**Inhibition of RecA Protein by the *Escherichia coli* RecX Protein**

**MODULATION BY THE RecA C TERMINUS AND FILAMENT FUNCTIONAL STATE**

**Julia C. Drees, Shelley L. Lusetti, and Michael M. Cox**

From the Department of Biochemistry, University of Wisconsin-Madison, Madison, Wisconsin 53706-1544

The RecX protein is a potent inhibitor of RecA activities. We identified several factors that affect RecX-RecA interaction. The interaction is enhanced by the RecA C terminus and by significant concentrations of free Mg$^{2+}$ ion. The interaction is also enhanced by an N-terminal His$_6$ tag on the RecX protein. We conclude that RecX protein interacts most effectively with a RecA functional state designated $A_0$, and that the RecA C terminus has a role in modulating the interaction. We further identified a C-terminal point mutation in RecA protein (E343K) that significantly alters the interaction between RecA and RecX proteins.

Homologous recombinational repair of damaged DNA mediated by the bacterial RecA protein is crucial in maintaining the integrity of the genome (1–6). *In vitro*, the RecA protein promotes a series of DNA pairing and strand exchange reactions that are thought to mimic the function of this protein *in vivo* (6, 7). RecA functions as a nucleoprotein filament. Filaments are assembled most readily on single-stranded DNA (ssDNA). However, filament assembly on duplex DNA (dsDNA) can also be achieved under some conditions, especially if a suitable nucleation site is available. RecA is a DNA-dependent ATPase, promoting ATP hydrolysis with a $k_{cat}$ of about 30 or 20 min$^{-1}$ when bound to ssDNA or dsDNA, respectively. RecA filaments assemble and disassemble in the 5’ to 3’ direction on ssDNA, with protomers added to one end and subtracted from the other under appropriate conditions (6, 7).

The activities of RecA protein must be regulated in the cell to target RecA to locations where it is needed and to avoid aberrant DNA transactions. The same must be true of all RecA homologs. In humans, aberrant recombination reactions could include the gross chromosomal rearrangements that lead to many human diseases, including cancer (8). Therefore, understanding the mechanisms by which RecA family recombinases are regulated is of utmost importance.

RecA itself provides one level of control. This involves autoregulation mediated by the RecA C terminus (9). In *Escherichia coli*, many additional proteins are known to regulate RecA function. The RecF, RecO, and RecR proteins have been implicated in the modulation of RecA filament assembly and disassembly (10–13). The DinI protein has been studied as a modulator of RecA function during the SOS response (14–18). The RecX protein is an inhibitor of RecA function both *in vivo* and *in vitro* (19). The RecX protein is among the least understood of these RecA modulator proteins. Recently, evidence has been obtained that RecX protein acts to cap the growing end of a RecA filament (20). This activity would serve to limit the length of RecA filaments formed *in vivo*, and we have proposed that this is the major mode of action of RecX (20).

The RecX protein (19.3 kDa) is encoded by a widespread bacterial gene, often but not always found as it is in *E. coli*, just downstream of the recA gene (21–26). A palindromic sequence separates the two *E. coli* genes (27). The resulting hairpin interrupts transcriptional read-through and limits the amount of *recA*-recX transcript to about 5–10% of the levels of the recA transcript alone. Protein levels of RecX were estimated to be 500 times less than those of the RecA protein (27). RecX interacts directly with the RecA protein (19), and direct binding to ssDNA is limited (19, 20).

Overexpression of RecA proteins in the absence of recX is toxic in *Pseudomonas aeruginosa*, *Streptomyces lividans*, *Mycobacterium smegmatis*, and *Xanthomonas oryzae* (23, 24, 28, 29). This provided early evidence that RecX is a negative modulator of RecA expression or function. Overexpression of the recX gene can reduce the induction of the SOS response (19), but there are no other clear phenotypes (27). When purified, RecX protein from both the *Mycobacterium* (30) and *E. coli* (19, 20) inhibit RecA functions *in vitro*. The RecX protein binds in the major helical groove of an AMPPNP-stabilized RecA filament (31). The effects of RecX are not identical in all bacteria, as RecX seems to enhance RecA activity in *Neisseria gonorrhoeae* (26).

Seifert and co-workers (19) found that *E. coli* RecX protein inhibits RecA protein ATPase activity *in vitro*, coprotease activity *in vivo*, and recombinase activity both *in vivo* and *in vitro*. The *in vitro* assays demonstrated inhibition at levels of RecX far below the concentration of RecA protein (19, 20), while the *in vivo* experiments employed a plasmid that overexpressed RecX protein. These same workers also demonstrated that RecA and RecX proteins physically interact using yeast two-hybrid analysis in *E. coli* (19). Electron Microscopy (EM) and the RecA crystal structure (32, 33) have been used to build three-dimensional reconstructions of RecA nucleoprotein filaments (stabilized by binding to AMPPNP) with bound *E. coli* RecX protein (31). Their data show RecX protein bound to the RecA filament, spanning the monomer-monomer interface from the C-terminal domain of one RecA monomer to the core domain of the second (31). These data suggest that the effects of RecX protein might involve an interaction with the C terminus of RecA.

In addition, RecX protein has several defined functional states that could affect the RecX-RecA interaction. When
bound to ssDNA, RecX is in a functional state designated A (active). There are two forms of the A state (9, 34). One of these, present at low Mg\(^{2+}\) concentrations, is relatively restricted in terms of allowed DNA pairing reactions (called A\(_{\text{p}}\), with the c reflecting a “closed” state). The other is present at higher Mg\(^{2+}\) concentrations and is unrestricted in DNA pairing (the open or A\(_{\text{a}}\) form). Addition of another strand of bound DNA to the RecA filament, either when RecA is bound to dsDNA or during DNA strand exchange, causes a transition to a state designated P (pairing) (7). In this report, we set out to determine how the RecA C terminus and the RecA functional state affected the interaction of RecA filaments with RecX protein.

MATERIALS AND METHODS

Enzymes and Biochemicals—The E. coli wild-type RecA, RecX E343K, and RecX\(_{\text{C17}}\) proteins were purified as described (9, 35). The RecA E343A mutant protein was purified identically to the RecX\(_{\text{A17}}\) protein (35), except that the final fraction was passed through a BioRex 70 column to remove a persistent nuclease activity. The concentration of the purified proteins was determined from the absorbance at 280 nm using the extinction coefficient 2.23 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1} (36). The E. coli SSB protein was purified as described (36). The concentration of the purified protein was determined from the absorbance at 280 nm using the extinction coefficient 2.83 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1} (38). Unless otherwise noted, all reagents were purchased from Fisher and were of the highest grade available. Xhol restriction endonuclease was purchased from Sigma. Hydroxyapatite and BioRex 70 resins were purchased from Bio-Rad. Isopropyl-1-thio-β-p-galactopyranoside was obtained from Gold Biotechnology, Inc. Ficol was obtained from Amersham Biosciences.

Buffers and Media—Phosphate buffer contained the indicated concentration of potassium phosphate (pH 6.8), 1 mM DTT, 0.1 mM EDTA, and 10% (v/v) glycerol. R buffer contained 20 mM Tris-HCl (80% cation, pH 7.5), 1 mM DTT, 0.1 mM EDTA, and 10% (v/v) glycerol. TAE buffer contained 40 mM Tris-OAc (80% cation) and 1 mM EDTA. Luria-Bertani medium (LB broth) is 10 g/liter tryptone, 5 g/liter yeast extract, and 10 g/liter NaCl, with the pH adjusted to 7.0.

DNA Substrates—Bacteriophage \(\phi X174\) circular single-stranded DNA (virion) was purchased from New England Biolabs. \(\phi X174\) RF I supercoiled circular duplex DNA was purchased from Invitrogen. Full-length linear duplex DNA was generated by digestion (38). The \(\phi X\) DNA (5386 bp) with the Xhol restriction endonuclease, using conditions suggested by the enzyme supplier. The digested DNA was extracted with phenol/chloroform/isoamyl alcohol (25:24:1), followed by ethanol precipitation. Circular single-stranded DNA from bacteriophage \(M13\)-mp8 (7229 nucleotides) was prepared using previously described methods (39). The concentration of the purified DNA was 19,274, in good agreement with the calculated mass of RecX (native RecX is \(1.59 \times 10^6 \text{ M}^{-1} \text{ cm}^{-1}\)).

Cloning and Overexpressing the RecX Protein—The recX gene was isolated from the \(E.\ coli\) genome (strain MG1655, a gift from George Weinstock, Ref. 40) by polymerase chain reaction. One DNA primer corresponded to the first 19 bases of the recX gene with the sequence 5'-CACCGTTT added to the 5'-end to provide a BspHI restriction site. The second DNA primer corresponded to the last 8 bases of the recX gene with the sequence 5'-CAGGATCCCCGATGCGT added to the 5'-end to provide a BamHI restriction site. This primers were used to amplify a DNA fragment of the appropriate size (529 bp) by the polymerase chain reaction. The recX gene fragment was digested with BspHI and BamHI and then ligated into the cloning vector pET21d (Novagen), which was digested with NcoI and BamHI (the NcoI and BspHI restriction sites have compatible cohesive ends). This plasmid containing the native recX gene under the control of the T7 RNA polymerase promoter was designated pEAW224. The integrity of the entire recX gene in this construct was verified by direct sequencing.

Competent cells of \(E.\ coli\) strain STL277/p77pol26 (35, 41) were transformed with plasmid pEAW224. Ten liters of culture were grown in LB broth to an OD\(_{600}\) of 0.7. RecX protein expression was induced by the addition of isopropyl-1-thio-β-p-galactopyranoside to 0.4 mM. Following a 3-h incubation at 37 °C, ~9 g of cells were harvested by centrifugation, flash-frozen in liquid N\(_2\), and stored at −80 °C.

The concentration of the RecX protein was determined from the absorbance at 280 nm using the extinction coefficient 2.57 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}. The RecX extinction coefficient \(e_{260} = 2.57 \times 10^4 \pm 0.027 \text{ M}^{-1} \text{ cm}^{-1}\) was determined during the course of the present work, using procedures described elsewhere (42, 43). The A\(_{260}\)/A\(_{280}\) ratio for the native RecX is 1.59 ± 0.031. The error in both numbers is ± 1 S.D. The protein was free of detectable nuclease activities and was determined to be greater than 99% pure by SDS-PAGE (Fig. 1). The identity of the purified protein was confirmed by matrix-assisted laser desorption/ ionization-time of flight mass spectrometry. The measured mass of the protein was 19,274, in good agreement with the calculated mass of RecX protein of 19,294 (with the initiator Met residue removed).

The RecX protein with a His\(_6\) N-terminal tag, used in one experiment, was generously provided by Elizabeth Stohl and Hank Seifert (Northwestern University). The purification of this protein has been described (19, 31).

In the course of these studies we noted that the native RecX protein was relatively unstable. RecX protein preparations stored at −80 °C exhibit a measurable loss of activity after four or more months of storage (data not shown). When thawed aliquots of the purified native RecX protein were kept at 4 °C for several days, its inhibitory effects declined (Fig. 2). For this reason, all of the experiments in this study made use of RecX aliquots thawed on the day of the experiment. Fur-

![Fig. 1. Purification of the native RecX protein.](Image 374x606 to 506x738)
RecA and RecA

cation, pH 7.4), 1 mM DTT, 3 mM potassium glutamate, the indicated

double-stranded DNA (labeled a coupling system (3 mM NADH and 10 units/ml lactate dehydro-

concentration of Mg(OAc)₂, 5% (w/v) glycerol, an ATP regeneration

sections were carried out as described under "Experimental Procedures," contain 6.7

tions were initiated by the addition of φX174 linear dsDNA to 20 μM. The reactions were incubated for 90 min. To stop the reaction, a 10-μl aliquot was removed and added to 5 μl of a solution containing 15% Ficoll, 0.25% bromphenol blue, 0.25% xylene cyanol, 72 mM EDTA, and 4% SDS. Samples were subjected to electrophoresis in 0.8% agarose gels with TAE buffer, stained with ethidium bromide, and exposed to ultraviolet light. Gel images were captured with a digital CCD camera utilizing GeiExpert software (Nucleotech). When indicated, the intensity of DNA bands was quantitated with the software package TotalLab version 1.10 from Phoretix.

RESULTS

The RecX Protein Inhibits the Circular ssDNA-dependent

ATPase Activity of the RecA Protein—The RecA protein hydro-

lyzes ATP when bound to DNA and the rate of ATP hydrolysis
generally correlates well to the amount of RecA bound to the

DNA (46). We previously showed that when RecX protein was

added to RecA filaments assembled on circular ssDNA, a slow
decline in ATP hydrolysis was observed that reflected a net
end-dependent disassembly of the filaments (20) and occurred
over a time span of 15–20 min. Analysis of this effect led to the

conclusion that RecX regulated the extension of RecA fil-

aments, capping the growing end (the 3’-proximal end) (20).

Here, we wished to examine the effects of Mg²⁺ and the RecA
C terminus on the inhibitory effects of RecX. These factors are

of interest since both of them modulate the functional state of

RecA filaments in vitro (9). We therefore determined the effects

of the RecX protein on the rate of the circular ssDNA-depend-

ent ATP hydrolysis activity of the wild-type RecA and

RecAΔC17 proteins, at two different Mg²⁺ concentrations. The

RecA and RecX proteins were added at the same time in all

experiments in this study, preincubated with the DNA for 10

min prior to the addition of ATP and SSB. Fig. 3 shows the

amount of ATP hydrolyzed by the wild-type (Fig. 3A) or the

RecAΔC17 (Fig. 3B) protein over time as a function of RecX

concentration. At lower concentrations of RecX protein, the

wild-type RecA-mediated ATP hydrolysis reaction is quite

robust initially, suggesting that RecA filaments have been able
to form. In the presence of 20–60 nM RecX protein, there is

then a slow decline in the rate of ATP hydrolysis, indicating a

relatively slow effect of RecX on the activity of the RecA fil-

ament. A significant inhibitory effect is obtained by the addition

of 10 nM RecX protein. The addition of 80 nM RecX brings about

almost complete inhibition in which the assembly of filaments

on the DNA appears to be limited. As demonstrated elsewhere

(20), the slow change reflects the slow and end-dependent dis-

sociation of assembled RecA protein filaments from the circular

ssDNA after the growing end of the filaments have been
capped.

In contrast, the RecAΔC17 protein is less inhibited by RecX

protein. There is only a slight decrease in the rate of ATP

hydrolysis at 10 nM RecX, and a comparable level of inhibition

of the RecAΔC17 protein requires about a 3-fold higher concen-

tration of RecX protein. Complete inhibition of ATP hydrolysis

occurs at RecX concentrations above 200 nM.

The concentration of Mg²⁺ was 10 mM in the experiments of

Fig. 3, A and B. This is the optimum Mg²⁺ concentration for the

DNA strand exchange mediated by the wild-type RecA protein,

but the RecAΔC17 protein promotes DNA strand exchange

better at lower Mg²⁺ concentrations (9). Mg²⁺ affects the func-

tional state of RecA filaments (9). To complete the data set, we

therefore carried out the same set of experiments under condi-

tions that entail Mg²⁺ concentrations stoichiometric with the

available ATP (9). At 3 mM Mg²⁺, the wild-type RecA protein

filaments appeared to be somewhat unstable even in the

absence of the RecX protein. However, the effect of RecX

protein was reduced relative to the results obtained at higher

Mg²⁺ concentrations (Fig. 3C). The inhibition of RecA-medi-

ated ATP hydrolysis was observed when RecX was present at

100 nM. Once again, at higher concentrations of RecX, complete

disassembly of the RecAΔC17 filaments was observed.

Limited stability of native RecX protein. A, reaction


diagram. In the first step, RecA forms a filament on circular single-

stranded DNA (labeled S₁). RecA then recruits complementary linear
double-stranded DNA (labeled S₂) and pairs the two molecules, aligning homology. A joint molecule intermediate (I) is formed while the identi-

cal strand is displaced. Finally, a nicked circular product (P) and a

linear single-stranded molecule result. Substrates, products, and inter-

mediates are distinguishable by agarose gel electrophoresis. B, reac-
tions were carried out as described under "Experimental Procedures," and contained 6.7 μM RecA protein, 20 μM φX174 circular ssDNA, 20 μM linear φX174 dsDNA, 10 mM Mg(OAc)₂, 3 mM ATP, and the indicated concentration of RecX protein. Reactions were incubated for 90 min. M is a marker of unreacted linear double-stranded DNA and circular

ssDNA. A thawed aliquot of RecX protein was stored at 4 °C for the

indicated time prior to use in these trials. The fresh aliquot was used 4 h after thawing.
Dependent ATPase activity of the wild-type RecA and RecA

Procedures" and contained 3 mM ATP, 5

Reactions were carried out as described under "Experimental

for the mutant RecA

ated ATP hydrolysis was alleviated to an even greater extent

the RecX protein, when the assay is carried out under the optimal conditions

from which C was derived to show the DNA complexes trapped in the

wells in two of the lanes. F, quantitation of nicked circular products for
each of the experiments A–D. The reaction is illustrated in Fig. 2, and
the gel labels are described in Fig. 2 and its legend.

The effect of RecX protein was significantly moderated when
the RecA protein had a point mutation near the C terminus
(Fig. 4B). The RecA C terminus features seven negatively
charged amino acid residues (35), and one of these is converted
to a positive charge in the mutation in question (E343K). The
RecE343K mutant protein is fully competent in DNA strand
exchange activities. Approximately 3–4-fold more RecX protein
is required to produce a given level of inhibition of DNA strand
exchange when this mutant protein is used.

Next, we assayed the effect of RecX on a C-terminal deletion
mutant of RecA protein that removed all seven of the nega-
tively charged amino acid residues. Assays were conducted
under the same conditions used for the wild-type RecA protein.
The RecAΔC17 protein-promoted DNA strand exchange reaction
(Fig. 4C) was also inhibited by the RecX protein, but to a
somewhat lesser extent than the wild-type RecA reaction. In
this case, about twice as much RecX protein was needed to
achieve a given level of inhibition.

As already noted, the optimum magnesium ion concentration
for DNA strand exchange promoted by RecAΔC17 is actually
much lower than the concentration optimal for wild-type RecA
protein (9), approximately stoichiometric with the available
ATP. We thus explored the effect of RecX at this lower (3 mM)
concentration of Mg2+. As indicated in panel D, the removal of
17 residues from the C terminus of RecA results in substantial
protection of RecA from the inhibitory effects of the RecX pro-
tein, when the assay is carried out under the optimal conditions
for RecAΔC17-mediated DNA strand exchange. Complete inhibi-
tion of the mutant-promoted reaction is not observed until
the ratio of RecX to RecAΔC17 is 1:1 (Fig. 4D). Thus, both free
Mg2+ and the RecA C terminus enhance the inhibitory effect
of the RecX protein on RecA protein activity.

In Fig. 4C, much of the loaded DNA seems to disappear in
the lanes containing 400 and 670 nM RecX protein. The missing
DNA is present in what appear to be DNA aggregates that
remain in the well of the gel. The full gel is shown in Fig. 4E.
Aggregated complexes of this kind have been observed previ-
ously with the RecAΔC17 mutant protein at higher Mg2+ con-
concentrations (9) and are thought to represent DNA networks that cannot be resolved. The added RecX protein may bring about the multiple strand invasions necessary to create these complex aggregates. Fig. 4F provides a quantitation of nicked circular products formed in the experiments shown in panels A–D.

The RecA E343K Mutation Facilitates Recovery from RecX Inhibition—Based on its moderating effect on DNA strand exchange (Fig. 4B), we decided to further explore the effects of RecA E343K on the RecX-RecA interaction. As shown in Fig. 5A, the RecA E343K mutant protein hydrolyzes ATP at rates similar to those seen for the wild-type protein, as does another mutant RecA protein with a neutral change at the same position, E343A. When the wild-type protein was preincubated with 100 nM RecX protein, the assembly of filaments was inhibited as before (Fig. 5A). Assembly of filaments by the RecA E343K mutant was also inhibited, but the ATPase activity of the mutant protein gradually recovered over a time course of 2 h. The wild-type RecA and the RecA E343A mutant exhibited little tendency to recover in the presence of bound RecX protein over this same time period. The C-terminal deletion mutant RecΔC17 was inhibited by the RecX protein (compare panels A and B in Fig. 3), but was somewhat more resistant to RecX inhibition of filament formation than was the wild-type protein. This was consistent with the greater resistance of this protein to inhibition of DNA strand exchange (Fig. 4C).

C-terminal deletion mutants of RecA protein have a greatly enhanced capacity to displace SSB protein (47), and the recovery of the RecA E343K mutant might be attributed to a similar capacity to nucleate enough filaments in the presence of bound SSB to circumvent the available RecX protein. We therefore examined SSB displacement by these mutants by precoating the ssDNA with the SSB before adding the RecA proteins. The RecA E343K did have a somewhat enhanced SSB displacement capacity, but it was very similar to the capacity exhibited by the RecA E343A mutant protein (Fig. 5B). As this latter protein did not recover in the presence of RecX we attributed the recovery of the RecA E343K mutant documented in Fig. 5A to an altered interaction between the RecX and RecA proteins.

RecX Inhibition of Wild-type RecA Is Highly Dependent on Magnesium Ion Concentration—Because RecX inhibition of RecΔC17-promoted DNA strand exchange is dependent on Mg$^{2+}$ concentration, we decided to look more closely at the Mg$^{2+}$ dependence of RecX inhibition of wild-type RecA. As Fig. 6 shows, wild-type RecA does not promote DNA strand exchange at 3 mM Mg$^{2+}$ either in the presence or absence of RecX. The maximum reaction in the presence of 25 mM RecX occurs at 10 mM Mg$^{2+}$, and the reaction is increasingly inhibited at higher Mg$^{2+}$ concentrations. The reaction at 10 mM Mg$^{2+}$ is inhibited in the presence of 67 mM RecX (Fig. 6). At magnesium ion concentrations above 15 mM, 25 mM RecX protein was sufficient to produce an almost complete inhibition of DNA strand exchange. Since Mg$^{2+}$ can influence the physical state of RecA filaments, it is possible that RecX interacts preferentially with the RecA filament state present at high Mg$^{2+}$ concentrations. Alternatively, RecX itself may be more active at high Mg$^{2+}$ concentrations.

A RecX N-terminal His$_6$ Tag Increases the Inhibitory Effects of RecX Inhibition—In a previous study, a RecX protein with an N-terminal His$_6$ tag was utilized, and this appeared to generate more substantial levels of inhibition of RecA activities in vitro than we have demonstrated here (19). We obtained a sample of the His-tagged RecX protein, and carried out the experiment shown in Fig. 7. This experiment was done under the conditions used for the ATPase inhibition studies of Fig. 3. Using wild-type RecA protein and 10 mM Mg$^{2+}$, we show that only about half as much His-tagged RecX protein is required to bring about a given level of inhibition of RecA-mediated ATP hydrolysis. This result suggests that the His$_6$ tag enhances the interaction between RecX and RecA.

**DISCUSSION**

As measured by the effects on RecA-mediated ATP hydrolysis, the interaction between RecX protein and RecA protein filaments is enhanced by at least three factors: (a) the presence of Mg$^{2+}$ concentrations in excess to those needed to chelate the available ATP, (b) the presence of the RecA C terminus or negatively charged amino acid residues therein, and (c) the
addition of a His tag on the N terminus of RecX protein. A RecA E343K mutant protein, with an alteration in one of its C-terminal residues, also appears to moderate the RecX-RecA interaction. Together, the observations indicate that the RecA-RecX interaction is sensitive to both the RecA C terminus and to the functional state of the RecA filament. The work also suggests that the RecA-RecX interaction is mediated at least in part by the RecA C terminus. This information should help illuminate future efforts to probe the interaction between these two proteins. The work also reinforces the notion, proposed previously (20), that the RecX protein limits RecA filament extension by directly interacting with the RecA protein.

The effects of magnesium ion concentration speak to the functional state of RecA protein to which RecX protein binds most avidly. RecA filaments formed on ssDNA exist in at least two functional states, designated A\textsuperscript{H} and A\textsubscript{o} (7, 9, 34). The two states are interconverted in response to changes in Mg\textsuperscript{2+} concentrations. The enhancement of the RecX inhibition by added Mg\textsuperscript{2+} ion in excess to that required to chelate the available ATP indicates that RecX protein interacts most effectively with a RecA A\textsubscript{o} state (7, 9, 34).

The C terminus has a role in modulating the interchanges between the A\textsuperscript{H} and A\textsubscript{o} states when RecA protein is bound to ssDNA (7, 9, 34). The moderating effects of a RecA C-terminal deletion within this groove, and interacts to a degree with the C-terminal domain of RecA (31).

When optimal conditions are used for both proteins, the RecA\textsubscript{ΔC17} mutant is not as sensitive to RecX protein as is the wild-type RecA protein. However, this compares the proteins under different conditions. We cannot compare the two RecA proteins at the lower Mg\textsuperscript{2+} ion concentrations that are optimal for RecA\textsubscript{ΔC17}, since the wild-type protein is normally inactive in the DNA strand exchange reaction under those conditions. At the higher Mg\textsuperscript{2+} concentrations, the C-terminal deletion mutant is still active, but in a functional state that is apparently different than it exhibits without the excess Mg\textsuperscript{2+} (9).

Here, we see that an increase in Mg\textsuperscript{2+} ion concentration changes the mutant RecA protein from a state that is relatively resistant to the effects of RecX, to one that is quite sensitive to RecX. This reinforces the previous observations indicating a Mg\textsuperscript{2+}-mediated change in functional state in the mutant protein (47). It again indicates that RecX protein binds most tightly to the A\textsuperscript{H} state of RecA filaments that exist at the higher Mg\textsuperscript{2+} concentration. RecX inhibition of wild-type RecA protein is also enhanced at higher Mg\textsuperscript{2+} concentrations (Fig. 6). Based on the clear effects of Mg\textsuperscript{2+} ion concentrations on RecA filament function (9, 34), we attribute most of the effects on changes in the functional state of RecA. However, we do not discount the possibility that the RecX protein itself is affected by Mg\textsuperscript{2+}, and this is an aspect of the problem that will require further investigation.

Elsewhere, we show that the effects of RecX protein on RecA activities are best explained by a RecX-mediated capping of the growing ends of RecA filaments (20). Within RecA filaments formed on circular DNAs, the capping would occur at short gaps that might exist in the filaments (20). This leads to a net dissociation of RecA from the unaffected disassembly end that can be monitored by changes in ATPase activity and by electron microscopy (20). The differences seen between the wild-type RecA and the RecA\textsubscript{ΔC17} proteins are best explained by a weaker affinity of RecX for the latter protein. It takes more RecX for a similar level of inhibition relative to the wild-type protein. In addition, there is a greater tendency for the RecA E343K protein to recover from the inhibition of RecX protein (Fig. 5) than is seen with the wild-type RecA protein. This further implicates the C terminus in the RecX-RecA interaction. However, if enough RecX protein is present, the assembly of RecA\textsubscript{ΔC17} protein filaments is strongly impeded, so that the RecX protein is clearly interacting with additional structural features of the RecA protein.

The RecX protein appears to be part of a growing suite of proteins that serves to modulate the assembly and disassembly of RecA filaments. With the RecFOR proteins (10–13), RecX protein would be present under normal growth conditions and would serve to modulate the formation and disassembly of RecA filaments for recombinational DNA repair and other functions. RecX is present at elevated levels during the SOS response. The DinI protein also appears to be a modulator, one that is specific to the SOS response (14–18). With its capacity to limit RecA filament extension, most likely by capping the filament (20), RecX would be expected to play a prominent role in limiting the length of RecA filaments in vivo. That role may complement the function of DinI, the RecFOR proteins, and perhaps other E. coli proteins.

Acknowledgments—We thank Elizabeth Wood for technical support in constructing the strains overproducing the native RecX protein. We also thank Hank Seifert (Northwestern University) and colleagues for the generous gift of His\textsubscript{6}-tagged RecX protein.

REFERENCES
1. Cox, M. M., Goodman, M. F., Kreuzer, K. N., Sherratt, D. J., Sandler, S. J., and Marians, K. J. (2000) Nature 404, 37–41
2. Cox, M. M. (2001) Proc. Natl. Acad. Sci. U. S. A. 98, 8173–8180
3. Cox, M. M. (2002) Mol. Cell. 10, 107–120
4. Kowalczykowski, S. C. (2000) Trends Biochem. Sci. 25, 156–165
5. Kuzminov, A. (1999) Microbiol. Mol. Biol. Rev. 63, 751–813
6. Luzetti, S. L., and Cox, M. M. (2002) Annu. Rev. Biochem. 71, 71–100
7. Cox, M. M. (2003) Annu. Rev. Microbiol. 57, 551–577
8. Yu, V. P., Koehler, M., Steinlein, C., Schmid, M., Hanakahi, L. A., van Gool, A. J., West, S. C., and Venkitaraman, A. R. (2000) Genes Dev. 14, 1400–1406
9. Luzetti, S. L., Shaw, J. J., and Cox, M. M. (2003) J. Biol. Chem. 278, 16381–16388
10. Shan, Q., Bork, J. M., Webb, B. L., Inman, R. B., and Cox, M. M. (1997) J. Mol. Biol. 269, 519–540
11. Umezu, K., and Kolodner, R. D. (1994) J. Biol. Chem. 269, 30005–30013
12. Webb, B. L., Cox, M. M., and Inman, R. B. (1997) Cell 91, 347–356
13. Morimoto, K., and Kowalczykowski, S. C. (2003) Mol. Cell 11, 1337–1347
RecX Inhibition of RecA

14. Voloshin, O. N., Ramirez, B. E., Bax, A., and Camerini-Otero, R. D. (2001) Genes Dev. 15, 415–427
15. Yasuda, T., Morimatsu, K., Kato, R., Usukura, J., Takahashi, M., and Ohmori, H. (2001) EMBO J. 17, 3207–3216
16. Yasuda, T., Morimatsu, K., Horii, T., Nagata, T., and Ohmori, H. (2001) EMBO J. 20, 1192–1202
17. Yeshimasu, M., Aihara, H., Ito, Y., Rajesh, S., Ishibe, S., Mikawa, T., Yokoyama, S., and Shibata, T. (2003) Nucleic Acids Res. 31, 1735–1743
18. Lusetti, S. L., Voloshin, O. N., Inman, R. B., Camerini-Otero, C. S., and Cox, M. M. (2004) J. Biol. Chem. 279, 30037–30046
19. Stohl, E. A., Brockman, J. P., Burkle, K. L., Morimatsu, K., Kowalczykowski, S. C., and Seifert, H. S. (2003) J. Biol. Chem. 278, 2278–2285
20. Drees, J. C., Lusetti, S. L., Chitteni-Pattu, S., Inman, R. B., and Cox, M. M. (2004) Mol. Cell 15, 789–798
21. De Mot, R., Schools, G., and Vanderleyden, J. (1994) Nucleic Acids Res. 22, 1313–1314
22. Papavinasasundaram, K. G., Movahedzadeh, F., Keer, J. T., Stoker, N. G., Colston, M. J., and Davis, E. O. (1997) Mol. Microbiol. 24, 141–153
23. Same, Y. (1993) J. Bacteriol. 175, 2451–2454
24. Vierling, S., Weber, T., Wohlbach, W., and Muth, G. (2000) J. Bacteriol. 182, 4005–4011
25. Yang, M. K., Chou, M. E., and Yang, Y. C. (2001) Curr. Microbiol. 42, 257–263
26. Stohl, E. A., and Seifert, H. S. (2001) Mol. Microbiol. 40, 1301–1310
27. Pages, V., Koffel-Schwartz, N., and Fuchs, R. P. (2003) DNA Repair 2, 273–284
28. Papavinasasundaram, K. G., Colston, M. J., and Davis, E. O. (1998) Mol. Microbiol. 30, 525–534
29. Sukchawalit, R., Vattanavivoon, P., Utamapongchai, S., Vaughan, G., and Mongkolsuk, S. (2001) FEMS Microbiol. Lett. 205, 83–89
30. Venkatesh, R., Ganesh, N., Guhan, N., Reddy, M. S., Chandrasekhar, T., and Munirajappa, K. (2002) Proc. Natl. Acad. Sci. U. S. A. 99, 12091–12096
31. vanLooch, M. S., Yu, X., Yang, S., Galkin, V. E., Huang, H., Rajan, S. S., Anderson, W. F., Stohl, E. A., Seifert, H. S., and Egelman, E. H. (2003) J. Mol. Biol. 333, 345–354
32. Story, R. M., Weber, I. T., and Seifert, T. A. (1992) Nature 355, 318–325
33. Story, R. M., and Stetz, T. A. (1992) Nature 355, 374–376
34. Haruta, N., Yu, X. N., Yang, S. X., Egelman, E. H., and Cox, M. M. (2003) J. Biol. Chem. 278, 52710–52723
35. Lusetti, S. L., Wood, E. A., Fleming, C. D., Modica, M. J., Korth, J., Abbott, L., Dwyer, D. W., Roca, A. I., Inman, R. B., and Cox, M. M. (2003) J. Biol. Chem. 278, 16372–16380
36. Craig, N. L., and Roberts, J. W. (1981) J. Biol. Chem. 256, 8039–8044
37. Shan, Q., Cox, M. M., and Inman, R. B. (1996) J. Biol. Chem. 271, 5712–5724
38. Lohnan, T. M., and Overman, L. B. (1985) J. Biol. Chem. 260, 3594–3603
39. Neuendorf, S. K., and Cox, M. M. (1986) J. Biol. Chem. 261, 8276–8282
40. Guyer, M. S., Reed, R. R., Steitz, J. A., and Low, K. B. (1981) Cold Spring Harbor Symp. Quant. Biol. 45, 135–140
41. Lovett, S. T., and Kolodner, R. D. (1989) Proc. Natl. Acad. Sci. U. S. A. 86, 2827–2831
42. Marrione, P. E., and Cox, M. M. (1995) Biochemistry 34, 9809–9818
43. Robu, M. E., and Cox, M. M. (2004) J. Biol. Chem. 279, 10973–10981
44. Lindsley, J. E., and Cox, M. M. (1990) J. Biol. Chem. 265, 9043–9054
45. Morrical, S. W., Lee, J., and Cox, M. M. (1986) Biochemistry 25, 1482–1494
46. Pugh, B. F., and Cox, M. M. (1988) J. Mol. Biol. 203, 479–493
47. Eggler, A. L., Lusetti, S. L., and Cox, M. M. (2003) J. Biol. Chem. 278, 16389–16396