THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 278, No. 4, Issue of January 24, pp. 2278–2285, 2003

Printed in U.S.A.

Escherichia coli RecX Inhibits RecA Recombinase and Coprotease Activities *in Vitro* and *in Vivo**

Received for publication, October 14, 2002
Published, JBC Papers in Press, November 9, 2002, DOI 10.1074/jbc.M210496200

Elizabeth A. Stohl‡§, Joel P. Brockman¶‖, Kristin L. Burkle‡, Katsumi Morimatsu¶, Stephen C. Kowalczykowski¶, and H. Steven Seifert‡ ‡‡**

From the ‡Department of Microbiology and Immunology, Feinberg School of Medicine, Northwestern University, Chicago, Illinois 60611 and the ¶Division of Biological Sciences, Section of Microbiology and of Molecular and Cellular Biology, Center for Genetics and Development, University of California, Davis, California 95616

In *Escherichia coli* the RecA protein plays a pivotal role in homologous recombination, DNA repair, and SOS repair and mutagenesis. A gene designated *recX* (or *oraA*) is present directly downstream of *recA* in *E. coli*; however, the function of RecX is unknown. In this work we demonstrated interaction of RecX and RecA in a yeast two-hybrid assay. *In vitro*, substoichiometric amounts of RecX strongly inhibited both RecA-mediated DNA strand exchange and RecA ATPase activity. *In vivo*, we showed that *recX* is under control of the LexA repressor and is up-regulated in response to DNA damage. A loss-of-function mutation in *recX* resulted in decreased resistance to UV irradiation; however, overexpression of RecX *in trans* resulted in a greater decrease in UV resistance. Overexpression of RecX inhibited induction of two *din* (damage-inducible) genes and cleavage of the UmuD and LexA repressor proteins; however, *recX* inactivation had no effect on any of these processes. Cells overexpressing RecX showed decreased levels of P1 transduction, whereas *recX* mutation had no effect on P1 transduction frequency. Our combined *in vitro* and *in vivo* data indicate that RecX can inhibit both RecA recombinase and coprotease activities.

The *Escherichia coli* RecA protein plays a central role in homologous recombination and is required for induction of the SOS pathway of DNA repair and mutagenesis. A fundamental step in both homologous recombination and SOS response induction is the formation of a RecA-ssDNA[1]-ATP nucleoprotein filament. In this form, RecA protein can act as a recombinase by mediating pairing and promoting strand exchange between single-stranded DNA and another homologous DNA molecule, a process important for phage transduction, conjugation, and DNA repair (1, 2). RecA also functions as a coprotease to activate the SOS response (3). The LexA protein represses transcription of over 30 SOS genes in the *E. coli* SOS regulon (4). DNA damage is believed to produce regions of ssDNA upon which RecA forms a nucleoprotein filament. RecA coprotease activity facilitates self-cleavage of the LexA repressor protein, enhancing expression of SOS genes. RecA coprotease activity is also responsible for cleavage of the UmuD protein, which is involved in SOS mutagenesis. It is assumed that the repair of damaged DNA gradually removes the signal (ssDNA) needed for nucleoprotein filament formation, resulting in the re-accumulation of LexA pools and subsequent repression of the SOS genes, thereby resetting the SOS system (3). In contrast to this essentially passive process, overexpression of the *E. coli* DinI protein has been shown to inhibit induction of the SOS response through inhibition of RecA coprotease activity (5), and a *dinI* knockout showed both increased UmuD cleavage and SOS mutagenesis, suggesting that DinI may play an active role in turning off or modulating the SOS response (5, 6).

An open reading frame, originally designated *oraA*, is located directly downstream of *recA* and upstream of *alaS* in *E. coli* (7) and shows sequence similarity to RecX proteins from Gram-positive and Gram-negative bacteria (8). *recX* genes are located downstream of *recA* or overlapping *recA* (8–12) or occasionally elsewhere in the chromosome, as in *Neisseria gonorrhoeae* and *Bacillus subtilis* (13). Overexpression of homologous RecA proteins from plasmid constructs is deleterious in the absence of *recX* in *Pseudomonas aeruginosa*, *Streptomyces lividans*, *Mycobacterium smegmatis*, and *Xanthomonas oryzae* (9, 11, 14, 15), suggesting a role for RecX in down-regulating RecA expression or activity. Consistent with these observations, *Mycobacterium tuberculosis* RecX was recently shown to inhibit RecA-promoted DNA strand exchange and ATP hydrolysis *in vitro* (16). Although a *recX* mutation in *S. lividans* did not affect homologous recombination or transcription of *recA* (11), mutation of *recX* in *X. oryzae* resulted in decreased RecA levels (15). In contrast, a *N. gonorrhoeae recX* mutant exhibited deficiencies in all RecA-mediated processes but did not affect RecA levels, leading to the conclusion that RecX enhances RecA activity in *N. gonorrhoeae* (13). This collection of phenotypes led us to investigate the role of *E. coli* RecX *in vitro* and *in vivo*. The data presented here demonstrate that *E. coli* RecX can strongly inhibit both RecA recombinase and coprotease activities *in vitro* and *in vivo*.

EXPERIMENTAL PROCEDURES

Strains, Plasmids, Media, and Chemicals—New strains were constructed using P1 transduction (17) or as described. Strains and mutant

* This work was supported in part by National Institutes of Health Grants RO1-AI44239 (to H. S. S.) and RO1-GM-62653 (to S. C. K.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

§ Supported by American Cancer Society postdoctoral fellowship PF-00-016-01-GMMC.

‖ Present address: Molecular Staging, Inc., 300 George St., Suite 701, New Haven, CT 06511.

** Present address: Dept. of Microbiology and Immunology, Loyola University Medical Center, Maywood, IL 60153.

‡‡ To whom correspondence should be addressed: Dept. of Microbiology and Immunology, Feinberg School of Medicine, Northwestern University, 303 E. Chicago Ave., Chicago, IL 60611. Tel.: 312-503-9788; Fax: 312-503-1339; E-mail: h-seifert@northwestern.edu.

[1] The abbreviations used are: ssDNA, single-strand DNA; dsDNA, double-strand DNA; IPTG, isopropyl β-D-thiogalactopyranoside; Kan, kanamycin; Erm, erythromycin; MMC, mitomycin C; HA, hemagglutinin; SSB, single-stranded DNA binding.

alleles used were DH5α (Invitrogen), BL21(DE3) (Novagen), AB1157 (18), AB1157Δ*recA* (19), Hfr Cavalli (20), DE192(*lexA51*) (R. Woodgate), DM49(*lexA3*) (21), *sulA*::*lacZ′YA*::*kan* from strain SY2 (22), and *dinD1*::Mud(Amp^R, *lacZ′YA*) from strain JH39 (23). All strains used in UmuD studies additionally contained *umuD* on a low copy number plasmid (pRW362) (24). Plasmids pKD4, pKD46, and pCP20 (25) were used for creation of a *recX* deletion strain. Media for *E. coli* were prepared as described previously (17). All media and plasmids used for yeast two-hybrid analyses were from Invitrogen. Antibiotics were added at the following concentrations: ampicillin (Amp), 100 μg/ml; kanamycin (Kan), 15 μg/ml for chromosomal markers, or 40 μg/ml for plasmid markers; spectinomycin, 50 μg/ml; streptomycin, 50 μg/ml; erythromycin (Erm), 250 μg/ml; chloramphenicol, 100 μg/ml. Isopropyl β-D-thiogalactopyranoside (IPTG) (Diagnostic Chemicals Ltd.) was used at 1 or 1.5 mM, mitomycin C (MMC) (Sigma) was used at 1 or 0.2 μg/ml, and 5-bromo-4-chloro-3-indolyl-α-D-galactopyranoside (X-α-gal) (Clontech) was used at 20 μg/ml. ATP was from Amersham Biosciences.

DNA Manipulations and Analysis—Standard procedures were performed as described previously (26). Extraction of DNA from bacteria and agarose gels was performed using Qiagen kits. Enzymes were used according to manufacturers' directions (Promega, New England BioLabs). Sequencing reactions were performed using the Big-Dye Terminator cycle sequencing kit (PerkinElmer Life Sciences), and sequencing products were separated on an ABI model 377 automated DNA sequencer.

Yeast Two-hybrid Analysis—Yeast two-hybrid analysis was performed using the Matchmaker GAL4 two-hybrid system 3 as described (Clontech). *E. coli recA* was amplified from plasmid pERA by PCR using *Pfu* polymerase (Stratagene) with primers ECRECAFOR 5′-TTACATATGGCTATCGACGAAAACAAACAG-3′, which introduces an *Nde*I site (underlined), and ECRECAREV 5′-GACTTAAAAATCTTCGTTAGTTTCTGC-3′. *E. coli recX* was similarly amplified using primers ECRECXFOR 5′-TTACCATGGACATGACAGAATCAACATCCC-3′, which introduces a *Nco*I site (underlined), and ECRECXREV 5′-TTATCAGTCGGCAAAATTTCGCC-3′. The gel-purified PCR fragments were cloned into pCR-Blunt (Invitrogen) and sequenced. The genes were further subcloned into *Nde*I/*Bam*HI-digested (for *recA*) and *Nco*I-*Eco*RI-digested (for *recX*) vectors pGADT7 and pGBKT7 (Clontech), yielding constructs pGADT7-RecX, pGADT7-RecA, pGBKT7-RecX, and pGBKT7-RecA. Clones were sequenced to verify maintenance of proper reading frame, and protein expression of all relevant constructs in yeast was demonstrated by Western blot analysis (data not shown).

Construction of Plasmids pET/HisRecX, pGCC4/recX, and pGCC4/HisRecX—Plasmid construct pET/HisRecX was used to overexpress RecX as an N-terminal His-tagged protein. The *recX* coding region was amplified by PCR from plasmid pERA[2] using primers ECRXFORNHE, which introduces a *Nhe*I site (underlined) (5′-GTAGCTAGCATGACAGAATCAACATCC-3′), and ECRX1R (5′-GCTGGTAACTGAAAAGTGGG-3′) with *Pfu* polymerase. The gel-purified PCR product was ligated to pCR-Blunt (Invitrogen) to yield pCRHisRecX, and the resulting clone was sequenced to verify that no mutations had been introduced. The *recX* insert was isolated by *Nhe*I-*Hin*dIII digestion and ligated to *Nhe*I-*Hin*dIII-digested pET28a vector (Novagen) to yield construct pET/HisRecX.

The *E. coli recX* gene was cloned under control of the *lac* promoter in plasmid pGCC4, a high copy number plasmid with a ColE1 origin of replication (27). *recX* was amplified from plasmid pERA by PCR using primers RECXFORPAC, which introduces a *Pac*I site (underlined) (5′-GTAGGTTAATTAAGTTGTAAGGATATGCCA-3′), and RECXREV (5′-AGTCGCTAGCAATACCGTATGCGTTCAGTCG-3′) using *Pfu* polymerase (Stratagene). The fragment was digested with *Pac*I, gel-purified, ligated to *Pac*I-*Pme*I-digested pGCC4, and the resulting clone was sequenced. Plasmid pGCC4/HisRecX was created by ligating the blunt-ended *Xba*I/*Hin*dIII fragment of pET/HisRecX to *Pme*I-digested pGCC4. Expression of similar levels of His-tagged RecX (HisRecX) and RecX proteins from vector pGCC4 in strain AB1157Δ*recX* resulted in similar levels of UV resistance, demonstrating the activity of HisRecX *in vivo* (data not shown).

Protein Purification—HisRecX protein was overexpressed in *E. coli* BL21(DE3) (pET/HisRecX) cells. Cultures (500 ml) were grown at 37 °C to mid-exponential phase ($A_{600} \sim 0.4$), IPTG (1 mM) was added to induce expression of HisRecX, and the culture was grown an additional 1.5 h. Cells were harvested by centrifugation (10,000 × *g*, 10 min) and resuspended in 1/10 volume column binding buffer (5 mM imidazole, 0.5 M NaCl, 20 mM Tris-HCl (pH 7.9)) amended with 0.1% Triton X-100. Cells were disrupted by sonication on ice for three 1-min pulses, with 1-min cooling between pulses, with a Vibra-Cell probe sonicator at output setting 4, 50% duty cycle (Sonics & Materials, Inc.). Soluble proteins were separated from cell debris and insoluble proteins by centrifugation (14,000 × *g*, 20 min) and by subsequent passage through a 0.45-μm filter. Clarified supernatant was applied to a HisBind Quick column (Novagen) that had been equilibrated with 15 ml of column binding buffer, and the column was washed with 50 ml of column binding buffer and 25 ml of column wash buffer (60 mM imidazole, 0.5 M NaCl, 20 mM Tris-HCl (pH 7.9)). HisRecX protein was eluted from the column with 100 mM EDTA, 0.5 M NaCl, 20 mM Tris-HCl (pH 7.9), and fractions containing HisRecX were identified via SDS-PAGE. HisRecX-containing fractions were combined and dialyzed against three changes of a 200-fold excess of 20 mM Tris-HCl (pH 7.9) over 24 h at 4 °C and subsequently concentrated in a Centriprep-40 concentrator (Millipore). Protein concentration was determined by BCA assay (Pierce), and protein purity was assessed to be >98% by quantitative scanning of Coomassie Blue-stained SDS-PAGE gels. For protein used in activity assays, glycerol was added to 20% final concentration and protein was flash-frozen on dry ice/EtOH. RecA and SSB proteins were prepared as described previously (28).

DNA Strand Exchange Assay—The agarose gel assay for DNA strand exchange was conducted and visualized as described (29). Reactions contained 25 mM Tris acetate (pH 7.5), 10 mM magnesium acetate, 0.1 mM dithiothreitol, 2 mM phosphoenolpyruvate, 10 units of pyruvate kinase per milliliter, 7 μM M13 ssDNA, 1 mM ATP, 0.64 μM SSB, 4.2 μM RecA, and 10 μM M13 replicative form dsDNA linearized with *Eco*RI, and varying concentrations of RecX.

ATP Hydrolysis Assay—The ssDNA-dependent hydrolysis of ATP by RecA was measured as described using a coupled enzymatic assay (30). Reactions contained 7 μM M13 ssDNA, 4.2 μM RecA, 0.64 μM SSB (where indicated), and varying amounts of RecX. RecA protein was added to M13 circular ssDNA either before RecX or following 10-min incubation of RecX with M13 circular ssDNA. When SSB was included, it was added 1 min after the addition of RecA.

Insertional Inactivation of recX—We created a 368-bp non-polar internal deletion of *recX* using the method described by Datsenko and Wanner (25). Gene deletions using this system are engineered to introduce stop codons in all six reading frames and an idealized ribosome binding site and start codon for downstream gene expression at the site of gene deletion. The gene disruptions created using this system have been shown to be non-polar (25). A PCR product was generated from plasmid pKD4 (25) using primers H15 (5′-AATCAACATCCCGTCGCCCGGCATATGCTCGCCTGTTGGATCGTGTGTAGGCTGGAGCTGCTTC-3′) and H23 (5′-GCAAAATTTCGCCAAATCTCCTGGATATCTTCCATCAGATAGCCCATATGAATATCCTCCTTAG-3′). This PCR product contains a *kan* marker flanked by short regions of homology to the *recX* gene at the 5′- and 3′-ends (underlined in primer sequences). We transformed strain AB1157 (pKD46) with this PCR product and selected for Kan^R colonies to identify insertions into *recX*, yielding strain AB1157Δ*recX*::*kan*. PCR analysis of Kan^R colonies using primer pairs ECRX1R (5′-GCTGGTAACTGAAAAGTGGG-3′) and ECRX2F (5′-AGGCGTAGCAGAAACTAACG-3′), just outside the *recX* coding region, and *kan*-specific primers k1 and k2 (25) confirmed the location and insertion of the *kan* gene (data not shown). The subsequent eviction of the *kan* gene from strain AB1157Δ*recX*::*kan*, using a curable helper plasmid encoding the FLP recombinase (pCP20), yielding strain AB1157Δ*recX*, was verified by PCR (data not shown).

UV Resistance Assays—Overnight cultures were diluted into LB or LB-Erm (1.5 mM IPTG) and grown to early stationary phase (A_{600} = 1.2), serially diluted, and spot-plated onto the appropriate LB agar. Plates were exposed to 0, 2.5, 5.0, 7.5, and 10 J/m^2 UV light (UV Stratalinker 1800, Stratagene).

P1 Phage Transduction—Overnight cultures were diluted into LB or LB-Erm containing 5.0 mM $CaCl_2$ and grown until late log phase (A_{600} = 0.8). IPTG (1.5 mM) was added to cultures containing plasmids, and cultures were grown for an additional 30 min. P1 transduction was performed as described (17) using phage grown on Hfr Cavalli.

LexA and UmuD Cleavage Assays—For LexA cleavage assays, overnight cultures were diluted into LB or LB-Erm (1.5 mM IPTG) and grown to A_{600} = 0.4, and cultures were supplemented with chloramphenicol (100 μg/ml) and incubated for an additional 10 min. For cultures containing plasmids pGCC4 and pGCC4/*recX*, aliquots were taken (t_0), the remainder of the culture was exposed to 8 J/m^2 UV irradiation, and additional aliquots were taken from the irradiated samples after 5, 15, 30, 60, and 90 min. For strains AB1157 and

[2] R. D. Porter, unpublished data.

AB1157Δ*recX*, t_0 aliquots were taken, both cultures were exposed to 4 J/m^2, and additional aliquots were taken after 2, 4, 6, 8, 10, 12, 15, 20, and 30 min. Protein concentration of samples was determined by BCA assay (Pierce).

For UmuD cleavage assays, overnight cultures were diluted into LB-Spc or LB-Erm-Spc (1.5 mM IPTG). For this analysis, all strains additionally contained plasmid pRW362 (*umuD*) to facilitate UmuD detection. Cells were grown to A_{600} = 0.4, a t_0 aliquot was taken, and MMC was added (0.2 μg/ml). Aliquots were taken after 80, 120, 160, 200, and 240 min. Protein concentration of samples was determined by BCA assay (Pierce).

Western Analysis—Western analysis and protein quantification was performed as described (13), except for exceptions noted. Samples were run on 15% SDS-PAGE gels (for RecX and LexA), 17% (for UmuD), or 12% (for RecA and all constructs in yeast), and subsequently developed using ECL or ECL Plus Western blotting protocols (Amersham Biosciences). Antibodies against HisRecX, raised in a rabbit using the Polyquik method (Zymed Laboratories), were used at a 1:600 dilution. Anti-LexA antibodies (Invitrogen), anti-UmuD antibodies (provided by R. Woodgate, National Institutes of Health, Bethesda, MD), anti-RecA antibodies (provided by M. Cox, University of Wisconsin-Madison), anti-c-myc and anti-HA antibodies (Roche Molecular Biochemicals) were used at 1:5,000 dilutions. Secondary goat anti-rabbit IgG, goat anti-mouse IgG, and rabbit anti-rat IgG antibodies conjugated to horseradish peroxidase were used at 1:5,000 to 1:15,000 dilutions (Roche Molecular Biochemicals). Membranes were either exposed to Kodak film or detected using a ChemiImager (Alpha Innotech Corp.). Subsequent densitometric analyses were performed using ImageQuaNT (Amersham Biosciences) or Alpha Ease (Alpha Innotech) software, respectively.

RESULTS

RecX and RecA Physically Interact—In several bacterial systems, overexpression of RecA proteins from plasmid constructs is deleterious in the absence of *recX* (9, 11, 14, 15), suggesting that RecX may down-regulate RecA activity or expression, possibly by interacting with the RecA protein. To investigate the direct association of *E. coli* RecX and RecA proteins, a yeast two-hybrid analysis was performed. Both *recA* and *recX* genes were cloned into vectors pGBKT7 (*TRP1* marker) and pGADT7 (*LEU2* marker), yielding constructs pGBKT7-RecA, pGBKT7-RecX, pGADT7-RecA, and pGADT7-RecX that express fusion proteins with Gal4 DNA binding or activation domains (described under "Experimental Procedures"). The yeast two-hybrid reporter strain AH109 contains *ADE2*, *HIS3*, and *MEL1* reporter genes that are expressed only when a functional Gal4 protein is reconstituted by an interaction between the activation domain and the DNA binding domain fusion proteins. AH109 cells carrying plasmid pairs pGBKT7-RecA/pGADT7-RecX or pGBKT7-RecX/pGADT7-RecA grew robustly on media lacking histidine, tryptophan, and leucine, and showed a blue color on media supplemented with X-α-gal, indicating both *MEL1* and *HIS3* reporter gene expression in these cells (Fig. 1 and data not shown). However, cells grew less well on media lacking adenine, histidine, tryptophan, and leucine, suggesting lower expression of the *ADE2* reporter gene, which demands strong protein-protein interactions for its expression (data not shown). AH109 cells carrying control plasmids with interacting gene products (pGADT7-T and pGBKT7–53) grew on media lacking histidine, tryptophan, and leucine, but AH109 carrying control plasmids with non-interacting gene products (pGADT7-T and pGBKT7-Lam), or AH109 co-transformed with the *recA* or *recX* plasmids and either pGADT7-T or pGBKT7-Lam plasmids did not grow on the selective media (Fig. 1 and data not shown). Together these data demonstrate a specific interaction of *E. coli* RecA and RecX proteins in yeast.

RecX Inhibits RecA Protein-promoted DNA Strand Exchange in Vitro—To investigate the functional significance of RecX interaction with RecA, we measured the effect of RecX on RecA recombinase activity *in vitro*. RecA-promoted DNA strand exchange was measured in the presence or absence of RecX. RecX completely abolished formation of joint molecules over a 60-min time course between homologous circular ssDNA and linear dsDNA molecules at a RecX:RecA molar ratio of 1:2.2 (Fig. 2). In the absence of RecA, RecX neither catalyzed the formation of joint molecules nor degraded DNA (data not shown), demonstrating that the effect of RecX on RecA activity is not simply due to degradation of DNA substrates. Titrating the level of RecX protein in the strand exchange reaction, complete inhibition of joint molecule formation was observed at a RecX:RecA molar ratio of 1:44, minimal joint molecule formation was seen at a 1:88 ratio, and resolution into a nicked circular form was never observed, even at a RecX:RecA molar ratio of 1:707 (Fig. 2). These data suggest that sub-stoichiometric levels of RecX inhibit both the initial pairing of homologous molecules, and, to a greater degree, subsequent branch migration.

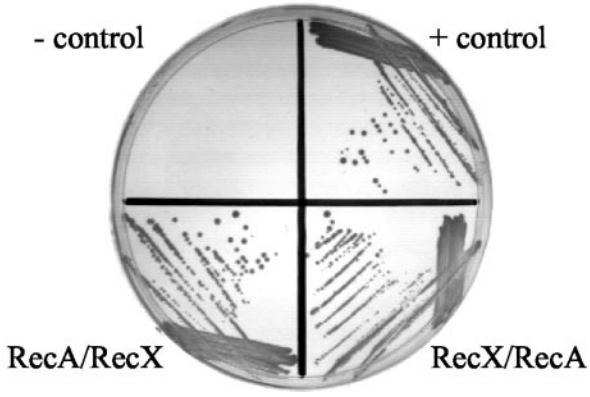

FIG. 1. **Yeast two-hybrid analysis of RecX and RecA interaction.** Yeast AH109 cells containing various constructs of pGBT7 and pGADT7 grown at 30 °C on complete synthetic media lacking tryptophan, leucine, and histidine. Each quadrant contains yeast cells streaked from a single transformant. *Top half of plate*, *left* to *right* are the negative (pGADT7-T/pGBKT7-Lam) and positive (pGADT7-T/pGBKT7–53) controls; *bottom half*, *left* to *right* are pGADT7-RecA/pGBKT7-RecX and pGADT7-RecX/pGBKT7-RecA.

RecX Inhibits RecA ssDNA-dependent ATPase Activity—Formation of a RecA-ssDNA-ATP nucleoprotein filament is accompanied by the subsequent hydrolysis of ATP by RecA (ATPase activity) and reflects the amount of active nucleoprotein filament formed. We therefore assessed the effect of RecX on RecA ATPase activity in the presence or absence of SSB, preincubating RecX with ssDNA before the addition of RecA. In the absence of SSB, RecX decreased ATPase activity 20% when present at a 1:70 RecX:RecA molar ratio (60 nM RecX), 50% when present at a 1:14 RecX:RecA molar ratio (300 nM RecX), and 85% when present at a 1:8 molar ratio (500 nM RecX) (Fig. 3). In the presence of SSB, the effects of RecX were more dramatic, with ATPase activity nearly completely abolished (decreased 98%) by a RecX:RecA molar ratio of 1:70, approximately the level where minimal joint molecule formation was seen in the *in vitro* strand exchange reaction. We observed the same effect of RecX on ATPase activity when RecX was added to the reaction after preincubation of RecA and ssDNA (data not shown). Therefore, the inhibitory effect of RecX is enhanced in the presence of SSB but is independent of the time of RecX addition to the reaction.

RecX Is Part of the SOS Regulon—The strong inhibitory effect of RecX on RecA activity *in vitro* suggested that RecX could have dramatic effects modulating RecA activities *in vivo* as well. Therefore, we began to characterize the *E. coli recX* gene to determine its role in the bacterial cell. In *E. coli*, *recX* is directly preceded by neither a canonical promoter sequence nor an SOS box, but it is located 76 bp downstream of the

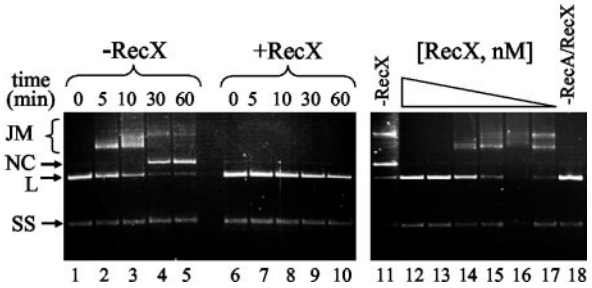

FIG. 2. **Effect of RecX on RecA-promoted DNA strand exchange.** Position of bands corresponding to ssDNA (*SS*), linearized dsDNA (*L*), nicked circular dsDNA (*NC*), and joint molecules (*JM*), are indicated. Time course of RecA-promoted DNA strand exchange in absence (*lanes 1–5*, RecX:RecA molar ratio 1:2.2) or presence (*lanes 6–10*) of RecX and titration of RecX inhibition of RecA-promoted DNA strand exchange over 30 min (*lanes 11–18*). Control *lanes 11* and *18* contain no RecX, no RecA or RecX, respectively. Molar ratios of RecX:RecA for *lanes 12–17* are as follows: 1:22, 1:44, 1:88, 1:177, 1:354, and 1:707.

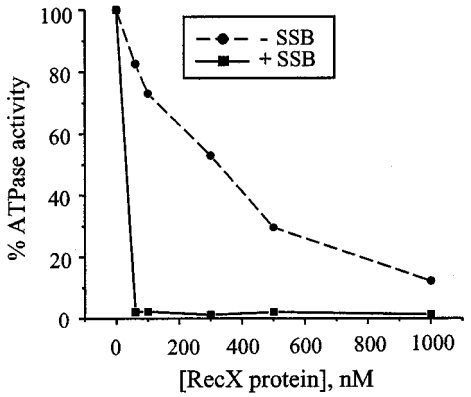

FIG. 3. **Effect of RecX on ssDNA-dependent ATP hydrolysis by RecA protein in the presence and absence of SSB.** Rates of hydrolysis are expressed as the percentage of control reactions without RecX. Molar ratios of RecX:RecA are as follows: 1:70 (60 nM RecX), 1:42 (100 nM RecX), 1:14 (300 nM RecX), 1:8 (500 nM RecX), and 1:4 (1000 nM RecX).

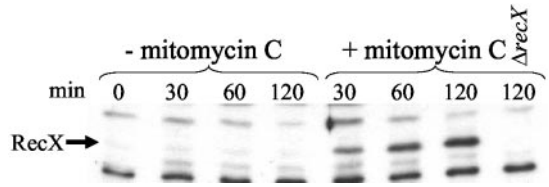

FIG. 4. **Western blot time course of RecX expression.** Cells were grown to A_{600} = 0.4 (min 0), and basal or MMC (1 μg/ml)-induced RecX expression was monitored over time in strain AB1157. AB1157Δ*recX* is included as a negative control. 10 μg of total protein was loaded per lane, blotted, and developed using polyclonal anti-RecX antisera.

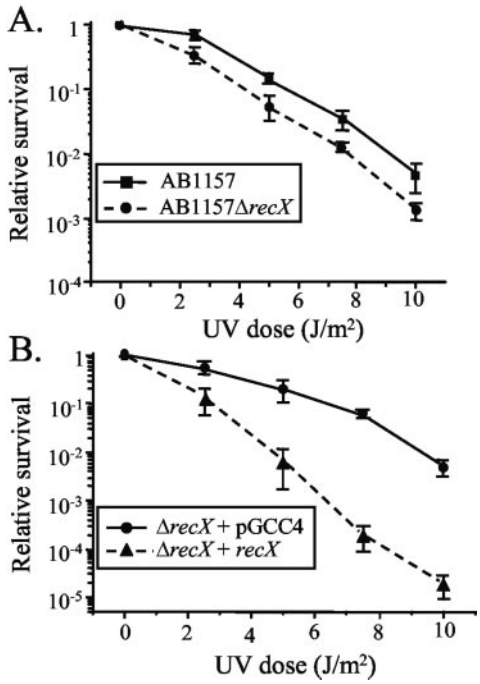

FIG. 5. **UV resistance of Δ*recX* mutant or overexpression strains.** *A*, relative survival of AB1157 and Δ*recX* mutant. *B*, AB1157Δ*recX* carrying pGCC4 (vector) or pGCC4/*recX*. IPTG (1.5 mM) was added to both cultures. *Error bars* represent the standard error of the mean of at least two independent experiments done in duplicate. Differences between strains AB1157 and Δ*recX* are statistically significant at all UV doses (excluding 10 J) at $p < 0.01$ by the Student's *t* test. Differences between strain AB1157Δ*recX* carrying pGCC4 or pGCC4/*recX* are statistically significant at all UV doses at $p < 0.05$ by the Student's *t* test.

SOS-regulated *recA* gene, suggesting that *recX* could be co-transcribed with *recA* and, therefore, under control of LexA. Western blot analysis revealed expression of a ~19-kDa band barely detectable in strain AB1157 that increased in intensity after treatment with mitomycin C (MMC) (Fig. 4) or exposure to UV light (data not shown). This band was undetectable in the MMC-treated Δ*recX* mutant, demonstrating that *recX* is induced upon DNA damage (Fig. 4). Robust expression of the ~19-kDa RecX band was detected in strain DE192, a *lexA*51(Def) strain that does not produce a functional LexA repressor, but not in the isogenic strain DE192Δ*recX* (data not shown). Moreover, RecX levels did not increase after addition of MMC to cultures of DM49(*lexA3*), which has a non-cleavable LexA repressor (data not shown). Finally, RecA and RecX proteins showed identical patterns of induction after UV treatment (data not shown), as was also found with *recA* and *recX* transcripts (31), and we demonstrated that *recA* and *recX* are present on the same transcript by RT-PCR (data not shown). Together these data demonstrate that *recX* is induced with *recA* in a LexA-dependent manner and is, therefore, an SOS response gene.

RecX Alters UV Resistance—Because *recX* was induced upon DNA damage, suggesting a possible role in DNA repair, we tested the effect of the Δ*recX* mutation on UV resistance in strain AB1157. Strain AB1157Δ*recX* showed a small, but statistically significant, decrease in UV resistance relative to AB1157 (Fig. 5*A*). Because the Δ*recX* mutation is non-polar (see "Experimental Procedures"), this phenotype is due to *recX* inactivation. To test this assumption, a functional copy of *recX* was introduced *in trans* (pGCC4/*recX*), with *recX* under control of *lac* regulatory elements, into strain AB1157Δ*recX*. Surprisingly, this plasmid conferred a striking reduction in UV resistance relative to strain AB1157Δ*recX* carrying pGCC4 alone (Fig. 5*B*). Titrating the amount of IPTG in the growth medium, we observed that increasing levels of IPTG induction resulted in decreased UV resistance, suggesting that the amount of RecX protein produced was affecting UV resistance (data not shown).

The amount of RecX protein present in various bacterial cells was determined in a semi-quantitative immunoblotting analysis. Serial dilutions of purified RecX protein were used as a standard for comparison against cell extracts made from a known quantity of bacterial cells. We estimated the basal number of RecX molecules in AB1157 to an average of <50 molecules per cell. After SOS induction of AB1157 with MMC (2 h), these averaged 800 molecules of RecX per cell. Cells carrying pGCC4/*recX* (induced with IPTG) averaged 10^5 RecX molecules

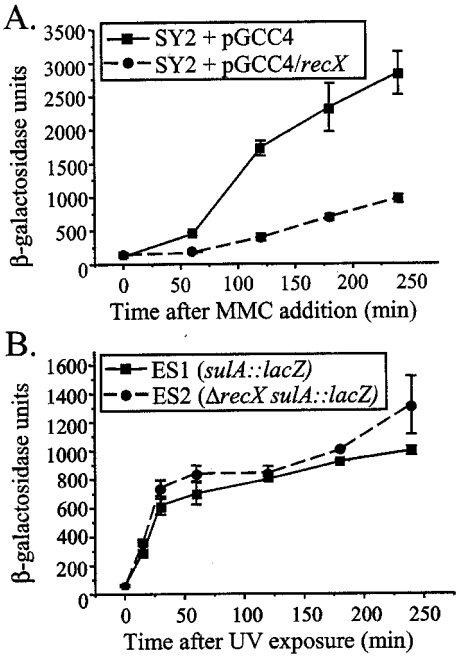

FIG. 6. ***sulA*::*lacZ* induction in *recX* mutant or overexpression strain.** *A*, strain SY2 (*sulA*::*lacZ′YA*::*kan*) carrying pGCC4 (vector) or pGCC4/*recX* was grown in LB-Erm (1.5 mM IPTG) to $A_{600} \sim 0.2$, MMC (0.2 μg/ml) was added, and samples were taken and assayed for β-galactosidase activity (17). *Error bars* represent the mean of at least two independent experiments done in duplicate. Differences between strains are statistically significant at all time points at $p < 0.01$ by the Student's *t* test. *B*, strains ES1 and ES2 (Δ*recX*), containing a chromosomal *sulA*::*lacZ* fusion, were grown in LB with 0.1% glucose to $A_{600} \sim 0.4$ and exposed to 4 J/m^2 UV light, and samples were taken and assayed for β-galactosidase activity. *Error bars* represent the mean of three independent experiments.

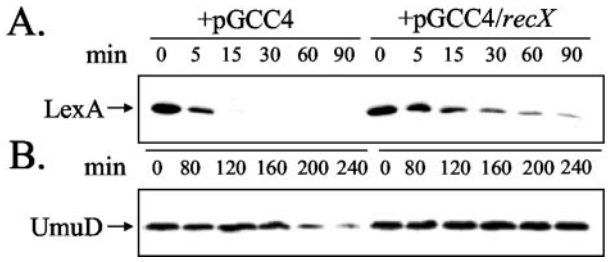

FIG. 7. **LexA and UmuD processing in RecX overexpression strains.** *A*, representative Western blot of LexA levels in strain AB1157Δ*recX* carrying pGCC4 (vector) or pGCC4/*recX* (both with 1.5 mM IPTG) at times indicated after exposure to 8 J/m^2 UV irradiation. 6 μg of total protein per lane was loaded for subsequent Western blot analysis using anti-LexA antisera. *B*, representative Western blot of UmuD levels in strain DE192Δ*recX* carrying pRW362 (*umuD*) and either pGCC4 or pGCC4/*recX* (both with 1.5 mM IPTG) at times indicated after addition of MMC (0.2 μg/ml). 60 μg of total protein was loaded per lane for subsequent Western blot analysis using anti-UmuD antisera.

per cell, whereas cells carrying pGCC4/*recX* (uninduced with IPTG) averaged 10^3 RecX molecules (data not shown). These results indicate that both overexpression and loss of RecX decrease UV resistance.

RecX Overexpression Inhibits Induction of the SOS Response—To determine whether RecX overexpression alters UV resistance by inhibiting induction of the SOS response, bacterial strains that contain SOS-regulated chromosomal fusions of either *sulA*::*lacZ* or *dinD1*::*lacZ* were transformed with either pGCC4 or pGCC4/*recX*, and gene expression was measured by quantifying β-galactosidase activity. After addition of MMC, cells carrying pGCC4/*recX* showed statistically significant lower induction of both *sulA* (3- to 4-fold; Fig. 6*A*) and *dinD1* (data not shown) relative to those carrying the vector alone. Therefore, overexpression of RecX inhibits induction of the SOS response. To determine whether a Δ*recX* strain would show increased SOS induction, the *sulA*::*lacZ* fusion was transduced into strains AB1157 and AB1157Δ*recX* to yield strains ES1 and ES2, respectively. After incubation with MMC (data not shown), or exposure to UV light (Fig. 6*B*), strain ES2 (Δ*recX*) showed the same increase in *sulA* expression as strain ES1. These results suggest that the chromosomal *recX* does not affect SOS induction.

The observed inhibition of SOS induction by RecX overexpression could be due to effects on either RecA activity or RecA levels. Although our *in vitro* experiments suggested that RecX affects RecA activity, we wanted to additionally test this *in vivo*. Western blot analyses showed no difference in basal RecA protein levels between strains AB1157 and AB1157Δ*recX* (data not shown). Strain AB1157Δ*recX* carrying pGCC4/*recX* (induced with IPTG) showed about 10% less RecA than cells carrying pGCC4 (data not shown), but these differences are too small to account for the large effect on SOS induction and UV resistance and suggest that the inhibitory effects of RecX are largely due to effects on RecA activity. Further experimental support for a direct and specific effect of RecX on RecA activity *in vivo* came from UV resistance studies in a *recA* deletion strain. The UV resistance of strain AB1157Δ*recA* carrying plasmids pGCC4 or pGCC4/*recX* (induced with IPTG) was the same (data not shown). Together these data strongly suggest that the phenotypes observed in cells overexpressing RecX are due to effects on RecA activity, not RecA levels. Moreover, these effects appear to be specifically mediated through RecA, an observation that is further supported by the interaction of RecX and RecA in a yeast two-hybrid assay, and are not simply due to some artifact of RecX protein overexpression.

RecX Overexpression Inhibits LexA and UmuD Cleavage—Because RecX has only minor effects on basal RecA protein levels, we tested the hypothesis that RecX overexpression inhibits SOS induction by inhibiting RecA coprotease activity. SOS induction requires activated RecA to function as a coprotease to facilitate LexA self-cleavage, thereby derepressing genes of the SOS regulon. RecA coprotease activity is also responsible for the cleavage that converts UmuD to UmuD′, the activated form of the protein that is involved in SOS-induced mutagenesis. We used Western blot analysis to monitor degradation of the LexA repressor protein after exposure to UV light under conditions where *de novo* protein synthesis was inhibited by chloramphenicol. In cells carrying pGCC4/*recX*, LexA was detected 90 min after UV exposure (Fig. 7*A*), whereas LexA was completely degraded in cells carrying the pGCC4 vector after 30 min. In contrast, there were no differences in the extent or rate of cleavage of LexA in strains AB1157 and AB1157Δ*recX* (data not shown).

The effect of *recX* on UmuD cleavage was measured using derivatives of strain DE192 (see "Experimental Procedures"), which carries a *lexA51* (Def) mutation resulting in constitutive expression of LexA-regulated genes. In these strains, DNA damage triggers cleavage of UmuD. We observed decreased cleavage of UmuD after 200-min treatment with MMC in cells carrying pGCC4/*recX* relative to cells carrying the vector alone (Fig. 7*B*). No differences in UmuD cleavage were observed between strains DE192 and DE192Δ*recX* (data not shown). Taken together, these results demonstrate that RecX overexpression inhibits the coprotease activity of RecA; however, the chromosomal *recX* does not have a measurable effect on coprotease activity *in vivo*.

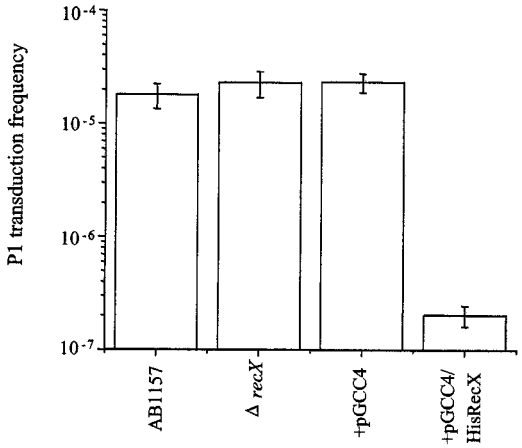

FIG. 8. **P1 transduction frequency of Δ*recX* or HisRecX overexpression strains.** Generalized transduction frequency of proline prototrophic marker in strains AB1157, AB1157Δ*recX*, and AB1157Δ*recX* carrying plasmids pGCC4 or pGCC4/HisRecX. *Error bars* represent the mean of three experiments done in triplicate (AB1157 and Δ*recX*) or the mean of two experiments done in duplicate (+pGCC4 and +pGCC4/HisRecX).

RecX Overexpression Inhibits P1 Transduction—Because we had observed inhibition of RecA-mediated DNA strand exchange by substoichiometric quantities of RecX *in vitro*, we wanted to test the effect of RecX on RecA recombinase activity *in vivo*. The effect of RecX on RecA-mediated homologous recombination was quantified *in vivo* with P1 transduction assays. The P1 transduction frequency of proline and leucine prototrophic markers was the same in strains AB1157 and AB1157Δ*recX* (Fig. 8; data not shown). However, the P1 transduction frequency of both markers was significantly reduced in AB1157Δ*recX* cells carrying plasmids pGCC4/HisRecX (>100-fold reduction) (Fig. 8; data not shown) or pGCC4/*recX* (data not shown) relative to those carrying the pGCC4 vector (Fig. 8; data not shown). Strain AB1157Δ*recX* carrying pGCC4/*recX* also showed decreased Hfr conjugation relative to the pGCC4 vector control strain (data not shown). These results indicate that RecX can inhibit RecA recombinase activity *in vivo* and support our observations that RecX inhibits DNA strand exchange *in vitro*.

DISCUSSION

The results presented in this study show that *E. coli* RecX can modulate RecA activities through direct physical interaction with RecA. *In vitro* studies established that RecX inhibits RecA recombinase and ATPase activities at substoichiometric levels and suggest mechanistic bases for this inhibition. *In vivo* studies where RecX was overexpressed corroborated these results, showing that RecX overexpression strongly inhibits RecA recombinase as well as RecA coprotease activities.

The inhibition of RecA ATPase and recombinase activities *in vitro* by substoichiometric amounts of RecX protein, coupled with the interaction of RecX and RecA in a yeast two-hybrid assay, suggest several mechanisms for RecX inhibition of RecA activities *in vitro*. Both RecA coprotease and recombinase activities require the formation of a RecA-ssDNA-ATP nucleoprotein filament. Therefore, affecting either the formation or integrity of the nucleoprotein filament will affect both activities of RecA. In gel-shift assays, we observed RecX binding to 100-mer ssDNA and dsDNA only at RecX concentrations of >1 μM (data not shown), a level too high to account for the observed inhibition of strand exchange. Therefore, we do not favor the models that RecX binds to ssDNA, thereby disrupting or impeding formation of the nucleoprotein filament, or that RecX binds to dsDNA, blocking homologous DNA exchange. During homologous recombination, dsDNA is believed to lie within the deep helical groove of the RecA nucleoprotein filament (32). The LexA repressor protein may also bind within this groove (33) and, possibly, the $UmuD_2'C$ complex (34) and UmuD protein (35). Therefore, a second model is that RecX interacts preferentially with RecA within the deep helical groove, thereby blocking access to or displacing the above substrates, as is believed to occur with the DinI protein (6). Although this model suggests how RecX could inhibit both RecA coprotease and recombinase activities through direct interaction with RecA, it accounts for neither the observed inhibition of RecA ATPase activity by RecX nor the effect of SSB on the reaction. Thus, a favored model is that RecX binds to RecA and diminishes the ability of RecA to bind ssDNA or ATP. The interaction of RecA and RecX may occur with either free RecA protein or RecA within the nucleoprotein filament. The nearly complete inhibition (>98%) of RecA ATPase activity by RecX in the presence of SSB, compared with the less dramatic inhibition (~20%) in the absence of SSB, suggests a role for SSB in the interaction of RecA and RecX as well. Similar to what was proposed to occur with the uncleavable LexA repressor protein (36), the interaction of RecX and RecA may disrupt the equilibrium of RecA and SSB binding to ssDNA, favoring the binding of SSB to ssDNA and resulting in collapse of the nucleoprotein filament and inhibition of ATPase activity. The observed effect of RecX on RecA recombinase activity *in vitro* could be due to the subsequent creation of gaps in the RecA-ssDNA-ATP nucleoprotein filament, blocking branch migration in particular, where a RecX:RecA molar ratio of 1:707 inhibited resolution of intermediates into a nicked-circular form (Fig. 2) but having a much smaller effect on nascent joint molecule formation, where a RecX:RecA molar ratio of 1:44 was required for inhibition (Fig. 2). RecX-mediated collapse of the nucleoprotein filament can also explain the decreased cleavage of LexA and UmuD proteins and the decreased P1 transduction frequency observed *in vivo* during RecX overexpression. The profound decrease in UV resistance when RecX was overexpressed could be due to a combination of suppression of SOS induction, through inhibition of RecA coprotease activity, and inhibition of recombinational DNA repair, both a result of nucleoprotein filament collapse.

E. coli RecX is a potent inhibitor of RecA activity *in vitro* when supplied at substoichiometric levels and *in vivo* when overexpressed, but the only phenotype observed in the Δ*recX* mutant was a small decrease in UV resistance. Therefore, one hypothesis is that RecX functions in DNA repair. Accordingly, we observed no differences in recombination ability, as measured by P1 transduction or Hfr conjugation (Fig. 8 and data not shown) between the Δ*recX* mutant and the parent strain. This is probably due to the exceedingly low basal levels of RecX, less than 50 molecules per cell. Using semi-quantitative immunoblotting, we calculated the basal level of RecA molecules to be ~15,000 molecules per cell (data not shown), which is consistent with previous reports (37, 38). Therefore, although RecX can completely abolish pairing of homologous DNA molecules (joint molecule formation) *in vitro* at a RecX:RecA molar ratio of 1:44, the RecX:RecA molar ratio inside the bacterial cell is significantly below this, at most 1:300. DNA damage resulted in increased levels of *recX* transcript in *E. coli* and other bacteria (11, 12, 15, 31, 39). In our studies, after treating *E. coli* with MMC, RecX and RecA protein levels increased to ~800 and 100,000 molecules per cell, respectively (data not shown), so the RecX:RecA ratio is 1:125, which is closer to the level where we saw inhibition of joint molecule formation *in vitro*. Therefore we propose that the biological role of RecX is manifest during the SOS response. It is possible that some threshold

level of RecX is reached or that additional factors influence the ability of RecX and RecA to interact under these circumstances, resulting in the observed phenotype of decreased UV resistance. In support of this hypothesis, the DinI protein, which also modulates RecA activity, showed increased affinity for the RecA protein *in vivo* at later stages of the SOS response (40). Moreover, because *recX* is directly downstream of and co-transcribed with *recA*, RecX and RecA are likely to be translated in the same region of the *E. coli* cell, allowing the local intracellular concentration of RecX to be higher, possibly driving interaction with the RecA protein.

An alternate role for RecX is suggested by studies in other bacteria. An *recX* mutant of *N. gonorrhoeae* was decreased in the RecA-mediated processes of DNA repair, pilus antigenic variation, and DNA transformation, suggesting that RecX may enhance RecA activity in this organism (13). Interestingly, *N. gonorrhoeae* is one of the few bacteria where *recX* is not found near *recA* (13), and *N. gonorrhoeae* lacks an SOS response (41). In *X. oryzae*, where *recX* is located downstream of *recA*, a *recX* mutant showed a 50% decrease in RecA levels relative to the parent strain (15). Finally, investigations of the *E. coli* RecX and RecA proteins heterologously expressed in *N. gonorrhoeae* suggested that RecX (or *recX*) may either stabilize the RecA protein or *recA* transcript when present at low levels (19). Therefore, RecX may either have multiple activities in a particular bacterial species, or RecX may show variable activity between species.

The RecX protein from *M. tuberculosis* (MtRecX) was recently characterized *in vitro* and was found to have profound inhibitory effects on RecA activity at substoichiometric levels and to interact with RecA (16). The MtRecX was also able to inhibit EcRecA activity *in vitro*, although not as efficiently as it did MtRecA activity (16). In combination with our current study, this work suggests that the *M. tuberculosis* and *E. coli* RecX proteins are homologues. However, the relative activity of MtRecX may differ slightly from that of *E. coli* RecX (EcRecX). Whereas we observed complete inhibition (>98% decrease) of ATPase activity at an EcRecX:EcRecA molar ratio of 1:70, ATPase activity was decreased 86% at a MtRecX:MtRecA molar ratio of 1:1.6 (16). The authors speculate that the biological role of MtRecX is to quell inappropriate recombinational repair during normal DNA metabolism; however, a *recX* mutant has not been generated in *M. tuberculosis*, so the function of MtRecX *in vivo* remains untested (16).

Although the exact biological role of RecX in any bacterium remains unclear, the importance of RecX for RecA activity is underscored by its conserved location with *recA* in myriad bacterial genomes (8–12, 15). Because *E. coli* RecX has potent inhibitory effects on RecA activities both *in vitro* and *in vivo*, and because RecX is up-regulated during the SOS response in many bacteria (11, 12, 15, 31, 39), this suggests that RecX has some regulatory role during the SOS response, which has yet to be elucidated. The identification of other proteins that modulate RecA activities emphasizes the biological importance of regulating both the SOS response and homologous recombination. *psiB*, which is found on many conjugative plasmids near *oriT*, the origin of conjugative transfer, possesses anti-SOS functions and anti-recombinase activities (42, 43). The location of *psiB* hints at its hypothesized function: to prevent ssDNA that is transferred upon conjugation from inducing the SOS response (44). Although the gene has not been identified, the *isfA* mutation of *E. coli* also has been found to suppress RecA coprotease-dependent cleavage of UmuD (45). Finally, overexpression of the DinI protein of *E. coli* inhibits both the coprotease and recombinase activities of RecA *in vivo* (5). A *dinI* mutant showed no decrease in UV resistance but exhibited increased cleavage of UmuD and higher SOS mutagenesis than the parent strain, suggesting that DinI may act specifically to down-regulate SOS mutagenesis (5). DinI also inhibits RecA activity *in vitro* but only when present in vast molar excess (17- to 30-fold) of RecA (5, 40). In contrast, *E. coli* RecX:RecA molar ratios of 1:44 and 1:70, respectively, were sufficient for complete inhibition of RecA-promoted DNA strand exchange and ATPase activity. Thus, RecX appears to be a stronger inhibitor of RecA activity than DinI *in vitro*. It is likely that a complicated network of interactions between the RecX, DinI, LexA, UmuD, SSB, RecA, and possibly other unidentified proteins, acts to regulate the RecA nucleoprotein filament and RecA function.

Acknowledgments—We thank A. Criss, K. Kline, and D. Tobiason for editorial suggestions for the manuscript. We also thank M. Cox for providing antibodies, P. Model and H. Ohmori for providing strains, and R. Woodgate for generously providing antibodies, strains, and technical assistance.

REFERENCES

1. Kowalczykowski, S. C., Dixon, D. A., Eggleston, A. K., Lauder, S. D., and Rehrauer, W. M. (1994) *Microbiol. Rev.* **94,** 401–465
2. Roca, A. I., and Cox, M. M. (1990) *Crit. Rev. Biochem. Mol. Biol.* **25,** 415–456
3. Walker, G. C. (1996) in *Escherichia coli and Salmonella: Cellular and Molecular Biology* (Neidhardt, F. C., Curtiss, R., III, Ingraham, J. L., Lin, E. C. C., Low, K. B., Magasamik, B., Reznikoff, W. S., Riley, M., Schaechter, M., and Umbarger, H. E., eds) pp. 1400–1416, American Society for Microbiology Press, Washington, D. C.
4. Fernandez de Henestrosa, A. R., Ogi, T., Aoyagi, S., Chafin, D., Hayes, J. J., Ohmori, H., and Woodgate, R. (2000) *Mol. Microbiol.* **35,** 1560–1572
5. Yasuda, T., Morimatsu, K., Horii, T., Nagata, T., and Ohmori, H. (1998) *EMBO J.* **17,** 3207–3216
6. Yasuda, T., Morimatsu, K., Kato, R., Usukura, J., Takahashi, M., and Ohmori, H. (2001) *EMBO J.* **20,** 1192–1202
7. Zaitsev, E., Alexseyev, A., Lanzov, V., Satin, L., and Clark, A. J. (1994) *Mutat. Res.* **323,** 173–177
8. De Mot, R., Schoofs, G., and Vanderleyden, J. (1994) *Nucleic Acids Res.* **22,** 1313–1314
9. Sano, Y. (1993) *J. Bacteriol.* **175,** 2451–2454
10. Papavinasasundaram, K. G., Movahedzadeh, F., Keer, J. T., Stoker, N. G., Colston, M. J., and Davis, E. O. (1997) *Mol. Microbiol.* **24,** 141–153
11. Vierling, S., Weber, T., Wohlleben, W., and Muth, G. (2000) *J. Bacteriol.* **182,** 4005–4011
12. Yang, M. K., Chou, M. E., and Yang, Y. C. (2001) *Curr. Microbiol.* **42,** 257–263
13. Stohl, E. A., and Seifert, H. S. (2001) *Mol. Microbiol.* **40,** 1301–1310
14. Papavinasasundaram, K. G., Colston, M. J., and Davis, E. O. (1998) *Mol. Microbiol.* **30,** 525–534
15. Sukchawalit, R., Vattanaviboon, P., Utamapongchai, S., Vaughn, G., and Mongkolsuk, S. (2001) *FEMS Microbiol. Lett.* **205,** 83–89
16. Venkatesh, R., Ganesh, N., Guhan, N., Reddy, M. S., Chandrasekhar, T., and Muniyappa, K. (2002) *Proc. Natl. Acad. Sci. U. S. A.* **99,** 12091–12096
17. Miller, J. H. (1992) *A Short Course in Bacterial Genetics*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY
18. DeWitt, S. K., and Adelberg, E. A. (1962) *Genetics* **47,** 577–585
19. Stohl, E. A., Blount, L., and Seifert, H. S. (2002) *Microbiology* **148,** 1821–1831
20. Bachmann, B. J. (1972) *Bacteriol. Rev.* **36,** 525–557
21. Little, J. W., Edmiston, S. H., Pacelli, L. Z., and Mount, D. W. (1980) *Proc. Natl. Acad. Sci. U. S. A.* **77,** 3225–3229
22. Ohmori, H., Saito, M., Yasuda, T., Nagata, T., Fujii, T., Wachi, M., and Nagai, K. (1995) *J. Bacteriol.* **177,** 156–165
23. Heitman, J., and Model, P. (1987) *J. Bacteriol.* **169,** 3243–3250
24. Frank, E. G., Gonzalez, M., Ennis, D. G., Levine, A. S., and Woodgate, R. (1996) *J. Bacteriol.* **178,** 3550–3556
25. Datsenko, K. A., and Wanner, B. L. (2000) *Proc. Natl. Acad. Sci. U. S. A.* **97,** 6640–6645
26. Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual*, 2nd Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY
27. Mehr, I. J., Long, C. D., Serkin, C. D., and Seifert, H. S. (2000) *Genetics* **154,** 523–532
28. Rehrauer, W. M., Bruck, I., Woodgate, R., Goodman, M. F., and Kowalczykowski, S. C. (1998) *J. Biol. Chem.* **273,** 32384–32387
29. Lavery, P. E., and Kowalczykowski, S. C. (1990) *J. Biol. Chem.* **265,** 4004–4010
30. Rehrauer, W. M., Lavery, P. E., Palmer, E. L., Singh, R. N., and Kowalczykowski, S. C. (1996) *J. Biol. Chem.* **271,** 23865–23873
31. Courcelle, J., Khodursky, A., Peter, B., Brown, P. O., and Hanawalt, P. C. (2001) *Genetics* **158,** 41–64
32. Story, R. M., Weber, I. T., and Steitz, T. A. (1992) *Nature* **355,** 318–325
33. Yu, X., and Egelman, E. H. (1993) *J. Mol. Biol.* **231,** 29–40
34. Frank, E. G., Cheng, N., Do, C. C., Cerritelli, M. E., Bruck, I., Goodman, M. F., Egelman, E. H., Woodgate, R., and Steven, A. C. (2000) *J. Mol. Biol.* **297,** 585–597

35. Lee, M. H., and Walker, G. C. (1996) *J. Bacteriol.* **178,** 7285–7294
36. Harmon, F. G., Rehrauer, W. M., and Kowalczykowski, S. C. (1996) *J. Biol. Chem.* **271,** 23874–23883
37. Moreau, P. L. (1987) *J. Mol. Biol.* **194,** 621–634
38. Sassanfar, M., and Roberts, J. W. (1990) *J. Mol. Biol.* **212,** 79–96
39. Van Dyk, T. K., DeRose, E. J., and Gonye, G. E. (2001) *J. Bacteriol.* **183,** 5496–5505
40. Voloshin, O. N., Ramirez, B. E., Bax, A., and Camerini-Otero, R. D. (2001) *Genes Dev.* **15,** 415–427
41. Black, C. G., Fyfe, J. A. M., and Davies, J. K. (1998) *Gene* (*Amst.*) **208,** 61–66
42. Bagdasarian, M., Bailone, A., Bagdasarian, M. M., Manning, P. A., Lurz, R., Timmis, K. N., and Devoret, R. (1986) *Proc. Natl. Acad. Sci. U. S. A.* **83,** 5723–5726
43. Bailone, A., Backman, A., Sommer, S., Celerier, J., Bagdasarian, M. M., Bagdasarian, M., and Devoret, R. (1988) *Mol. Gen. Genet.* **214,** 389–395
44. Bagdasarian, M., Bailone, A., Angulo, J. F., Scholz, P., and Devoret, R. (1992) *Mol. Microbiol.* **6,** 885–893
45. Bebenek, A., and Pietrzykowska, I. (1996) *Mol. Gen. Genet.* **250,** 674–680