

# SSB Antagonizes RecX-RecA Interaction\*

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The RecX protein of *Escherichia coli* inhibits the extension of RecA protein filaments on DNA, presumably by binding to and blocking the growing filament end. The direct binding of RecX protein to single-stranded DNA is weak, and previous reports suggested that direct binding to DNA did not explain the effects of RecX. We now demonstrate that elevated concentrations of SSB greatly moderate the effects of RecX protein. High concentrations of the yeast RPA protein have the same effect, suggesting that the effect is not species-specific or even specific to bacterial SSB proteins. A direct SSB-RecX interaction is thus unlikely. We suggest that SSB is blocking access to single-stranded DNA. The evident competition between RecX and SSB implies that the mechanism of RecX action may involve RecX binding to both RecA protein and to DNA. We speculate that the interaction of RecX protein and RecA may enable an enhanced DNA binding by RecX protein. The effects of SSB are increased if the SSB C terminus is removed.

The bacterial RecA protein is the prototype of a nearly ubiquitous class of recombinase proteins (1–11). These enzymes promote DNA strand exchange reactions that lie at the heart of all recombination and recombinational DNA repair processes (11–16). The recombinase forms a right-handed helical filament, usually on single-stranded DNA (ssDNA).<sup>2</sup> A homologous duplex DNA is then aligned with this bound single strand, and one strand (the one complementary to the bound single strand) is transferred from the duplex substrate to form a new duplex within the filament. This process is used to reconstruct stalled replication forks, repair double strand breaks, facilitate meiotic cross-overs in eukaryotes, and facilitate genetic exchanges of many types in almost every organism. Typical reactions are illustrated in Fig. 1.

The assembly and disassembly of RecA protein filaments on DNA has been studied in some detail, although understanding is not complete. Nucleation occurs most readily on ssDNA. There are discrete nucleation and extension phases in the assembly process, with extension proceeding primarily 5' to 3' relative to the ssDNA (17, 18). Disassembly also proceeds primarily 5' to 3', with monomeric subunits being added on one

end and subtracted from the other (19, 20). Dissociation at the disassembly end is coupled to ATP hydrolysis (21, 22).

RecA-promoted reactions are important to cell viability. *Escherichia coli* strains lacking a functional *recA* gene are viable but highly sensitive to DNA-damaging agents (23–25). Recombinational DNA repair is thus important in bacteria, especially for the repair of stalled or collapsed replication forks (26–30). However, recombination can also have deleterious consequences and must be regulated. Recombination between repeated sequences in the genome, for example, could result in the deletion of essential genes.

In the past decade, multiple new RecA regulatory mechanisms have been elucidated. The expression of the *recA* gene is regulated as part of the SOS response (31–35). The native RecA protein is auto-regulated by the 17 amino acid residues at its own C terminus, because virtually every RecA activity is enhanced when those 17 amino acids are deleted (36–41). Finally, RecA function is regulated by a growing array of regulatory proteins.

There are at least eight proteins in a typical *E. coli* cell that modulate RecA protein function to some extent. Much of the regulation is focused on RecA filament assembly and disassembly. One modulator of RecA filament dynamics is SSB. SSB strongly inhibits the nucleation phase of RecA filament assembly (42) but stimulates the extension phase, removing secondary structure in ssDNA that would otherwise impede filament growth (43–46). Although ordinarily SSB protein prevents filament nucleation, single RecA monomers can easily be added to an existing filament and displace SSB from DNA at the rate of filament extension (47).

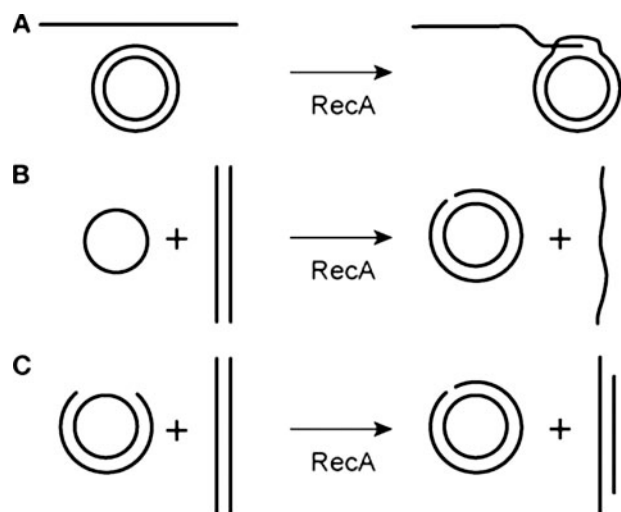
Additional proteins affect other aspects of RecA protein filament function, often by interacting directly with RecA. The nucleation barrier represented by SSB is overcome by the RecO and RecR proteins (48, 49), perhaps with help from the RecF protein (50). Another protein, DinI, stabilizes RecA protein filaments and prevents filament dissociation (51). The RecX protein blocks RecA filament extension (52). The RdgC protein can block RecA protein activities by excluding RecA from duplex DNA (53). The PsiB protein, encoded by some F plasmids, may also block RecA function (54–56). The RecF protein can facilitate RecA protein filament extension by antagonizing RecX function (57).

The RecX protein is the focus of the present report and an inhibitor of RecA function both *in vivo* and *in vitro* (58). Seifert and co-workers (58) demonstrated that RecA and RecX proteins physically interact using yeast two-hybrid analysis in *E. coli*. The RecX protein binds deep within the major helical groove of a RecA filament (59). Electron microscopy image reconstructions indicate that when RecX protein is bound to an

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<sup>2</sup> The abbreviations used are: ssDNA, single-stranded DNA; gDNA, gapped DNA substrate.



**FIGURE 1. DNA pairing and strand exchange promoted by the RecA protein.** Three typical reactions are shown. *A*, D-loop formation. The reaction involves the insertion of a linear single-strand DNA (or short oligonucleotide) into a duplex DNA, with a strand of the duplex displaced over the length of the paired inserted DNA. For highest yields, the duplex DNA is often a supercoiled circle. *B*, the three strand exchange reaction. *C*, the four-strand exchange reaction (initiated as a three-strand reaction in the gap of the DNA circle).

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 (59). RecX possesses a limited ssDNA binding activity (52). The effect of RecX is actually greater where RecX protein is added to an experiment prior to RecA protein (52). The inhibition of RecA ATPase activity by RecX is greater in the presence of SSB than in the absence of SSB (58).

The RecX protein is effective in halting RecA filament extension, and a previous study suggested that RecX acts via a capping mechanism (52). The binding of RecX to the growing end of a RecA filament would simply preclude further RecA protein additions at that end (52). The apparently weak binding of RecX to ssDNA suggested that RecX did not simply bind to the DNA and act as an inert barrier to extension but instead interacted directly with the RecA protein. The function of RecX binding elsewhere along the RecA filament is unclear.

In the present study, we demonstrate that elevated concentrations of SSB interfere with RecX function. The patterns of interference indicate a direct competition between SSB and RecX for a ligand, presumably ssDNA, and suggest that an interaction of RecX with ssDNA may be part of the mechanism by which RecX halts RecA filament extension. The RecX-ssDNA interaction may be affected by RecA protein and thus underestimated in the earlier work.

## EXPERIMENTAL PROCEDURES

**Enzymes and Reagents**—The *E. coli* WT RecA protein was overexpressed and purified as described previously (40). The concentration of the purified protein was determined from the absorbance at 280 nm using the extinction coefficient  $2.23 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$  (60). The *E. coli* SSB protein was purified as described (61). The concentration of the purified protein was determined from the absorbance at 280 nm using the extinction

coefficient  $2.83 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$  (62). The RecX protein was purified as described (63). The concentration of RecX protein was determined from the absorbance at 280 nm using the extinction coefficient  $2.57 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$  (63). The concentrations of RPA protein was determined by UV absorption at 280 nm using the extinction coefficients of  $8.8 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$  (64). Unless otherwise noted, all of the reagents were purchased from Fisher and were of the highest grade available. Dithiothreitol was obtained from Research Organics. Phosphoenolpyruvate, pyruvate kinase, lactate dehydrogenase, ATP, and NADH were purchased from Sigma.

**DNA Substrates**—Circular ssDNA from bacteriophage M13mp8 (7229 nucleotides) was prepared using previously described methods (65–67). Gapped DNA substrate (gDNA) was prepared by annealing four oligonucleotides ( $10 \mu\text{M}$  nucleotides each) to M13mp8 circular single-stranded DNA ( $500 \mu\text{M}$  nucleotides) as described previously (50). The sequences of the oligonucleotides are complementary to the M13mp8 ssDNA sequences corresponding to nucleotides 224–255, 1005–1039, 2514–2545, and 4728–4760, with reference to the standard map of the bacteriophage (68). Poly(dT) (average length, 229 nucleotides; lot GG0076) was purchased from Amersham Biosciences. The concentrations of bacteriophage ssDNA and gDNA were determined by absorbance at 260 nm, using  $36 \mu\text{g ml}^{-1} \text{ A}260^{-1}$  as conversion factor. All of the DNA concentrations are reported in the form of total nucleotides ( $\mu\text{M}$ ).

**ATPase Assay**—A coupled spectrophotometric enzyme assay (20, 69) was used to measure the DNA-dependent ATPase activities of the wild type RecA. The regeneration of ATP from phosphoenolpyruvate and ADP was coupled to the oxidation of NADH and followed by the decrease in absorbance of NADH at 380 nm. The assays were carried out on a Varian Cary 300 dual beam spectrophotometer equipped with a temperature controller. The cell path length and band pass were 1 cm and 2 nm, respectively. The NADH extinction coefficient at 380 nm of  $1.21 \text{ mM}^{-1} \text{ cm}^{-1}$  was used to calculate the rate of ATP hydrolysis. The reactions were carried out at  $37^\circ\text{C}$  in 25 mM Tris-OAc (80% cation, pH 7.4), 1 mM dithiothreitol, 3 mM potassium glutamate, 10 mM  $\text{Mg}(\text{OAc})_2$ , 5% (w/v) glycerol with an ATP regeneration system (20 units/ml pyruvate kinase and 4 mM phosphoenolpyruvate), a coupling system (3 mM NADH and 20 units/ml lactate dehydrogenase), 3 mM ATP and  $5 \mu\text{M}$  M13mp8 circular single-stranded DNA or gDNA. The aforementioned components and RecA protein ( $3 \mu\text{M}$ ) were incubated together for 5 min prior to the addition of the SSB protein. SSB was added to the indicated concentration, and the reaction was challenged 7 min later by the addition of RecX protein. The addition of RecX protein defines  $t = 0$  for each experiment unless otherwise noted.

In the experiments with poly(dT), the concentrations of ssDNA and RecA protein were reduced to 4 and  $2.4 \mu\text{M}$ , respectively. The incubation time before ATP and RecX addition was 10 min each. The concentrations of the components of the regeneration system and coupling system were 10 units/ml pyruvate kinase, 3.5 mM phosphoenolpyruvate, 10 units/ml lactate dehydrogenase, and 1.5 mM NADH.

## Regulation of RecA by RecX

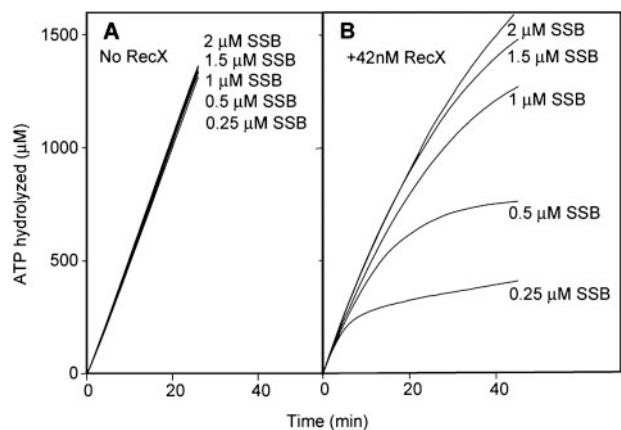


FIGURE 2. **SSB protein antagonizes RecX function.** The reactions were carried out as described under "Experimental Procedures" and contained 3  $\mu\text{M}$  RecA protein, 5  $\mu\text{M}$  M13mp8 ssDNA, 3 mM ATP, and (where indicated) 42 nM RecX. The SSB protein concentration was varied as indicated.

### RESULTS

**Experimental Rationale**—The RecA protein hydrolyzes ATP when bound to DNA, and the rate of ATP hydrolysis generally correlates well to the amount of RecA bound to the DNA (20, 69–71). The purpose of the experiments is to examine the effects of SSB on RecX function. RecA-mediated ATP hydrolysis is used as a real time indicator of RecA filament status. RecX and SSB concentrations are varied, and several different orders of addition are employed.

**SSB Protein Antagonizes RecX Function**—In the absence of SSB protein, the ATP hydrolysis rate was constant and slow (data not shown). This reflects the limited binding of RecA to the ssDNA, where formation of a complete and contiguous RecA filament is precluded by secondary structure in the DNA. The binding that does occur is maintained at a constant steady state. SSB facilitates RecA filament extension by removing secondary structure in the ssDNA, leading to the rapid stimulation of ATP hydrolysis. When there was no RecX present, but SSB was added, ATP hydrolysis proceeded at a steady state reflecting a  $k_{\text{cat}}$  of just over 30  $\text{min}^{-1}$  (Fig. 2A). The RecA filaments appear to be largely contiguous under these conditions, although a few short gaps or breaks are generally present in each filament where exchange of RecA monomers between the bound and free pool is occurring (52, 72). The final steady state rates of ATP hydrolysis are unaffected by increasing SSB concentration (Fig. 2A).

In Fig. 2B, the effects of RecX protein are shown, with 42 nM RecX protein added after RecA filaments had been formed. The addition of RecX protein resulted in a time-dependent decline of ATP hydrolysis. This reflects the net end-dependent disassembly of RecA filaments that occurs when filament extension is prevented by RecX protein (52). To test the effect of SSB protein on the function of RecX protein, the concentration of SSB protein was varied from 0.25 to 2  $\mu\text{M}$  while maintaining the same RecX protein concentration of 42 nM. When SSB protein was present at low concentration, the rate of ATP hydrolysis declines over a period of several minutes. The effect of the added RecX protein diminishes as SSB protein concentration increases. The apparent protection afforded by SSB protein with respect to RecX function appears to saturate as SSB con-

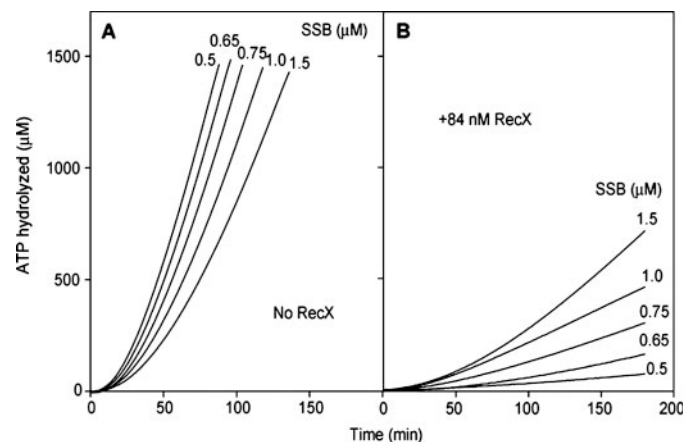


FIGURE 3. **SSB and RecX were bound to the ssDNA prior to addition of the RecA.** RecA, ssDNA, and ATP concentrations were identical to those in Fig. 2. RecX concentration was 84 nM (where indicated). The SSB concentration was varied. SSB and RecX protein (when present) were preincubated with the ssDNA and ATP during the 10 min prior to introduction of RecA protein. The RecA protein addition defines the zero time point.

centration approaches 1.5–2  $\mu\text{M}$ . There were no SSB concentrations that could completely protect RecA filaments from 42 nM RecX protein, although the effects of RecX are greatly diminished at high SSB concentrations. This suggests that the mechanism by which RecX protein affects RecA protein filaments is SSB-dependent.

A similar experiment was carried out in which SSB was bound to the ssDNA prior to the addition of RecA (Fig. 3). SSB impedes RecA protein loading onto ssDNA (73), and higher SSB concentrations increase the long lag in RecA protein binding as seen in Fig. 3A. When 84 nM RecX protein was also added to the reaction prior to RecA (Fig. 3B), the formation of RecA filaments was impeded much more. However, increasing SSB concentrations had a positive, rather than a negative effect in this experiment. The capacity of RecX to block RecA filament formation was substantially decreased by the higher SSB concentrations.

The apparently robust effects observed when RecX is added prior to RecA protein likely do not reflect the prebinding of RecX to the ssDNA. The same result is obtained if RecA and RecX are preincubated together prior to the addition of ssDNA and ATP + SSB in rapid succession (data not shown).

The actual molecular effects of RecX on RecA are the same regardless of when RecX is added. RecA filament extension is blocked. The observed effects of RecX on RecA-mediated ATP hydrolysis, which reflects the amount of bound RecA, are simply greater if RecX can act early in the RecA filament assembly process. RecX, added early, can minimize the amounts of RecA that bind to the ssDNA and thus suppress the observed ATP hydrolysis. When RecX is added to fully formed filaments, the effects appear less dramatic because the resulting reduction in ATP hydrolysis reflects the slow disassembly of the RecA filaments.

**The Protective Effects of SSB Are Not Species-specific**—We wished to determine whether the protective effects of SSB reflected a specific interaction between SSB and either RecA or RecX protein. We therefore carried out a new set of experiments, adding the yeast RPA protein in place of SSB. RPA

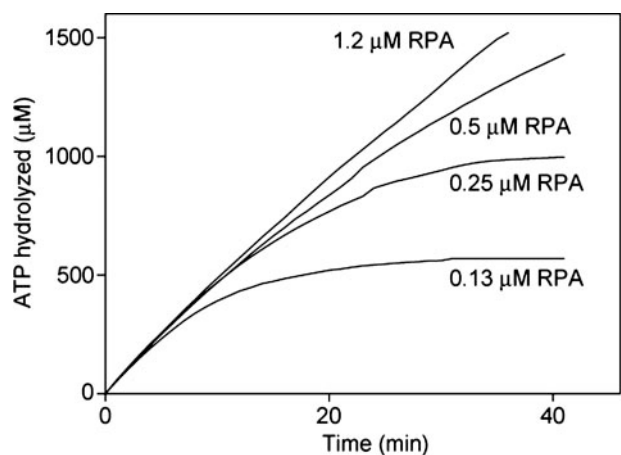


FIGURE 4. The protective effects of SSB are not species-specific. The reaction conditions were as in Fig. 2, but with RPA replacing SSB. RPA protein concentration was varied as indicated.

removes secondary structure in ssDNA and stimulates the extension phase of RecA filaments as effectively as does SSB (74). We used several concentrations of RPA and 42 nM RecX protein (Fig. 4). When RecX was added to preformed RecA filaments in the presence of low concentrations of RPA, a decline in the rate of hydrolysis was observed, reflecting RecA filament disassembly. As was the case for SSB, higher RPA concentrations moderated the effects of RecX protein. However, a lower concentration of RPA (relative to SSB) was required to counter the inhibitory effects of RecX. The same protective effect on RecA filaments was found with T4 gene 32 protein (data not shown). The data presented here suggest that SSB does not specifically interact with RecX protein. This implies that the effect of SSB could be mediated by a simple competition between RecX and SSB. In principle, one could consider three kinds of competition mechanisms: (a) a binding of RecX protein directly to ssDNA with competition with SSB, (b) a binding of RecX to RecA by a capping mechanism facilitated by the binding of RecX to ssDNA, with the latter function blocked by SSB, and (c) a steric inhibition of RecX capping of RecA filaments by SSB bound downstream of the growing end of a RecA filament that does not involve RecX binding to ssDNA.

**The Protective Effect of SSB Is Reduced on gDNA**—Because RecX inhibition of ATPase is dependent on SSB concentration, we decided to look at the RecX inhibition under conditions in which the DNA substrate included short duplex regions. SSB will not bind to the double-stranded DNA regions. However, RecA filaments are more dynamic when bound to double-stranded DNA (72) and should be somewhat more accessible to RecX protein in these locations. The gDNA substrates were produced by annealing either two or four short synthetic oligonucleotides to circular M13mp8 ssDNA. The oligonucleotides restricted SSB binding over just their 32-nucleotide lengths relative to the phage DNA (7229 nucleotides). The rate of RecA-mediated ATPase approached the same steady state level after SSB addition as in the experiments with ssDNA. The final gDNA-dependent steady state rates did not change with increasing SSB concentration over 40 min. As was shown before (22, 75), the RecA filaments can rapidly propagate from single-stranded into double-stranded regions. We added 42 nM RecX protein to

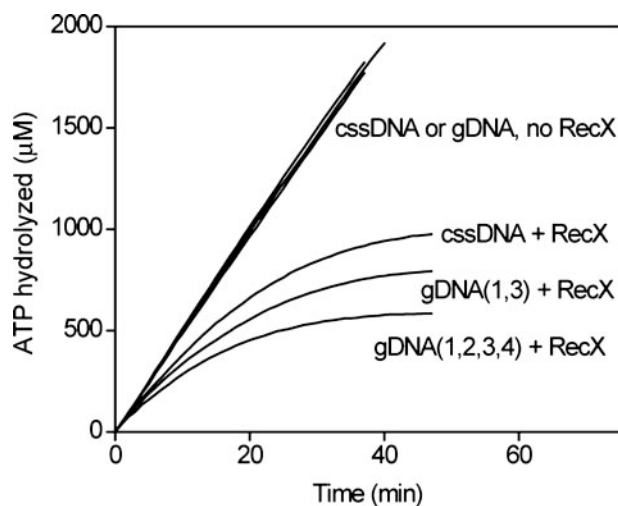


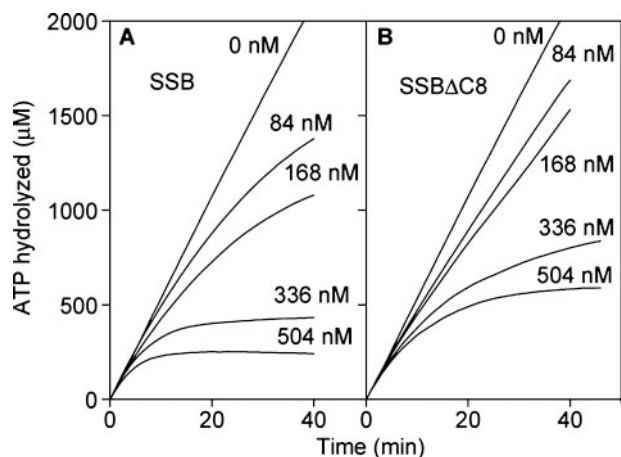
FIGURE 5. The protective effect of SSB is reduced on gDNA. RecA, ssDNA, and ATP concentrations were identical to those in Fig. 2. RecX concentration was 84 nM (where indicated). In all cases, the SSB was present at 1.5 μM.

reactions containing fully formed filaments. When RecX was added, the decline in the rate of RecA-mediated hydrolysis was substantially greater on the gDNA than when ssDNA was used (Fig. 5). A dose effect was evident, with the gDNA possessing four annealed oligonucleotides exhibiting a greater effect than the gDNA possessing two annealed oligonucleotides. The dose effect was observed in four separate experiments with consistent results. We interpret this as a reduction in an SSB-RecX competition, with RecX functioning more effectively in the duplex regions of the DNA substrate. We assume that the very short regions of double-stranded DNA (less than 2% of the total DNA available for RecA binding) do not notably affect the net rates of RecA protein filament disassembly on their own.

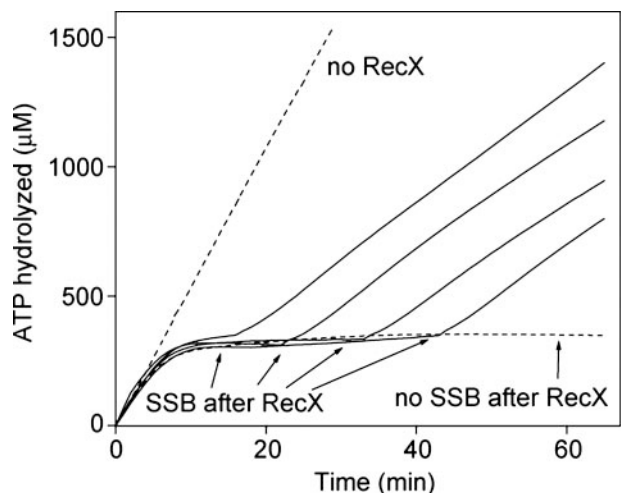
**RecX Protein Can Overcome the Effects of SSB Concentration**—We next wanted to determine whether RecX protein could overcome the effects of a high SSB concentration sufficient to provide optimal protection of RecA filaments. We titrated RecX protein into reactions containing the RecA filaments formed with 1.8 μM SSB (Fig. 6A). The effect of RecX protein on ATPase activity gradually increased with increasing RecX concentration, reinforcing the conclusion that there is a direct competition between SSB and RecX.

**Deleting 8 Amino Acid Residues from the SSB C Terminus Decreases the Inhibitory Effects of RecX**—The effect of RecX protein was moderated to an even greater extent when the SSB protein had a C-terminal deletion. In the experiments of Fig. 6B, the SSB protein is replaced with SSBΔC8 (76). C-terminally deleted variants of SSB, in which the C-terminal domain (up to 42 residues) has been removed, bind somewhat more tightly to ssDNA than does wild type SSB (77, 78). In trials to date, no ssDNA binding deficiency (relative to wild type SSB) has been detected with the SSBΔC8 variant (76, 79). Four of the final eight residues of *E. coli* SSB are aspartate residues, and many proteins interact with this acidic SSB C terminus, (73, 79–91). Mutations in the six to eight C-terminal amino acids of the SSB protein can render a bacterial cell inviable (85). Approximately 2–3-fold more RecX protein is required to produce a given level of inhibition of RecA-mediated ATPase, relative to the results

## Regulation of RecA by RecX



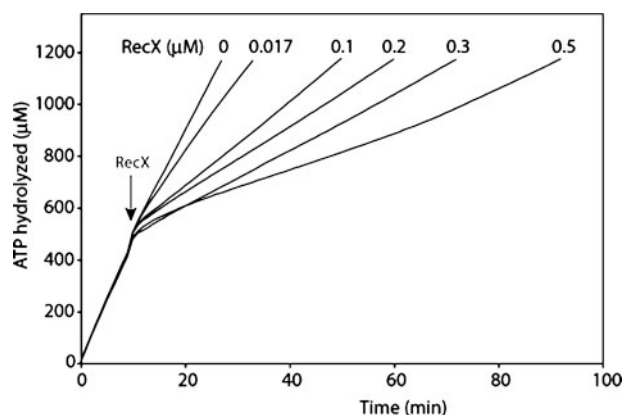
**FIGURE 6. RecX protein can overcome the effects of SSB concentration.** RecA, ssDNA, and ATP concentrations were identical to those in Fig. 2. SSB concentration was  $1.8 \mu\text{M}$ . RecX protein concentration was varied as indicated. The SSB $\Delta$ C8 protein used in panel B has a C-terminal truncation of eight amino acid residues that eliminates an interaction point used by multiple proteins in DNA metabolism. See text for details.



**FIGURE 7. SSB can reverse the effects of RecX protein.** RecA, ssDNA, and ATP concentrations were identical to those in Fig. 2. RecX concentration was  $42 \text{ nM}$ . SSB protein was added at two different times. The first portion was added after RecA protein, at a concentration of  $0.25 \mu\text{M}$ . The second addition (as indicated by an arrow) was added at various times thereafter, after RecX. The final concentration of SSB (total) was  $3.5 \mu\text{M}$ . Time 0 corresponds to the time of the RecX addition.

obtained with wild type SSB (Fig. 6A). There are at least two possible explanations: (a) SSB $\Delta$ C8 simply binds more tightly to ssDNA and therefore competes better with RecX or (b) SSB $\Delta$ C8 is less resistant to RecA filament extension. We prefer mechanisms that do not involve a direct RecX-SSB interaction because of the results in Fig. 4, although the C terminus of SSB is a known interaction point for many proteins as noted above.

**SSB Can Reverse the Effects of RecX Protein**—Additional experiments were carried out to determine whether the effects of RecX could be reversed by SSB after RecX had already bound and halted RecA filament extension. In the experiment shown in Fig. 7, RecA filaments were formed with  $0.25 \mu\text{M}$  SSB, followed by the addition of  $42 \text{ nM}$  RecX protein at the point indicated as  $t = 0 \text{ min}$ . At various times thereafter,  $3.25 \mu\text{M}$  SSB protein was added (to give a final concentration of  $3.5 \mu\text{M}$ ). As shown in Fig. 7, RecA filaments recovered, as indicated by



**FIGURE 8. RecX inhibits RecA protein filaments bound to poly(dT).** The poly(dT) and RecA protein concentrations were  $4$  and  $2.4 \mu\text{M}$ , respectively. ATP was present at  $3 \text{ mM}$ . RecX protein concentrations (in  $\mu\text{M}$ ) are indicated in the figure. RecA was incubated with poly(dT) and ATP and then challenged with the indicated concentration of RecX protein at the point denoted with the vertical arrow. The apparent small but abrupt increase in hydrolyzed ATP seen upon RecX addition is an artifact associated with the volume change accompanying the RecX addition.

increased ATP hydrolysis, in all cases. The results suggest a highly dynamic competition between RecX and SSB, in a system where binding to ssDNA and (with RecX) to RecA protein filament ends is reversible.

**RecA Filaments Bound to Poly(dT) Are Inhibited by RecX**—The enhancement of RecX-mediated inhibition when the DNA includes short duplex regions (Fig. 5) suggests that the RecX protein might itself bind to regions of secondary structure. If so, then the effects of RecX should be eliminated when RecA protein is bound to poly(dT). We therefore carried out a series of experiments in which RecA protein was bound to poly(dT) with an average length of 229 nucleotides. The results are presented in Fig. 8.

The kinetic effects of RecX are predictably different when poly(dT) is substituted for the long circular ssDNAs used in Figs. 2–7. In the earlier figures, RecX addition after RecA filaments are assembled leads to a slow disassembly of the few long RecA filaments on each DNA molecule. Because SSB is present, it replaces the RecA as disassembly proceeds, impeding RecA rebinding and leading eventually to a steady state in which relatively little RecA is bound.

On shorter linear ssDNA, RecA filaments form and disassemble rapidly. The total ssDNA (in terms of total nucleotides) is comparable in Fig. 8 ( $4 \mu\text{M}$ ) with the ssDNA concentration in the other figures ( $5 \mu\text{M}$ ). However, the distribution of RecA protein onto many short oligonucleotides rather than a relatively few long ssDNAs means that there are many more RecA filament ends to interact with in the poly(dT) experiment. A filament with 70 RecA monomeric subunits (enough to coat one of these poly(dT) molecules) should disassemble completely in about 1 min, given the measured end-dependent disassembly rates of  $60\text{--}70 \text{ subunits min}^{-1}$  (21). When SSB was added to these filaments, the rate of ATP hydrolysis declined rapidly. With sufficient SSB ( $3 \mu\text{M}$ ), the rate went to zero within 5 min, reflecting the expected replacement of the disassembling RecA with SSB (data not shown). SSB was thus not added to the experiments in Fig. 8, because the effects of RecX could not be monitored with SSB present.

When RecX protein was added to preassembled RecA filaments on poly(dT), the rates of ATP hydrolysis did not decline slowly but assumed a new steady state within a minute or so (Fig. 8). The new steady state seen after RecX addition declined as the concentration of RecX protein increased. The concentration of poly(dT) oligonucleotides in molecules (as opposed to total nucleotides) was  $\sim 17$  nM, which also approximates the concentration of RecA filament ends available to RecX protein. The observed steady state of RecA-mediated ATP hydrolysis continues to decline well past the point at which RecX protein concentration is stoichiometric with RecA filament ends. These results are readily interpreted within the filament capping model proposed previously (52) (perhaps augmented by an interaction of RecX with ssDNA as implied by Figs. 2–7). If RecX can interact only with a preformed RecA filament and not with free RecA, the inhibition by RecX on poly(dT) could occur only after filaments were nucleated. As filaments dissociated and then renucleated, RecX addition would prevent new filament extension from going to completion. Higher RecX concentrations would increase the probability of capping at an earlier point in filament formation and decrease the average filament length in the population. The shorter filaments would dissociate in a shorter time, and the steady state concentration of bound RecA protein (and associated ATP hydrolysis) would decline. RecA-mediated ATP hydrolysis is not suppressed entirely even at very high RecX concentrations, because RecX can only act after some RecA has bound to the DNA (and every DNA binding event by RecA will result in ATP hydrolysis). To the extent that RecX protein interacts with the DNA as it caps a RecA filament, it does not bind exclusively to duplex DNA or to DNA hairpins (secondary structure), because RecX can inhibit RecA even on poly(dT).

## DISCUSSION

We conclude that SSB antagonizes the inhibitory effects of RecX protein on RecA protein filaments. The simplest interpretation is that RecX and SSB protein compete for a ligand to which both may bind, almost certainly single-stranded DNA. Further, the ssDNA-RecX interaction would then represent at least part of the mechanism by which RecX halts RecA filament extension. The RecX-SSB competition is dynamic, and the effects of each protein are readily overcome by increasing the concentration of the other. Whereas RecX protein appears to have a limited capacity to bind directly to ssDNA on its own (52), the effects of SSB may indirectly reveal an important RecX-ssDNA interaction. This RecX protein interaction with DNA does not involve duplex DNA or secondary structure in the ssDNA (hairpins), at least not exclusively, because RecX has a substantial inhibitory effect on RecA filaments even when those filaments are bound to poly(dT). Given the relatively weak binding of RecX protein to ssDNA observed to date, we note that we cannot yet exclude an alternative mechanism presented as mechanism *c* under "Results." In this alternative, SSB antagonizes RecX action via a steric effect in which bound SSB is so close to the 3'-proximal end of RecA filaments (where RecX capping must occur) that it impedes RecX binding to RecA without an interaction of RecX with ssDNA.

When RecX protein is added to formed RecA filaments, a net disassembly ensues. The dissociating RecA protein is not replaced by growth of existing filaments, presumably because RecX is blocking filament growth on the 3'-proximal end where extension occurs. The results led to the RecA filament capping mechanism for RecX action (52). The general effects of RecX remain the same; however, the effects of SSB add a new dimension. We infer that the relevant competition between RecX and SSB is occurring at the 3'-proximal (extension) end of a RecA filament. Regardless of the precise mechanism by which SSB antagonizes RecX function, the results reinforce the idea that the primary effect of RecX occurs at the extending end of the RecA filament.

If direct binding of RecX protein to ssDNA is weak yet SSB can interfere with RecX function, then the capping of a RecA filament may involve a RecX-ssDNA interaction. As noted above, the effects of SSB could also reflect a steric effect that does not require a RecX-ssDNA interaction. In the first scenario, it is reasonable to speculate that the interaction between RecX and RecA may facilitate a RecX-ssDNA interaction that is otherwise relatively cryptic. Given the filamentous form of RecA protein on ssDNA, it is difficult to measure the RecX-ssDNA interaction in the presence of RecA. In typical DNA binding assays, the background rendered by the RecA-ssDNA interaction has so far blocked any direct measurement of RecX binding to ssDNA.

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