from ssDNA. In contrast to its effect on ATP hydrolysis, LexA S119A repressor inhibited RecA protein-mediated DNA strand exchange independent of SSB protein, presumably by physically blocking a site required by RecA protein for this reaction. Collectively, these results demonstrate that LexA repressor blocks DNA strand exchange directly, and they argue that recombination and SOS induction are mutually exclusive processes.

MATERIALS AND METHODS

Reagents—Chemicals were reagent-grade and all solutions were prepared using Barnstead Nanopure water. ATP, dATP, and ADP were purchased from Pharmacia Biotech, Inc., and were dissolved as concentrated stock solutions at pH 7.5. Nucleotide concentrations were determined spectrophotometrically using an extinction coefficient of 1.54×10^4 M^{-1} cm^{-1} at 260 nm. PEP and NADH were purchased from Sigma. Low molecular weight (10,000–30,000) PVA was purchased from Sigma, dissolved in water as a 30% (w/v) stock, and used without further purification.

Proteins—RecA protein was purified from *E. coli* strain JC12772, obtained from Dr. A. John Clark (University of California, Berkeley), using a preparative protocol based on spermidine acetate precipitation (25). RecA protein concentration was determined spectrophotometrically using an extinction coefficient of 2.7×10^4 M^{-1} cm^{-1} at 280 nm. SSB protein was purified from *E. coli* strain RLM727 as described (26), and its concentration was determined spectrophotometrically using an extinction coefficient at 280 nm of 3.0×10^4 M^{-1} cm^{-1} (27). The wild-type LexA repressor was purified from strain JL652 using the preparative protocol of Schnarr *et al.* (28) with modifications as described previously (22). LexA S119A repressor was a kind gift of Dr. Hazel Holden (University of Wisconsin, Madison). Both wild-type and LexA S119A repressor concentrations were determined using an extinction coefficient of 7300 M^{-1} cm^{-1} at 280 nm (29). Lactate dehydrogenase and pyruvate kinase were purchased from Sigma.

DNA—Single- and double-stranded DNA were prepared from bacteriophage M13mp7 using the procedure described by Messing (30). The duplex DNA was linearized by digestion with *Eco*RI restriction endonuclease (New England BioLabs). The molar nucleotide concentrations of single- and double-stranded DNAs were determined using the extinction coefficients at 260 nm of 8784 and 6500 M^{-1} cm^{-1}, respectively. ϵM13 ssDNA was prepared from M13mp7 ssDNA as described (31), and the concentration was determined using an extinction coefficient of 7000 M^{-1} cm^{-1} at 260 nm. Poly(dT) was purchased from Pharmacia, and the concentration was determined using an extinction coefficient of 8520 M^{-1} cm^{-1} at 260 nm.

LexA Repressor Cleavage Assay—Proteolytic cleavage reactions were conducted at 37 °C in buffer containing 25 mM Tris-HCl (pH 7.5), 10 mM $MgCl_2$, 50 mM NaCl, 0.1 mM DTT, 1 mM ATP, and an ATP regenerating system consisting of 8 mM PEP and 12.5 units of pyruvate kinase/ml. The RecA protein (1 μM) was preincubated for 3 min with M13 ssDNA (6 μM) prior to initiating the reaction time course with the addition of a prepared mixture of wild-type and LexA S119A repressors at the indicated concentrations. When present, SSB protein (0.25 μM) was added 3 min after the RecA protein and 3 min before commencing the time course by addition of LexA repressor; in SSB protein-containing reactions, the concentration of M13 ssDNA was 3 μM.

LexA repressor cleavage was measured using a SDS-polyacrylamide gel assay as described previously (22, 32–34). In all reactions, the initial amount (at zero time) of the wild-type LexA repressor substrate was measured and found to be ≥85% intact. Since the wild-type and uncleavable mutant LexA repressors displayed no quantitative differences in staining (data not shown), the amount of intact LexA repressor attributable to the wild-type protein in a given lane was determined by subtracting the integrated optical density contributed by the uncleavable LexA S119A repressor. The rates of wild-type repressor proteolysis in the presence of the LexA S119A repressor are the average of two independent determinations with experimental error of ±5.2 and 6.6%, respectively.

ATP Hydrolysis Assay—The ssDNA-dependent or high salt-induced hydrolysis of ATP and dATP promoted by RecA protein was monitored at 37 °C as described previously (35). Unless otherwise noted, the buffer employed was 25 mM Tris acetate (pH 7.5), 10 mM magnesium acetate, 0.1 mM DTT, 3 mM PEP, 1 mM nucleotide cofactor, and 16 units/ml each of pyruvate kinase and lactate dehydrogenase. Standard conditions were 3 μM M13 ssDNA, 1 μM RecA protein, 0.45 μM SSB protein (where indicated), and LexA S119A repressor as indicated. The pyruvate kinase concentration was doubled for dATP hydrolysis assays (36). LexA S119A repressor was preincubated with RecA protein and ssDNA for 2 min prior to starting the reactions with addition of ATP. SSB protein, when present, was added after the reaction had reached a steady state rate. High salt-induced ATP hydrolysis was carried out in a buffer same as above except 1.8 M NaCl was present (37).

SSB Protein-displacement Assay—The displacement of SSB protein from M13mp7 ssDNA was monitored by ATPase assays as described previously (34). The assays were carried out at 37 °C in a buffer that consisted of 25 mM Tris-HCl (pH 7.5), 10 mM $MgCl_2$, 0.1 mM DTT, and 1 mM of either ATP or dATP. Standard conditions were 3 μM M13 ssDNA, 1 μM RecA protein, 0.45 μM SSB protein, and LexA S119A repressor at the indicated concentrations. Reactions contained a regenerating system consisting of 16 units/ml pyruvate kinase and 3 mM PEP except in those assays using dATP where pyruvate kinase was increased to 32 units/ml. Lactate dehydrogenase was present at 16 units/ml.

DNA Strand Exchange Assay—The agarose gel assay for DNA strand exchange was conducted and visualized as described previously (38). Reactions were carried out at 37 °C in a standard buffer consisting of 25 mM Tris acetate (pH 7.5), 6 mM magnesium acetate, 0.1 mM DTT, 3 mM PEP, 1 mM ATP, and 20 units of pyruvate kinase/ml. Standard conditions were 3 μM RecA protein, 5 μM M13 ssDNA, 0.45 μM SSB protein, and 10 μM M13 RF DNA linearized with the restriction endonuclease *Eco*RI. LexA S119A repressor was added to the indicated concentrations. SSB protein-independent reactions were carried out with 7.5% (w/v) PVA (39) and standard buffer except it contained 1 mM magnesium acetate. In the "magnesium-jump" assays, complete presynaptic filaments were formed in standard buffer with 1 mM magnesium acetate but lacking PVA, and then the reaction was initiated by the simultaneous addition of linear M13 dsDNA and additional magnesium acetate to a final concentration of 10 mM (40). In all reactions, LexA S119A repressor was preincubated in assay buffer for 5 min with the RecA protein, SSB protein (when present), and ssDNA prior to starting the reaction with linear M13 dsDNA. Bands corresponding to substrates, homology-dependent DNA networks, and products in the ethidium bromide-stained gel were quantified using the BioImage image acquisition and analysis system (Millipore). In the SSB protein-independent assays, the dsDNA was 5′-end labeled using T4 polynucleotide kinase (New England BioLabs) and [γ-^{32}P]ATP (ICN) and the extent of joint molecule formation was determined using a Betascope 603 β-particle counting system (Betagen Corp., Waltham, MA).

DNA Binding Assay—Both the binding of RecA protein to ϵM13 DNA and the stability of the resultant complex to dissociation by NaCl were monitored fluorometrically as described previously (31, 41). The concentration of nucleotide cofactors was 1 mM. A regeneration system consisting of 10 units of pyruvate kinase/ml and 3 mM PEP was present in all reactions with ATP cofactors. The assays were carried out in a buffer consisting of 25 mM Tris-HCl (pH 7.5), 6 mM $MgCl_2$, and 0.1 mM DTT at 25 °C with constant stirring. Standard reactions contained 1 μM RecA protein, 3 μM ϵM13 DNA, and LexA S119A repressor as indicated. The RFI was determined as the difference between the ϵM13 DNA fluorescence induced by the fully bound RecA protein complex and the complex completely dissociated by addition of NaCl. The STMP was determined as the NaCl concentration required to reduce the RecA protein-induced increase in intrinsic fluorescence of ϵM13 DNA by one-half.

RESULTS

LexA S119A Repressor Competitively Inhibits RecA Protein-promoted Cleavage of LexA Repressor—As a consequence of being uncleavable, the LexA S119A repressor is potentially an ideal analogue for investigating the mechanism by which the wild-type repressor is able to inhibit RecA protein-promoted activities, under certain conditions (22). Previously, it was demonstrated that the uncleavable LexA S119A repressor inhibits the rate of RecA protein-stimulated proteolysis of the wild-type LexA repressor (24). The observed inhibition was

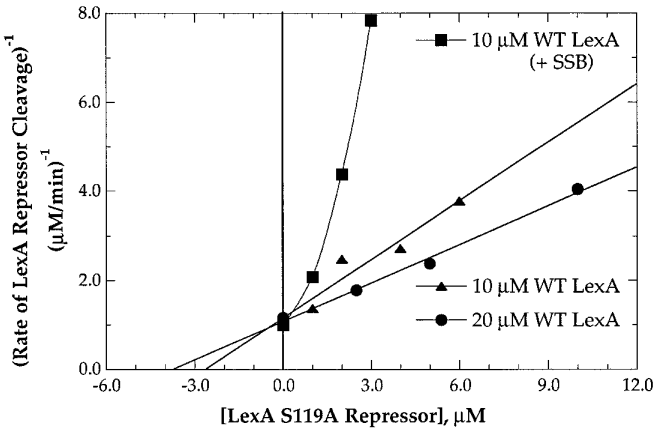

FIG. 1. **LexA S119A repressor is a competitive inhibitor of RecA protein-stimulated proteolysis of the wild-type LexA repressor.** Reactions were conducted and rates were determined as described under "Materials and Methods." The rate of proteolytic cleavage was determined at two different concentrations of wild-type LexA repressor (10 μM *triangles* and 20 μM *circles*) in the absence of SSB protein; assays done in the presence of 10 μM wild-type LexA repressor and the SSB protein are indicated by *squares*. In all cases, rates were measured as a function of increasing amounts of the LexA S119A repressor. Linear regression was used to fit data sets corresponding to 10 and 20 μM wild-type LexA repressor, in the absence of the SSB protein; the linear functions correspond to $y = 1.1508 + 0.4378x$, $y = 1.0761 + 0.28818x$, respectively. The point of intersection of the linear functions defines an apparent K_i for the LexA S119A repressor of approximately 0.5 μM that is in reasonable agreement with that previously reported (10).

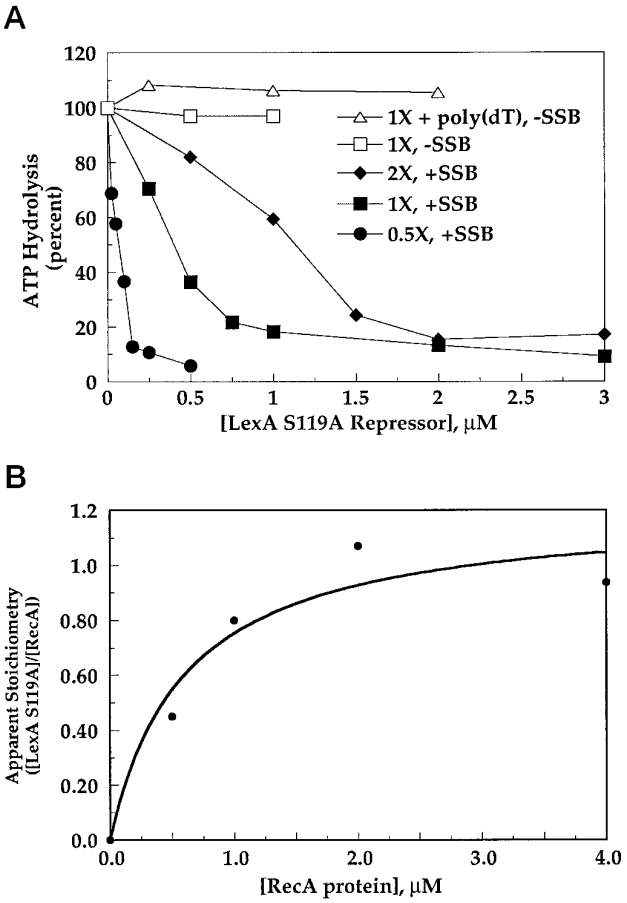

FIG. 2. **LexA S119A repressor inhibits the ssDNA-dependent ATP hydrolysis activity of RecA protein when SSB protein is present.** *A*, LexA S119A repressor titration of ssDNA-dependent ATP hydrolysis reactions. Rate of hydrolysis is expressed as the percentage of control reactions without LexA S119A repressor. Reactions were carried out in the buffer described in "Materials and Methods." Initial experiments were performed using standard ATP hydrolysis reaction conditions (*1*×). Reactions labeled 2× and 0.5× had RecA protein, SSB protein, and DNA concentrations that were double or half, respectively, of that in standard ATP hydrolysis reactions. *+SSB* indicates the presence of SSB protein and *−SSB* indicates the absence of SSB protein. Experimental uncertainty is ±5%. Rates of hydrolysis in μM ATP min^{-1} for the reactions without LexA S119A repressor were as follows: 8.0 (*0.5*×,*+SSB*), 24.3 (*1*×,*+SSB*), 56.4 (*2*×,*+SSB*), 11.7 (*1*×,*−SSB*), and 25.2 (*poly(dT),−SSB*). *B*, apparent stoichiometry of LexA S119A repressor-mediated inhibition of ssDNA-dependent ATP of RecA protein hydrolysis. The apparent stoichiometry for inhibition by LexA S119A repressor was determined as outlined in the text. All data were taken from the curves in *A* except for the reactions in which the protein and DNA concentrations were quadrupled (4 μM RecA) for which the data are not shown. The *line* corresponds to a hyperbolic fit of the data with the plateau corresponding to an apparent stoichiometry of 1.2 (±0.2) μM and the midpoint 0.6 (±0.3) μM LexA S119A repressor.

proposed to be caused by reversible binding of the mutant to the RecA protein ternary complex; therefore, LexA S119A repressor should act as a competitive inhibitor of wild-type LexA repressor cleavage (24). As expected, the preparation of LexA S119A repressor used here inhibits the rate of LexA repressor cleavage stimulated by the RecA protein in the presence M13 ssDNA and ATP (Fig. 1). An increase in the LexA S119A repressor concentration at a given wild-type LexA repressor concentration results in a greater degree of inhibition; conversely, an increase in the wild-type protein at a constant concentration of uncleavable mutant protein decreases the amount of inhibition. These characteristics indicate that LexA S119A repressor acts as a competitive inhibitor of wild-type LexA repressor cleavage. In addition, the calculated K_i of 0.5 μM agrees with the K_m previously reported for the binding of wild-type LexA repressor to the RecA protein filament (10).

LexA S119A Repressor Inhibits ssDNA-dependent ATP Hydrolysis by RecA Protein—Before investigating the effect of LexA S119A repressor on RecA protein-promoted DNA strand exchange, it was determined whether LexA S119A repressor affected the activities of RecA protein in a manner similar to that observed for the wild-type repressor (22). Initially, the ssDNA-dependent ATP hydrolysis activity of RecA protein was monitored in the presence of LexA S119A repressor. Increasing concentrations of LexA S119A repressor inhibited the ATPase activity of RecA protein, provided that SSB protein was present (*closed squares* Fig. 2*A*, Table I). At 1 μM RecA protein, an equimolar concentration of LexA S119A repressor reduced the rate of ATP hydrolysis by 80%; higher LexA S119A repressor concentrations had a slight additional inhibitory effect on the rate of ATP hydrolysis. The degree of inhibition observed was the same if RecA protein and LexA S119A repressor were preincubated together or if LexA S119A repressor was added following initiation of the reaction (data not shown). As indicated above, the inhibitory effect of LexA repressor required the presence of SSB protein (compare *open squares* to *closed squares* in Fig. 2*A*, Table I). When SSB protein was added to ongoing ATPase assays, the rate of ATP hydrolysis decreased; prior to the addition of SSB protein the hydrolysis rate for reactions with and without LexA S119A repressor was identical (data not shown). In agreement, cleavage of wild-type LexA repressor by RecA protein is inhibited by LexA S119A repressor to a greater extent in the presence of SSB protein than in its absence (compare *squares* to *triangles* Fig. 1).

The effect of LexA S119A repressor on ATPase activity stimulated by other types of ssDNA was also examined. There was no effect of added LexA S119A repressor when either poly(dT) (Fig. 2*A*) or ϵM13 DNA was the ssDNA cofactor (data not shown). LexA S119A repressor also had no effect on ATP hydrolysis under reaction conditions (1 mM Mg^{2+}) that allow formation of a contiguous RecA protein filament on M13 ssDNA

TABLE I
Comparison of ssDNA-dependent and DNA-independent ATP hydrolysis activity of RecA protein in the presence of LexA S119A repressor, SSB protein, and either ATP or dATP

Reactions were performed as described under "Materials and Methods" using standard conditions. Experimental uncertainty is ±5%.

[LexA S119A]	Steady state rate (percent activity)[a]		
	ATP		dATP
	−SSB	+SSB	+SSB
μM		μM/min	
0	11.7 (100)	24.3 (100)	33.6 (100)
0.5	11.4 (97)	10.4 (43)	28.4 (85)
1.0	11.2 (96)	5.3 (22)	17.8 (53)
0[b]	6.2 (100)	5.9 (100)	
0.5[b]	7.3 (118)[c]	6.5 (110)[c]	

[a] Percent activity was determined by normalizing to the reactions without LexA S119A protein.
[b] DNA-independent RecA protein ATP hydrolysis induced by addition of 1.8 M NaCl (37).
[c] The increase in this rate is likely due to volume exclusion effect since bovine serum albumin protein added at this same concentration had a similar effect (F. G. Harmon and S. C. Kowalczykowski, unpublished observations).

in the absence of SSB protein (data not shown). The combined effects of SSB protein and LexA S119A repressor cannot be tested using these ssDNA substrates or conditions because SSB protein alone inhibits ATP hydrolysis by competing with RecA protein for binding to the ssDNA (35).

If LexA repressor is mediating its effects through specific contacts on the RecA protein filament, these sites should be saturable and should yield a fixed stoichiometry for inhibition. Using electron micrographic reconstructions of uncleavable LexA K156A repressor bound to dsDNA-RecA protein filaments, the binding stoichiometry has been calculated as one LexA repressor monomer to two RecA protein protomers (17). To determine if a fixed stoichiometry exists for LexA S119A repressor-mediated inhibition of ssDNA-dependent ATP hydrolysis, LexA S119A repressor titrations were performed at RecA protein, SSB protein and ssDNA concentrations that were one-half, 2-fold and 4-fold of those used above (Fig. 2*A*; data for 4-fold assays not shown). The initial slope was extrapolated to the X-intercept to obtain the estimated LexA S119A repressor concentration at 100 percent inhibition. This LexA S119A repressor concentration was then used to calculate the apparent binding stoichiometry at each RecA protein concentration examined. Because these experiments at the lower protein concentrations were below the estimated K_d for the LexA-RecA protein interaction, these apparent stoichiometries underestimate the true stoichiometry. For this reason, these calculated apparent binding stoichiometries were plotted *versus* the corresponding RecA protein concentration (Fig. 2*B*). As can be seen, the apparent stoichiometry approaches a plateau at 1.2 (±0.2):1 (LexA S119A:RecA), suggesting an approximate limiting stoichiometry of 1:1 for the interaction of LexA S119A repressor with RecA protein. The stoichiometry is 1:1 only at higher RecA protein concentrations (*i.e.* 4 μM) because these are conditions under which the binding of LexA repressor to RecA protein is stoichiometric, since the LexA S119A repressor concentrations used are well above the K_d of 0.5 μM for the binding of LexA repressor to RecA protein (10). Additionally, the approximate K_d calculated from these data is 0.6 (±0.3) μM, which is in close agreement with the reported value and that obtained in Fig. 1. Although this stoichiometry does not agree with the previously determined 1:2 stoichiometry it suggests that LexA S119A repressor associates with the RecA protein filament at sites that can be saturated (see below).

The effect of both SSB protein and LexA S119A repressor on the DNA-independent, high-salt activated ATPase activity of RecA protein (37) was tested to determine whether the inhibition could be explained entirely as a direct effect on RecA protein or if it is mediated *via* the ssDNA. LexA S119A repressor does not inhibit the ATP hydrolysis activity of RecA protein at 1.8 M NaCl (Table I). Furthermore, LexA S119A repressor has no inhibitory effect in the presence of SSB protein under these conditions. These results suggest that competition for binding to ssDNA represents an important aspect of the mechanism of LexA S119A inhibition (see below).

TABLE II
Effect of LexA S119A repressor on displacement on SSB protein by RecA protein

Reactions were performed as under "Materials and Methods" using standard conditions.

[LexA S119A]	ATP		dATP	
	Lag[a]	Steady state rate	Lag[a]	Steady state rate
μM	s	μM/min	s	μM/min
0	880	12.1[b]	210	39.6
0.5	1000	11.1	215	37.3
1.0	—[c]	—[c]	350	21.5

[a] Lag times were determined as the intersection of two lines, one drawn parallel to the final steady state rate and the second parallel to the initial lag period.
[b] Not steady state, but final velocity at 1 h.
[c] A measurable rate was not achieved in these reactions after 1 h.

LexA S119A Repressor Reduces the Ability of RecA Protein to Compete with SSB Protein for ssDNA Binding Sites—As indicated above, inhibition of ssDNA-dependent ATPase activity by LexA S119A repressor is completely dependent on the SSB protein. Since both RecA protein and SSB proteins compete for ssDNA binding sites, any conditions that favor SSB protein binding would reduce steady state levels of RecA protein bound to DNA, resulting in decreased ATP hydrolysis activity. Therefore, it is possible that LexA S119A repressor changes the DNA binding affinity of either RecA protein or SSB protein, which either hinders binding of RecA protein or favors binding of SSB protein.

To characterize the effect of LexA S119A repressor on the ability of RecA protein to compete with SSB protein, the rate and extent of RecA protein-promoted displacement of SSB protein from ssDNA was measured using an ATP hydrolysis assay (34). Upon addition of RecA protein to SSB protein-ssDNA complexes, there is a lag period where no ATP hydrolysis is observed until a certain proportion of the SSB protein is displaced. This lag time is a measure of the ability of RecA protein to compete with SSB protein and the final observed rate of ATP hydrolysis a measure of the final extent of SSB protein-displacement (34).

The presence of LexA S119A repressor slowed or completely inhibited SSB protein-displacement by RecA protein (Table II). When 0.5 μM LexA S119A repressor was present, the lag time for ATP hydrolysis increased by 120 s over the control (Table II). At 1 μM LexA S119A repressor, a measurable rate of ATP hydrolysis was not achieved within an hour, indicating that the ATP-RecA protein complex could not displace enough SSB protein to form a filament competent for ATP hydrolysis. Thus, the presence of LexA S119A repressor moderated the ability of RecA protein to displace SSB protein from ssDNA.

The ability of RecA protein to displace SSB protein can be enhanced by using dATP as the nucleotide cofactor; the dATP-RecA protein complex competes better with SSB protein for ssDNA binding sites (36). Therefore, if LexA S119A repressor attenuates RecA protein's ability to compete with SSB, as suggested above, dATP should partially relieve this LexA repressor-mediated inhibition. In agreement, dATP decreased the inhibitory effects of LexA S119A repressor; at a LexA S119A

TABLE III
The binding of RecA protein to ϵM13 ssDNA in the presence of LexA S119A repressor

Reactions were performed as under "Materials and Methods" using standard conditions. The STMP and RFI were determined as described under "Materials and Methods." The experimental uncertainty is ±50 mM NaCl and ±5% RFI, respectively.

Nucleotide cofactor	[LexA S119A]	STMP	RFI
	μM	mM	
No cofactor	0	270	1.8
	1.0	250	2.1
ADP	0	125	2.0
	1.0	130	2.0
ATP	0	1000	2.9
	1.0	1000	3.0

concentration of 0.5 μM, the lag time was unchanged from the dATP control reaction, and the final steady state rate was within 6% of the control (Table II). In the presence of 1.0 μM LexA S119A repressor, the lag time was only 150 s longer, and the final steady state rate was 50% of the reaction without LexA S119A repressor (Table II). It is clear from these experiments that when RecA protein is better able to compete with SSB protein (*i.e.* when dATP is present as nucleotide cofactor), the LexA S119A repressor-mediated effects on RecA protein function are less severe. Therefore, LexA S119A repressor lessens the ability of RecA protein to compete with SSB protein for ssDNA binding sites.

LexA S119A Repressor Does Not Alter the Apparent ssDNA Binding Affinity of RecA Protein—LexA S119A repressor could lessen RecA protein's ability to compete with SSB protein and, therefore, ATP hydrolysis activity by decreasing its affinity for ssDNA either by affecting the induction of RecA protein to its high affinity DNA-binding state or by reducing the DNA-binding affinity of RecA protein in this state. Both of these effects would result in a RecA protein species that could not compete as well with SSB protein for limited DNA binding sites. To determine if LexA S119A repressor affects either of these DNA binding properties, the stability of RecA protein-ϵM13 DNA complexes to disruption by NaCl ("salt titrations") was measured (31, 41). The relative maximal increase in intrinsic ϵM13 DNA fluorescence upon RecA protein binding to the DNA substrate, or RFI, is characteristic of the affinity state of RecA protein, higher values indicate that more RecA protein has assumed the high affinity DNA-binding state as a result of binding nucleotide cofactors such as ATP (31, 41). The relative affinity of RecA protein for ssDNA can be estimated by the STMP, which is the concentration of salt required to dissociate one-half the ϵM13 DNA-RecA protein complexes. If the DNA binding properties of RecA protein are altered in the presence of LexA S119A repressor, then either the STMP or RFI should reflect these changes.

In the absence of cofactor and LexA S119A repressor, the STMP and RFI for the ϵM13 DNA-RecA protein complex were 270 mM NaCl and 1.8, respectively (Table III). LexA S119A repressor added to 1 μM had little effect on these two parameters (Table III). LexA S119A repressor at this concentration also did not affect either the STMP or RFI of complexes formed in the presence of 1 mM ADP (Table III). Since neither the STMP nor the RFI changed in its presence, LexA S119A repressor appears not to affect the low affinity DNA binding state of RecA protein. In addition, the STMP and RFI of the RecA protein-ATP complex was not changed in the presence of 1.0 μM LexA S119A repressor (Table III), although inhibition of ATP hydrolysis (in the presence of SSB protein) was maximal under similar conditions (Fig. 2*A*). It is apparent from these experiments that LexA S119A repressor does not either alter the equilibrium binding affinity of RecA protein for ssDNA or affect the ability of RecA protein to achieve the high affinity DNA binding state.

Since the salt titrations necessarily measure apparent DNA binding affinity at conditions of elevated NaCl concentrations, it was possible that any effects of LexA S119A repressor escaped detection because the LexA-RecA protein complex is unstable at these concentrations. For this reason, the kinetics of association with and dissociation from ssDNA were measured. In the absence of LexA S119A repressor, the kinetics of association between RecA protein (0.1 μM) and ϵM13 DNA (6 μM) are complete in approximately 90 s (at 25 °C with 150 mM NaCl present; data not shown). When LexA S119A repressor is present at either 0.05 or 0.5 μM in similar assays, the kinetics and extent of RecA protein association paralleled that of the control reactions (data not shown). Additionally, LexA S119A repressor did not affect the dissociation of RecA protein from ϵM13 DNA; the dissociation rate constant obtained for the transfer of RecA protein from ϵM13 DNA to poly(dT) was the same in the presence and absence of 1.0 μM LexA S119A repressor (data not shown). Thus, under these conditions, LexA S119A repressor does not measurably affect RecA protein's equilibrium ssDNA binding affinity or rates of association and dissociation with ssDNA.

LexA S119A Repressor Inhibits RecA Protein-promoted DNA Strand Exchange—To directly address whether the co-protease and DNA strand exchange activities are competitive, the effect of LexA S119A on RecA protein-promoted DNA strand exchange was examined using linear M13mp7 dsDNA and circular M13mp7 ssDNA as substrates (8). In the presence of SSB protein and no LexA S119A repressor, RecA protein converted approximately 60% of the initial linear duplex substrate and circular ssDNA substrate to gapped heteroduplex product in 60 min (Fig. 3*A*, *B*, and *D*). When 1.0 μM LexA S119A repressor was present (LexA S119A:RecA protein ratio of 1:3), no DNA strand exchange was detectable (Fig. 3, *B* and *D*). DNA strand exchange was also completely inhibited at 0.5 μM LexA S119A (LexA S119A:RecA protein ratio of 1:6; Fig. 3*A*). At 0.25 μM LexA S119A repressor (LexA S119A:RecA protein ratio of 1:12), an intermediate level of inhibition was observed; substrate uptake was inhibited 95%, and the few joint molecules that formed were not resolved to final product (data not shown). At these concentrations, LexA S119A repressor appears to inhibit presynaptic complex formation, similar to that seen for inhibition of ATP hydrolysis activity. In contrast, at 0.1 μM LexA S119A repressor (LexA S119A:RecA protein ratio of 1:30), gapped heteroduplex product was formed, but the extent of product formation was inhibited by 40% (Fig. 3, *A* and *D*). At LexA S119A repressor concentrations as low as 0.05 μM (LexA S119A:RecA protein ratio of 1:60), the extent of product formation was inhibited by 20% (Fig. 3*D*). In addition, the rate of product formation at these two LexA repressor concentrations was reduced (Fig. 3, *A* and *D*). Interestingly, the ATPase activity of RecA protein at these LexA S119A repressor concentrations (0.1–0.05 μM) was essentially unaffected by the inhibitor (*closed squares* Fig. 2*A*). LexA S119A repressor at 0.025 μM concentration (RecA:LexA S119A repressor ratio of 120:1) had little observable effect on DNA strand exchange (data not shown). Apparently, the DNA strand exchange activity of RecA protein is more sensitive to LexA S119A repressor than is the ATPase activity.

When LexA S119A repressor is present at concentrations of 0.05 and 0.1 μM, the rate and extent of linear duplex substrate uptake are the same as the control reaction (Fig. 3, *A* and *B*), but extensive homology-dependent DNA networks (which ap-

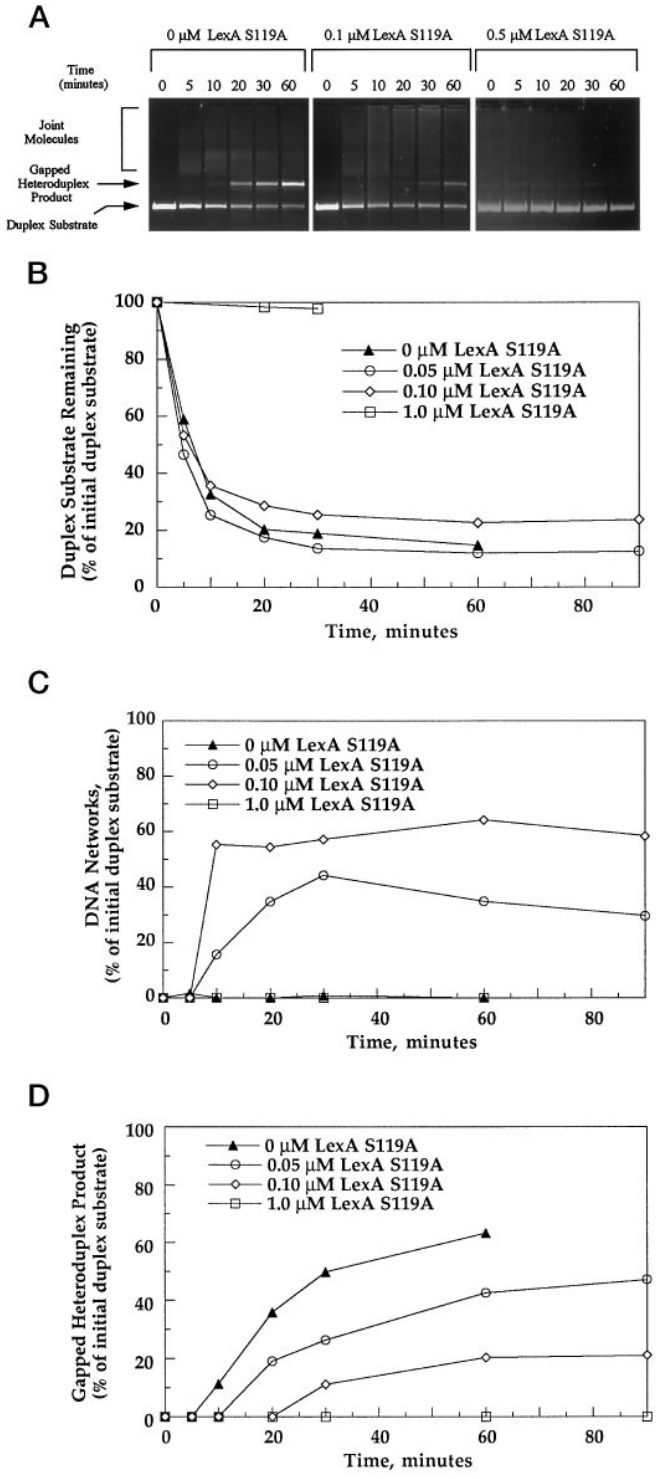

FIG. 3. **LexA S119A repressor inhibits RecA protein-promoted DNA strand exchange in the presence of SSB protein.** *A*, time course of the RecA protein-promoted DNA strand exchange reaction in the absence and presence of 0.1 and 0.5 μM LexA S119A repressor, as indicated. The position of bands corresponding to duplex DNA substrate, joint molecules, and gapped heteroduplex product are indicated. *B*, uptake of linear duplex DNA substrate into joint molecules. *C*, formation of homology-dependent DNA networks. Networks appear as DNA that is trapped in the wells, see *middle panel* in *A*. *D*, formation of gapped heteroduplex DNA product. All data in *B–D* are normalized to the concentration of duplex DNA substrate at 0 min. Reactions were carried out using standard conditions.

pear as DNA trapped in the wells of the gel) are formed at the later time points (Fig. 3, *A* and *C*). These networks result from invasion of a joint molecule by a second DNA molecule (42). The presence of these networks indicates that either RecA protein has an increased ability to remove SSB protein from the displaced strand of the joint or the joint molecules are unusually long lived, allowing time for RecA protein to displace SSB protein. The first possibility is unlikely since RecA protein is unable to do so in the presence of LexA S119A repressor (see above). Thus, the longevity of the joint molecules appears to be increased in the presence of LexA S119A repressor. These results suggest that LexA S119A repressor at these concentrations does not affect initial pairing and joint molecule formation, but once a joint molecule is made RecA protein promotes branch migration slowly or not at all. In support of this, 0.05 or 0.1 μM LexA S119A repressor added 15 min after the initiation of the DNA strand exchange reaction inhibits gapped heteroduplex product formation to the same extent as when LexA repressor is preincubated with RecA protein and M13 ssDNA (data not shown). Thus, at LexA S119A:RecA protein ratios $<$1:12, inhibition by LexA S119A repressor is manifest at the branch migration stage; whereas at ratios $>$1:12, inhibition by LexA S119A repressor is manifest early at either presynaptic complex formation or initial pairing.

Inhibition of DNA Strand Exchange by LexA S119A Is SSB Protein-independent—Since LexA repressor is believed to bind within the deep helical groove of the RecA protein presynaptic complex (see above), inhibition of DNA strand exchange may simply result from LexA S119A repressor blocking the secondary DNA binding site. If this were the case, then inhibition of DNA strand exchange should be independent of SSB protein. To determine if the inhibition of DNA strand exchange by LexA S119A repressor is dependent upon SSB protein, the effect of LexA S119A repressor on DNA strand exchange was examined in the absence of SSB protein. In the presence of a volume-occupying agent such as polyvinyl alcohol (PVA), RecA protein promotes DNA strand exchange in the absence of SSB protein (39). When 7.5% (w/v) PVA is present, the rate and extent of joint molecule formation is similar to that seen in the presence of SSB protein, but no product is formed and extensive DNA networks accumulate (39). In agreement, our assays without LexA S119A repressor showed both joint molecule (*open triangles* Fig. 4*A*) and network formation but no product formation within 60 min (data not shown). The number of joint molecules present decreases in the later time points as joints take part in formation of DNA networks (*triangles* and *circles* Fig. 4*A*).

In the presence of LexA S119A repressor and 7.5% PVA, DNA strand exchange was inhibited (Fig. 4*A*), similar to the results observed in the presence of SSB protein. At LexA S119A repressor concentrations $\geq$0.5 μM (LexA S119A:RecA protein ratios of $>$1:12), RecA protein could not promote joint molecule formation. At 0.25 μM LexA S119A repressor (LexA S119A:RecA protein ratio of $>$1:12), joint molecule formation was half of that in the absence of inhibitor and DNA networks did not form. At 0.1 μM LexA S119A repressor (LexA S119A:RecA protein ratio of 1:30) joint molecule formation was inhibited by approximately 10%, and few joint molecules were incorporated into homology-dependent DNA networks (Fig. 4*A*). In the presence of 0.05 μM LexA S119A repressor (RecA:LexA S119A repressor ratio of 60:1), RecA protein formed 10% less joint molecules but incorporated these into DNA networks, as manifest by a decrease in joint molecules at the later time points (*open circles*, Fig. 4*A*).

To demonstrate that this was not an effect specific to PVA, DNA strand exchange reactions using the "magnesium-jump" protocol were also performed (40). Presynaptic filaments were formed at 1 mM magnesium acetate, and then DNA strand exchange was initiated by bringing the magnesium ion concentration up to 10 mM upon the addition of linear dsDNA. The

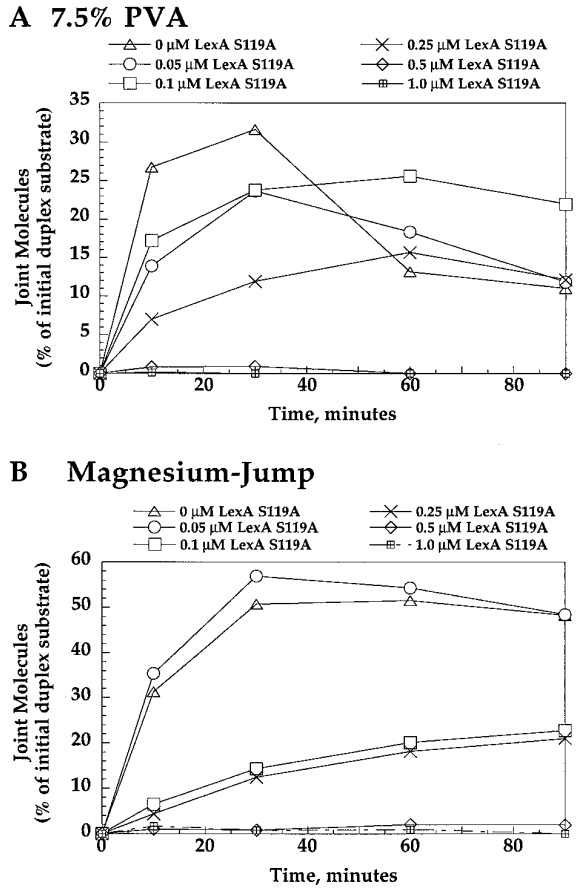

FIG. 4. **LexA S119A repressor inhibits RecA protein-promoted DNA strand exchange in the absence of SSB protein.** *A*, joint molecule formation in the presence of 7.5% PVA (w/v). *B*, formation of joint molecules in "magnesium-jump" experiments. Standard concentrations of protein and DNA were used except that SSB protein was absent from all reactions.

inhibition by LexA S119A repressor in these reactions was similar to those with 7.5% PVA (Fig. 4*B*). In agreement with the results from DNA strand exchange reactions carried out in the presence of SSB protein, LexA S119A repressor completely inhibited the SSB protein-independent pairing reactions at LexA S119A:RecA protein ratios >1:12 but allowed joint molecule formation at ratios ≤1:12. In contrast, LexA S119A repressor did not inhibit the ATP hydrolysis activity of RecA protein in the presence of 7.5% PVA or at 1 mM magnesium ion (data not shown). Clearly, LexA S119A repressor-mediated inhibition of DNA strand exchange is independent of SSB protein, unlike the effect of LexA S119A repressor on RecA ssDNA-dependent ATP hydrolysis. This difference in the requirement for SSB protein suggests the presence of two distinct modes of LexA S119A repressor-mediated inhibition (see below).

dATP Relieves LexA S119A Repressor-dependent Inhibition of RecA Protein ssDNA-dependent ATP Hydrolysis but Not of DNA Strand Exchange—As described above, dATP allows RecA protein to compete better with SSB protein for ssDNA binding sites. If LexA S119A repressor inhibits DNA strand exchange and ATP hydrolysis by different mechanisms, then the use of dATP should partially relieve the LexA S119A repressor-mediated inhibition of dATP hydrolysis but not necessarily the inhibition of DNA strand exchange. As predicted, hydrolysis of dATP by RecA protein is less sensitive to the presence of LexA S119A repressor than is the hydrolysis of ATP (Table I); however, the inhibition remains dependent upon the presence of SSB protein (data not shown).

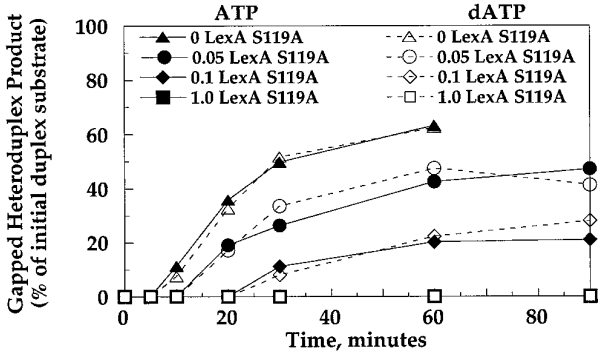

FIG. 5. **Comparison of DNA strand exchange in the presence of either ATP or dATP.** Reaction conditions are described under "Materials and Methods" except that 1 mM dATP was substituted for ATP where indicated.

Since DNA strand exchange is inhibited in an SSB-independent manner, dATP might not alleviate the inhibitory effects of LexA S119A repressor. In accord, dATP does not change the pattern of inhibition of DNA strand exchange by LexA S119A repressor; inhibition of gapped heteroduplex product formation is similar to that observed with ATP (compare *closed symbols* to *open symbols* Fig. 5). Substrate uptake is also similar for the dATP and ATP reactions, except that at 0.1 μM LexA S119A repressor uptake with dATP is slightly less (data not shown). Taking into account that LexA S119A repressor inhibits DNA strand exchange in the absence of SSB protein, the inability of dATP to rescue this activity of RecA protein argues against a ssDNA-dependent effect and, instead, suggests that LexA repressor and dsDNA bind to the same site on the RecA protein filament. This is in contrast to the apparent requirement for ssDNA for the inhibition of RecA protein ATP hydrolysis activity (see above, Table I).

DISCUSSION

The data presented here demonstrate that in the presence of LexA S119A repressor, RecA protein has reduced ssDNA-dependent ATP hydrolysis activity and is unable to complete DNA strand exchange. Inhibition of ssDNA-dependent ATP hydrolysis by LexA S119A repressor is dependent upon the presence of *E. coli* SSB protein, but inhibition of DNA strand exchange is not. Additionally, LexA S119A repressor hinders displacement of SSB protein from ssDNA by RecA protein. dATP relieves the LexA S119A repressor-dependent inhibition of both ATP hydrolysis and displacement of SSB protein but does not rescue DNA heteroduplex formation. Lastly, LexA S119A repressor does not measurably affect either the kinetics or apparent affinity of RecA protein binding to ssDNA.

Three models can be advanced to explain LexA S119A repressor-mediated inhibition of RecA protein function. The first model, which is applicable only to the DNA strand exchange reaction, is that LexA S119A repressor binds to the homologous duplex DNA thereby blocking branch migration (Fig. 6*A*). The second proposes that the binding of LexA S119A repressor directly to RecA protein within the presynaptic filament blocks binding of dsDNA and, therefore, DNA strand exchange (Fig. 6*B*). The third model, which would account for both ATP hydrolysis and DNA strand exchange inhibition, suggests that LexA repressor alters the steady state level of RecA protein bound to ssDNA when SSB protein is present (Fig. 6*C*).

In the first model, LexA S119A repressor blocks DNA strand exchange by binding to the duplex DNA (Fig. 6*A*). LexA S119A repressor bound along the duplex DNA could block branch migration past the region to which it is bound. LexA S119A repressor could conceivably be binding to an SOS operator, but a sequence search of M13mp7 failed to find a consensus SOS

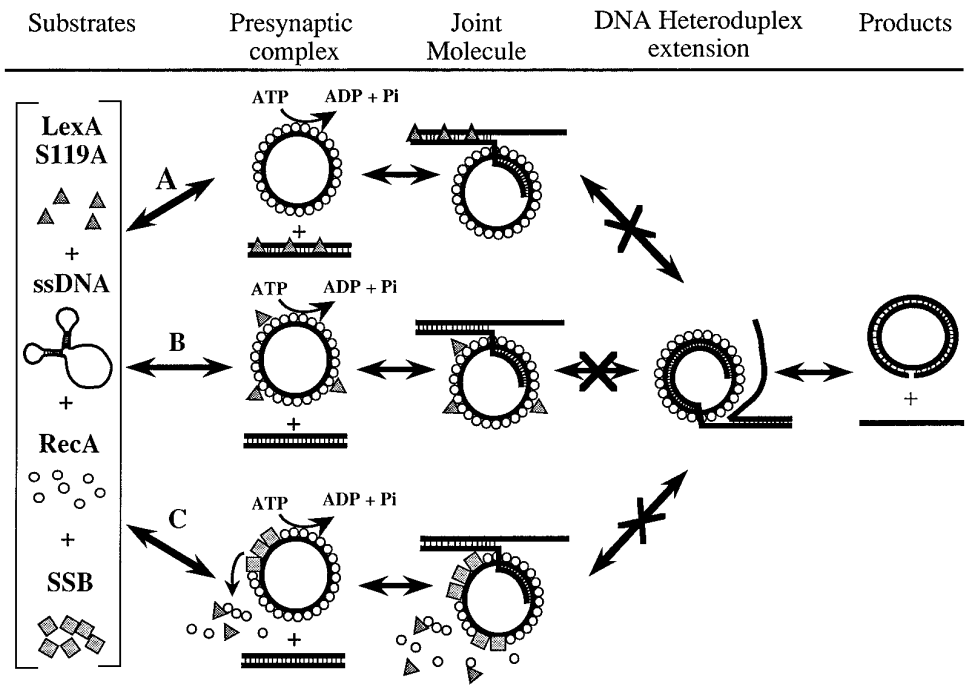

FIG. 6. **Models for LexA S119A repressor inhibition of RecA protein-mediated activities.** *A*, LexA S119A repressor binds to duplex DNA and blocks branch migration. *B*, LexA S119A repressor binds directly to the RecA protein filament and blocks binding of a second DNA strand. *C*, LexA S119A repressor disrupts the integrity of the RecA protein filament. The major steps in RecA protein-promoted DNA strand exchange are indicated. An *X* indicates that this step is blocked when LexA S119A repressor is present.

box, although several sites had a very limited homology (6 out of 16 bases) to the SOS box sequence. Alternatively, LexA S119A repressor could be bound to the DNA nonspecifically since LexA repressor binds to poly(dA·dT) in a cooperative manner (29). In agreement, LexA S119A repressor displayed nonspecific binding to *Ase*I-digested M13 dsDNA in polyacrylamide gel electrophoresis mobility-shift assays,[3] but only at protein concentrations (>1 μM LexA S119A repressor) that were over 2-fold higher than needed for maximal inhibition of DNA strand exchange and 20-fold higher than needed for the onset of inhibition. Therefore, it is unlikely that binding of LexA S119A repressor to the duplex DNA blocks DNA strand exchange.

An alternative to this first model is illustrated in Fig. 6*B* and proposes that binding of LexA repressor to the presynaptic filament occurs at a site that is essential for homologous pairing with the dsDNA. Such a model readily explains the observed inhibition of DNA strand exchange and is consistent with the proposal that LexA repressor binds within the deep helical groove of the RecA protein filament, believed to also harbor the secondary DNA binding site (17, 18, 43). Therefore, it is possible that LexA S119A repressor bound to the RecA protein filament blocks dsDNA binding which impedes DNA strand exchange (Fig. 6*B*). At low LexA S119A concentrations, where the protein would be bound infrequently, the RecA protein filament could bind and pair the duplex DNA but would be unable to extend the joint molecule past the sites to which LexA S119A repressor had bound. Higher LexA S119A repressor concentrations would block the majority of DNA binding sites, thereby inhibiting initial pairing. In complete accord, the data presented here demonstrate that LexA S119A repressor blocked DNA heteroduplex extension, but not DNA pairing at LexA S119A:RecA protein ratios that were between 1:12 and 1:60, and completely abolished pairing at higher ratios (Fig. 3). Theoretically, to completely block joint molecule formation the ratio of LexA S119A repressor to RecA protein would have to be ≥1:6 (calculated using a LexA:RecA binding stoichiometry of 1:2 since it is not clear how ATP hydrolysis is inhibited; see below) since RecA protein requires approximately three monomers (8 homologous nucleotide bases) to initiate homologous pairing (44). The results presented here agree with this estimate; LexA S119A completely blocked joint molecule formation at ratios of 1:6 and 1:3. In addition, if LexA repressor is simply blocking dsDNA binding, the LexA S119A repressor-mediated inhibition of DNA strand exchange should be independent of SSB protein, as observed here (Fig. 4). In support of the view that inhibition of DNA strand exchange is SSB protein-independent, dATP does not allow RecA protein to overcome this inhibition of DNA strand exchange (Fig. 5). This result argues that, unlike inhibition of ATP hydrolysis, competition between SSB protein and RecA protein for ssDNA binding sites is not the basis of inhibition by LexA S119A repressor (see below). Since the inhibitory effects of LexA S119A repressor on DNA strand exchange are parallel with and without SSB protein, this same mode of inhibition must be operating in both the presence and absence of SSB protein. Taken together, these results suggest that the SOS induction and recombinational activities of RecA protein share a common site on the RecA protein filament as indicated in model B (Fig. 6*B*, see below). This model, however, explains neither the inhibition of ATP hydrolysis nor the absolute need for SSB protein for inhibition of ATP hydrolysis (see below).

A third model is advanced to explain primarily the need for SSB protein to observe inhibition of ATPase activity of RecA protein by LexA S119A repressor. This model proposes that LexA S119A repressor has a role in altering the equilibrium between SSB protein and RecA protein binding to ssDNA (Fig. 6*C*). In this model, the presence of LexA S119A repressor favors SSB protein binding over that of RecA protein leading to gaps in the RecA protein filament. Gaps such as these would cause

[3] F. G. Harmon and S. C. Kowalczykowski, unpublished observations.

a reduction in the observed rate of ATP hydrolysis. The strongest support for this model is the difference in the LexA S119A repressor-mediated inhibition of ATP hydrolysis when dATP was present in place of ATP (Table I). In addition, LexA S119A repressor has a lesser effect on RecA protein-promoted displacement of SSB protein from ssDNA when dATP is present (Table II). Clearly, the inhibitory effect of LexA S119A repressor is lower when conditions favor binding of RecA protein over that of SSB protein (*i.e.* using dATP as nucleotide cofactor) (Tables I and II). LexA S119A repressor also did not inhibit the high salt-induced DNA-independent ATP hydrolysis activity of RecA protein, even though wild-type LexA repressor can be cleaved under these conditions (43). These results suggest that the ssDNA is required for inhibition and are consistent with RecA and SSB proteins competing sites on the DNA. Based on this evidence, it is clear the LexA S119A repressor inhibits ssDNA-dependent ATP hydrolysis of RecA protein by altering its ability to compete with SSB protein for ssDNA binding sites, which leads to a decrease in the steady state level of bound RecA protein.

Theoretically, LexA S119A could alter the DNA binding equilibrium between RecA protein and SSB protein in three ways: 1) binding of LexA S119A repressor to RecA protein weakens the binding of RecA protein to ssDNA; 2) LexA S119A repressor acts by influencing the binding of SSB protein to ssDNA; and 3) LexA S119A repressor competes directly for ssDNA binding sites with both RecA protein and SSB proteins. In support of the first proposal LexA S119A displayed an apparent 1:1 stoichiometry for its inhibition of ATP hydrolysis (alternatively, the binding stoichiometry could be 1:2, with one-half of our LexA S119A repressor being inactive, but two different LexA S119A preparations yielded identical results; the possibility that dimerization of the LexA repressor in solution is affecting the observed stoichiometry is unlikely given a dimerization constant of 50 μM (28), and that dimerization occurs only after binding to its target DNA sequence (45)), which is consistent with LexA repressor binding to specific sites that can be saturated. Furthermore, both biochemical and electron microscopic experiments have clearly established that LexA repressor binds to RecA protein. But, LexA S119A repressor did not have a measurable effect on the equilibrium or the kinetics of RecA protein-ssDNA binding, which argues that binding of LexA S119A repressor to the RecA protein filament does not weaken the ssDNA binding affinity of individual RecA protomers, at least under the conditions that we employed. On the other hand, it is formally possible inhibition reflects LexA S119A repressor binding to SSB protein. However, gel filtration of ssDNA-SSB protein complexes formed in the presence of wild-type LexA repressor showed that LexA repressor was not associated with either the bound or free SSB protein.[3] In addition, LexA S119A repressor did not change the binding affinity of SSB protein for a 32-mer oligonucleotide or affect the apparent site size of SSB protein tetramers bound to ϵM13 DNA.[3] Therefore, there is no experimental support for proposal two. Finally, although LexA S119A repressor binds nonspecifically to dsDNA, there are no reports that this protein binds nonspecifically to ssDNA. In addition, the migration of M13mp7 ssDNA in an agarose gel was not retarded by the presence of LexA S119A repressor.[3] Proposal three, therefore, is also unlikely to explain the effect that LexA S119A repressor has on RecA protein. At this point, the manner by which LexA S119A repressor affects the equilibrium ssDNA binding of RecA protein and SSB protein binding remains unclear. The most attractive explanation is proposal one in which a direct interaction between the LexA S119A and RecA proteins affects the ssDNA binding of RecA protein. This interaction does not necessarily have to take place once RecA protein has bound to the ssDNA, LexA S119A repressor could be interacting with free RecA protein monomers to produce an inactive RecA protein species or one that is slow in filament formation. Whatever the mechanism, it is clear that in the presence of LexA S119A repressor, RecA protein has a decreased ability to compete with SSB protein. Although discussion of this model has been couched in terms of ATP hydrolysis inhibition, this effect would also contribute to the inhibition of DNA strand exchange observed at higher LexA S119A repressor concentrations by creating gaps in the filament that could block both pairing and branch migration.

The most complete model for LexA S119A-mediated inhibition of both ATP hydrolysis and DNA strand exchange is one that combines models B and C. Model B must represent the mode of LexA S119A repressor-mediated inhibition of DNA strand exchange at low LexA S119A repressor concentrations and when SSB protein is absent, since the inhibition of DNA strand exchange in the presence and absence of SSB protein is similar. The gaps predicted in model C would also contribute to inhibition of DNA strand exchange but only at higher LexA S119A repressor concentrations when SSB protein is present. On the other hand, LexA S119A repressor-mediated inhibition of RecA protein ATP hydrolysis is clearly due only to model C.

This study further illustrates the dynamic state of DNA binding that exists between RecA protein and SSB protein. Any condition that shifts this equilibrium can alter the DNA-dependent activities of RecA protein, an effect most clearly demonstrated with RecA mutants such as RecA803 and RecA730 proteins (34, 46). These proteins have an increased ability to compete with SSB protein and as a result display increased recombination and SOS induction activity *in vitro* and *in vivo* (34–36, 47, 48); presumably, these mutant proteins would be more refractory to inhibition by LexA repressor than is the wild-type RecA protein. Analogous to our studies, *in vitro* the RecF, RecO, and RecR proteins alter the activities of RecA protein by influencing the ability of RecA protein to compete with SSB protein (49). RecF protein, a ssDNA binding protein, inhibits RecA protein ssDNA-dependent ATP hydrolysis and DNA strand exchange in an SSB protein-dependent manner (49). In addition, RecF protein inhibits displacement of SSB protein by RecA protein. On the other hand, the RecO and RecR proteins together stimulate RecA protein-promoted joint molecule formation when the ssDNA substrate is coated with SSB protein, consistent with the idea that these two proteins increase the ability of RecA protein to compete with SSB protein. Recent evidence demonstrates that RecO and RecR act by binding directly to SSB protein (50). Similarly, we have demonstrated that LexA S119A acts in a similar manner to alter the ability of RecA protein to compete with SSB protein. *In vivo*, suppressors of *recF*, *recO*, and *recR* mutants are mutations in RecA protein (*i.e.* RecA803 and RecA703 proteins) that allow it to compete better with SSB protein for ssDNA binding sites (46, 51, 52). Interestingly, these mutant RecA proteins are also co-protease-constitutive. Thus, proteins that alter the ability of RecA protein to compete with SSB protein, RecF, RecO, RecR, and now presumably LexA repressor, have a parallel impact on RecA protein-promoted activities *in vitro* and *in vivo.*

This study also addresses the question of whether the SOS induction and recombinational activities of RecA protein are competitive. Previous studies have proposed that both the secondary DNA and LexA repressor binding sites lie within the deep helical groove of the RecA protein filament (17, 18, 43). If these two sites actually overlap, RecA protein should be unable to take part in both LexA repressor co-cleavage (*i.e.* binding of LexA repressor) and recombination simultaneously, as ob-

served here. Apparently, the site to which LexA S119A repressor binds in the deep helical groove overlaps the secondary DNA binding site. Thus, using uncleavable LexA S119A repressor we have demonstrated that these two activities of RecA protein are indeed competitive. In parallel, RecA protein-promoted cleavage of LexA repressor is inhibited by the presence of dsDNA (22), which reinforces the argument that these two activities of RecA protein are competitive. Although this evidence is indirect, it suggests that the RecA protein filament utilizes either the same sites or sites very close to one another to conduct both SOS induction and recombination. As a result, these two functions of RecA protein must be viewed as competing processes for individual RecA protein filaments.

In vivo support for the competitive nature of recombination and the SOS-induced activities of RecA protein is provided by results that suggest that formation of the UmuD′C protein complex, which is essential for SOS mutagenesis, inhibits recombination in favor of error-prone DNA replication (53). Increasing concentrations of UmuD′C protein complex in the cell reduces conjugal recombination up to 50-fold. Thus, it appears that the cell must stop recombination in order to initiate some SOS activities. Based on our study, RecA protein-mediated SOS induction may represent one such activity. Prior to cleavage, binding of LexA repressor to RecA protein filaments formed at the onset of SOS induction may act to momentarily halt the recombination process. This temporary interruption of recombination may be important to enhance LexA repressor cleavage, since the binding of LexA repressor and a second DNA molecule are mutually exclusive events. In addition, binding of LexA repressor could serve as a temporary block before the UmuD′C complex binds to disrupt ongoing recombination.

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