A RecA Filament Capping Mechanism for RecX Protein

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Summary

The RecX protein is a potent inhibitor of RecA protein activities. RecX functions by specifically blocking the extension of RecA filaments. In vitro, this leads to a net disassembly of RecA protein from circular single-stranded DNA. Based on multiple observations, we propose that RecX has a RecA filament capping activity. This activity has predictable effects on the formation and disassembly of RecA filaments. In vivo, the RecX protein may limit the length of RecA filaments formed during recombinational DNA repair and other activities. RecX protein interacts directly with RecA protein, but appears to interact in a functionally significant manner only with RecA filaments bound to DNA.

Introduction

The bacterial RecA protein plays an important role in recombinational DNA repair in bacteria (Cox, 2001; Cox et al., 2000; Kowalczykowski, 2000; Kuzminov, 1999; Lusetti and Cox, 2002). In vitro, RecA protein is a DNA-dependent ATPase and promotes DNA strand exchange reactions that mimic its presumed role in recombinational DNA repair (Cox, 2003; Lusetti and Cox, 2002; Roca and Cox, 1997). In addition, the RecA protein regulates the induction of the SOS response by facilitating the autocatalytic cleavage of the LexA repressor (Kim and Little, 1993; Nastri and Knight, 1994). RecA functions as a nucleoprotein filament, which assembles on the DNA in several steps (Lusetti and Cox, 2002; Roca and Cox, 1997). A slow nucleation step is followed by rapid extension 5' to 3', such that additional RecA monomers are added at one filament end (Register and Griffith, 1985; Shan et al., 1997). Disassembly of RecA filaments is also unidirectional and end dependent, occurring on the end opposite to that at which filament extension occurs (Bork et al., 2001a; Lindsley and Cox, 1990; Shan et al., 1997). Assembly and disassembly both proceed 5' to 3' (Figure 1).

To bring about efficient recombinational DNA repair, it is essential for RecA filaments to form when and where they are needed. It is also important that RecA filaments do not form where they are not needed, to avoid aberrant DNA rearrangements. Thus, RecA filament assembly and disassembly are regulated. One level of control involves the autoregulation of RecA function mediated by the C terminus of the RecA protein (Lusetti et al., 2003a). The C terminus largely blocks direct binding of RecA protein to duplex DNA, effectively directing RecA protein

filament nucleation to single strand gaps (Benedict and Kowalczykowski, 1988; Lusetti et al., 2003b; Tateishi et al., 1992). Also, the C terminus modulates the intrinsic capacity of RecA protein to displace SSB (Eggler et al., 2003). RecA is also regulated by a variety of additional proteins in Escherichia coli. The RecFOR proteins mediate RecA filament assembly on SSB-coated single-stranded DNA, and may limit RecA filament extension on duplex DNA (Bork et al., 2001b; Morimatsu and Kowalczykowski, 2003; Shan et al., 1997; Webb et al., 1995, 1997). The DinI protein stabilizes RecA filaments and may play a role in regulating RecA function during the SOS response (Lusetti et al., 2004; Venkatesh et al., 2002; Voloshin et al., 2001; Yasuda et al., 1998, 2001). Among the least well understood of the proteins that modulate RecA function is the RecX protein.

The RecX protein (19.4 kDa) is encoded by a widespread bacterial gene often (as in E. coli) found just downstream of the recA gene (De Mot et al., 1994; Sano, 1993; Vierling et al., 2000; Yang et al., 2001). In a very few cases, the gene is found in another region of the chromosome (Stohl and Seifert, 2001). In E. coli, the recX gene is expressed from the recA promoter via a 5%–10% transcriptional readthrough of a hairpin sequence separating the two genes (Pages et al., 2003). In some bacteria, RecX protein is necessary to overcome deleterious effects of overexpression of RecA protein, implying that RecX is a negative modulator of RecA (Papavinasasundaram et al., 1998; Sano, 1993; Sukchawalit et al., 2001; Vierling et al., 2000). Deletion of the recX gene in E. coli produces no clear phenotype (Pages et al., 2003), although overexpression can reduce the induction of the SOS response (Stohl et al., 2003). When purified, both the Mycobacterium RecX (Venkatesh et al., 2002) and the E. coli RecX protein (Stohl et al., 2003) inhibit the ATPase and strand exchange activities of RecA protein in vitro. The RecX protein binds deep within the major helical groove of a RecA filament (Van-Loock et al., 2003). EM image reconstructions indicate that when RecX protein is bound to an intact RecA filament, it spans the monomer-monomer interface, binding from the C-terminal domain of one RecA monomer to the core domain of a second monomer (VanLoock et al., 2003).

The mechanism by which RecX inhibits RecA functions is not understood. As we began to further explore RecX function, we found that RecX inhibition of RecA activities is both slow and highly selective. Some RecA activities are affected at quite low RecX concentrations, while other RecA activities are not affected unless an excess of RecX protein (relative to the available RecA) is added. In this report, we rationalize these observations and demonstrate that RecX acts primarily by blocking the growing end of RecA filaments.

Results

Experimental Design and Hypothesis
To explain the experiments, it is convenient to first introduce the hypothesis they lead to. Based on the observa-
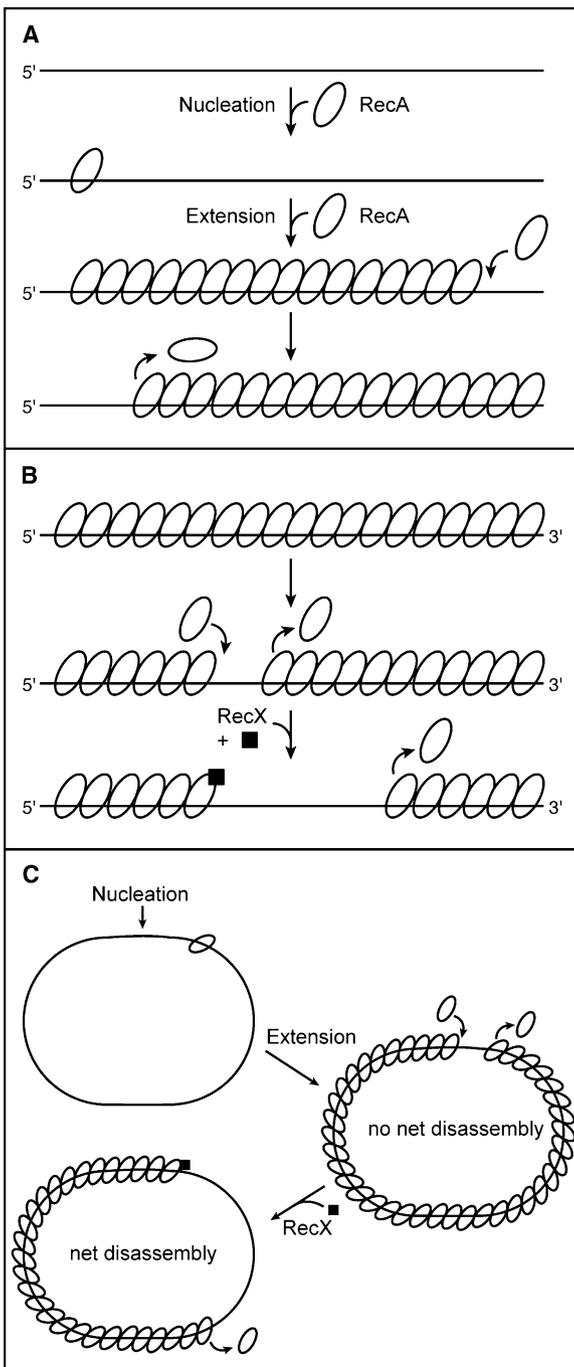
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Figure 1. Model for the Inhibition of RecA Function by the RecX Protein

(A) RecA protein binds to single-stranded DNA (ssDNA) in two major steps. A slow nucleation is followed by a rapid 5′ to 3′ extension that encompasses the available DNA. Disassembly is also 5′ to 3′, proceeding from the end opposite to that where assembly occurs. Disassembly, but not assembly, requires ATP hydrolysis.

(B) In a RecA filament, if an interior monomer dissociates (e.g., at a break in the filament), the filament gap is quickly filled in by extension of the trailing filament. We hypothesize that RecX protein caps the assembly end of the filament, leading to net disassembly.

(C) On a circular ssDNA, the balance between disassembly and assembly keeps the net binding of RecA and its attendant ATP hydrolysis at a steady state level. Capping the assembly ends at any point where there is a break in the filament and preventing

tions we report here, we propose that the RecX protein acts by capping the growing end of a RecA filament and blocking further extension. When RecA protein forms a filament on a *circular* single-stranded DNA, it will nucleate at one site. The 5′ to 3′ extension will then coat the available DNA around the circle. The filament will also begin a slower end-dependent disassembly from the point of nucleation. Filament extension on the growing end is faster than RecA dissociation on the disassembly end. When the extending end of the filament "catches up" with the disassembling end (Figures 1B and 1C), a short gap may exist where RecA monomers at the disassembling end are dissociating (Figure 1B). Additional gaps may occur transiently in an otherwise contiguous filament (Morrical and Cox, 1990). Limited exchange of RecA between free and bound forms in these filaments has been documented (Shan and Cox, 1996). On a circular ssDNA, there is no *net* disassembly of RecA filaments under normal conditions, and the rate of ATP hydrolysis is constant. Even if there are several filament gaps at a particular moment, any dissociation of RecA at an exposed disassembly end will be quickly filled in by extension of the trailing filament. With RecA protein bound to circular ssDNA, a measurable decline in ATP hydrolysis could occur if (a) the ATP was consumed (our experiments have an ATP regeneration system), (b) a molecule was added that inhibited the intrinsic ATPase activity, (c) a reagent was added that completely destroyed the integrity of the filament and released it from DNA (such as a denaturing agent), or (d) something blocked the extension of RecA filaments.

Since it is clear that some RecA is moving into and out of RecA filaments at steady state (Shan and Cox, 1996), we assume that there are a few short filament breaks such as those illustrated in Figures 1B and 1C even when RecA is bound to circular DNA. If the assembly end of a RecA filament were blocked or capped by RecX protein so that no further addition of RecA on that end could occur, the filament gap created by filament disassembly would cease to be filled in. Net disassembly of the filament would follow. If disassembly were occurring only at one or a few locations in the filament, the decline in the bound RecA and its associated ATP hydrolysis would have a predictable profile. The rate of end-dependent filament disassembly from ssDNA proceeds at 60–70 monomers per minute per filament end (Arenson et al., 1999). The time required for disassembly of a single contiguous filament formed on M13mp8 DNA (7229 nucleotides; with approximately 7229/3 or 2409 RecA monomers bound) should take 35 to 40 min, assuming there is only one disassembling end. If RecX dissociates to allow further RecA filament extension, or if there is binding of free RecA to the vacated DNA, this will lessen the observed effect of RecX on the filaments and decrease the net rate of disassembly. If, on the other hand, there are multiple gaps in the filament, and RecX caps the exposed assembly ends at more than one location on a filament, the observed rate of disassembly

extension will lead to net disassembly of the filament. If re binding is largely blocked (such as by the SSB protein), the filaments may dissociate completely.
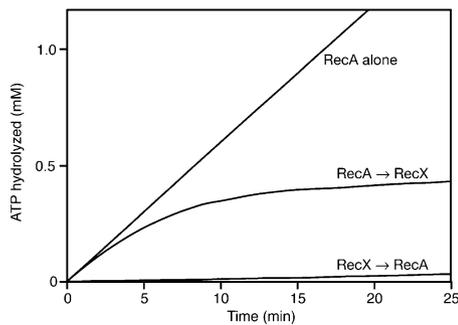
Figure 2. The Effect of the RecX Protein on RecA Protein-Mediated ATP Hydrolysis

Reactions contained 3 μM RecA protein, 5 μM M13mp8 ssDNA, 0.5 μM SSB, and 3 mM ATP. RecA filaments assembled on a circular ssDNA hydrolyze ATP at a steady rate. Addition of RecX protein (100 nM added at t = 0 min) to the preassembled RecA filaments (RecA → RecX curve) leads to a slow decline in ATP hydrolysis that is completed in 15-20 min. Addition of the same amount of RecX protein 10 min prior to adding the RecA protein to the ssDNA (RecX → RecA curve) leads to a general suppression of RecA filament formation.

Figure 3. Effect of RecX Concentration on the Inhibition of RecA-Mediated ATP Hydrolysis

(A) RecA protein (1.2 μM) was incubated with M13mp8 ssDNA (2 μM) for 10 min at 37°C, followed by addition of ATP and SSB to initiate ATP hydrolysis. After 15 min, RecX protein was added at the concentration indicated beside each curve (t = 0 min), and the ATPase activity was monitored.

(B) These experiments are similar to those in (A), except that the RecX protein was present during the preincubation with RecA and ssDNA (5 min), prior to the addition of ATP and SSB (t = 0 min).

would increase. SSB is present in these trials, both to facilitate the formation of RecA filaments at the beginning of the reaction and to suppress re-nucleation of new RecA filaments as existing filaments disassemble. We examined the effects of RecX protein on RecA filament assembly and disassembly while varying the concentration of RecX and the order of addition of reaction components. We also monitored the effects of RecX on other RecA protein functions, including DNA strand exchange and LexA cleavage. For a number of experiments, we utilized the RecA-mediated ATPase activity as an indirect measure of RecA binding to DNA. In the absence of agents that affect the intrinsic ATPase of RecA, this has proved to be a reliable measure of RecA binding (Lindsley and Cox, 1990; Lusetti et al., 2003b; Roca and Cox, 1997; Shan et al., 1997; Morimatsu and Kowalczykowski, 2003). This was complemented by the direct observation of filament status by electron microscopy and direct DNA binding assays.

Addition of RecX Protein Leads to Net Filament Disassembly on Circular Single-Stranded DNA

The general effects of RecX on RecA filaments, examined indirectly by monitoring RecA-mediated ATP hydrolysis, are presented in Figure 2. When there was no RecX present, ATP hydrolysis proceeded at a steady state reflecting a k_{cat} of just over 30 min^{-1}. As much as 1.67 μM of the 3 μM RecA protein is bound to the 5 μM ssDNA present. Addition of RecX protein (100 nM) after the RecA filaments had formed resulted in a time-dependent decline of ATP hydrolysis that required nearly 15 min to complete. The decline is brought about by quite low concentrations of RecX protein. In this experiment, the RecA protein is present in 30-fold excess relative to the RecX protein. When RecX was present before RecA filaments were able to form on the DNA, a different result was obtained. The ATP hydrolysis was nearly completely suppressed, suggesting that filament formation is largely blocked (Figure 2).
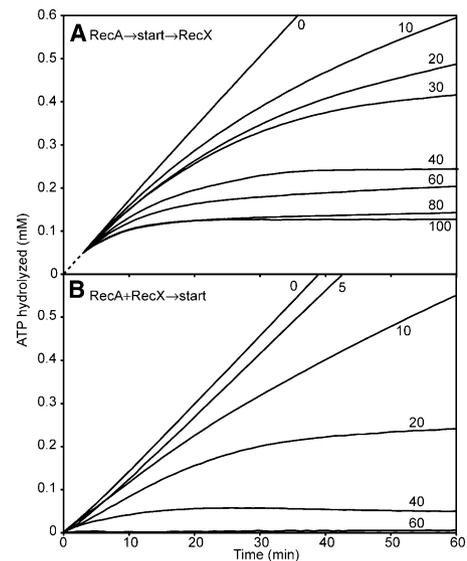
These results are expanded in Figure 3. In Figure 3A, the RecX protein was added after RecA filaments had been formed. Significant effects of RecX protein are seen with as little as 10 nM RecX protein. The RecA protein concentration in these experiments is 1.2 μM, with up to 0.67 μM bound to DNA. Thus, RecX can produce substantial effects when it is present at very low concentrations relative to RecA. The effect of RecX protein appears to saturate as the RecX concentration approaches 80–100 nM, a concentration well below that of the bound RecA protein. This effect would be expected if the number of exposed RecA filament ends such as those in Figures 1B and 1C, where exchange of RecA monomers was occurring, was limited to an average of 2–3 per filament. The approximately 15 min required for suppression of the ATP hydrolysis would correspond to the disassembly of just under half the length of the RecA filament that could coat an M13 mp8 ssDNA. It could be accounted for if there were about 2–3 actively disassembling ends per nucleoprotein filament, each disassembling at the previously measured rate of approximately 70 monomers of RecA per filament end per minute (Arenson et al., 1999).

In Figure 3B, the effect of RecX protein is examined under conditions where it is present prior to the formation of RecA filaments. Here, the effects were substantially greater at any given RecX concentration, and the addition of only 60 nM RecX completely abolished ATP hydrolytic activity. This is consistent with a mechanism in which the RecX is interfering with RecA filament formation, by limiting the filament extension process. In this scenario, each time a nucleation event occurs, the
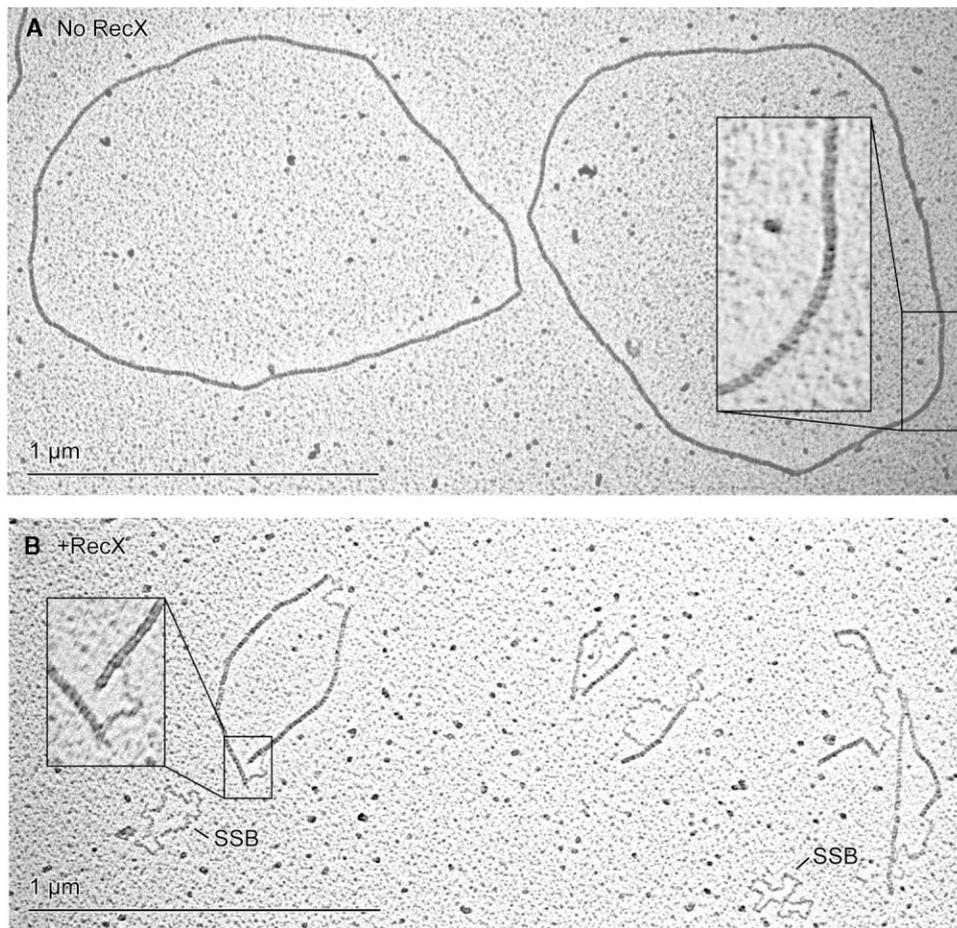
Figure 4. Electron Microscopy of RecA Protein Filaments on ssDNA, with and without Treatment by RecX Protein

Electron micrographs show RecA filaments formed on circular ssDNA in the absence of the RecX protein (A) and after the addition of RecX (B). Reactions include 1.2 μM RecA, 2 μM M13mp8 circular ssDNA, 0.2 μM SSB protein, and 3 mM ATP. RecX protein (to 60 nM) or the equivalent volume of RecX storage buffer ([B] and [A], respectively) were added after RecA nucleoprotein filaments were established. After 10 min the filaments were fixed with ATPγS (to 3 mM). Reaction mixtures were diluted 7-fold (A) or 5-fold (B) before adhesion to the electron microscopy grid.

extension of the filament is suppressed when sufficient RecX protein is present.

The RecX-Mediated Decline in ATP Hydrolysis Reflects RecA Filament Disassembly

In principle, the results of Figures 2 and 3A might be explained if RecX bound to the outside of a RecA filament, and directly affected its ATP hydrolytic activity. Such a mechanism would have to involve a very slow conformational change or other transition to explain the kinetics of the inhibition. This mechanism is rendered unlikely since the effects are seen when RecX is added in low substoichiometric amounts relative to bound RecA protein. Rather than base our conclusions solely on kinetic arguments, we examined RecX-treated RecA filaments directly by electron microscopy to test our prediction that the RecX was triggering a disassembly of the filaments. When RecA filaments were assembled on M13mp8 ssDNA circles, and incubated without RecX protein prior to spreading, a major portion were present as fully filamented circles (Figure 4A). Of 107 molecules examined at random 79 (74%) were full filaments such as those in Figure 4A. Of the remainder, 5 (5%) were nearly complete circular filaments with very short SSB-bound breaks, 15 (14%) were linear filaments (broken circles), and 8 (7%) were single-stranded DNAs to which only SSB was bound. When RecX protein (60 nM) was added to the pre-formed RecA filaments, the effect on the filaments was dramatic (Figure 4B). After 10 min of incubation, there were no complete circular filaments observed among the 192 molecules examined. Over half of the molecules (103 or 54%) exhibited partially disassembled filaments (Figure 4B). Most of the remainder (81 or 42%) contained only bound SSB. There were also 8 (4%) broken or linearized molecules in this sample. No intact circular RecA filaments were observed even in the course of a much more extensive, albeit nonquantitative, examination of the grids. The insets in Figure 4 show that the characteristic striations are present in the RecA filaments both with and without RecX treatment. Thus at this resolution there is no evidence that RecX produces any gross deformations of the structure of any intact segments of filaments that are disassembling in its presence. The compact structure of the DNA
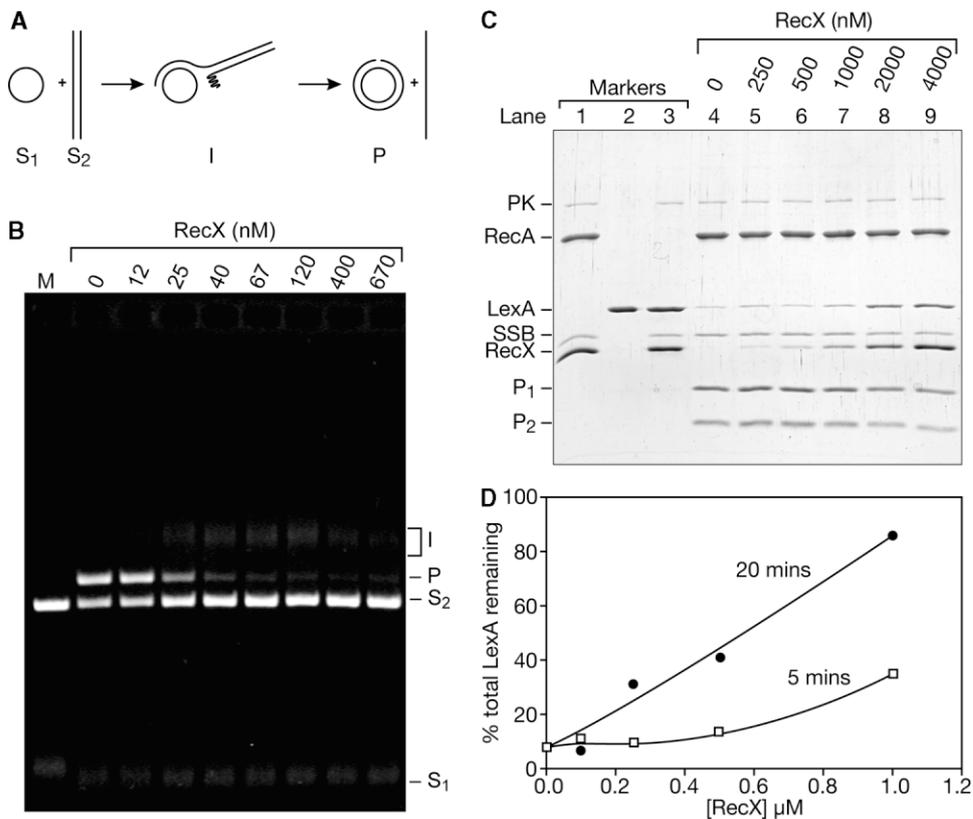
Figure 5. Inhibition of RecA-Mediated DNA Strand Exchange and LexA Cleavage by RecX Protein

(A) Diagram of the DNA three strand exchange reaction. First, a RecA filament is formed on the circular ssDNA (S_1). The linear duplex DNA (S_2) is then aligned with the bound single strand, and exchange is initiated to form a joint molecule intermediate (I). Completion of branch migration generates a nicked circular product (P) and releases the displaced single strand.

(B) Increasing concentrations of RecX protein inhibits the RecA-promoted DNA strand exchange reaction. RecX inhibits formation of nicked circular products (P) while joint molecule intermediates (I) continue to form.

(C) RecX significantly inhibits RecA-mediated LexA cleavage only at RecX concentrations in excess of RecA (3 μM). LexA protein is cleaved into 2 cleavage products (P_1 and P_2). PK (pyruvate kinase) is a component of the ATP regeneration system. DNA strand exchange and LexA cleavage experiments were carried out as described under Experimental Procedures.

(D) Quantitation of LexA cleavage reactions carried out in the presence of RecX protein. RecX is added either 5 min or 20 min (as indicated), prior to the addition of LexA protein.

vacated by RecA filament disassembly in Figure 4B (see the inset and the small DNAs labeled "SSB") is a characteristic of ssDNA that is bound by the SSB protein (Bork et al., 2001a; Shan et al., 1997), which replaces the dissociating RecA protein.

The Inhibition of RecA Protein Activities by RecX Protein Reflects the Presence of Partial (Disassembling) RecA Filaments

We examined the RecA protein-mediated DNA strand exchange reaction in the presence of a range of concentrations of RecX protein (Figures 5A and 5B). In this reaction, the circular single-stranded DNA is first paired to a linear dsDNA to form an intermediate (I in Figure 5A) called a joint molecule, and then this intermediate is resolved by slow branch migration to generate a nicked circular duplex product (P in Figure 5A). A partial RecA filament on the DNA would allow the formation of joint molecules, but the gaps in the filament would preclude the formation of the final products. As RecX protein is titrated into the reaction, a substantial diminution of full product formation is seen at quite low concentrations of RecX (Figure 5B). At the same time, the amounts of joint molecule intermediates (that cannot be resolved) increases. At very high RecX concentrations, the entire strand exchange reaction is abolished, but only at RecX concentrations that are stoichiometric relative to the RecA protein (data not shown).

Effects on the RecA-facilitated LexA repressor cleavage reaction are seen only when substantially higher RecX concentrations are present (Figure 5C). Whereas DNA strand exchange requires a contiguous RecA filament on the ssDNA, relatively short RecA filament tracts can mediate LexA cleavage. As seen in lane 4, LexA is almost completely cleaved into two products (P1 and P2 in Figure 5C) in the absence of RecX within 15 min. When incubated with RecA filaments for 5 min prior to LexA addition, RecX protein does not completely inhibit the LexA cleavage reaction even when it is present in excess of RecA (Figure 5C). However, previous figures demonstrate that complete filament disassembly in the presence of RecX generally requires at least 15 min. When RecX protein was added to the RecA filaments
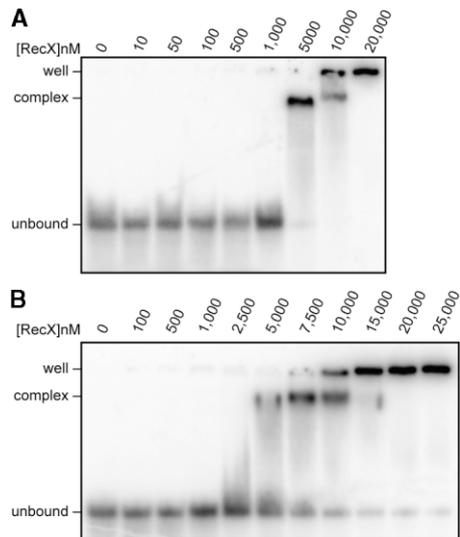
Figure 6. RecX Protein Binding to ssDNA

RecX protein binds to single-stranded DNA only at concentrations in excess of 1 μM. A 51-mer oligonucleotide labeled with ^{32}P (51 nM nucleotides) was incubated with varying concentrations of RecX under reaction conditions similar to those used in ATPase assays. EMSA was used to assess binding. The two panels are similar. (B) is included to provide more data at the higher RecX concentrations.

Figure 7. The RecX Protein Does Not Functionally Interact with RecA Protein Unless the RecA Is Bound to ssDNA

Reactions were carried out as described in the legend to Figure 3. All reactions contained a total of 2.4 μM RecA except the one labeled 1.2 μM RecA. The ssDNA concentration was 2 μM. Pre-incubations were for 5 min at 37°C. Reactions were started by the addition of ATP and SSB, which allows formation of complete RecA filaments. The t = 0 min point is the point at which the last addition was made. RecA at either 1.2 μM or 2.4 μM was in excess of the available RecA binding sites on the DNA (0.67μM), and therefore the rate of ATP hydrolysis was similar in each case. There was little difference in the amount of RecX inhibition whether RecX (60 nM final concentration) was preincubated with half of the 2.4 μM RecA and added after filament formation (RecA→start→RecA/RecX) or whether RecX was added alone after filament formation (RecA→start→RecX).

20 min prior to the addition of LexA protein, the effects of RecX were substantially enhanced (Figure 5D). This experiment suggests that RecX does not inhibit the activities of RecA filaments or segments of filaments by directly altering the function of the bound monomers. Instead, RecA function is lost only as the RecA filaments disassemble.

The results of Figures 2–5 provide evidence that RecX protein is suppressing the extension of RecA filaments, allowing end-dependent filament disassembly to proceed. This results in net disassembly. Two additional experiments were carried out to supplement the available information needed to evaluate potential mechanisms.

The RecX Protein Has a ssDNA Binding Activity
In principle, the effect of RecX could be mediated by a simple binding of RecX to ssDNA that resisted displacement by a growing RecA filament. A limited DNA binding activity has been mentioned in the literature but no data has been provided (Stohl et al., 2003). We therefore used an electrophoretic mobility shift assay (EMSA) to explore RecX binding to ssDNA (Figure 6). We used labeled single strand oligonucleotides, 51 nucleotides in length, at a concentration of 1 nM molecules (51 nM total nucleotides). Although this is lower than the concentration of ssDNA present when RecX is used to challenge RecA filaments as described above, most of the DNA in the experiments of Figures 2–5 begins the assay coated with RecA protein and is unavailable for direct RecX binding. If RecX can bind directly to the very limited ssDNA available in these experiments, it must compete with RecA protein and SSB for binding sites. In addition, any RecX interaction with DNA must compete with the demonstrated RecX binding to RecA protein filaments
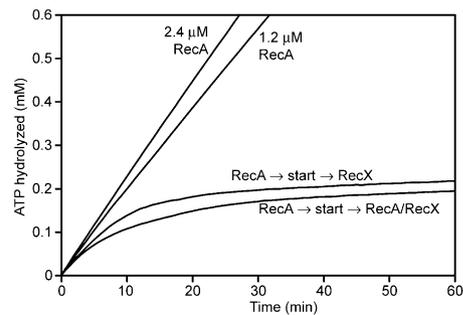
(VanLoock et al., 2003). Thus, the experiment in Figure 6 is designed to explore RecX binding to a relatively low concentration of DNA, under ideal conditions in which there is no competition for the RecX-ssDNA interaction. RecX protein binds to the ssDNA when its concentration exceeds 1 μM (Figure 6). This is consistent with the DNA binding activity of RecX described previously (Stohl et al., 2003). Over 50 times more RecX protein is required to generate a discrete RecX-ssDNA complex than is needed to trigger RecA filament disassembly in Figures 2-5. As detailed in the Discussion, we conclude that the direct ssDNA binding activity of RecX, by itself, is insufficient to explain the blockage of RecA filament extension.

RecX Protein Does Not Functionally Interact with Free RecA Protein
We carried out a somewhat more complex series of experiments to determine if RecX protein interacted with free RecA protein in a manner that would prevent its subsequent interaction with assembled RecA filaments. We divided the RecA solution to be added to an experiment into two parts, adding RecX protein to one of them. In two controls, we added either all (1.2 μM) or twice (2.4 μM) the normal allocation of RecA protein to the DNA to determine the baseline of ATP hydrolysis (Figure 7). Note that even the normal allocation of RecA is in nearly 2-fold excess relative to the 0.67 μM binding sites available in the 2 μM ssDNA. In a third experiment, we added 60 nM RecX protein to the assembled RecA filaments (with 2.4 μM RecA), as before (Figure 7). This produced the gradual decline in ATP hydrolysis seen in the earlier figures. In a fourth experiment, we added 1.2 μM RecA to the DNA, and assembled filaments on the DNA. With the filaments in place, there is still considerable excess (and thus free) RecA present as noted

above. We then challenged these filaments not with RecX, but with a mixture of RecX and free RecA (preincubated without DNA) so as to bring the final RecA and RecX concentrations in the reaction to 2.4 μM and 60 nM respectively. If the RecX protein had bound to the 20-fold excess of free RecA present during the preincubation, it would presumably not be available to affect the pre-assembled RecA filaments or the free RecA (unexposed to RecX) present in the original reaction prior to the challenge. However, the same decline in ATP hydrolysis was observed, indicating that the RecX was completely active in blocking RecA filament extension even though it had been preincubated with a large excess of free RecA protein.

Discussion

We conclude that the RecX protein blocks the normal extension of RecA filaments, as illustrated in Figure 1. We propose that this blockage involves the binding of RecX protein to RecA filaments to cap the assembly ends of the filaments. The disassembly ends are unaffected, and the addition of RecX thereby results in net filament disassembly.

The conclusion that RecX blocks RecA filament extension is based on multiple observations. First, RecX exerts a considerable inhibitory effect on RecA filaments at concentrations that are much lower than the RecA concentration. The RecX protein clearly does not have to interact with every RecA monomer to exert its effect. Second, the decline in RecA filaments is slow, occurring over tens of minutes in a manner consistent with normal end-dependent filament disassembly. This implies that normal filament disassembly is proceeding. Further, if *net* disassembly is occurring on circular single-stranded DNA, then RecA filament extension must be blocked (see Figure 1). Third, the effects of RecX are amplified if it is added prior to RecA filament formation rather than after the filaments are formed (Figures 2 and 3). This further implicates RecX in the inhibition of some aspect of RecA filament assembly. Fourth, RecX addition has an obvious and catastrophic effect on RecA filaments as seen by electron microscopy (Figure 4). Fifth, much less RecX protein is required to inhibit DNA strand exchange (which requires intact RecA filaments) than is needed to inhibit the LexA cleavage reaction (which requires only short segments of RecA filament) (Figure 5).

RecX appears to interact functionally only with RecA filaments on DNA. The inhibitory effects of RecX were not measurably lessened by preincubation of the RecX protein with a 20-fold excess of free RecA protein (Figure 7).

We base the proposal of a RecA filament capping activity for RecX on the observations enumerated above, additional data presented here and in the literature, and a general consideration of possible mechanisms for suppressing filament extension. In principle, one could consider three kinds of mechanisms that might reasonably explain the RecX-imposed block to RecA filament extension: (a) a RecX-mediated change in the overall filament conformation to a form unable to support extension, (b) a binding of RecX protein directly to ssDNA so as to provide a passive barrier to RecA filament extension, or (c) the proposed capping mechanism.

In mechanism (a), RecX protein would act indirectly on the extension process by altering the filament as a whole. There is evidence that RecX binds along the length of a RecA filament, at least when high levels of RecX are present (VanLoock et al., 2003). However, several observations argue against an indirect effect of RecX on filament extension: First, RecX exerts its maximum effect at low, sub-stoichiometric levels relative to the RecA concentration. If RecX affects RecA filament extension by binding along the filament, it must often act at a substantial distance. However, when RecA is bound to ssDNA, cooperativity is limited. For example, the ATP hydrolytic cycle in a monomer within such filaments has little effect on the hydrolytic cycle of its neighbors (Arenson et al., 1999, Shan and Cox, 1996). Second, there are no major changes elicited in the core structure of RecA filaments by RecX binding, as seen in electron microscopy image reconstructions (VanLoock et al., 2003). Third, although the RecX protein is triggering RecA filament disassembly, the fragments that remain as the disassembly proceeds are active. These fragments promote DNA pairing (Figure 5), LexA cleavage (Figure 5), and ATP hydrolysis (Figures 2–3; the ATPase does not simply stop when RecX is added, but declines at a pace reflecting filament disassembly). Thus, if RecX affects filament conformation, the change is not manifested in filament activity.

The DNA binding data of Figure 6 argues against mechanism (b). RecX possesses a readily detected ssDNA binding activity (Figure 6). However, the binding appears insufficient to explain the efficient blockage of RecA filament extension that RecX brings about. Since SSB is readily displaced by growing RecA filaments, RecX binding to ssDNA would presumably have to occur with higher affinity than the SSB-ssDNA interaction if it is to block RecA filament extension. There is little free ssDNA in the pre-assembled RecA filaments used in this study, and no RecX binding to ssDNA is observed at RecX concentrations that produce a dramatic disassembly of RecA filaments. Thus, a model envisioning a passive block to RecA filament disassembly due to RecX binding to ssDNA does not satisfactorily explain most of the data in this study. At the same time, there is ample evidence that RecX interacts directly with RecA protein. This includes published evidence both in vitro and in vivo (Stohl, et al., 2003, VanLoock et al., 2003). In addition, multiple RecA mutant proteins have been identified that moderate the effects of RecX on RecA (J.C.D., unpublished results), consistent with the notion that the effects of RecX involve a direct interaction of RecX with RecA filaments.

In those few experiments where RecX protein is added to an experiment with or prior to RecA protein (e.g., Figure 3B), there is potentially greater opportunity for RecX to bind DNA and form the passive block envisioned in model (b). The effect of RecX is actually greater when this order of addition is applied. However, this does not provide an argument in favor of model (b). Any RecX mechanism that blocks RecA filament extension will lead to a more robust inhibitory effect if RecX is added before RecA filaments have formed (since the observed inhibition is then not limited by slow filament disassem-

bly). Further, model (b) provides no role for the documented interaction between RecX and RecA described above. Finally, a viable model must explain all of the data, not part of it.

Model (c) readily explains all of the data in this and previously published studies. The very low concentrations at which RecX protein acts are rationalized. The direct interaction between RecX and RecA is required for model (c). We thus feel that a RecA filament capping function, involving an interaction of RecX at the growing end of assembled RecA filaments, provides the best interpretation of the available data, as well as a clean and internally consistent explanation of RecX function.

When RecX is present at higher concentrations than those used in the present study, and when RecA filaments are fixed by incubation with the non-hydrolyzed ATP analog AMPPNP, RecX binding can be observed along the length of RecA filaments (VanLoock et al., 2003). The RecX monomers bind so as to span the interface between adjacent RecA monomers in the filament. We do not preclude the possibility that RecX thus bound may have additional effects on RecA function. The RecX might destabilize monomer-monomer interfaces so as to create new filament ends, alter the interaction of other proteins with RecA filaments, or have other effects. Further study is needed to evaluate these possibilities.

The RecX protein regulates RecA filament assembly. RecX will limit the length of RecA filaments on ssDNA, and genetic studies indicate that RecX should be present most of the time. RecX now takes its place within the growing repertoire of bacterial proteins that modulate the assembly and disassembly of RecA filaments. These proteins include the RecFOR proteins (Bork et al., 2001b; Morimatsu and Kowalczykowski, 2003; Shan et al., 1997; Webb et al., 1995; Webb et al., 1997), the DinI protein (Lusetti et al., 2004; Voloshin et al., 2001; Yasuda et al., 1998; Yasuda et al., 2001), and probably the F plasmid-encoded PsiB protein (Bagdasarian et al., 1992; Bailone et al., 1988; Golub et al., 1988). While the PsiB protein has not yet been characterized, all of the other proteins affect RecA filaments in a unique way, forming a complementary network that provides a potentially exquisite fine-tuning of the filament assembly and disassembly processes. Such regulation is typical of other filamentous systems, including microtubules (Bokoch, 2003; Drewes et al., 1998; Wasteneys and Galway, 2003) and actin filaments (McGough et al., 2003; Schafer and Cooper, 1995). The capping function we propose for RecX is unprecedented for RecA-family proteins, but analogous to the capping of actin filaments by the gelsolin family of proteins (McGough et al., 2003; Schafer and Cooper, 1995).

Experimental Procedures

Enzymes and Biochemicals
The E. coli wild-type RecA protein was purified as described (Lusetti et al., 2003b). The concentration of the purified protein was determined from the absorbance at 280 nm using the extinction coefficient 2.23×10^4 M^{-1} cm^{-1} (Craig and Roberts, 1981). The E. coli SSB protein was purified as described (Shan et al., 1996). The concentration of the purified protein was determined from the absorbance at 280 nm using the extinction coefficient 2.83×10^4 M^{-1} cm^{-1} (Lohman and Overman, 1985). The purification of the native RecX protein and the determination of its extinction coefficient will

be described elsewhere (J.C.D., S.L.L., and M.M.C., unpublished data). RecX protein is stored in 20 mM Tris-HCl (80% cation, pH 7.5), 1 mM DTT, 0.1 mM EDTA, 100 mM potassium glutamate, and 50% (w/v) glycerol. The concentration of the purified protein was determined from the absorbance at 280 nm using the extinction coefficient of $\epsilon = 2.57 \times 10^4$ M^{-1} cm^{-1}. Our work with native RecX protein has shown that RecX is somewhat unstable during storage at $-80°C$, with measurable loss of activity evident after 4 months. All experiments reported here were carried out within 12 weeks of the purification of a given RecX preparation. In addition, fresh aliquots were thawed and used on the same day of any given experiment.

Unless otherwise noted, all reagents were purchased from Fisher and were of the highest grade available. XhoI restriction endonuclease and T4 polynucleotide kinase were purchased from MBI Fermentas. DTT was obtained from Research Organics. Phosphoenolpyruvate (PEP), pyruvate kinase, ATP, bromphenol blue, lactate dehydrogenase, HEPES, and NADH were purchased from Sigma. Ficoll was from Pharmacia.

DNA Substrates
Bacteriophage ϕX174 circular single-stranded DNA (virion) was purchased from New England Biolabs. ϕX174 RF I supercoiled circular duplex DNA was purchased from Invitrogen. Full-length linear duplex DNA was generated by the digestion of ϕX174 RF I DNA (5386 bp) with XhoI endonuclease, using conditions suggested by the supplier. Circular single-stranded DNA from bacteriophage M13mp8 (7229 nucleotides) was prepared using previously described methods (Neuendorf and Cox, 1986). The concentrations of ssDNA and dsDNA were determined by absorbance at 260 nm, using 36 and 50 μg ml^{-1} A$_{260}$$^{-1}$, respectively, as conversion factors. All DNA concentrations are given in μM nucleotides.

ATPase Assay
A coupled spectrophotometric enzyme assay (Lindsley and Cox, 1990; Morrical et al., 1986) was used to measure the DNA-dependent ATPase activities of the RecA protein. The regeneration of ATP from PEP and ADP was coupled to the oxidation of NADH and followed by the decrease in absorbance of NADH at 380 nm (380 nm wavelength was used so that the signal remained within the linear range of the spectrophotometer for the duration of the experiment). The assays were carried out on a Varian Cary 300 dual beam spectrophotometer equipped with a temperature controller and a 12-position cell changer. The cell path length and band pass were 1 cm and 2 nm, respectively. The NADH extinction coefficient at 380 nm of 1.21 mM^{-1} cm^{-1} was used to calculate the rate of ATP hydrolysis.

The reactions were carried out at 37°C in 25 mM Tris-OAc (80% cation, pH 7.4), 1 mM DTT, 3 mM potassium glutamate, 10 mM Mg(OAc)$_2$, 5% (w/v) glycerol, an ATP regeneration system (10 units/ml pyruvate kinase and 3.5 mM phosphoenolpyruvate), a coupling system (1.5 mM NADH and 10 units/ml lactate dehydrogenase), and 2 μM M13mp8 circular single-stranded DNA. The aforementioned components were incubated for 10 min. The figure legends note the time of addition of wild-type RecA protein (1.2 μM), the RecX protein (concentration indicated in the figure legends), the SSB protein (to 0.2 μM), and ATP (to 3 mM). For Figure 2 only, these conditions were altered slightly to 3 mM NADH, 3 μM RecA, 5 μM DNA, and 0.5 μM SSB. A cell path length of 0.5 cm was used in these experiments.

DNA Three-Strand Exchange Reaction Promoted by the RecA Protein
Three-strand exchange reactions were carried out in 25 mM Tris-OAc (80% cation, pH 7.4), 1 mM DTT, 5% (w/v) glycerol, 3 mM potassium glutamate, and 10 mM Mg(OAc). An ATP regeneration system (10 units/ml pyruvate kinase and 2 mM phosphoenolpyruvate) was also included. All incubations were carried out at 37°C. The following are final concentrations. The wild-type RecA protein (6.7 μM) was preincubated with 20 μM ϕX174 circular ssDNA for 10 min. SSB protein (2 μM) and ATP (3 mM) were then added, followed by another 6 min of incubation. RecX protein was added to the reactions at the concentrations given in the figure legends and incubated for 6 min before the reactions 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, 0.24% bromphenol blue, 0.24% xylene cyanol, and 4% SDS. Samples were subjected to electrophoresis in 0.8% agarose gels with 1× TAE buffer, stained with ethidium bromide, and exposed to ultraviolet light. Gel images were captured with a digital CCD camera utilizing GelExpert software (Nucleotech).

RecA Protein-Facilitated LexA Cleavage Assay
Reactions were carried out in 25 mM Tris-OAc (80% cation, pH 7.4), 1 mM DTT, 5% (w/v) glycerol, 3 mM potassium glutamate, 3 mM Mg(OAc)$_2$, and an ATP regeneration system (2 mM phosphoenolpyruvate and 10 units/ml pyruvate kinase). The following are final concentrations. All incubations were at 37°C. The wild-type RecA protein (3 μM) was preincubated with 9 μM ϕX174 circular ssDNA for 5 min. The SSB protein (0.9 μM) and ATP (3 mM) were then added and the reactions were incubated for 5 min. RecX was added to the reactions at concentrations listed in figure legends and incubated for 5 or 20 min. The LexA protein (3 μM) was added to start the reaction. All reactions were incubated for 15 min. Laemmli sample buffer (250 mM Tris-Cl pH 6.8, 4% SDS, 20% w/v glycerol, 10% β-mercaptoethanol, and 0.1% w/v bromphenol blue) was added to stop the reaction. Samples were subjected to SDS-PAGE electrophoresis on 17% acrylamide gels and stained with Coomassie brilliant blue.

Electron Microscopy
A modified Alcian method was used to visualize RecA filaments. Activated grids were prepared as described previously (Lusetti et al., 2003b). Samples for electron microscopy were prepared by preincubating 1.2 μM RecA and 2 μM M13mp8 circular ssDNA, 25 mM Tris-OAc (80% cation) buffer, 1 mM DTT, 5% (w/v) glycerol, 3 mM potassium glutamate, and 10 mM Mg(OAc)$_2$ for 10 min. All incubations were at 37°C. An ATP regeneration system of 3 mM phosphoenolpyruvate and 10 units/ml pyruvate kinase was also included in the preincubation. ATP and SSB were added to 3 mM and 0.2 μM, respectively, followed by a 15 min incubation. The RecX protein or the equivalent volume of RecX storage buffer was added, followed by another 10 min incubation. ATPγS was then added to 3 mM, followed by another 3 min incubation. The reaction mixture described above was diluted as indicated in the figure legend with 200 mM ammonium acetate, 2 mM HEPES (pH 7.5), and 10% glycerol. The sample was prepared for analysis as described (Lusetti et al., 2004).

Electrophoretic Mobility Shift Assays
A single-stranded 51-mer oligonucleotide was ^{32}P end-labeled using T4 polynucleotide kinase. The labeled DNA was used at 51 nM (in total nucleotides) in DNA binding reactions containing 25 mM Tris-OAc (80% cation, pH 7.4), 1 mM DTT, 3 mM potassium glutamate, 10 mM Mg(OAc)$_2$, 5% (w/v) glycerol, 3.5 mM phosphoenolpyruvate, 1.5 mM NADH, and 3 mM ATP. The aforementioned components were incubated at 37°C with varying concentrations of RecX protein or RecX storage buffer. After 10 min, 10 μl of each reaction were added to 5 μl of loading dye (9% ficoll, 10 mM Tris-OAc 80% cation, and 0.125% xylene cyanol) and the reactions were loaded onto a native 8% polyacrylamide gel and subjected to electrophoresis in TBE buffer (90 mM Tris-borate and 10 mM EDTA).

References

Arenson, T.A., Tsodikov, O.V., and Cox, M.M. (1999). Quantitative analysis of the kinetics of end-dependent disassembly of RecA filaments from ssDNA. J. Mol. Biol. 288, 391–401.

Bagdasarian, M., Bailone, A., Angulo, J.F., Scholz, P., and Devoret, R. (1992). PsiB, an anti-SOS protein, is transiently expressed by the F sex factor during its transmission to an Escherichia coli K-12 recipient. Mol. Microbiol. 6, 885–893.

Bailone, A., Backman, A., Sommer, S., Celerier, J., Bagdasarian, M.M., Bagdasarian, M., and Devoret, R. (1988). PsiB polypeptide prevents activation of RecA protein in Escherichia coli. Mol. Gen. Genet. 214, 389–395.

Benedict, R.C., and Kowalczykowski, S.C. (1988). Increase of the DNA strand assimilation activity of RecA protein by removal of the C terminus and structure-function studies of the resulting protein fragment. J. Biol. Chem. 263, 15513–15520.

Bokoch, G.M. (2003). Biology of the p21-activated kinases. Annu. Rev. Biochem. 72, 743–781.

Bork, J.M., Cox, M.M., and Inman, R.B. (2001a). RecA protein filaments disassemble in the 5′ to 3′ direction on single-stranded DNA. J. Biol. Chem. 276, 45740–45743.

Bork, J.M., Cox, M.M., and Inman, R.B. (2001b). The RecOR proteins modulate RecA protein function at 5′ ends of single-stranded DNA. EMBO J. 20, 7313–7322.

Cox, M.M. (2001). Historical overview: searching for replication help in all of the rec places. Proc. Natl. Acad. Sci. USA 98, 8173–8180.

Cox, M.M. (2003). The bacterial RecA protein as a motor protein. Annu. Rev. Microbiol. 57, 551–577.

Cox, M.M., Goodman, M.F., Kreuzer, K.N., Sherratt, D.J., Sandler, S.J., and Marians, K.J. (2000). The importance of repairing stalled replication forks. Nature 404, 37–41.

Craig, N.L., and Roberts, J.W. (1981). Function of nucleoside triphosphate and polynucleotide in Escherichia coli recA protein-directed cleavage of phage lambda repressor. J. Biol. Chem. 256, 8039–8044.

De Mot, R., Schoofs, G., and Vanderleyden, J. (1994). A putative regulatory gene downstream of recA is conserved in gram-negative and gram-positive bacteria. Nucleic Acids Res. 22, 1313–1314.

Drewes, G., Ebneth, A., and Mandelkow, E.M. (1998). Maps, marks and microtubule dynamics. Trends Biochem. Sci. 23, 307–311.

Eggler, A.L., Lusetti, S.L., and Cox, M.M. (2003). The C terminus of the Escherichia coli RecA protein modulates the DNA binding competition with single-stranded DNA-binding protein. J. Biol. Chem. 278, 16389–16396.

Golub, E., Bailone, A., and Devoret, R. (1988). A gene encoding an SOS inhibitor is present in different conjugative plasmids. J. Bacteriol. 170, 4392–4394.

Kim, B., and Little, J.W. (1993). LexA and lambda CI repressors as enzymes: specific cleavage in an intermolecular reaction. Cell 73, 1165–1173.

Kowalczykowski, S.C. (2000). Initiation of genetic recombination and recombination-dependent replication. Trends Biochem. Sci. 25, 156–165.

Kuzminov, A. (1999). Recombinational repair of DNA damage in Escherichia coli and bacteriophage lambda. Microbiol. Mol. Biol. Rev. 63, 751–813.

Lindsley, J.E., and Cox, M.M. (1990). Assembly and disassembly of RecA protein filaments occurs at opposite filament ends: relationship to DNA strand exchange. J. Biol. Chem. 265, 9043–9054.

Lohman, T.M., and Overman, L.B. (1985). Two binding modes in Escherichia coli single strand binding protein-single stranded DNA complexes. J. Biol. Chem. 260, 3594–3603.

Lusetti, S.L., and Cox, M.M. (2002). The bacterial RecA protein and the recombinational DNA repair of stalled replication forks. Annu. Rev. Biochem. 71, 71–100.

Lusetti, S.L., Shaw, J.J., and Cox, M.M. (2003a). Magnesium ion-dependent activation of the RecA protein involves the C terminus. J. Biol. Chem. 278, 16381–16388.

Lusetti, S.L., Voloshin, O.N., Inman, R.B., Camerini-Otero, C.S., and Cox, M.M. (2004). The DinI protein stabilizes RecA protein filaments. J. Biol. Chem. *279*, 30037–30046.

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. (2003b). C-terminal deletions of the *Escherichia coli* RecA protein. J. Biol. Chem. *278*, 16372–16380.

McGough, A.M., Staiger, C.J., Min, J.K., and Simonetti, K.D. (2003). The gelsolin family of actin regulatory proteins. FEBS Lett. *552*, 75–81.

Morimatsu, K., and Kowalczykowski, S.C. (2003). RecFOR proteins load RecA protein onto gapped DNA to accelerate DNA strand exchange. Mol. Cell *11*, 1337–1347.

Morrical, S.W., and Cox, M.M. (1990). Stabilization of RecA protein-ssDNA complexes by the single-stranded DNA binding protein of *Escherichia coli*. Biochemistry *29*, 837–843.

Morrical, S.W., Lee, J., and Cox, M.M. (1986). Continuous association of *Escherichia coli* single-stranded DNA binding protein with stable complexes of RecA protein and single-stranded DNA. Biochemistry *25*, 1482–1494.

Nastri, H.G., and Knight, K.L. (1994). Identification of residues in the L1 region of the RecA protein which are important to recombination or coprotease activities. J. Biol. Chem. *269*, 26311–26322.

Neuendorf, S.K., and Cox, M.M. (1986). Exchange of RecA protein between adjacent RecA protein-single-stranded DNA complexes. J. Biol. Chem. *261*, 8276–8282.

Pages, V., Koffel-Schwartz, N., and Fuchs, R.P. (2003). recX, a new SOS gene that is co-transcribed with the recA gene in *Escherichia coli*. DNA Repair (Amst.) *2*, 273–284.

Papavinasasundaram, K.G., Movahedzadeh, F., Keer, J.T., Stoker, N.G., Colston, M.J., and Davis, E.O. (1997). Mycobacterial recA is cotranscribed with a potential regulatory gene called recX. Mol. Microbiol. *24*, 141–153.

Register, J.C., III, and Griffith, J. (1985). The direction of RecA protein assembly onto single strand DNA is the same as the direction of strand assimilation during strand exchange. J. Biol. Chem. *260*, 12308–12312.

Roca, A.I., and Cox, M.M. (1997). RecA protein: structure, function, and role in recombinational DNA repair. Prog. Nuc. Acid Res. Mol. Biol. *56*, 129–223.

Sano, Y. (1993). Role of the recA-related gene adjacent to the recA gene in Pseudomonas aeruginosa. J. Bacteriol. *175*, 2451–2454.

Schafer, D.A., and Cooper, J.A. (1995). Control of actin assembly at filament ends. Annu. Rev. Cell Dev. Biol. *11*, 497–518.

Shan, Q., and Cox, M.M. (1996). RecA protein dynamics in the interior of RecA nucleoprotein filaments. J. Mol. Biol. *257*, 756–774.

Shan, Q., Cox, M.M., and Inman, R.B. (1996). DNA strand exchange promoted by RecA K72R. J. Biol. Chem. *271*, 5712–5724.

Shan, Q., Bork, J.M., Webb, B.L., Inman, R.B., and Cox, M.M. (1997). RecA protein filaments: end-dependent dissociation from ssDNA and stabilization by RecO and RecR proteins. J. Mol. Biol. *265*, 519–540.

Stohl, E.A., and Seifert, H.S. (2001). The recX gene potentiates homologous recombination in *Neisseria gonorrhoeae*. Mol. Microbiol. *40*, 1301–1310.

Stohl, E.A., Brockman, J.P., Burkle, K.L., Morimatsu, K., Kowalczykowski, S.C., and Seifert, H.S. (2003). *Escherichia coli* RecX inhibits RecA recombinase and coprotease activities in vitro and in vivo. J. Biol. Chem. *278*, 2278–2285.

Sukchawalit, R., Vattanaviboon, P., Utamapongchai, S., Vaughn, G., and Mongkolsuk, S. (2001). Characterization of *Xanthomonas oryzae* pv. oryzae recX. FEMS Microbiol. Lett. *205*, 83–89.

Tateishi, S., Horii, T., Ogawa, T., and Ogawa, H. (1992). C-terminal truncated *Escherichia coli* RecA protein RecA5327 has enhanced binding affinities to single- and double-stranded DNAs. J. Mol. Biol. *223*, 115–129.

VanLoock, 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). Complexes of RecA with LexA and RecX differentiate between active and inactive RecA nucleoprotein filaments. J. Mol. Biol. *333*, 345–354.

Venkatesh, R., Ganesh, N., Guhan, N., Reddy, M.S., Chandrasekhar, T., and Muniyappa, K. (2002). RecX protein abrogates ATP hydrolysis and strand exchange promoted by RecA. Proc. Natl. Acad. Sci. USA *99*, 12091–12096.

Vierling, S., Weber, T., Wohlleben, W., and Muth, G. (2000). Transcriptional and mutational analyses of the *Streptomyces lividans* recX gene and its interference with RecA activity. J. Bacteriol. *182*, 4005–4011.

Voloshin, O.N., Ramirez, B.E., Bax, A., and Camerini-Otero, R.D. (2001). A model for the abrogation of the SOS response by an SOS protein, Genes Dev.elop. *15*, 415–27.

Wasteneys, G.O., and Galway, M.E. (2003). Remodelling the cytoskeleton for growth and form. Annu. Rev. Plant Biology *54*, 691–722.

Webb, B.L., Cox, M.M., and Inman, R.B. (1995). An interaction between the Escherichia coli RecF and RecR proteins dependent on ATP and double-stranded DNA. J. Biol. Chem. *270*, 31397–31404.

Webb, B.L., Cox, M.M., and Inman, R.B. (1997). Recombinational DNA repair—the RecF and RecR proteins limit the extension of RecA filaments beyond single-strand DNA gaps. Cell *91*, 347–356.

Yang, M.K., Chou, M.E., and Yang, Y.C. (2001). Molecular characterization and expression of the recX gene of Xanthomonas campestris pv. citri. Curr. Microbiol. *42*, 257–263.

Yasuda, T., Morimatsu, K., Horii, T., Nagata, T., and Ohmori, H. (1998). Inhibition of *Escherichia coli* RecA coprotease activities by DinI. EMBO J. *17*, 3207–3216.

Yasuda, T., Morimatsu, K., Kato, R., Usukura, J., Takahashi, M., and Ohmori, H. (2001). Physical interactions between DinI and RecA nucleoprotein filament for the regulation of SOS mutagenesis. EMBO J. *20*, 1192–1202.