## Mechanism of the concerted action of recA protein and helixdestabilizing proteins in homologous recombination

(single-strand-binding protein/single-stranded DNA secondary structure/sequence specificity)

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ABSTRACT Secondary structure in single-stranded DNA impedes the presynaptic association of recA protein and consequently blocks the formation of joint molecules as evidenced by effects of temperature, nucleotide sequence, and ionic conditions. *Escherichia coli* single-strand-binding protein eliminates sequence-specific "cold spots" by removing folds even from sites of strong secondary structure. Thus, destabilization of secondary structure in single-stranded DNA is critical for the action of recA protein, whereas specific interactions directly between helix-destabilizing proteins and recA protein are unimportant.

The product of the recA gene in Escherichia coli is essential for homologous recombination and for the induction of the "SOS" pathway of DNA repair. The recA protein, which has a molecular weight of 38,000, is both an ATP-dependent protease that specifically cleaves certain repressors and a DNAdependent ATPase that promotes homologous pairing and strand exchange (1). The product of the ssb gene is essential for replication and plays important roles as well in homologous recombination, induction of the SOS system, and control of DNA degradation after UV irradiation (2-5). The protein, called single-strand-binding protein (SSB), which has a molecular weight of 19,000, is a member of the class of helixdestabilizing proteins (6). In vitro, SSB extensively coats single-stranded DNA (7) in a way that enables it to promote the renaturation of complementary single strands, as well as to direct the site-specific priming of DNA synthesis (8).

In vitro, recA protein promotes the homologous pairing of single-stranded or partially single-stranded DNA with duplex DNA in three sequential phases: (i) a slow presynaptic phase during which recA protein polymerizes on singlestranded DNA to form presynaptic complexes (1, 9–11); (ii) synapsis, the rapid pairing of single-stranded DNA with its complementary strand in duplex DNA to produce a nascent heteroduplex joint (12–14); and (iii) strand exchange, the slow unidirectional displacement of a strand from the duplex and its concomitant replacement by a new strand to produce lengthy regions of heteroduplex DNA (15–17).

SSB accelerates 2- to 3-fold both the formation of joint molecules by recA protein and subsequent strand exchange (1, 18, 19). The kinetics of formation of joint molecules by recA protein exhibit a characteristic lag that can be eliminated either by preincubation of single strands with recA protein or by the addition of SSB (19). The presence of SSB stabilizes presynaptic complexes and inhibits exchange of bound and free recA protein (18). By electron microscopy, Flory and Radding (9) observed that SSB strikingly accelerated the formation of filaments consisting largely of recA protein plus single-stranded DNA, and it inhibited the formation of large aggregates. Soltis and Lehman (11) reported that when complexes that were formed in the presence of

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SSB and recA protein were stabilized by the addition of adenosine 5'-[ $\gamma$ -thio]triphosphate, little or no labeled SSB was detectable in the reisolated complexes. Although these and other significant observations have been made on the ability of SSB to favor the formation of joint molecules by recA protein (20-22), the nature of the interaction has remained enigmatic. Is there a specific protein-protein interaction, or does SSB act primarily via the DNA? Experiments reported here support the latter explanation.

## **METHODS**

**Enzymes and DNA.** recA protein was purified to homogeneity and its concentration was determined as described (23). The  $\beta$  protein of phage  $\lambda$  was purified as described before (24) except that it was further purified by chromatography on a Sephacryl S-200 column to remove residual  $\lambda$  exonuclease activity and an associated protein. *E. coli* SSB was the generous gift of John Chase (Dept. of Molecular Biology, Albert Einstein College of Medicine, New York).

Circular single-stranded and circular duplex DNA from phage M13 were prepared as described (25). All the preparations of circular single-stranded DNA contained less than 5% linear molecules as determined by electrophoresis on 1.8%agarose gels. Superhelical DNA (form I) was purified by banding in CsCl/ethidium bromide gradients. It contained less than 2% nicked molecules as estimated by the method of Kuhnlein et al. (26). Form I DNA was cleaved at a single site by respective restriction endonucleases under standard conditions described by the supplier. Restriction endonucleases Hpa I, Acc I, BamHI, HincII, and Sau96I were obtained from New England Biolabs. After digestion, DNA was extracted with phenol and ether and precipitated by ethanol. The pellet was redissolved in 10 mM Tris·HCl (pH 7.5) containing 1 mM EDTA and dialyzed against the same buffer overnight at 4°C. The concentration of DNA is expressed in moles of nucleotide residues.

Assay for Joint Molecules. The assay for joint molecules is based on the method of Beattie et al. (27) and measures the retention on nitrocellulose filters of <sup>3</sup>H-labeled linear duplex molecules bound to homologous single strands. Standard reaction mixtures (30  $\mu$ l) contained 2.5  $\mu$ M linear duplex [<sup>3</sup>H]DNA (M13 supercoiled DNA cleaved with restriction endonuclease Hpa I), 8  $\mu$ M circular single strands, SSB, and recA protein in 31 mM Tris·HCl (pH 7.5)/1.2 mM ATP/12.5 mM MgCl<sub>2</sub>/1.8 mM dithiothreitol/88  $\mu$ g of bovine serum albumin (nuclease free, obtained from Bethesda Research Laboratories) per ml/6 mM phosphocreatine/10 units of creatine kinase per ml. Reaction mixtures were prepared in Eppendorf tubes (0.5 ml) at 0°C warmed for 1-2 min at their respective temperatures before the reaction was initiated by the addition of circular single strands. Incubation for formation of joint molecules was for 20 min and the reaction was terminated by transferring it to 0.3 ml of 25 mM EDTA (pH

Abbreviation: SSB, single-strand-binding protein.

9.0) at 0°C. After taking an aliquot for the determination of total input radioactivity, we diluted the reaction mixtures in 3 ml of ice-cold 1.5 M NaCl/0.15 M sodium citrate and immediately filtered it through a nitrocellulose filter (Millipore, HAWP, 0.45- $\mu$ m pore diameter) that had been soaked in the above buffer. The filters were rinsed with 8 ml of the same ice-cold buffer, dried, and assayed for bound radioactivity.

## RESULTS

**Experimental Design.** Preincubation of single-stranded DNA with recA protein or addition of *E. coli* SSB have equivalent effects in eliminating a lag in the formation of joint molecules and accelerating the initial rate of the reaction. Under normal circumstances, the presynaptic phase limits the rate of formation of joint molecules (19). Accordingly, one can study the presynaptic phase either by varying conditions during preincubation of single strands with recA protein or by varying conditions when single-stranded DNA is the last ingredient added. Both protocols have been used in the experiments described here.

A Sharp Thermal Transition in the Formation of Joint Molecules: Effect of SSBs. When we added M13 circular single strands last to otherwise complete reaction mixtures already equilibrated at various temperatures, no joint molecules formed below 29°C even after 20 min of incubation. However, between 30°C and 33°C there was a sharp increase in the yield of joint molecules measured after 20 min of incubation (Fig. 1). We will speak of this sharp effect of temperature as a thermal transition.

When we included 0.55  $\mu$ M SSB in the reaction mixtures before adding circular single strands, we found that the entire thermal transition was shifted 5-6°C toward lower temperatures (Fig. 1). The effect of SSB was not unique, since T4 gene 32 protein caused a shift of the same magnitude (unpublished observations), and the  $\beta$  protein of phage  $\lambda$  caused a similar but smaller shift (Fig. 1). At 27°C in the absence of SSB, joint molecule formation was not detectable even after 60 min of incubation (Fig. 1 *Inset*).

The thermal transition was also shifted  $5-6^{\circ}C$  toward lower temperatures by preincubating single strands with recA protein or with both recA protein and SSB (data not shown). The experiment shows that the thermal transition is related to the slow interaction of recA protein with single-stranded DNA, which is accelerated by SSB and other helix-destabilizing proteins.

Sequence-Specific Formation and Decay of Presynaptic Complexes. We prepared different linear duplex substrates by cleaving superhelical M13 DNA at nucleotide 2220 with restriction endonuclease BamHI, at 5724 with Sau96I, at 6090 with Acc I, and at 6405 with Hpa I. Although a circular single strand can pair at any position within a linear duplex molecule, strand exchange and subsequent stabilization of the joint molecule require pairing at one end produced by each of these restriction enzymes (13, 14). In the singlestranded DNA at the site corresponding to the end created by BamHI there is no evident secondary structure, whereas the Sau96I site corresponds to a potential hairpin structure in the single strand that is flanked on either side by two other hairpins (28). We allowed these different linear substrates to pair with circular single-stranded M13 DNA in reactions promoted by recA protein at various temperatures. The substrates produced by cutting with Hpa I and Acc I showed identical thermal transitions (Fig. 2). Hpa I generates a flush end, whereas Acc I generates two unpaired bases at the 5' end. The substrate produced by BamHI, which has four unpaired bases at the 5' end, showed a similar but somewhat broader transition. However, the substrate produced by Sau96I, which has three unpaired bases at the 5' end, was associated with a shift of the thermal transition of 3-5°C toward higher temperatures. The addition of SSB shifted all of



Incubation temperature, °C

FIG. 1. Sharp thermal transition in the formation of joint molecules by recA protein. Single-stranded DNA was added last to reaction mixtures that had been equilibrated at the temperatures indicated. •, In the presence of 5  $\mu$ M recA protein alone; •, 5  $\mu$ M recA protein plus 0.55  $\mu$ M SSB; •, 5  $\mu$ M recA protein plus 3  $\mu$ M  $\beta$  protein. (*Inset*) Time course of formation of joint molecules at 27°C. Reaction mixtures (90  $\mu$ l) were prepared and incubated as described above. Aliquots (8  $\mu$ l) were taken at the indicated time intervals and added to 0.3 ml of 25 mM EDTA (pH 9.0) at 0°C. •, In the presence of 5  $\mu$ M recA protein alone; •, 5  $\mu$ M recA protein plus 0.55  $\mu$ M SSB.

the thermal transitions toward lower temperatures and made them indistinguishable. For the substrate made by Sau96I, the downward shift of the thermal transition caused by SSB was as large as  $10^{\circ}C$ .

Unpublished work has demonstrated that presynaptic complexes made at  $37^{\circ}$ C in the absence of SSB and held at  $30^{\circ}$ C, where they cannot rapidly form, lose much of their activity in the course of 30 min. We compared the time course of this decay when the presynaptic complexes were paired with two different linear duplex substrates. The very same presynaptic complexes lost their activity more rapidly when they were subsequently paired with the *Sau*96I substrate than when they were paired with the *Hin*cII substrate



Incubation temperature, °C

FIG. 2. Effect of nucleotide sequence on the formation of joint molecules in the presence and absence of SSB. Reaction mixtures (30  $\mu$ l) contained 3.5  $\mu$ M linear duplex [<sup>3</sup>H]DNA, 8  $\mu$ M circular single strands, and 5  $\mu$ M recA protein. The reaction was initiated by the addition of circular single-stranded DNA and terminated after 20 min of incubation at the temperature indicated. M13 form I [<sup>3</sup>H]DNA linearized with restriction endonuclease Hpa I:  $\odot$ , 5  $\mu$ M recA protein plus 0.55  $\mu$ M SSB. M13 form I DNA cleaved with restriction endonuclease Acc I:  $\times$ , 5  $\mu$ M recA protein alone;  $\bullet$ , 5  $\mu$ M recA protein plus 0.55  $\mu$ M SSB. M13 form I DNA cleaved with restriction endonuclease Acc I:  $\times$ , 5  $\mu$ M recA protein alone;  $\bullet$ , 5  $\mu$ M recA protein alone;  $\bullet$ , 5  $\mu$ M recA protein alone;  $\bullet$ , 5  $\mu$ M recA protein plus 0.55  $\mu$ M SSB. M13 form I DNA cleaved with restriction endonuclease Acc I:  $\times$ , 5  $\mu$ M recA protein alone;  $\bullet$ , 5  $\mu$ M recA protein alone;  $\bullet$ , 5  $\mu$ M recA protein alone;  $\bullet$ , 5  $\mu$ M recA protein plus 0.55  $\mu$ M SSB. M13 form I DNA cleaved with restriction endonuclease Acc I:  $\times$ , 5  $\mu$ M recA protein alone;  $\bullet$ , 5  $\mu$ M recA p

(Fig. 3) (*HincII* is an isoschizomer of Hpa I). This loss of activity, which is a function of the time during which presynaptic complexes are held at 30°C, must be attributed to a change in the structure of the presynaptic complex itself. SSB inhibits or prevents the loss of activity (Fig. 3).

The high thermal transition associated with the Sau96I substrate, and the corresponding rapid loss of activity of presynaptic complexes, correlates with the potential secondary structure in the single-stranded DNA at the Sau96I site (28). Since in addition there is no correlation between the formation of joint molecules and the physical nature of the ends made by restriction endonucleases (Fig. 2 and unpublished data), we interpret these observations as evidence of sequence specificity in the formation and decay of presynaptic complexes.

Effects of Preheating Circular Single-Stranded DNA and of Using Fragments. Incubation of circular single strands in Tris/EDTA at  $60^{\circ}$ C for 10 min prior to their addition to the reaction mixture at  $30^{\circ}$ C enhanced the rate and yield of the reaction 2- to 3-fold (Fig. 4). On the other hand, the formation of joint molecules was even less than the control values when the heated single strands were subsequently chilled on ice before they were added to the reaction (Fig. 4).

At 30°C, efficient formation of joint molecules occurred when we used single-stranded fragments (average length of 600 nucleotides) rather than intact circular single strands. Both the initial rate of formation and final yields were greater with fragments (Fig. 4). Preheating of fragments had no effect (data not shown). The activity of fragments at  $30^{\circ}$ C and their insensitivity to preheating is further evidence that the thermal transition is attributable to the structure of the single-stranded DNA. Fragmentation of the circular singlestranded DNA greatly increases the number of separate single-stranded molecules, any of which can react with linear duplex DNA to form a joint molecule. Presumably, some



FIG. 3. Sequence-specific decay of presynaptic complexes. M13 circular single-stranded DNA was preincubated with recA protein or recA protein and SSB at 37°C for 10 min in the standard reaction mixture. After preincubation, reaction mixtures were shifted to 30°C for the indicated times before the addition of M13 linear duplex DNA to begin the formation of joint molecules at 30°C for 10 min. The reaction was quenched by mixing the reaction mixture with 0.3 ml of 25 mM EDTA (pH 9.0) at 0°C. The amount of joint molecules obtained after 2 min at 30°C was taken as 100%. Linear duplex DNA obtained by cleaving form I DNA with endonuclease Sau96I: 0, 1.5  $\mu$ M single strands, 5  $\mu$ M linear duplex DNA, and 4.2  $\mu$ M recA protein; •, 8  $\mu$ M single strands, 3  $\mu$ M linear duplex DNA, and 5  $\mu$ M recA protein; ■, 8 µM single strands, 3 µM linear duplex DNA, 5  $\mu$ M recA protein, and 0.44  $\mu$ M SSB. Linear duplex DNA obtained by cleaving form I DNA with endonuclease  $HincII: \triangle, 8 \mu M$  circular single strands, 3  $\mu$ M linear duplex DNA, and 5  $\mu$ M recA protein:  $\blacktriangle$ , 1.5  $\mu$ M circular single strands, 5  $\mu$ M linear duplex DNA, and 4.2  $\mu$ M recA protein.



FIG. 4. Effects of preheating single strands and of fragments on the formation of joint molecules at 30°C. Reaction mixtures (90  $\mu$ l) contained 2.0 µM M13 linear duplex [3H]DNA (M13 form I DNA cleaved with restriction endonuclease Hpa I), 8 µM single-stranded DNA, and 5  $\mu$ M recA protein. Aliquots were taken at the indicated time intervals and added to 0.3 ml of 25 mM EDTA (pH 9.0) at 0°C. o, Single-stranded fragments, not heated; •, circular single strands in 10 mM Tris·HCl (pH 7.5)/1 mM EDTA were heated at 60°C for 10 min and added directly to the reaction mixture to form joint molecules;  $\triangle$ , untreated circular single strands;  $\Box$ , control: untreated circular single strands plus an equal volume of buffer kept at 60°C were added to the reaction mixture;  $\blacktriangle$ , circular single strands, treated at 60°C for 10 min, and subsequently chilled on ice for 20 min before being added to the reaction mixture. Fragments of single-stranded DNA were prepared as described before. The treatment of circular single strands at 60°C did not produce either fragments or linear molecules as judged by agarose gel electrophoresis.

fraction of the population of fragments will be relatively free of secondary structure.

Effect of the Concentration of  $Mg^{2+}$  on Secondary Structure in Single-Stranded DNA. Low concentrations of  $Mg^{2+}$  favor the disruption of secondary structure in single-stranded DNA (29), but optimal formation of joint molecules by recA protein occurs at concentrations of  $Mg^{2+}$  above 10 mM (30). By varying the order of addition of  $MgCl_2$  and recA protein relative to single-stranded DNA we were able to distinguish the effect of  $Mg^{2+}$  on the secondary structure of single strands from its role as a cofactor.

We incubated single strands for 10 min at  $37^{\circ}$ C with 1 mM MgCl<sub>2</sub> prior to shifting aliquots to various temperatures and allowing them to equilibrate for 1–2 min. We then started the reaction by adding recA protein plus linear duplex DNA and more MgCl<sub>2</sub>. The control was a reaction mixture that contained 12 mM Mg<sup>2+</sup> and in which the reaction was started by adding single-stranded DNA that had not been preincubated in any way.

When we incubated single strands in 1 mM Mg<sup>2+</sup> and added them to mixtures containing 11 mM Mg<sup>2+</sup>, the temperature transition shifted to the left (Fig. 5A). When recA protein was included in the preincubation of single-stranded DNA with 1 mM MgCl<sub>2</sub>, a very similar shift was seen (Fig. 5A). The effect of preincubating single strands in a low concentration of Mg<sup>2+</sup> was greatest at 30°C and was minimal at 37°C.

If instead of increasing the final concentration of MgCl<sub>2</sub> to 12 mM we increased it to 20 mM, there was no shift in the thermal transition (Fig. 5A). This observation indicates that MgCl<sub>2</sub> caused a rapid change in conformation of the single strands. To study this effect further, we incubated single strands at 37°C in 1 mM MgCl<sub>2</sub>, shifted the reaction to 30°C, and added recA protein, duplex DNA, and additional MgCl<sub>2</sub>, in that order. Under these conditions, the optimal final concentration of MgCl<sub>2</sub> differed when the linear DNA was made by cutting form I DNA with endonuclease Sau96I as com-



FIG. 5. Effects of  $Mg^{2+}$ ; sequence-specific  $Mg^{2+}$  optima. (A) Standard reaction mixtures (30  $\mu$ l) contained 3.5  $\mu$ M linear duplex [<sup>3</sup>H]DNA (M13 form I DNA linearized with restriction endonuclease Sau96I), 8 µM circular single strands, and 5 µM recA protein. 0, Circular single strands were incubated at 37°C for 10 min with 1 mM Mg<sup>2+</sup> followed by 1- to 2-min equilibration at the indicated temperatures prior to the addition of recA protein, linear duplex DNA, and 11 mM  $Mg^{2+}$  to begin the formation of joint molecules.  $\Box$ , Circular single strands incubated with 1 mM  $Mg^{2+}$ , then adjusted to 20 mM  $Mg^{2+}$  before the addition of recA protein and linear duplex DNA.  $\triangle$ , No preincubation; the reaction mixture contained 12 mM Mg<sup>2+</sup>. The reaction was initiated by the addition of circular single strands. •, Circular single strands incubated at 37°C for 10 min with 1 mM Mg<sup>2</sup> and recA protein followed by 1–2 min at the indicated temperature prior to the addition of 11 mM  $Mg^{2+}$  and linear duplex DNA to begin the formation of joint molecules. After the addition of the last component, all mixtures were incubated for 10 min at the indicated temperatures. (B) Reaction mixtures (30  $\mu$ l) contained 3.0  $\mu$ M linear duplex DNA, 8  $\mu$ M M13 circular single strands, 5  $\mu$ M recA protein, and indicated concentrations of  $Mg^{2+}$ . Circular single strands were incubated at 37°C for 10 min and subsequently shifted to 30°C and allowed to stand for 1 min before the rest of the assay components were added, in the order indicated, and incubation was continued for 10 min. The names of restriction enzymes listed below indicate which enzyme was used to make linear duplex DNA from form I DNA: (i) recA protein was added first, followed by linear duplex DNA, and the mixture was adjusted to the indicated amount of Mg<sup>2+</sup>. The time involved between each addition was roughly 30 sec. ●, Sau961; ▲, HincII. (ii) Mg<sup>2+</sup> was added first to obtain the desired final concentration, followed by recA protein and linear duplex DNA. The time intervals were as described above.  $\circ$ , Sau96I;  $\triangle$ , HincII. (iii) No preincubation. The standard reaction mixture contained 12 mM Mg<sup>2+</sup> and the reaction for joint molecules was initiated by the addition of circular single strands.  $\Box$ , Sau961;  $\nabla$ , HincII. HincII is an isoschizomer of Hpa I.

pared with *HincII* (Fig. 5B). Consistent with the effect of secondary structure in the single-stranded DNA at the Sau96I site (see above), the  $Mg^{2+}$  optimum for duplex DNA cut at the Sau96I site was lower than the optimum for DNA cut at the *HincII* site.

Strikingly, we found that if we raised the final concentration of MgCl<sub>2</sub> to any level from 4 to 20 mM before we serially added recA protein and duplex DNA, no joint molecules were formed at all in 10 min at 30°C (Fig. 5B). As expected, an inactive reaction mixture produced by this order of addition was immediately reactivated by the addition of SSB (data not shown). The single-stranded DNA was inactivated by first adding the required amount of Mg<sup>2+</sup>, whether the duplex DNA added later was cleaved by Sau96I or HincII. The latter observation shows that the effect of secondary structure in the single-stranded DNA is not localized solely at sites of strong secondary structure, but is also a global effect.

Melting curves of single-stranded M13 DNA in the buffers used for recA reactions showed that increases of  $Mg^{2+}$  concentration in the range of 1 to 10 mM were accompanied by hypochromic shifts at all temperatures below 75°C (data not shown). These observations confirm directly that changes in secondary structure occurred under the conditions of our experiments.



FIG. 6. Secondary structure in the single-stranded DNA of presynaptic complexes. M13 circular single strands (50  $\mu$ M) were incubated for various times at 37°C in a standard assay mixture (60  $\mu$ l) containing concentrations of recA protein and SSB as indicated below. After incubation, the reaction mixture was adjusted to 4 mM NaCl, 100 µg of bovine serum albumin per ml, and 6 mM 2-mercaptoethanol, followed by 2 units of restriction endonuclease Hae III. After 20 min of incubation with endonuclease Hae III at 37°C, the reaction mixtures were extracted with phenol and ether and precipitated by ethanol. The pellet was washed with 70% (vol/vol) ethanol and resuspended in 10 mM Tris·HCl (pH 7.5) containing 1 mM EDTA, to which glycerol was added to 10% (vol/vol). The individual samples were loaded on a 2% agarose gel and subjected to electrophoresis in 89 mM Tris/borate containing 2 mM EDTA (pH 8.3) for 4 hr at 200 V. After electrophoresis, the gel was stained for 30 min with ethidium bromide at 0.5  $\mu$ g/ml. SSO marks the position of circular single-stranded DNA, and SS that of linear single strands. Lane a, M13 circular single strands; lane b, M13 circular single strands incubated without recA protein, SSB and restriction endonuclease; lane c, incubated with restriction endonuclease in the absence of recA protein or SSB. All of the following samples were treated with restriction endonuclease: Lane d, incubated with 31  $\mu$ M recA protein for 2.5 min (1 molecule of recA protein per 1.6 nucleotide residues); lane e, same as d, but 10 min of incubation; lane f, same as d, but 30 min of incubation; lane g, incubated with 6.25  $\mu$ M SSB for 2.5 min (1 molecule of SSB per 8 nucleotide residues); lane h, same as g, but 10 min of incubation; lane i, incubated with 25  $\mu$ M recA protein and 3.5 µM SSB for 2.5 min (1 molecule of recA protein per 2 nucleotide residues and 1 molecule of SSB per 14.3 residues); lane j, same as i, but incubation for 10 min.

Secondary Structure in the Single Strands of Presynaptic Complexes. Certain restriction endonucleases cleave singlestranded DNA by virtue of secondary structure in the DNA (31). Restriction endonuclease Hae III cleaves M13 singlestranded DNA into at least 12 fragments (Fig. 6, lane c). All but one of the restriction sites were protected when recA protein was incubated with single-stranded DNA for 30 min before Hae III was added. The unprotected site is presumably the one in the region of strong secondary structure (28). By contrast, either SSB or SSB plus recA protein protected all sites from cleavage by Hae III (Fig. 6, lanes g-j). This experiment shows that recA protein only slowly masks sites in M13 DNA that have secondary structure and that at least one site resists unfolding by recA protein, whereas SSB rapidly masks all Hae III sites, by unfolding secondary structure. These observations correlate with those which show that SSB accelerates the formation of presynaptic complexes and shifts to lower temperatures the thermal transition that characterizes the formation of joint molecules (Figs. 1 and 2).

## DISCUSSION

Helix-destabilizing proteins enable recA protein to promote homologous pairing by overcoming factors that favor secondary structure in single-stranded DNA; such factors include palindromic sequences, low temperature, and Mg<sup>2+</sup>. Since secondary structure in the single-stranded DNA is critical, there should be sequence-specific effects on the formation of joint molecules. We found such effects by using linear duplex DNA that was produced by cutting superhelical DNA with different restriction endonucleases, one of which cuts duplex DNA at a site corresponding to a strong hairpin region in single-stranded DNA. Evidence that the observed differences are attributable to effects of nucleotide sequence on the single-stranded DNA came particularly from the observation that the loss of activity of presynaptic complexes incubated alone at 30°C depended upon which duplex molecule was subsequently added as a substrate for the formation of joint molecules. To the extent that any given region of single-stranded DNA has potential secondary structure, the random dissociation of recA protein from that region would cause the DNA to assume a folded conformation that is unfavorable for the reassociation of recA protein. It is simplest to suppose that SSB prevents the decay of presynaptic complexes (Fig. 3) in the same way that it favors their initial formation, namely, by removing folds from the single strand and permitting recA protein to associate.

The role of helix-destabilizing proteins in promoting DNA-DNA interaction, namely, the pairing of complementary single strands, has long been known (32, 33). More recent studies on replication *in vitro* describe two ways in which *E. coli* SSB influences the interactions of other proteins with single-stranded DNA. By coating DNA, SSB determines the site-specific priming of DNA synthesis on single-stranded templates of M13, G4, and  $\phi$ X174 phage DNA by three different enzyme systems. This site specificity has been attributed to residual secondary structure in the coated DNA. Second, in the priming of  $\phi$ X174 replication, SSB is required for the binding of protein n to single-stranded DNA; and sedimentation studies showed that the two proteins can form a complex in the absence of DNA (34).

The effect of SSB on the interaction of recA protein with single-stranded DNA is different in several respects. The binding of recA protein is not site-specific or even localized, and it is favored by the elimination of secondary structure from single-stranded DNA. There is no published evidence of a direct interaction between SSB and recA protein; moreover, the ability of several different helix-destabilizing proteins to promote the action of recA protein argues against a specific protein-protein interaction. Further research is required to understand more precisely how helix-destabilizing proteins, acting on the structure of single-stranded DNA, can facilitate rather than obstruct the access of another protein to single-stranded DNA.

The binding of recA protein to single-stranded DNA that is stimulated by SSB also activates the protease function of recA protein and, consequently, the induction of the SOS pathway (3, 4, 35, 36). Thus, the destabilization of secondary structure in single-stranded DNA, which enables recA protein to act effectively on those strands, is likely to be an essential common step in recombination, inducible repair, and control of postirradiation DNA degradation.

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