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# recA protein-catalyzed strand assimilation: Stimulation by *Escherichia coli* single-stranded DNA-binding protein

[filter binding assay/ATP hydrolysis/single-stranded and duplex DNA binding competition/adenosine 5-*O*-(3-thiotriphosphate)/*lexC* mutation]

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**ABSTRACT** **The single-stranded DNA-binding protein of *Escherichia coli* significantly alters the strand assimilation reaction catalyzed by recA protein [McEntee, K., Weinstock, G. M. & Lehman, I. R. (1979) *Proc. Natl. Acad. Sci. USA* 76, 2615–2619]. The binding protein (*i*) increases the rate and extent of strand assimilation into homologous duplex DNA, (*ii*) enhances the formation of a complex between recA protein and duplex DNA in the presence of homologous or heterologous single-stranded DNA, (*iii*) reduces the rate and extent of ATP hydrolysis catalyzed by recA protein in the presence of single-stranded DNA, (*iv*) reduces the high concentration of recA protein required for strand assimilation, and (*v*) permits detection of strand assimilation in the presence of the ATP analog, adenosine 5′-*O*-(3-thiotriphosphate). Single-stranded DNA-binding protein purified from a binding protein mutant (*lexC*) is considerably less effective than wild-type binding protein in stimulating strand assimilation, a result which suggests that single-stranded DNA-binding protein participates in general recombination *in vivo*.**

Considerable genetic (1–3) and biochemical evidence (4, 5) supports the idea that the protein specified by the *recA* gene of *Escherichia coli* (recA protein) participates in one or more early steps in the exchange of strands between homologous chromosomes. Two enzymatic activities associated with the homogeneous recA protein have recently been described that further implicate it in the initiation of general recombination: (*i*) recA protein catalyzes the annealing of complementary single strands to form duplex DNA, accompanied by hydrolysis of ATP to ADP and $P_i$ (6), and (*ii*) it promotes the annealing of a single-stranded DNA (ss DNA) chain into homologous duplex DNA (strand assimilation) (7, 8). At least one product of this reaction is a "D-loop" structure, a locally triple-stranded region containing the paired exogenous strand and a displaced single-stranded loop from the duplex (7, 8). Strand assimilation, like the renaturation of single strands, requires ATP hydrolysis. However, there are markedly different requirements for recA protein in these two reactions. Renaturation of single strands is efficiently catalyzed by recA protein when it is present at a ratio of one monomer per 250 nucleotides (6), whereas assimilation of ss DNA into duplexes cannot be detected until this ratio is increased by some 50-fold (7, 8). Because of this difference we have sought additional proteins or factors that might enhance the efficiency of the strand assimilation reaction. We reasoned that, *in vivo*, ss DNA is likely to be complexed with proteins such as the ss DNA-binding protein (SSB), which binds tightly and specifically to ss DNA (9, 10). SSB is required for the replication *in vivo* of *E. coli* DNA (11) and for the replication *in vitro* of single-stranded phage DNAs (10). Mutations in the SSB structural gene, designated *ssb* and allelic to *lexC* (11–13), have been described and shown to produce both UV-sensitive and recombination-deficient phenotypes (12, 14). Thus, SSB may participate in DNA repair and recombination as well as in DNA replication. In this paper we demonstrate that SSB increases the rate and extent of strand assimilation catalyzed by recA protein. Analysis of this stimulatory effect indicates that SSB influences several features of the interaction between recA protein and DNA. These findings have clarified certain biochemical aspects of strand assimilation and further implicate SSB in general recombination *in vivo*.

## MATERIALS AND METHODS

recA protein was purified to homogeneity as described (6). Protein concentrations were determined by using an $E_{280}^{1\%}$ of 4.4 based upon the amino acid composition of recA protein (T. Ogawa and H. Ogawa, personal communication).

$^3$H-Labeled P22 duplex DNA was prepared as described (15). P22 DNA was denatured by heating at 100°C for 3 min followed by cooling on ice. DNA concentrations are expressed as total nucleotides. SSB, purified from *E. coli* B, was a generous gift of Robert Fuller of this department. Mutant SSB was purified from strain PAM5779 (*lexC113*) through the heating step and subsequent dialysis (10). The material, judged to be approximately 50% pure by electrophoresis in polyacrylamide gels, contained neither DNase nor ATPase activities.

The assay used for detection of single-strand assimilation depends upon the ability of duplex DNA molecules to be retained on nitrocellulose filters as a consequence of the single-stranded regions that they acquire. Assimilation was measured essentially as reported earlier (7) with the following modifications. The reaction mixtures (200 μl) contained 20 mM Tris·HCl, pH 8/10 mM $MgCl_2$/20 mM KCl/0.1 mM EDTA/0.1 mM dithiothreitol and the amounts of recA protein, ATP, P22 duplex, and ss DNA and SSB indicated in the legends. The reactions were stopped by addition of 1/20th vol of 20% Sarkosyl or 20% sodium dodecyl sulfate; the samples were filtered, washed, and assayed for radioactivity as originally described. All reactions were performed in 1.5-ml plastic Eppendorf microfuge tubes.

Formation of recA protein–DNA complexes was measured by using alkali-treated nitrocellulose filters (Millipore, type HAWP 45 μm) (16). Filters were soaked in 0.5 M KOH for 20 min at 22°C, washed extensively with distilled $H_2O$, then washed for 45 min in 100 mM Tris·HCl (pH 7.5) and stored in the same buffer at 4°C. Neither ss DNA nor duplex DNA is retained by alkali-washed filters (<2%), but protein–DNA complexes are efficiently retained. The conditions for forming recA protein–DNA complexes were those used for strand as-

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Abbreviations: ss DNA, single-stranded DNA; SSB, single-stranded DNA-binding protein; ATP[γ-S], adenosine 5′-*O*-(3-thiotriphosphate); ADP[NH]P, adenyl-5′-yl imidodiphosphate; ADP[$CH_2$]P, adenyl-5′-yl methylenediphosphate.

similation except that adenosine 5′-*O*-(3-thiotriphosphate) (ATP[γ-S]) (100 μM) was substituted for ATP. After incubation, the samples were applied directly to alkali-treated filters, washed, and assayed for radioactivity as described (7).

Hydrolysis of ATP was measured during strand assimilation by including [$^3$H]ATP (50 Ci/mol, 1 Ci = $3.7 \times 10^{10}$ becquerels) in the reaction. Samples (1 μl) were taken at the indicated times and applied to polyethyleneimine (PEI)-cellulose as described (6).

## RESULTS

**SSB Stimulates Strand Assimilation Catalyzed by recA Protein.** recA protein must interact with both ss and duplex DNA for strand assimilation to occur. As shown in Fig. 1, the overall reaction was influenced strongly by the ratio of recA protein to single strands. In the presence of 70 pmol of recA protein, optimal assimilation into $^3$H-labeled P22 duplex DNA (approximately 10% of input duplex DNA retained on filters) occurred with 200 pmol of ss DNA (1 recA monomer per 3 nucleotides). Increasing the ratio of ss DNA to recA protein eliminated strand assimilation. When the amount of recA protein was raised to 350 pmol, optimal strand assimilation (40%) occurred in the presence of 500 pmol ss DNA (1 recA monomer per 1.5 nucleotides). Again, inhibition was observed upon increasing the ratio of ss DNA to recA protein. A plausible explanation for these findings is that the ss DNA must be saturated with recA protein for optimal strand assimilation to occur. Direct binding measurements indicate that ss DNA is saturated with recA protein at a ratio of 1 recA monomer per 5 nucleotides (unpublished data). Inhibition of the assimilation reaction under conditions of excess ss DNA suggests that ss DNA com-

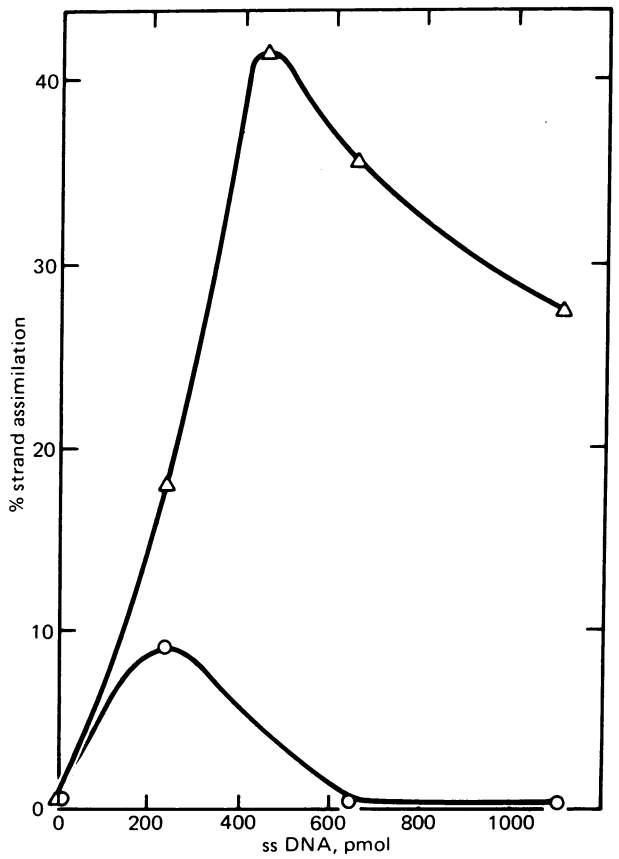


FIG. 1. Inhibition of recA-protein-catalyzed strand assimilation by excess ss DNA. $^3$H-Labeled P22 duplex DNA (1.45 nmol), 500 μM ATP, 70 (○) or 350 (△) pmol of recA protein, and the indicated amounts of unlabeled P22 ss DNA were incubated for 40 min at 37°C. The amount of strand assimilation, expressed as percent of the input $^3$H-labeled duplex DNA retained on nitrocellulose filters, was determined.

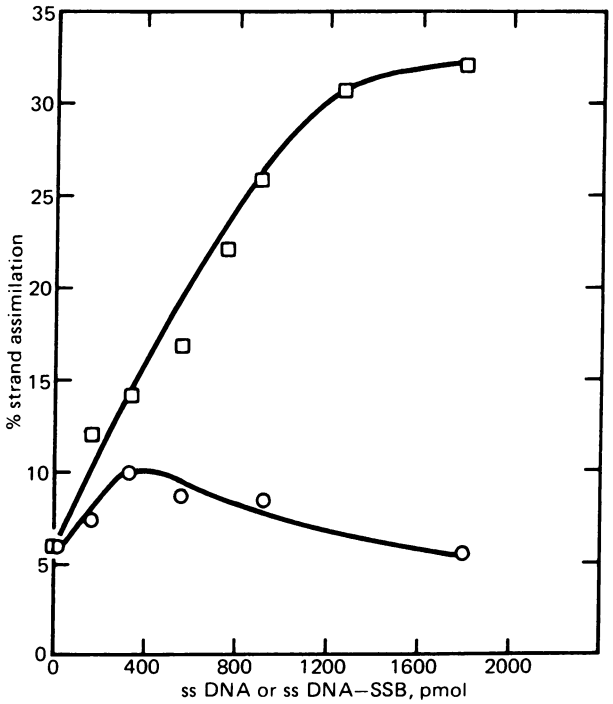


FIG. 2. Single-strand assimilation in the presence (□) and absence (○) of SSB. recA protein (85 pmol) was incubated with 1.05 nmol of $^3$H-labeled P22 duplex DNA, 920 μM ATP, and the indicated amounts of P22 ss DNA or P22 ss DNA complexed with SSB at a ratio of 1 SSB monomer per 8 nucleotides. Reactions were started by addition of recA protein; the mixtures were incubated at 37°C for 20 min and strand assimilation was determined.

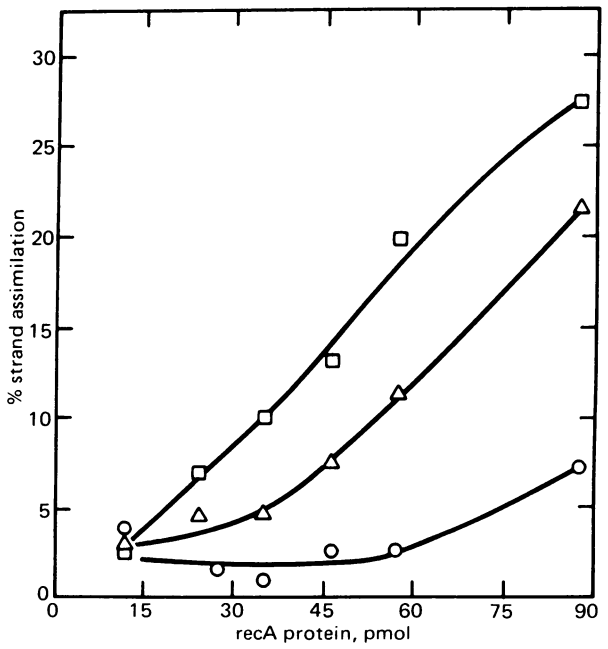


FIG. 3. Requirement for recA protein during strand assimilation in the presence of SSB. The indicated amounts of recA protein, 1.05 nmol of $^3$H-labeled P22 duplex DNA, 1.05 nmol of unlabeled P22 ss DNA, 920 μM ATP, and 1 monomer of SSB per 20 nucleotides of ss DNA (△) or 1 monomer of SSB per 8 nucleotides of ss DNA (□) were incubated for 30 min at 37°C, and strand assimilation was determined. The requirement for recA protein in the absence of SSB is also shown (○).

petes with duplex DNA for binding to recA protein, resulting in the formation of nonproductive ss DNA–recA protein complexes. Direct binding competition experiments (unpublished data) have, in fact, shown that recA protein has a greater affinity for ss than for duplex DNA.

Because of the inhibition of strand assimilation by excess ss DNA, we investigated the effects of SSB on this reaction. We reasoned that SSB would bind to free ss DNA and thereby prevent inhibition by excess ss DNA. The experiment shown in Fig. 2 confirms this expectation; in the presence of SSB, inhibition of strand assimilation did not occur even at a substantial excess of ss DNA (1 recA protein monomer per 24 nucleotides). Furthermore, in the presence of SSB, both the rate and extent of assimilation were enhanced. Under these conditions, approximately 30% of the input $^3$H-labeled P22 duplex DNA was retained on filters. In the absence of SSB, only 5–7% of the duplex DNA was retained. By increasing the amount of recA protein from 85 pmol to 350 pmol, 100% of the duplex DNA could be retained (not shown). Thus, SSB significantly increases the efficiency of recA protein-promoted strand assimilation.

In the absence of SSB, strand assimilation shows a nonlinear dependence on recA protein concentration (7). However, at a ratio of 1 SSB monomer per 8 nucleotides of ss DNA, strand assimilation increased linearly with recA protein concentration. Under these conditions, little or no strand assimilation occurred in the absence of SSB, a result that is consistent with our earlier findings (Fig. 3). At intermediate levels of SSB (1 SSB monomer per 20 nucleotides), the amount of recA protein required to promote strand uptake increased. Increasing the SSB concentration beyond saturation had only a slight effect on the reaction (see below). A plausible interpretation of this result is that SSB binds to ss DNA and inhibits formation of nonproductive recA protein–ss DNA complexes.

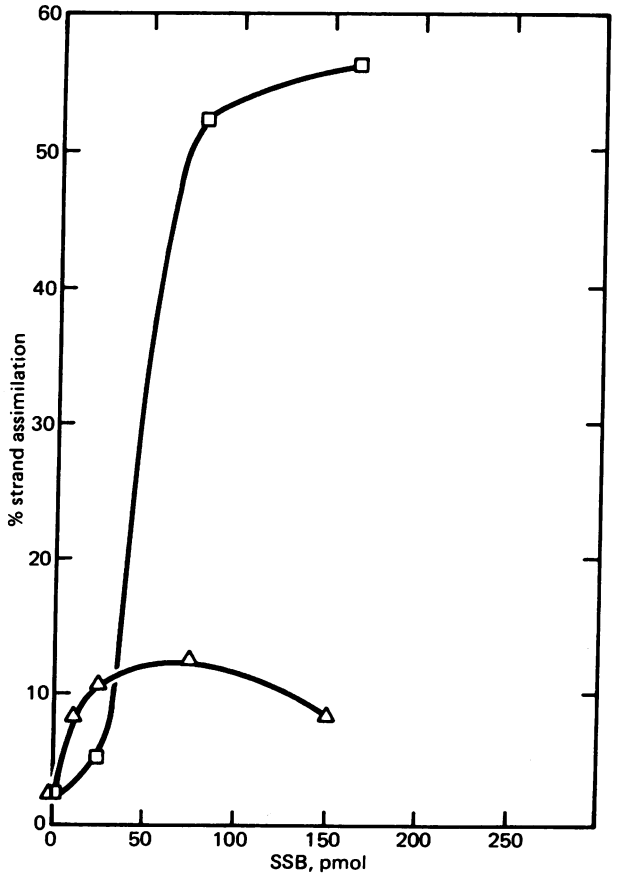


FIG. 4. Stimulation of recA protein-catalyzed strand assimilation by SSB. Reaction mixtures contained 173 pmol of recA protein, 1.05 nmol of $^3$H-labeled P22 duplex DNA, 450 μM ATP, 1.05 nmol of P22 ss DNA, and the indicated amounts of wild-type SSB (□) or SSB purified from the *lexC113* mutant PAM5779 (△). Incubations were performed at 37°C for 15 min, and strand assimilation was determined.

**Dependence of Strand Assimilation on SSB Is Nonlinear.** The rate of strand assimilation catalyzed by recA protein depended nonlinearly upon the ratio of SSB to ss DNA (Fig. 4). Stimulation was maximal at a ratio of approximately 1 SSB monomer per 8 nucleotides, a value at which the ss DNA is saturated with SSB (10, 17). Increasing the amount of SSB beyond this value produced only a slight stimulation. Below saturation, however, the rate of assimilation was markedly dependent upon SSB, a result consistent with the cooperative binding of SSB to ss DNA (10, 17).

SSB purified from the *lexC* mutant strain was significantly less effective in stimulating strand assimilation than the wild-type SSB. The mutant protein binds cooperatively to M13 single strands and stimulates the replication of phage G4 DNA at 30°C *in vitro* (ref. 10 and unpublished results). Hence, the failure of the *lexC* mutant protein to stimulate recA protein-catalyzed assimilation is not due to an inability to bind cooperatively to ss DNA.

**Requirements for recA Protein-Catalyzed Strand Assimilation in Presence of SSB.** The requirements for recA protein-catalyzed strand assimilation in the presence of SSB are given in Table 1. The reaction requires recA protein; retention of duplex DNA on filters did not occur in the presence of SSB alone, a result consistent with the ss DNA binding specificity of this protein and with the lack of DNase activity in the SSB preparation (R. Fuller, personal communication). Homologous single strands were required although heterologous ss DNA can stimulate the ATPase activity of recA protein (6) and promote formation of complexes of recA protein with duplex DNA (ref. 18 and below). The reaction requires ATP; the nonhydrolyzable analogs adenyl-5′-yl imidodiphosphate (ADP[NH]P) and adenyl-5′-yl methylenediphosphate (ADP[$CH_2$]P) could not

Table 1. Requirements for strand assimilation catalyzed by recA protein in the presence of SSB

| Reaction mixture | Complex formed, % |
|---|---|
| Complete | 100 |
| −recA | 5.0 |
| −SSB | 3.0 |
| −P22 ss DNA | 1.5 |
| −P22 ss DNA + poly(dT) | 3.2 |
| −P22 ss DNA + poly(dA) | 2.0 |
| −P22 ss DNA + calf thymus ss DNA | 2.7 |
| −ATP | 7.0 |
| −ATP + ATP[γ-S] | 41 |
| −ATP + ADP[NH]P | 4.7 |
| −ATP + ADP[$CH_2$]P | 5.0 |

Strand assimilation was measured as described in *Materials and Methods* with the indicated additions or omissions. The complete reaction mixture contained 173 pmol of recA protein, 1.05 nmol of $^3$H-labeled P22 duplex DNA, 1.05 nmol of P22 ss DNA, 2.5 μg of SSB, and 460 μM ATP; 1.05 nmol of either poly(dT) or poly(dA) or 1.2 nmol of calf thymus ss DNA was added as indicated. The concentration of ATP[γ-S] was 100 μM; that of ADP[NH]P and ADP[$CH_2$]P, 200 μM. Reaction mixtures were incubated for 30 min at 37°C. Fifty-six percent of the added $^3$H-labeled P22 duplex DNA was retained on nitrocellulose filters in the complete reaction.

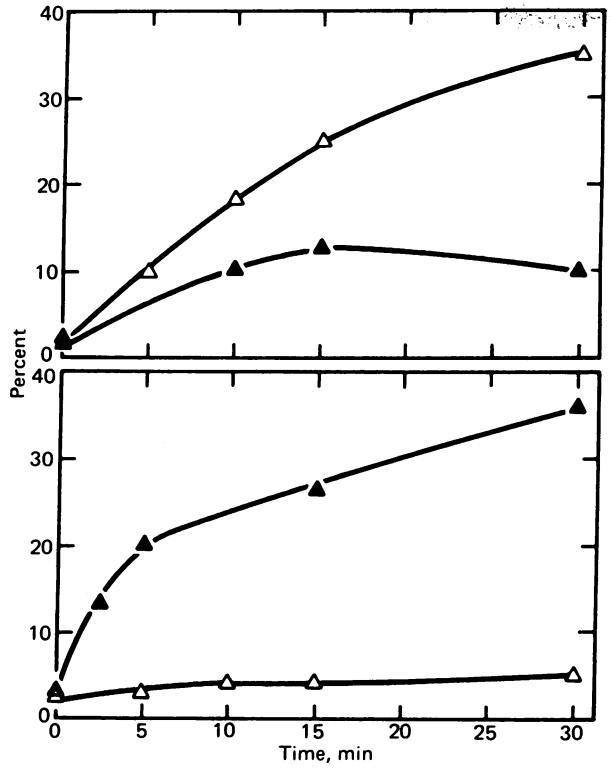


FIG. 5. Kinetics of strand assimilation and ATP hydrolysis in the presence (*Lower*) and absence (*Upper*) of SSB. Reaction mixtures contained 173 pmol of recA protein, 1.05 nmol of $^3$H-labeled P22 duplex DNA, 1.05 nmol of unlabeled P22 ss DNA, 450 $\mu$M [$^3$H]ATP (50 Ci/mol), and either no SSB or 130 pmol of SSB (1 SSB monomer per 8 nucleotides). Forty-microliter samples were taken after the indicated times at 37°C for determination of strand assimilation. One-microliter samples were also applied to PEI-cellulose sheets for determination of ATP hydrolysis (6). ▲, Strand assimilation; △, ATP hydrolysis.

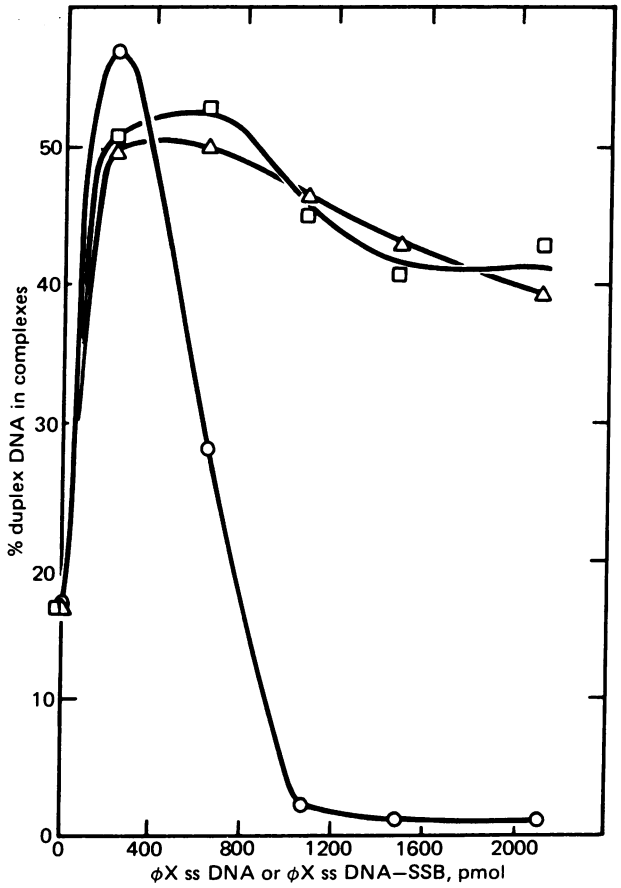


FIG. 6. Formation of recA protein duplex DNA complexes in the presence of heterologous ss DNA. Formation of complexes of recA protein and P22 duplex DNA was measured by filtration through alkali-treated Millipore filters. Reaction mixtures contained 85 pmol of recA protein, 1.05 nmol of $^3$H-labeled P22 duplex DNA, 100 $\mu$M ATP[$\gamma$-S], and the indicated amounts of $\phi$X174 (+) strands (○) or $\phi$X174 (+) strands complexed with wild-type (□) or *lexC* (△) SSB (1 monomer of SSB per 8 nucleotides). Incubation was for 15 min at 37°C.

substitute for ATP as a cofactor. These requirements are completely analogous to those for the reaction promoted by high levels of recA protein in the absence of SSB (7).

An important difference between the SSB-stimulated and -independent reactions is the effect of the analog ATP[$\gamma$-S]. In the presence of SSB, ATP[$\gamma$-S] permitted considerable single-strand assimilation into $^3$H-labeled duplex DNA (Table 1). Like ATP-dependent strand assimilation, this reaction requires recA protein and homologous single strands (data not shown). In contrast, ATP[$\gamma$-S] not only fails to serve as a cofactor in the SSB-independent reaction, but also inhibits the reaction in the presence of ATP (7, 18). Kinetic analysis of the effect of ATP[$\gamma$-S] on the nucleoside triphosphatase activity of recA protein suggests that this analog may not be a simple competitive inhibitor (18). Instead, it may irreversibly inhibit the ATPase activity of the recA protein during the first round of hydrolysis (unpublished data). Thus, strand assimilation may require only limited ATP hydrolysis (see below).

**SSB Inhibits ATP Hydrolysis Catalyzed by recA Protein.** During strand assimilation in the absence of SSB, recA protein catalyzes extensive hydrolysis of ATP as a result of the interaction of recA protein with ss DNA. As shown in Fig. 5, ATP hydrolysis persisted after strand assimilation had reached a maximum, indicating that much of the hydrolysis is due to formation of nonproductive recA protein–ss DNA complexes. In contrast, ATP hydrolysis was sharply reduced in the presence of saturating levels of SSB at the same time that strand assimilation was strongly stimulated (Fig. 5). Under these conditions, the assimilation reaction had not reached a plateau after 30 min of incubation. However, even early in the reaction, there was considerable strand assimilation (greater than 20% at 7 min) accompanied by very little ATP hydrolysis (<1%). These results are consistent with the notion that SSB prevents the nonproductive interaction between recA protein and ss DNA that leads to extensive ATP hydrolysis.

**SSB Stimulates Formation of Complexes Containing recA Protein, Duplex DNA, and Heterologous ss DNA.** In the presence of the analog ATP[$\gamma$-S], ss DNA stimulates the binding of recA protein to duplex DNA (ref. 18 and unpublished data). Unlike strand assimilation, this binding can be stimulated by heterologous ss DNA or certain polyribonucleotides (unpublished data). The complex of recA protein, duplex DNA, and ss DNA that is formed under these conditions may represent an intermediate in strand assimilation. As shown in Fig. 6, complex formation in the absence of SSB was extremely sensitive to the ratio of recA protein to ss DNA and was inhibited by an excess of ss DNA, a characteristic of the overall assimilation reaction. When SSB was present at a concentration that saturated the single strands, complex formation was still stimulated; however, only slight inhibition occurred at high concentrations of ss DNA. As with strand assimilation, complex formation required recA protein (less than 5% of the duplex DNA was retained in the absence of recA protein). ATP also stimulated

complex formation in the presence of SSB although in the absence of SSB, complex formation could not be detected (data not shown). We conclude that SSB prevents ss DNA from competing with duplex DNA for binding to recA protein.

The SSB purified from the *lexC* mutant is also effective in stimulating complex formation and preventing inhibition by ss DNA, a result that is consistent with the ability of the mutant protein to bind ss DNA. The failure of the mutant SSB to stimulate strand assimilation as effectively as the wild-type protein implies that SSB may play a role in strand assimilation that is more complex than simply coating ss DNA.

## DISCUSSION

**Effects of SSB on recA Protein-Catalyzed Strand Assimilation *in Vitro*.** SSB stimulates the rate and extent of recA protein-mediated strand assimilation and prevents inhibition of this reaction by excess ss DNA. When the SSB–ss DNA complex is used in place of free ss DNA, the concentration of recA protein required for strand assimilation is significantly reduced. Because strand assimilation promoted by the recA protein is complex, SSB may influence more than one step in the overall process. The mechanism by which recA protein promotes strand assimilation involves formation of a complex between duplex and ss DNA molecules (ref. 18 and these results). A similar mechanism might operate in the renaturation of ss DNA catalyzed by recA protein (6), although in this case the complex formed would involve two ss DNA chains. Such recA protein–ss DNA complexes would be nonproductive in strand assimilation and would sequester the recA protein required for the formation of the duplex DNA–ss DNA–recA protein complexes, which serve as intermediates in the assimilation pathway. We propose that at least one effect of SSB in stimulating strand assimilation is to prevent formation of such nonproductive recA protein–ss DNA complexes. We have, in fact, observed that SSB inhibits renaturation of ss DNA catalyzed by recA protein, a result to be expected if SSB prevented recA protein from complexing with two ss DNA chains (unpublished data). Furthermore, stimulation of strand assimilation by SSB does not result solely from the removal of secondary structure from ss DNA since such a mechanism would be expected to promote renaturation as well. We cannot, however, rule out the possibility that SSB can have more subtle effects upon strand assimilation: for example, by influencing the distribution of recA protein along a ss DNA molecule or interacting with recA protein directly. That SSB inhibits the recA protein-dependent hydrolysis of ATP to ADP and $P_i$ in the presence of ss DNA may also bear on the assimilation reaction since ADP inhibits the interaction of recA protein with duplex DNA (unpublished data). Our finding that SSB purified from the *lexC* mutant also stimulates formation of complexes of recA protein, duplex DNA, and ss DNA, but is relatively ineffective in stimulating strand assimilation, further suggests that SSB may influence more than one step in the assimilation reaction. A more detailed analysis of the genetic and biochemical properties of this mutant will provide additional insight into the role of SSB in strand assimilation.

**Requirement for ATP Hydrolysis in Strand Assimilation.** SSB greatly reduces the extensive ATP hydrolysis that accompanies strand assimilation. We infer that in the absence of SSB most of the ATP hydrolysis results from the nonproductive interaction of recA protein with free ss DNA and that SSB inhibits this interaction. Hence, only limited ATP hydrolysis is required for assimilation. Consistent with this finding, SSB allows strand assimilation to occur with ATP[$\gamma$-S] as cofactor. We have, in fact, found that this analog may undergo a single round of hydrolysis (unpublished data). On the other hand, neither ADP[NH]P nor ADP[$CH_2$]P, which act as nonhydrolyzable competitive inhibitors of the ATPase activity of recA protein (unpublished data), support strand assimilation. Therefore, a relatively small (though undetermined) number of hydrolytic events may be required for the reaction. Inasmuch as several thousand monomers of recA protein are added per duplex molecule, and a single D-loop would result in retention of the duplex on a nitrocellulose filter, it is conceivable that a single hydrolytic event per monomer of recA protein could promote significant assimilation. The results obtained with ATP[$\gamma$-S] as cofactor in strand assimilation would be compatible with this explanation.

**Significance *in Vivo* of SSB-Stimulated Strand Assimilation.** There is genetic evidence that SSB plays a significant role in recombination and in DNA repair (12–14). Our findings indicate that one function of SSB is to stimulate strand assimilation, a process likely to be a central feature of recombination and postreplication repair *in vivo*. The UV sensitivity and recombination deficiency of *lexC* mutants are not as severe as in *recA* mutants, suggesting that SSB is not absolutely essential for these processes *in vivo*. In agreement with these observations, we find that the *in vitro* assimilation reaction, though absolutely dependent on recA protein, is significantly enhanced by, but not dependent upon, SSB. The high levels of recA protein that accumulate after induction of the *recA* gene (19) may permit assimilation without SSB *in vivo* in a manner analogous to the SSB-independent reaction, which requires high levels of recA protein *in vitro* (7, 8).

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