Effects of Various Single-Stranded-DNA-Binding Reactions Promoted by RecA Protein

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To relate the roles of Escherichia coli SSB in recombination in vivo and in vitro, we have studied the mutant proteins SSB-1 and SSB-113, the variant SSB,1 produced by chymotryptic cleavage, the partially homologous variant F SSB (encoded by the E. coli sex factor), and the protein encoded by gene 32 of bacteriophage T4. All of these, with the exception of SSB-1, augmented both the initial rate of homologous pairing and strand exchange promoted by RecA protein. From these and related observations, we conclude (i) that SSB stimulates the initial formation of joint molecules by nonspecifically promoting the binding of RecA protein to single-stranded DNA; (ii) that SSB plays no role in synopsis of the RecA nucleoprotein filament with duplex DNA; (iii) that stimulation of strand exchange by SSB is similarly nonspecific; and (iv) that all members of the class of proteins represented by SSB, F SSB, and gene 32 protein may play equivalent roles in making single-stranded DNA more accessible to RecA protein.

A ubiquitous class of proteins exists the members of which bind specifically to single-stranded DNA. These proteins, variously called single-strand-binding proteins or helixdestabilizing proteins, have been shown to function in replication, recombination, and repair in procaryotes. Among these proteins is the single-stranded DNA-binding protein of Escherichia coli, designated Eco SSB, which is an important participant in DNA replication, recombination, and induction of the SOS response. When irradiated with UV light, ssb mutants show decreased survival, decreased mutagenesis, decreased RecA protein synthesis, decreased induction of lambda prophage, and increased DNA degradation (7).

In vitro, SSB and other helixdestabilizing proteins exhibit activities that may be related to their roles in recombination and to the role of SSB in the SOS response. Helixdestabilizing proteins promote the renaturation of DNA in vitro, in part at least, by destabilizing secondary structure in single strands and thereby removing a kinetic barrier to interstrand pairing. SSB plays a similar role in making single-stranded DNA more accessible to RecA protein under conditions that otherwise favor intrastrand folding and thereby impede the binding of RecA protein (16, 19, 20, 24, 25, 32). This action of SSB may be related to its role in both recombination and induction of the SOS response, since single-stranded DNA plays a key role in the activities of RecA protein.

RecA protein is the exemplar of a class of proteins that act rather differently on DNA. At subsaturating concentrations, RecA protein promotes renaturation of DNA (3, 22, 36); at saturating concentrations, RecA protein promotes the pairing of single-stranded or partially single-stranded DNA with duplex DNA and subsequent strand exchange, a set of reactions that is called strand invasion. In addition, RecA protein, when bound to single-stranded DNA, promotes the specific cleavage of several repressors. In this reaction, ATP is an essential cofactor for the binding of RecA protein to single-stranded DNA, but the hydrolysis of ATP is not required. recA mutants are usually pleiotropic by virtue of the dual nature of RecA protein: its ability, on the one hand, to promote homologous pairing, and its ability, on the other hand, to inactivate the lexA repressor. Mutations in the recA gene markedly reduce homologous recombination and the functions associated with the inducible SOS pathway of repair which is repressed by the lexA repressor (for reviews, see references 26 and 34; see also M. M. Cox and I. R. Lehman, Annu. Rev. Biochem., in press).

In vitro, RecA protein promotes the pairing of a single strand with duplex DNA in the following three distinguishable phases (27). During a presynaptic phase, the protein polymerizes on single-stranded DNA in the presence of ATP to produce a helical nucleoprotein filament which is an intermediate in the succeeding reaction (10, 32). The single-stranded filament, which contains many copies of RecA protein, is polyvalent with regard to binding sites for duplex DNA. Consequently the polyvalent filament forms large but dynamic networks with duplex DNA within which the molecules rearrange to come into homologous alignment (15, 17, 31). This synaptic phase is followed by postsynaptic strand exchange, during which RecA protein displaces a strand from the original duplex molecule in a 5'-to-3' direction and creates heteroduplex regions that can be thousands of base pairs in length. Hydrolysis of ATP is essential for strand exchange (9).

In vitro, SSB plays an auxiliary role in the presynaptic phase, making single-stranded DNA more accessible to RecA protein as described above. SSB also plays a role in strand exchange that is manifested by a doubling or tripling of the rate of exchange (11, 18) and by an action that helps RecA protein to drive strand exchange past heterologous regions that are up to hundreds of base pairs in length (2).
The protein products of several mutant alleles of ssb have been studied in detail, including SSB-1 and SSB-113 (4, 40). In addition, proteolysis of SSBind with chymotrypsin produces a polypeptide, designated SSB, which contains amino acid residues 1 to 135 and has greatly increased helix-destabilizing activity relative to SSB (41).

F SSB, a protein encoded by the E. coli sex factor F, has been found to share extensive homology with SSB (5), especially within the NH₂-terminal region, which contains the DNA-binding domain. Of the first 115 residues of this protein, 87 exactly correspond to those of SSB. In vivo, F SSB can complement the sbb-l and sbb-113 mutations (13).

The protein encoded by gene 32 of bacteriophage T4 (gp32) is functionally analogous to SSB, although the proteins are not homologous in amino acid sequence. Like SSB, gp32 has an acidic carboxy-terminal end that is not essential for binding to DNA (7).

In this paper we report studies in which we examined the action of this set of mutant and variant forms of helix-destabilizing proteins on the strand invasion reaction promoted by RecA protein.

MATERIALS AND METHODS

Proteins. Single-stranded DNA-binding proteins were purified by published procedures: E. coli wild-type SSB and SSB-113 mutant proteins were purified by the method of Chase et al. (4, 6); SSB-1 mutant protein was purified by the method of Williams et al. (40); a partial proteolysis product of SSB (SSB₁)₄ was purified by the method of Williams et al. (41); and the SSB-like single-stranded DNA-binding protein from the E. coli F plasmid was purified by the method of Chase et al. (5). Bacteriophage T4 gp32 was the generous gift of Kenneth R. Williams, Yale University. RecA protein was prepared as described previously (30). Restriction endonuclease HpaI or Sau96I, from New England Biolabs, was used as recommended by the supplier to cleave M13 replicative form (RF) DNA at a unique site. Completeness of digestion was ascertained by electrophoresis on 1% agarose gels.

DNA. M13 RF DNA and bacteriophage DNA were prepared in the same manner as the respective fd DNAs (12). In all experiments described in this paper, we used as substrates single-stranded circular DNA and the double-stranded RF DNA linearized by restriction enzyme cleavage.

Joint molecule assays. Reaction mixtures contained the following: 7.5 μM unlabeled M13 single-stranded circular DNA, 2 μM M13 RF [³H]DNA linearized by HpaI digestion, 5 μM RecA protein, 30 mM PIPES (piperazine-N,N'-bis(2-ethanesulfonic acid)) (pH 7.5), 12 mM MgCl₂, 1.3 mM ATP, and an ATP-regenerating system consisting of 10 U of creatine phosphokinase (from rabbit muscle; Sigma Chemical Co.) per ml, and 3 mM phosphocreatine.

Assays for joint molecules were done by the following method based on previously published D-loop assays (15). Aliquots of 10 μl of the reaction mixture were added to 200 μl of cold 25 mM EDTA (pH 9.4). After all aliquots had been taken, 4 ml of cold 10× SSC (1× SSC is 0.15 M sodium chloride plus 0.015 M sodium citrate) was added to each sample tube. Nitrocellulose filters (diameter, 25 mm; pore size, 0.45 μm; Sartorius) were wet in distilled water and washed with 2 ml of 10× SSC before the samples were filtered through them. The tube and filter were then washed twice with 2.5 ml of 10× SSC, and the filter was washed once with 5 ml of 10× SSC and 5 ml of 2× SSC. Filters were dried under a heat lamp, and the activity was counted in vials containing Betafluor (National Diagnostics).

Strand exchange. The method used for strand exchange was based on previously used assays (42). Reaction mixtures contained (in 400 μl) 6 μM single-stranded circular M13 DNA, 4 μM M13 RF [³H]DNA linearized by HpaI digestion, 5 μM RecA protein, 30 mM Tris hydrochloride (pH 7.5), 12 mM MgCl₂, 1 mM dithiothreitol, 100 μg of bovine serum albumin per ml, 6 mM phosphocreatine, and 10 U of creatine phosphokinase per ml. After a 10-min preincubation at 37°C, reactions were started by adding 3 mM ATP and 0.5 μM SSB simultaneously. After the indicated times at 37°C, 40-μl samples were added to Eppendorf tubes containing 5 μl of 10% sodium dodecyl sulfate and 2 μl of 320 mM EDTA (pH 8), and the tubes were kept on ice. One such quenched reaction sample was boiled for 10 min to obtain total counts.

To all samples were added 400 μl of S1 cocktail consisting of 0.1 M sodium acetate, 0.2 M NaCl, and 2 mM ZnSO₄ (pH 4.3), followed by 20 U of S1 nuclease (Sigma) per ml. The digestion was allowed to proceed for 30 min at 37°C before the tubes were transferred to ice and the DNA was precipitated by addition of 30 μl of 2-ml/g/ml chicken blood DNA as carrier and 500 μl of 10% trichloroacetic acid. After 15 min on ice, the tubes were centrifuged for 10 min in an Eppendorf Microfuge. Supernatant samples of 600 μl were counted in 5 ml of Liquisint (National Diagnostics). The counts per minute obtained from the boiled, S1 nuclease-digested sample was divided by 2. This number was taken to be the maximum attainable counts per minute in the strand exchange assay, or 100% heteroduplex DNA. It represents the point at which one complete strand of the linear M13 RF [³H]DNA has come free and its partner strand has become half of an S1 nuclease-resistant heteroduplex.

Aggregation assays. Experiments were performed as described by Tsang et al. (31). Reaction mixtures of 30 μl contained 33 mM Tris hydrochloride (pH 7.5), 1.8 mM dithiothreitol, 1.3 mM ATP, 88 μg of bovine serum albumin per ml, 10 U of phosphocreatine kinase per ml, 3 mM phosphocreatine, and 13 mM MgCl₂. Concentrations of [³H]DNA, RecA protein, and SSB were as stated for each experiment. After a 10-min incubation at 37°C in 0.5-ml Eppendorf tubes, reaction mixtures were centrifuged for 2 min at room temperature in an Eppendorf Microfuge. Aliquots of the supernatant and of the resuspended pellet were counted in a liquid scintillation counter to estimate the percentage of single-stranded DNA aggregated in the pellet.

RESULTS

Effects of single-stranded DNA-binding proteins on aggregation of single strands by RecA proteins. As reported previously, incomplete coating of single-stranded DNA by RecA protein results in aggregation of the single strands with RecA protein in complexes that sediment at more than 10,000s. Addition of SSB promotes the coating of single strands by RecA protein and rapidly dissolves such aggregates (31, 32). Coated single strands that do not aggregate are active intermediates that nonspecifically bind duplex DNA and promote the search for homology. As reasoned previously (18, 25), and as supported further by data in this paper, this action of SSB constitutes the major mechanism by which SSB promotes the initial formation of joint molecules by RecA protein. Thus the suppression by SSB of the RecA protein-dependent aggregation of single strands provides a rapid assay for a function of SSB that may be significant for its action in vitro. When we tested variants of SSB, we found
that the mutant SSB-1 protein failed to suppress aggregation of single strands, but that the mutant SSB-113 protein and the chymotryptic derivative SSB* were active in suppressing aggregation (Table 1). As shown below, these observations correlate with the action of these proteins in promoting the formation of joint molecules and subsequent strand exchange.

**Effects of mutant single-stranded DNA-binding proteins and other variants on the formation of joint molecules by RecA protein.** The formation of joint molecules by RecA protein depends strikingly upon temperature: below 30°C the rate of formation of joint molecules becomes vanishingly small (18, 25). When SSB is added, however, RecA protein forms joint molecules efficiently at temperatures 5 to 6°C lower (Fig. 1). This temperature dependence has been related to secondary structure in single-stranded DNA (25), which impedes the binding of RecA protein (31).

SSB-113, like SSB, lowered by 5 to 6°C the threshold temperature for the formation of joint molecules (Fig. 1A). SSB-1, by contrast, did not lower the threshold, but appears to have raised it by a few degrees.

SSB* has been shown to lower the melting point of poly(dA-dT) significantly more than SSB does (41). Similarly, SSB* lowered the threshold temperature for formation of joint molecules by about 8°C, compared with 5 to 6°C for SSB (Fig. 1B). The action of the helix-destabilizing protein of phage T4, gp32, was similar to that of SSB. The helix-destabilizing protein encoded by the *E. coli* sex factor, F SSB, also lowered the temperature threshold.

In addition to being able to lower the threshold temperature for the formation of joint molecules, SSB can eliminate a lag in the reaction which is attributable to the relatively slow polymerization of RecA protein on single-stranded DNA (18, 32). As another measure of how well these helix-stabilizing proteins could facilitate strand invasion, we tested them for their ability to shorten or eliminate the lag in reactions at 34°C, a temperature that accentuates the lag (Fig. 2). All of the tested helix-destabilizing proteins reduced the lag except SSB-1, which slightly increased the lag and decreased the rate when used at 1 μM. At this concentration SSB-1 is mostly in the monomer form, which is unstable at high temperatures (40). Therefore we also tested SSB-1 at 10 μM, at which concentration the protein is a tetramer that binds to single-stranded DNA with an affinity somewhat lower than that of SSB (40). SSB-1 at 10 μM reduced the lag slightly, but did not improve the rate beyond that seen in the absence of any SSB (data not shown). These observations on the time course agree with those described above on the temperature threshold.

**Effects of single-stranded DNA-binding proteins on strand exchange promoted by RecA protein.** The addition of SSB to a reaction mixture before or during strand exchange promoted by RecA protein increases the rate at least twofold (11, 18, 37). We tested other helix-destabilizing proteins to see whether they had an effect similar to that of SSB. Except for SSB-1, all stimulated the rate of strand exchange (Fig. 3). SSB*, as in experiments on joint molecule formation, had the largest effect. The other proteins also showed relative efficiencies consistent with their performance in joint molecule formation. SSB-1, when present from the start of the reaction or when added after strand exchange had already begun (data not shown), had little or no effect on the rate of strand exchange.

**Further observations on SSB-113.** The *ssb-113* mutation in *E. coli* has several effects including extreme UV sensitivity even at low temperatures permissive for growth (38). We wondered whether a defect in recombinational repair might contribute to the UV sensitivity of *ssb-113*. In the tests described above of effects on the formation of presynaptic complexes, on homologous pairing, and on strand exchange, SSB-113 showed behavior nearly indistinguishable from that of SSB. The observations of Cohen et al. (8) indicated that SSB-113 might have a higher affinity for single-stranded DNA than SSB did. When they added labeled RecA protein to a limiting amount of single-stranded DNA in the presence of SSB-113 or SSB, they found that SSB-113 allowed less RecA protein to bind per nucleotide residue than SSB did. Therefore we looked for evidence that at high temperature, SSB-113 may bind so tightly to single-stranded DNA that it interferes with RecA protein binding and recombination. However, over a range of NaCl concentrations from 0 to 150

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**TABLE 1. Inhibition by SSB of single-stranded DNA aggregation caused by RecA protein**

<table>
<thead>
<tr>
<th>Reaction mixture</th>
<th>Aggregation (%)</th>
<th>Stimulation of pairing and strand exchange*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Expt 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (no SSB)</td>
<td>86</td>
<td></td>
</tr>
<tr>
<td>+ SSB</td>
<td>2</td>
<td>+</td>
</tr>
<tr>
<td>+ SSB-1</td>
<td>81</td>
<td>-</td>
</tr>
<tr>
<td>+ SSB-113</td>
<td>10</td>
<td>+</td>
</tr>
<tr>
<td>Expt 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (no SSB)</td>
<td>92</td>
<td></td>
</tr>
<tr>
<td>+ SSB</td>
<td>9</td>
<td>+</td>
</tr>
<tr>
<td>+ SSB*</td>
<td>5</td>
<td>+</td>
</tr>
</tbody>
</table>

* As assessed by the data shown in fig. 1 to 3.
* Reaction conditions were as given in Materials and Methods for aggregation assays. For experiment 1, 8 μM single-stranded DNA and 5.5 μM RecA protein were used. SSB proteins were added within 30 s after RecA protein, at the following concentrations: SSB, 0.67 μM; SSB-113, 0.67 μM; SSB-1, 0.77 μM.
* In experiment 2, 8 μM single-stranded DNA from phage dX174 and 3.3 μM RecA protein were used. SSB or SSB* was added to 0.33 μM.

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**FIG. 1.** Effect of helix-destabilizing proteins on the threshold temperature for strand invasion promoted by RecA protein. Reaction mixtures (30 μl) lacking single-stranded DNA were warmed to the temperatures indicated, and reactions were started by adding the DNA. After 10 min of incubation, 10-μl aliquots were assayed for joint molecules as described in Materials and Methods, except that 10 μl SSC was added immediately after samples were quenched in EDTA. The final concentration of helix-destabilizing proteins was 1 μM. Symbols: ○, no helix-destabilizing protein; ●, SSB; △, SSB-1; ▲, SSB-113; □, F SSB; ■, SSB*; ◇, gp32.
mM, we saw no difference between SSB and SSB-113 in the formation of joint molecules at 42°C (data not shown).

In another experiment we tested whether SSB-113 would be as good as SSB at promoting the formation of active presynaptic complexes. Our most stringent test of this activity has been that of whether single-stranded circles of M13 DNA can pair with M13 RF DNA linearized by cutting at the unique Sau96I site. The bases in the single-stranded circle homologous to this site are in a region of persistent secondary structure (the origin of replication) that apparently must be disrupted before RecA protein can bind to the region and initiate joint molecule formation (25). This test showed that between 28 and 42°C, SSB and SSB-113 were equally effective in stimulating the formation of joint molecules (data not shown). Therefore the two proteins were equally competent at helix destabilization.

**Lack of effect of SSB on synopsis.** Saturation binding of RecA protein to single-stranded DNA in the presynaptic step is necessary for optimal rates of synaptic complex formation. This can be accomplished either by adding SSB to the single-stranded DNA-RecA protein complexes or by allowing RecA protein to bind to the single-stranded DNA in the presence of a low concentration of Mg^{2+} (32). We used a 5-min preincubation at 37°C in the presence of 1 mM Mg^{2+} to allow RecA protein to bind to single-stranded M13 DNA before adding, together, linear duplex M13 DNA, SSB (or buffer), and additional MgCl_2 to bring its final concentration to 12 mM. Once presynaptic complexes were fully formed by preincubation of single-stranded DNA with RecA protein in 1 mM Mg^{2+}, the addition of SSB made no detectable difference in the initial rate of formation of joint molecules (Fig. 4). As seen previously (25, 32), when secondary structure in single-stranded DNA is favored by the higher concentration of divalent cation (12 mM Mg^{2+}), the presence of SSB strongly stimulates the formation of joint molecules (Fig. 4, solid versus open squares), and the reaction in 12 mM MgCl_2-SSB was slightly faster than reactions in which single-stranded DNA was preincubated with RecA protein in 1 mM MgCl_2.

**DISCUSSION**

Examination of the mutant forms of SSB showed that the SSB-1 protein was defective in lowering the temperature threshold for homologous pairing, in blocking the aggregation of single strands by RecA protein, and in stimulating the rates of formation of joint molecules and of strand exchange. SSB-1 has a tyrosine-for-histidine substitution at amino acid residue 55. This change adversely affects interactions between monomers such that the tetramer form is unstable. At concentrations normally found in the cell (0.5 to 1 μM), SSB-1 is in a monomer form that is unstable to elevated temperatures. At concentrations 10 times as great, the equilibrium shifts to the tetramer conformation, but the tetramer is still not as effective a helix DESTABILIZING protein as SSB (40). Since at 34°C, SSB-1 was inhibitory in joint molecule formation at 1 μM, a concentration at which most of the protein is in monomer form, it may be binding to the single-stranded DNA in a way that interferes with the binding of RecA protein.

Surprisingly, our observations in vitro on the pairing and strand exchange reactions of RecA protein failed to reveal any defect in the potentiating functions of SSB-113 protein. Previous observations on a partially purified preparation of SSB-113 found that it was defective in the formation of joint molecules (23); possibly some other factor was involved.

![FIG. 2. Effect of helix-destabilizing proteins on the time course of formation of joint molecules by RecA protein at 34°C. Conditions and methods were as described in the legend to Fig. 1, except that all reactions were at 34°C and aliquots were assayed at the times indicated. Symbols: ○, no helix-destabilizing protein; ●, SSB; △, SSB-1; ▲, SSB-113; □, F SSB; ■, SSBc*; ○, gp32.](image)

**FIG. 3. Acceleration of strand exchange by helix-destabilizing proteins. Reaction mixtures and conditions are described in Materials and Methods. All reactions were at 37°C. Formation of heteroduplex DNA results in the displacement of one labeled strand from the parental duplex molecule, which becomes sensitive to digestion by S1 nuclease: 100% heteroduplex DNA corresponds to the sensitivity of 50% of the original duplex [3H]DNA to S1 nuclease (see Materials and Methods). Symbols: ○, no helix-destabilizing protein; ●, SSB; △, SSB-1; ▲, SSB-113; □, F SSB; ■, SSBc*; ○, gp32.**
The effects of the ssb-113 mutation in vivo are numerous and include failure to amplify RecA protein synthesis (1) and to induce lambda prophage (33), as well as an extreme sensitivity to UV radiation (21, 35, 39). An increased affinity of SSB-113 protein for single-stranded DNA has been noted, and this difference has been suggested to account for the effects of the ssb-113 mutation (4, 8, 28). SSB-113 would compete with RecA protein more effectively than SSB would for DNA sites necessary to activate RecA protein as a specific protease for the LexA (and lambda repressor) protein. Failure to induce the SOS response would result. A recent experiment demonstrates that, at least for the ssb-l mutation, failure to induce the SOS response cannot entirely account for the UV sensitivity of the cells (14). Therefore SSB may participate directly in the repair of UV-damaged DNA. For the severely UV-sensitive ssb-113 strains, the more life-threatening defect may be the inability to interact with an enzyme involved in DNA repair, rather than a decrease in the efficiency of induction of the SOS response.

SSB*, results from proteolytic removal of 42 amino acids from the carboxyl terminus. Experiments described in this paper have shown that this proteolytic fragment is superior to all the other proteins tested, with regard to augmenting RecA protein-mediated strand assimilation reactions. The relative decreases in the denaturation temperature of poly(dA-dT) caused by SSB or by SSB*, binding suggest that SSB*, can bind to single-stranded DNA about 100 times as strongly as SSB can (41). The stronger binding activity of the amino-terminal end of SSB without its other end provides evidence for two domains of different function. The carboxyl terminus may be required to reduce the helixdestabilizing potential or to interact with proteins involved in DNA repair or replication, although probably not with RecA protein.

F SSB behaved much like SSB in strand assimilation assays. F SSB and SSB share very little sequence homology within their carboxyl terminal regions, but six of the last seven amino acid residues of F SSB are identical to those at the end of SSB (5). This finding, along with the severe effects caused by the ssb-113 mutation, which changes the penultimate amino acid residue, suggests that this region of the protein interacts specifically with some other protein. F SSB is a member of a family of homologous proteins encoded by conjugative plasmids belonging to 12 different incompatibility groups. The plasmid-encoded ssb genes were able, at least partially, to complement ssb-l in E. coli (13). The function of the plasmid ssb genes and the reason for their conservation are unknown, although it has been shown that they have no effect on efficiencies of transfer (14).

To understand how SSB and other helixdestabilizing proteins facilitate the action of RecA protein, it is important to bear in mind the three distinct phases of the recombination reaction that RecA protein promotes in vitro (see Introduction). Previous studies have shown that SSB stimulates the first phase, presynaptic polymerization of RecA protein on single-stranded DNA, and the last phase, strand exchange (26, 27; Cox and Lehman, in press). In the present experiments, we looked for a role of SSB in the intermediate synaptic phase by providing optimal conditions for the binding of RecA protein to single-stranded DNA in the absence of SSB. Once presynaptic filaments were formed in the presence of 1 mM MgCl2, subsequent addition of SSB had no detectable effect on the rate of homologous pairing and thus no detectable effect on the conjunction and homologous alignment of presynaptic filaments and duplex DNA.

Varous studies have led to the conclusion that the presynaptic polymerization of RecA protein is hindered by secondary structure in single-stranded DNA and that SSB helps to overcome that barrier (19, 20, 24, 25). The degree to which SSB remains associated with the active RecA nucleoprotein filament has been the subject of differing observations and interpretations, but at least some of the current evidence suggests that there is a metastable but regular and stoichiometric association of SSB with the RecA–single-stranded DNA filament (16, 19, 24, 32; K. Muniyappa, K. Williams, J. Chase, and C. Radding, submitted for publication). However, the present results show, as suggested previously (20, 29), that specific interaction of the SSB polypeptide chain with the RecA polypeptide chain plays no important role in either presynaptic polymerization of RecA protein or postsynaptic strand exchange. Thus the interaction of SSB and RecA protein via the DNA is novel and particularly interesting.

In the present study we tested SSB, two mutant forms of SSB, a partial proteolysis product of SSB, the closely related F SSB, and the unrelated product (gp32) of gene 32 of phage
T4. With the exception of SSB-1, all eliminate the lag in the formation of joint molecules, lower the temperature at which RecA protein can form joint molecules, increase the rate of formation of joint molecules, and increase the rate of strand exchange. The order of the relative efficiencies of the different helix-stabilizing proteins in each of these functions was about the same, suggesting that there is one function common to these various proteins that does not require specific interactions with RecA protein.

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