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# A Postsynaptic Role for Single-stranded DNA-binding Protein in recA Protein-promoted DNA Strand Exchange*

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**The single-stranded DNA-binding protein (SSB protein) is required for efficient genetic recombination *in vivo*. One function for SSB protein in DNA strand exchange *in vitro* is to remove secondary structure from single-stranded DNA (ssDNA) and thereby aid in the formation of recA protein-saturated presynaptic complexes. In the preceding paper (Lavery, P. E., and Kowalczykowski, S. C. (1992) *J. Biol. Chem.* 267, 9307–9314) we demonstrated that DNA strand exchange can occur in the presence of volume-occupying agents at low magnesium ion concentration, where secondary structures are reduced. Our results suggest that SSB protein is not acting during presynapsis under these conditions, yet the DNA strand exchange reaction is stimulated by the addition of SSB protein. In this study we present biochemical evidence which suggests that SSB protein stimulates DNA strand exchange by binding to the ssDNA displaced from joint molecules, thereby stabilizing them and allowing branch migration to extend the region of heteroduplex DNA. Therefore, our results indicate dual roles for SSB protein at elevated magnesium ion concentration; it functions during presynapsis, removing secondary structure from ssDNA, as indicated previously, and it also functions postsynaptically, binding to the ssDNA displaced from joint molecules.**

In the accompanying paper (Lavery and Kowalczykowski, 1992) we demonstrate that the addition of polyvinyl alcohol (PVA)[1] or polyethylene glycol (PEG) enables the recA protein of *Escherichia coli* to promote DNA strand exchange between a linear duplex DNA molecule and a homologous circular ssDNA molecule at an otherwise nonpermissive magnesium ion concentration (see Cox and Lehman, 1982; Shibata *et al.*, 1979). We found that both the rate of recA protein association with ssDNA and the steady-state affinity of recA protein for ssDNA are increased in the presence of these macromolecules. Consequently, the ability of recA protein to compete with SSB protein for ssDNA and to bind ssDNA containing secondary structures is enhanced. Thus, at 1 mM magnesium acetate, the addition of PVA or PEG to the DNA strand exchange reaction alleviates the inhibitory effects of SSB protein on the formation of recA protein-ssDNA presynaptic complexes. Furthermore, in the presence of these polymers the subsequent pairing and strand exchange between presynaptic complexes and duplex DNA is no longer dependent on elevated magnesium ion concentration. Because the ability of recA protein to bind to ssDNA with secondary structures is enhanced under these conditions, joint molecule formation occurs quite well when SSB protein is omitted from the reaction.

Previous studies of recA protein-promoted DNA strand exchange have emphasized a presynaptic role for SSB protein (McEntee *et al.*, 1980; Cox *et al.*, 1983; Kahn and Radding, 1984; Tsang *et al.*, 1985; Morrical *et al.*, 1986). At elevated magnesium ion concentration, SSB protein is required to remove secondary structure from ssDNA, thereby allowing recA protein to form saturated presynaptic complexes (Muniyappa *et al.*, 1984; Kowalczykowski and Krupp, 1987). This requirement for SSB protein in an early step of DNA strand exchange has obscured identification of an additional role for SSB protein. At 1 mM magnesium acetate in the presence of PVA or PEG, SSB protein does not appear to aid recA protein in the formation of saturated presynaptic complexes, *i.e.* there is no stimulation of ssDNA-dependent ATPase activity by SSB protein. However, SSB protein does have a stimulatory effect on DNA strand exchange under these conditions. It has been suggested that SSB protein binds to the ssDNA displaced from joint molecules (Soltis and Lehman, 1983; Stasiak *et al.*, 1984; Register *et al.*, 1987; Chow *et al.*, 1988); however, it has not been clear whether this binding is an essential component of the DNA strand exchange process or merely a consequence of strand displacement. Electron microscopic analysis of DNA strand exchange has led Stasiak and Engelman (1988) to propose that the binding of SSB protein pulls the strand being displaced from the recA protein-covered region of DNA alignment and in doing so drives branch migration. In this study we present biochemical evidence that supports a postsynaptic function for SSB protein. We conclude that by binding to the displaced ssDNA, SSB protein stabilizes joint molecules, inhibits the reinitiation reactions that lead to the formation of homology-dependent DNA networks (Chow *et al.*, 1988), and favors the extension of heteroduplex DNA via branch migration.

## MATERIALS AND METHODS

All materials and methods are the same as described in the accompanying paper (Lavery and Kowalczykowski, 1992) with the following exceptions.

*ATPase Assay*—Reactions were carried out in TMD buffer (25 mM Tris acetate, 1 mM magnesium acetate, 1 mM dithiothreitol, and PVA or PEG as indicated) and contained 0.2 mg of NADH/ml, 0.5 mM ATP, 3 mM phosphoenolpyruvate, 30 units/ml each of pyruvate

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[1] The abbreviations used are: PVA, polyvinyl alcohol; PEG, polyethylene glycol; ssDNA, single-stranded DNA; dsDNA, double-stranded DNA; SSB protein, *E. coli* single-stranded DNA-binding protein.

kinase and lactate dehydrogenase, 2.5 μM M13 ssDNA, and varying recA protein concentrations.

*DNA Strand Exchange Assay*—Reactions were performed in TD buffer (25 mM Tris acetate and 1 mM dithiothreitol) containing either 1 or 6 mM magnesium acetate and PVA or PEG as indicated. Reactions in which the concentration of M13 dsDNA was varied contained 0.5 mM ATP, 3 mM phosphoenolpyruvate, 10 units of pyruvate kinase/ml, 10 μM M13 ssDNA, 6 μM recA protein, and SSB protein as indicated.

## RESULTS

*recA Protein Concentration Dependence of ssDNA-dependent ATPase Activity*—Because SSB protein does not stimulate the ATPase activity of recA protein at 1 mM magnesium acetate in volume-occupied solution (Lavery and Kowalczykowski, 1992), SSB protein does not appear to facilitate the saturation of ssDNA by recA protein. To address this supposition further we looked at the recA protein concentration dependence of ssDNA-dependent ATPase activity. The observed rate of ATP hydrolysis increases with increasing recA protein concentration until the available DNA is fully saturated, and then it becomes independent of recA protein concentration (Fig. 1). At 7.5% PVA, saturation of the ATPase rate occurs at about 0.7 μM recA protein, corresponding to an apparent stoichiometry of 3.5 nucleotides/recA protein monomer; the apparent $k_{cat}$ for ATP hydrolysis is 33/min under these conditions. A stoichiometry of 3.5 nucleotides/recA protein monomer is in agreement with values determined previously at elevated magnesium ion concentration using ssDNA that contains no secondary structures (*i.e.* poly(dT) and etheno M13 ssDNA) or using ssM13 in the presence of SSB protein (Menetski and Kowalczykowski, 1989). Thus, at 1 mM magnesium acetate and 7.5% PVA, recA protein appears to be able to saturate ssM13 in the absence of SSB protein.

At 7.5% PEG, saturation of the observed rate of ATP hydrolysis occurs at about 0.4 μM recA protein (Fig. 1), corresponding to an apparent stoichiometry of 6.25 nucleotides/recA protein monomer; the apparent $k_{cat}$ for ATP hydrolysis is 33/min. At 10% PEG, saturation of the observed rate of ATP hydrolysis occurs at a higher recA protein concentration (0.5 μM, corresponding to an apparent stoichiometry of 5 nucleotides/recA protein monomer) (Fig. 1); the apparent $k_{cat}$ for ATP hydrolysis is 32/min. Because the apparent $k_{cat}$ values are the same under all of these conditions, the data indicate that differences in the rate of ATP hydrolysis at saturation result from differences in the amount of recA protein bound to ssDNA. Thus, recA protein can saturate ssDNA better in buffer containing PVA than in buffer containing PEG.

*The Effect of SSB Protein on DNA Strand Exchange in the Presence of PVA*—The preceding results imply that SSB protein is not required for recA protein to form saturated presynaptic complexes at 1 mM magnesium acetate and 7.5% PVA. However, the presence of SSB protein does have a stimulatory effect on DNA strand exchange under these conditions (Fig. 2). Fig. 3, which is derived from densitometric scanning of data such as those in Fig. 2, shows the average time courses for the appearance of joint molecule intermediate DNA species, gapped circular heteroduplex DNA product molecules (form II DNA), and homology-dependent DNA

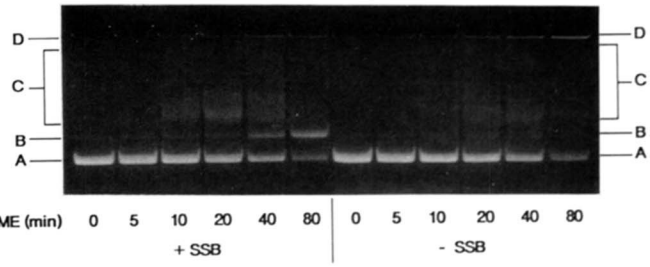


FIG. 2. **recA protein-promoted DNA strand exchange in the presence and absence of SSB protein.** Reactions were performed as described in Lavery and Kowalczykowski (1992) and contained 1 mM magnesium acetate, 7.5% PVA, 0.5 mM ATP, 3 mM phosphoenolpyruvate, 10 units of pyruvate kinase/ml, 5 μM M13 ssDNA, 10 μM M13 dsDNA, 3 μM recA protein, and 0.45 μM SSB protein as indicated. *Band A* indicates linear M13 dsDNA substrate molecules; *band B* indicates gapped circular heteroduplex DNA product molecules (form II DNA); *region C* indicates joint molecule intermediate DNA species; *band D* indicates homology-dependent DNA networks. *Lanes 1–6* are 0-, 5-, 10-, 20-, 40-, and 80-min time points, respectively, in a reaction containing SSB protein. *Lanes 7–12* are 0-, 5-, 10-, 20-, 40-, and 80-min time points, respectively, in a reaction that does not contain SSB protein.

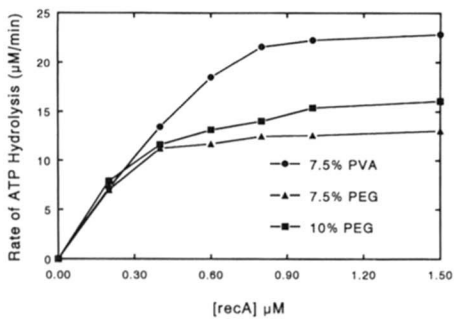


FIG. 1. **recA protein concentration dependence of ssDNA-dependent ATPase activity.** The hydrolysis of ATP was measured as described in Lavery and Kowalczykowski (1992). Reactions contained 2.5 μM M13 ssDNA and recA protein as indicated. *Circles* indicate reactions performed at 1 mM magnesium acetate and 7.5% PVA; *triangles* indicate reactions performed at 1 mM magnesium acetate and 7.5% PEG; *squares* indicate reactions performed at 1 mM magnesium acetate and 10% PEG.

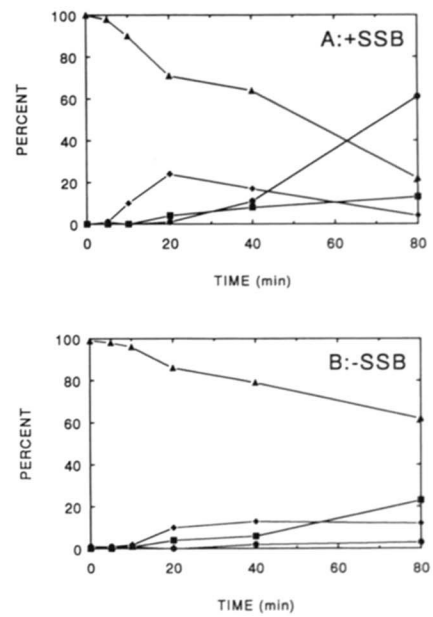


FIG. 3. **Time courses for recA protein-promoted DNA strand exchange in the presence and absence of SSB protein.** Reactions were performed as described in Fig. 2. *Triangles* indicate linear M13 dsDNA substrate molecules; *diamonds* indicate joint molecule intermediate DNA species; *circles* indicate gapped circular heteroduplex DNA product molecules; *squares* indicate homology-dependent DNA networks. *Panel A* indicates reactions performed in the presence of SSB protein; *panel B* indicates reactions performed in the absence of SSB protein.

networks, in the presence and absence of SSB protein. The rate of joint molecule formation is decreased, and the conversion of joint molecule intermediate species to form II DNA product molecules is greatly inhibited when SSB protein is omitted from the reaction. Even though joint molecule formation is reduced in the absence of SSB protein, homology-dependent DNA networks appear at a slightly greater rate than observed in the presence of SSB protein. These networks are formed when recA protein reinitiates successive rounds of pairing between the ssDNA partially displaced from one joint molecule and the duplex region of another, and the omission of SSB protein allows recA protein to bind this ssDNA easily (Chow *et al.*, 1988).

*SSB Protein Concentration Dependence of Joint Molecule Formation in Volume-occupied Solution*—Because SSB protein is often found associated with the ssDNA displaced from joint molecules (Soltis and Lehman, 1983; Register *et al.*, 1987), it is conceivable that the binding of SSB protein to this ssDNA is responsible for the effect we observe on DNA strand exchange at 7.5% PVA. To address this possibility we investigated whether a relationship exists between the concentration of dsDNA (*i.e.* the maximum amount of ssDNA which could be displaced from joint molecules) and the concentration of SSB protein required for optimal DNA strand exchange (see Kodadek, 1990; see "Discussion"). Fig. 4 shows the SSB protein concentration dependence of joint molecule formation at three dsDNA concentrations; the reactions employ constant ssDNA and recA protein concentrations that are in excess of the amounts required to pair with the dsDNA. In the absence of SSB protein, the rates of joint molecule formation are essentially the same at all three dsDNA concentrations, and in each case the addition of SSB results in an increase in the rate at which joint molecules are formed. However, the amount of SSB protein required to optimize joint molecule formation is greater at higher dsDNA concentrations; an additional 0.1 μM SSB protein is required for each 5 μM increase in dsDNA concentration. This is consistent with the hypothesis that SSB protein acts by binding to the ssDNA displaced from joint molecules. For each 5 μM increase in dsDNA concentration, 2.5 μM more ssDNA can be displaced from joint molecules, and 0.1 μM more SSB protein is required to bind to it; this corresponds to an apparent stoichiometry of 25 nucleotides/SSB protein monomer.

At 10% PEG, the addition of SSB protein also has a stimulatory effect on DNA strand exchange without stimulating the observed rate of ssDNA-dependent ATP hydrolysis (Lavery and Kowalczykowski, 1992). When the SSB protein concentration dependence of joint molecule formation is examined at varying dsDNA concentrations under these conditions, results similar to those seen at 7.5% PVA are obtained (Fig. 5). More SSB protein is required for optimal joint molecule formation as the concentration of dsDNA increases; again, an additional 0.1 μM SSB protein is required for each 5 μM increase in dsDNA concentration. However, the SSB protein concentration dependence curves are slightly sigmoid in appearance at 10% PEG, and slightly more SSB protein is required for optimal joint molecule formation at each dsDNA concentration.

*SSB Protein Concentration Dependence of Joint Molecule Formation at Elevated Magnesium Ion Concentration*—When DNA strand exchange is performed at an elevated magnesium ion concentration, the stimulatory effect of SSB protein on the reaction has been attributed to the removal of secondary structure from ssDNA, which enables recA protein to form saturated presynaptic complexes (Muniyappa *et al.*, 1984). To determine if SSB protein plays an additional role in DNA strand exchange under these conditions (*e.g.* binds to the ssDNA displaced from joint molecules), we again looked at the relationship between the concentration of dsDNA and the amount of SSB protein required for DNA strand exchange (Fig. 6). As is true in volume-occupied solution, the concentration of SSB protein required to optimize joint molecule formation increases with increasing dsDNA concentration; and once again, 0.1 μM more SSB protein is required for each 5 μM increase in dsDNA concentration. However, at 6 mM magnesium acetate, the SSB protein concentration dependence curves are very sigmoid in appearance. At all three dsDNA concentrations, significant stimulation of joint molecule formation is not observed until the SSB protein concentration exceeds 0.2 μM; presumably, this SSB protein is required during presynapsis. SSB protein added above this

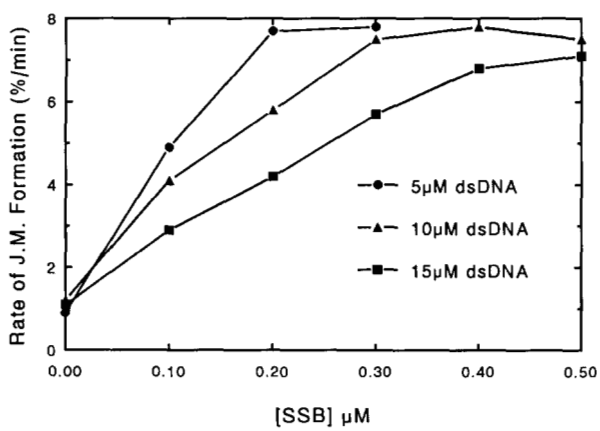


FIG. 4. **SSB protein concentration dependence of joint molecule formation at 1 mM magnesium acetate and 7.5% PVA.** DNA strand exchange reactions were performed as described in Lavery and Kowalczykowski (1992) and contained 6 μM recA protein, 10 μM M13 ssDNA, M13 dsDNA as indicated, and SSB protein as indicated. The maximum rate of joint molecule formation was determined (percent substrate utilized/min) from a reaction time course. *Circles* indicate reactions performed at 5 μM M13 dsDNA; *triangles* indicate reactions performed at 10 μM M13 dsDNA; *squares* indicate reactions performed at 15 μM M13 dsDNA.

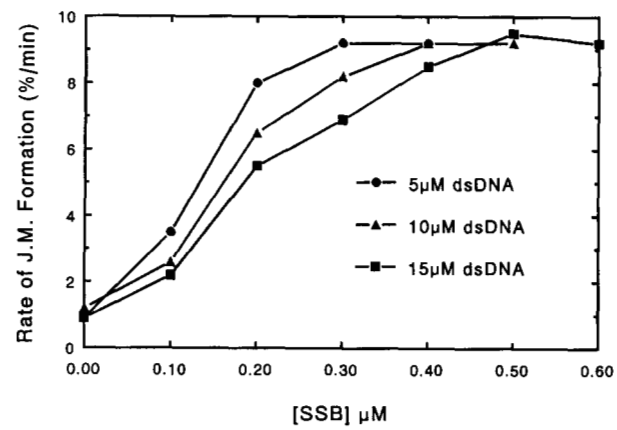


FIG. 5. **SSB protein concentration dependence of joint molecule formation at 1 mM magnesium acetate and 10% PEG.** DNA strand exchange reactions were performed as described in Lavery and Kowalczykowski (1992) and contained 6 μM recA protein, 10 μM M13 ssDNA, M13 dsDNA as indicated, and SSB protein as indicated. The maximum rate of joint molecule formation was determined (percent substrate utilized/min) from a reaction time course. *Circles* indicate reactions performed at 5 μM M13 dsDNA; *triangles* indicate reactions performed at 10 μM M13 dsDNA; *squares* indicate reactions performed at 15 μM M13 dsDNA.

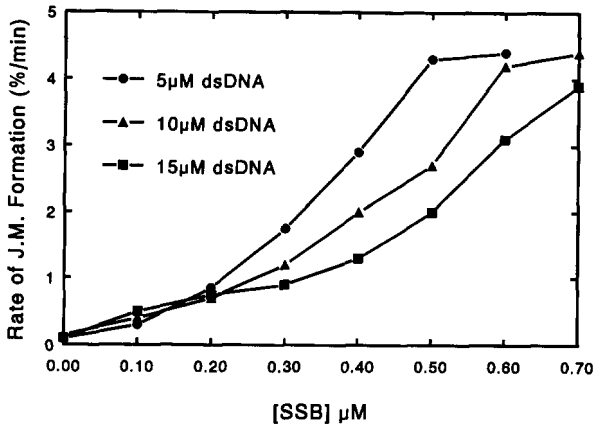


FIG. 6. **SSB protein concentration dependence of joint molecule formation at 6 mM magnesium acetate.** DNA strand exchange reactions were performed as described in Lavery and Kowalczykowski (1992) and contained 6 μM recA protein, 10 μM M13 ssDNA, M13 dsDNA as indicated, and SSB protein as indicated. The maximum rate of joint molecule formation was determined (percent substrate utilized/min) from a reaction time course. *Circles* indicate reactions performed at 5 μM M13 dsDNA; *triangles* indicate reactions performed at 10 μM M13 dsDNA; *squares* indicate reactions performed at 15 μM M13 dsDNA.

concentration is likely binding to the ssDNA displaced from joint molecules.

## DISCUSSION

At elevated magnesium ion concentrations, SSB protein stimulates joint molecule formation by removing secondary structure from ssDNA and allowing recA protein to form saturated presynaptic complexes (Muniyappa *et al.*, 1984). Although DNA strand exchange can occur at 1 mM magnesium acetate in the presence of PVA or PEG, our examination of ATPase activity suggests that SSB protein does not aid recA protein in the saturation of ssDNA under these conditions; yet SSB protein does stimulate DNA strand exchange. In this study we present biochemical evidence that suggests that SSB protein stimulates DNA strand exchange, both under these conditions and at elevated magnesium ion concentrations, by binding to the ssDNA displaced from joint molecules, thereby stabilizing them and allowing branch migration to produce the gapped circular heteroduplex DNA molecules of complete strand exchange.

The binding of recA protein to ATP and ssDNA results in the formation of a presynaptic complex that can participate in joint molecule formation. Because a consequence of this binding is the hydrolysis of ATP, the formation of presynaptic complexes can be monitored by measuring ssDNA-dependent ATPase activity. At an elevated magnesium ion concentration, the recA protein concentration dependence of ssDNA-dependent ATPase activity indicates that more recA protein is involved in the hydrolysis of ATP when SSB protein is present (Kowalczykowski and Krupp, 1987); *i.e.* presynaptic complexes are more fully saturated with recA protein. Our examination of the recA protein concentration dependence of ssDNA-dependent ATP hydrolysis at 1 mM magnesium acetate and 7.5% PVA suggests that recA protein is able to saturate the ssDNA fully in the absence of SSB protein. Consistent with this, there is no increase in the observed rate of ATP hydrolysis upon addition of SSB protein under these conditions (Lavery and Kowalczykowski, 1992).

The preceding suggests that SSB protein is not acting during the formation of presynaptic complexes at 1 mM magnesium acetate and 7.5% PVA; however, SSB protein does have an effect on recA protein-promoted DNA strand exchange under these conditions. This paradox is similar to that posed by the T4 bacteriophage system for DNA strand exchange. In this system, the *in vitro* activity of the strand transferase, uvsX protein, is strongly stimulated by the addition of gene 32 protein, a helix-destabilizing protein. However, examination of ATPase activity shows that uvsX protein is not troubled by secondary structure in ssDNA (Harris and Griffith, 1989), and the concentration of gene 32 protein optimal for pairing reactions inhibits ssDNA-dependent ATPase activity by uvsX protein (Formosa and Alberts, 1986). Thus, gene 32 protein does not appear to be aiding presynapsis in this system. Kodadek (1990) has demonstrated a correlation between the concentration of duplex DNA and the concentration of gene 32 protein required for efficient heteroduplex DNA formation in uvsX protein-mediated pairing reactions. He has proposed that gene 32 protein acts either by binding directly to the ssDNA displaced from the duplex, thereby stabilizing the product, or by displacing uvsX protein from the invading strand and allowing it to bind to the displaced strand, similarly resulting in stabilization.

Here we demonstrate that SSB protein plays a comparable role in the recA protein-promoted DNA strand exchange reaction (see Fig. 7). At 1 mM magnesium acetate and 7.5% PVA, the amount of SSB protein required for optimal joint molecule formation is dependent on the concentration of dsDNA present in the reaction. If the role of SSB protein were presynaptic, the concentration of SSB protein required for DNA strand exchange would be expected to be dependent on ssDNA concentration and independent of dsDNA concentration. Because this is not the case, the results do not support the idea that SSB protein is acting during presynapsis. Instead, they suggest that SSB protein is binding to the linear ssDNA displaced from joint molecules; this follows because the amount of ssDNA which is displaced from the joint molecule is proportional to the input dsDNA concentration,

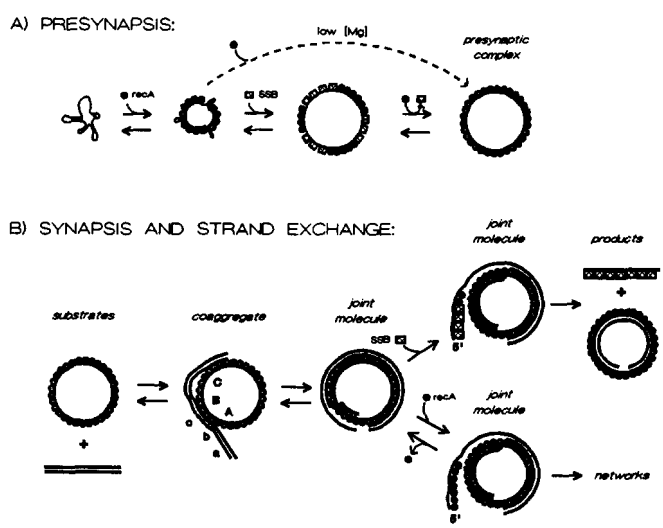


FIG. 7. **Model for the role of SSB protein in DNA strand exchange.** *Squares* indicate SSB protein, and *circles* indicate recA protein. *Line A* of the model depicts SSB protein acting during presynapsis, removing secondary structure from ssDNA at elevated magnesium ion concentration; at low magnesium ion concentrations SSB protein is not required during presynapsis. *Line B* depicts a postsynaptic role for SSB protein; binding to the ssDNA displaced from joint molecules, SSB protein drives the reaction to the formation of the form II DNA products of complete strand exchange. When recA protein binds to this displaced ssDNA it promotes the reinitiation reactions that lead to the formation of extensive homology-dependent DNA networks (see Chow *et al.*, 1988).

as a result using a concentration of invading circular ssDNA which is in excess of the dsDNA concentration. Unlike the experiments performed with uvsX protein and gene 32 protein (Kodadek, 1990), we can exclude the alternative interpretation that stimulation of joint molecule formation by SSB protein is an indirect effect, resulting from displacement of recA protein from the invading ssDNA and followed by binding of this recA protein to the displaced ssDNA. This conclusion follows because in our experiments excess DNA strand transfer protein is employed, *i.e.* the concentration of recA protein is more than sufficient to bind both the invading ssDNA and the displaced ssDNA. Because of this excess recA protein, SSB protein cannot be acting via displacement of the recA protein from the invading ssDNA; thus, SSB protein must be acting by binding directly to the displaced strand.

Similar to the results obtained at 7.5% PVA, the concentration of SSB protein required for joint molecule formation is dependent on dsDNA concentration at 1 mM magnesium acetate and 10% PEG. This indicates a postsynaptic role for SSB protein under these conditions as well; however, the SSB protein concentration dependence curves are slightly sigmoid in appearance at 10% PEG. The ssDNA-dependent ATPase data suggest that presynaptic complexes are not fully saturated with recA protein under these conditions. Thus, some of the SSB protein added to the DNA strand exchange reaction is likely binding to presynaptic complexes and therefore is not available for binding to the ssDNA displaced from joint molecules. This would account for the sigmoid appearance of the SSB protein concentration dependence. It would also explain why, under these conditions, slightly more SSB protein is required than is required at 7.5% PVA, where presynaptic complexes are apparently saturated with recA protein.

This postsynaptic role for SSB protein is seen not only at 1 mM magnesium acetate in volume-occupied solution, but at elevated magnesium ion concentrations as well. Under these conditions, SSB protein plays a crucial role in DNA strand exchange by removing secondary structure from presynaptic complexes (see Fig. 7*A*). However, in addition to this presynaptic role, SSB protein must also be binding to the ssDNA displaced from joint molecules; the concentration of SSB protein required for optimal joint molecule formation is dependent on dsDNA concentration. The significantly sigmoid appearance of the SSB protein concentration dependence curves is consistent with some SSB protein being required to act on secondary structure during the formation of presynaptic complexes. At all three dsDNA concentrations examined, there is no significant stimulation of joint molecule formation until SSB protein exceeds a common concentration. Because ssDNA concentration is constant in these reactions, this amount of SSB protein is presumably required for presynapsis. SSB protein added above this concentration is most likely binding to the ssDNA displaced as a result of joint molecule formation.

By binding to the 5′ end of the ssDNA displaced from a joint molecule, SSB protein imparts a 5′ to 3′ directionality upon branch migration; it prevents backward recA protein-mediated reversal or thermal branch migration (Fig. 7*B*). In doing so, SSB protein stabilizes the joint molecule and drives the overall reaction in the forward direction. The addition of SSB protein not only increases the rate of joint molecule formation via this stabilization, but it also increases formation of the form II DNA products of complete strand exchange and decreases formation of homology-dependent DNA networks (see Lavery and Kowalczykowski, 1992). These networks are formed when recA protein reinitiates pairing between the ssDNA displaced from one joint molecule and the duplex region of another, and their formation is inhibitory to product molecule formation (see Chow *et al.*, 1988; Lavery and Kowalczykowski, 1990). SSB protein limits recA protein's access to displaced ssDNA strands, thereby inhibiting the reinitiation reactions that lead to the formation of networks and favoring the extension of heteroduplex DNA via branch migration (Fig. 7*B*).

Previous studies on the role of SSB protein in recA protein-promoted DNA strand exchange have emphasized the notion that SSB protein acts during presynapsis (McEntee *et al.*, 1980; Cox *et al.*, 1983, 1984; Kahn and Radding, 1984; Muniyappa *et al.*, 1984; Tsang *et al.*, 1985; Morrical *et al.*, 1986). This crucial function for SSB protein has obscured identification of a second postsynaptic role for SSB protein. Our examination of DNA strand exchange at 1 mM magnesium acetate in volume-occupied solution, in which SSB protein does not appear to be acting during presynapsis (see Fig. 7*A*, *low [Mg] pathway*), has illuminated a postsynaptic role for SSB protein. Our analysis of the DNA strand exchange reaction at elevated magnesium ion concentration, in turn, supports dual roles for SSB protein; it plays a presynaptic role, removing secondary structure from ssDNA (Fig. 7*A*), and it plays a postsynaptic role, binding to the ssDNA displaced from joint molecules, stabilizing them and favoring the extension of the heteroduplex DNA by branch migration (Fig. 7*B*). As expected for this model, recA protein-promoted reciprocal DNA strand exchange reactions (dsDNA × dsDNA reactions, in which no ssDNA is displaced) are not affected by the addition of SSB protein (West *et al.*, 1982; Chiu *et al.*, 1990).

Our *in vitro* observations suggest that, *in vivo*, SSB protein would be expected to play a postsynaptic role in recA protein-promoted pairing reactions between ssDNA and dsDNA. Whether or not SSB protein also plays a presynaptic role would depend on the intracellular concentration of free magnesium ion. If the magnesium ion concentration is low enough (*i.e.* 1 mM), recA protein should be able to form effective presynaptic complexes in the absence of SSB protein. If not, SSB protein might also be required to remove secondary structure from ssDNA during presynapsis.

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