

# recA Protein-promoted DNA Strand Exchange

## EFFECT OF IONIC STRENGTH\*

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The addition of from 80 to 120 mM NaCl to a recA protein-promoted DNA strand exchange results in a significant increase in both the initial rate and extent of reaction. This increase results from the formation of a relatively stable recA protein-single-stranded DNA complex that is similar to the complex formed in the presence of the single-stranded DNA-binding protein. In addition to increasing the rate and extent of strand exchange, the amount of recA protein and ATP hydrolysis needed to promote these reactions efficiently is reduced. However, single-stranded DNA-binding protein is significantly more effective than addition of 100 mM NaCl in promoting stable complex formation and stimulating strand exchange. These findings support the hypothesis that a stable recA protein-single-stranded DNA complex is required for efficient DNA strand exchange.

A functional recA gene is essential for homologous recombination in *Escherichia coli*. *In vitro*, the product of the recA gene, the recA protein, promotes a variety of DNA pairing, transfer, and strand-exchange reactions that very likely reflect its role *in vivo* (1-5). One of these reactions involves the transfer of a strand from a linear duplex to a circular ssDNA<sup>1</sup> to generate a circular duplex with a single-strand interruption (RFII) and a linear single strand (4). The rate and extent of the reaction are stimulated by the single-stranded DNA-binding protein of *E. coli*, SSB (4, 6-8), which promotes the formation of a stable complex between the recA protein and circular ssDNA.

In the present study, we have examined the effect of ionic strength on strand exchange in the absence of SSB. We have found that addition of 100 mM NaCl results in a significant enhancement of both the rate and extent of reaction, by a mechanism similar to that observed with SSB.

### EXPERIMENTAL PROCEDURES

#### Materials

recA protein (9), <sup>35</sup>S-labeled recA protein (10), and SSB (10) were prepared as described previously. Labeled and unlabeled  $\phi$ X circular single-stranded and linear duplex DNAs were prepared, and their concentrations were determined as described (4, 8). The <sup>3</sup>H-labeled

ssDNA had a specific radioactivity of >45,000 cpm/ $\mu$ g of nucleotide. All DNA concentrations are given in nucleotides.

The restriction endonucleases *Pst*I, *Xho*I, and *Stu*I were purchased from New England Biolabs. [<sup>3</sup>H]ATP was obtained from Amersham, and [<sup>3</sup>H]thymidine was purchased from New England Nuclear. Phosphoenolpyruvate, pyruvate kinase, phosphocreatine, creatine phosphokinase, and S1 nuclease were purchased from Sigma. ATP $\gamma$ S was obtained from Boehringer Mannheim.

#### Methods

All reactions were performed in 25 mM Tris·HCl (pH 7.2), 10 mM MgCl<sub>2</sub>, 1 mM dithiothreitol, and 5% glycerol. NaCl was added to the reaction mixtures as indicated. Two different ATP regeneration systems were used and added at final concentrations of either 4.2 mM phosphoenolpyruvate and 33  $\mu$ g/ml pyruvate kinase, or 4 mM phosphocreatine and 20  $\mu$ g/ml creatine phosphokinase. Reactions were started by first preincubating the recA protein with the ssDNA for 5 min, then adding the ATP and where indicated, SSB; after 5 additional min, the linear duplex DNA was added. In reactions with NaCl but not SSB, the linear duplex DNA was added after 20 min of preincubation of all other components. All reactions were performed at 37 °C.

**Assay for Heteroduplex Formation**—The incorporation of <sup>3</sup>H-labeled ssDNA into S1 nuclease-resistant heteroduplex was measured as previously described (8) except that S1 nuclease digestions were performed at 45 °C.

**Assay for ATP Hydrolysis**—ATP hydrolysis was measured as previously described (2). In reactions in which the linear duplex DNA was omitted, the reactions were started by adding ATP or a mixture of ATP and SSB to a preincubation containing recA protein and ssDNA.

**Sucrose Density Gradient Sedimentation**—Reactions were performed and the products were sedimented and analyzed as previously described (10) except that the reaction was incubated for 20 min before addition of ATP $\gamma$ S.

### RESULTS

**Effect of Ionic Strength on Strand Exchange**—Addition of from 80 to 120 mM NaCl to a recA protein-promoted DNA strand exchange in the absence of SSB produced a 3-fold increase in the initial rate of the reaction (Fig. 1). There was also a 2-fold increase in the extent (data not shown). Similar results were obtained with other salts, including KCl, LiCl, NH<sub>4</sub>Cl, sodium acetate, and (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> (data not shown), indicating that the stimulation was the result of an increase in the ionic strength rather than a specific effect of NaCl. The ionic strength of the reaction mixture with 100 mM NaCl added is equivalent to 150 mM NaCl. This value is within the range of estimated physiological ionic strengths (11); hence, the stimulation may be relevant to reactions promoted by the recA protein *in vivo*.

This reaction differed from strand exchange at lower ionic strengths in two respects. First, to obtain optimal initial rates it was necessary to preincubate the recA protein with the ssDNA and ATP for at least 20 min. In the absence of added NaCl, or in the presence of SSB, only 3 to 5 min of preincubation were required (data not shown). The extended prein-

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<sup>1</sup> The abbreviations used are: ssDNA, single-stranded DNA;  $\phi$ X, bacteriophage  $\phi$ X174; RFII, double-stranded circular DNA containing a single-strand break; SSB, *E. coli* single-stranded DNA-binding protein; ATP $\gamma$ S, adenosine 5'-O-(3-thiophosphate).

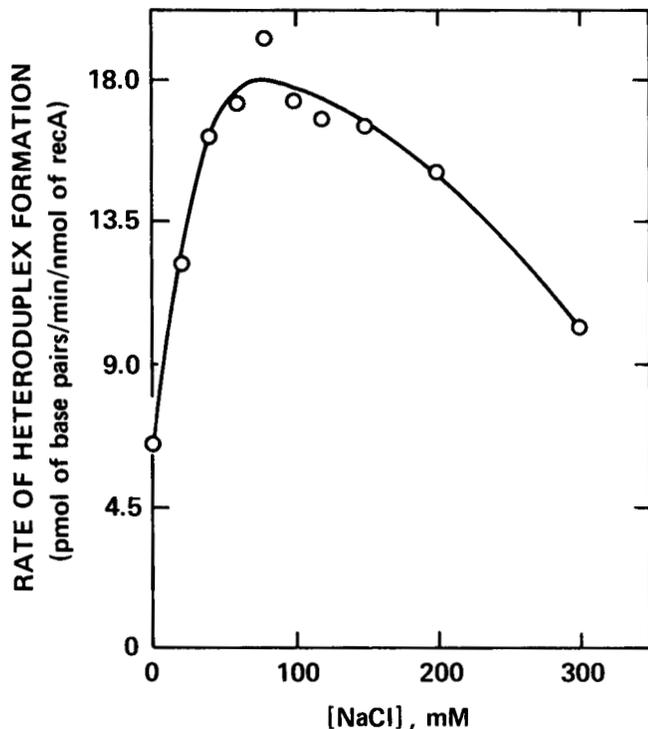


FIG. 1. Effect of ionic strength on the initial rate of heteroduplex formation. Reactions were carried out as described under "Experimental Procedures." The reaction mixtures contained  $4.5 \mu\text{M}$  recA protein,  $4.5 \mu\text{M}$   $^3\text{H}$ -labeled  $\phi\text{X}$  ssDNA,  $9.1 \mu\text{M}$  linear duplex  $\phi\text{X}$  DNA, and  $13 \text{ mM}$  ATP.

incubation period may be necessary to allow the recA protein to interact stably with the ssDNA at the high ionic strength. Second, the reaction required high concentrations of ATP to obtain maximum initial rates and extents. Thus, optimal rates were observed at  $9 \text{ mM}$  ATP, as compared with  $2 \text{ mM}$  ATP in the absence of added NaCl, or with SSB (data not shown). Lowering the ATP concentration to  $4 \text{ mM}$  and adding sufficient levels of an ATP-regenerating system to maintain this ATP concentration during the reaction performed in the presence of  $100 \text{ mM}$  NaCl caused a reduction in the initial rate of reaction. These results suggest that the higher concentration of ATP is necessary to initiate and maintain strand exchange performed in the presence of  $100 \text{ mM}$  NaCl, and may indicate that recA protein binds ATP less well under these conditions. Alternatively, at the higher ionic strengths, ATP hydrolysis may be less efficiently coupled to strand exchange than in the presence of SSB.

**Formation of recA Protein-ssDNA Complexes**—A strand-exchange reaction in the presence of added NaCl was challenged at various times with ssDNA to determine whether the recA protein was sequestered in a complex as observed in reactions with SSB (8, 10). The recA protein was preincubated with the ssDNA, ATP, and  $100 \text{ mM}$  NaCl for 20 min prior to starting the reaction with the linear duplex. The reaction was then challenged with another equivalent of ssDNA at various times. By using  $^3\text{H}$ -labeled ssDNA in the preincubation with recA protein or as the challenging DNA, reactions with both ssDNA substrates could be followed independently. The amount of recA protein present in these experiments was sufficient to allow the reaction on only one of the ssDNA substrates to reach completion. An ATP-regenerating system was added to ensure that ATP was not limiting. As the reaction with the first ssDNA proceeded, that with the challenging DNA decreased, indicating that the recA protein was

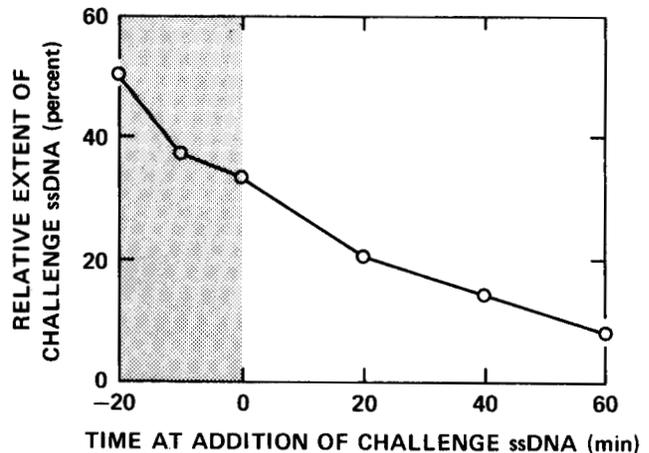


FIG. 2. Sequestering of recA protein in the presence of added  $100 \text{ mM}$  NaCl. Reactions were performed as described under "Experimental Procedures." The reaction mixtures contained  $2.3 \mu\text{M}$  recA protein,  $4.5 \mu\text{M}$   $^3\text{H}$ -labeled or unlabeled  $\phi\text{X}$  ssDNA,  $9.1 \mu\text{M}$  linear duplex  $\phi\text{X}$  DNA,  $9.5 \text{ mM}$  ATP,  $100 \text{ mM}$  NaCl,  $4.2 \text{ mM}$  phosphoenolpyruvate, and  $33 \mu\text{g/ml}$  pyruvate kinase. The challenging ssDNA was also added at a concentration of  $4.5 \mu\text{M}$ .

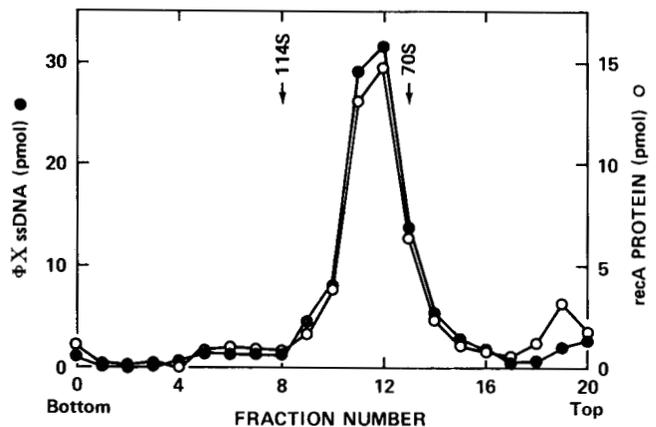


FIG. 3. recA protein-ssDNA complex formation in the presence of added  $100 \text{ mM}$  NaCl. Sedimentation analysis was performed as described under "Experimental Procedures." The reaction mixture contained  $2.3 \mu\text{M}$   $^{35}\text{S}$ -labeled recA protein,  $4.5 \mu\text{M}$   $^3\text{H}$ -labeled  $\phi\text{X}$  ssDNA,  $9.5 \text{ mM}$  ATP, and  $100 \text{ mM}$  NaCl. The migration of normal ( $114 \text{ S}$ ) and eclipsed ( $70 \text{ S}$ ) forms of bacteriophage  $\phi\text{X}174$  are also indicated.

incorporated into a complex with the first ssDNA, analogous to that formed in the presence of SSB (8, 10) (Fig. 2). However, since a significant fraction of the recA protein could react with the challenging DNA, the recA protein-ssDNA complex formed at the higher ionic strength is not as stable as that formed in the presence of SSB.

As shown in Fig. 3, a recA protein-ssDNA complex could be detected by sedimentation in sucrose density gradients after stabilization of the recA protein-DNA interactions with ATP- $\gamma\text{S}$ . This complex had a sedimentation coefficient of  $78 \text{ S}$ , which is similar to that found for the complex formed in the presence of SSB (10). In the absence of both SSB and added NaCl, however, significantly less recA protein was found in association with the ssDNA (10).

**Effect of Duplex DNA Termini on Strand Exchange**—The effect of duplex DNA termini on strand exchange in the presence of  $100 \text{ mM}$  NaCl, but in the absence of SSB, is shown in Fig. 4. Linear duplex DNA with either 4 unpaired bases at the  $5'$  termini generated by *Xho*I cleavage or a blunt end generated by *Stu*I were essentially inactive, in contrast to

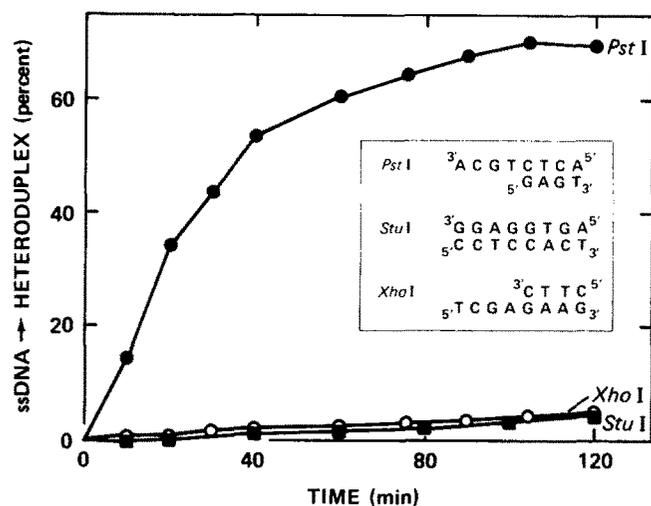


FIG. 4. Effect of duplex DNA termini on heteroduplex formation in the presence of added 100 mM NaCl. The reactions were carried out as described under "Experimental Procedures." The reaction mixtures contained 2.2  $\mu$ M recA protein, 4.5  $\mu$ M  $^3$ H-labeled  $\phi$ X ssDNA, 9.1  $\mu$ M linear duplex  $\phi$ X DNA, 9.5 mM ATP, 100 mM NaCl, 4.2 mM phosphoenolpyruvate, and 33  $\mu$ g/ml pyruvate kinase. The linear duplex DNAs were generated by cutting with the restriction endonucleases *Pst*I, *Stu*I, and *Xho*I.

*Pst*I-cleaved RFI. These results are similar to those reported previously for strand exchange in the presence of both SSB and 100 mM NaCl (12) and emphasize the requirement for an unpaired 3' terminus for the efficient initiation of DNA strand exchange. These results also imply that strand exchange in the presence of added 100 mM NaCl proceeds with the same polarity as the reaction performed with SSB at low ionic strength (13). In fact, reactions performed under both of these conditions showed a similar 3'  $\rightarrow$  5' polarity (data not shown).

*Comparison of Effects of Ionic Strength and SSB on DNA Strand Exchange*—The addition of SSB to a recA protein-promoted DNA strand-exchange reaction results in a marked increase in both the initial rate and extent of heteroduplex formation (4, 7). A similar, although less extensive, stimulation occurred when 90 mM NaCl was added (Fig. 5). As seen in Table I, the addition of SSB resulted in a 30-fold increase in the initial rate of heteroduplex formation. In contrast, there was only an approximately 6-fold increase in rate upon addition of 90 mM NaCl.

The addition of 90 mM NaCl to a strand-exchange reaction in the presence of SSB inhibited both the initial rate and extent of heteroduplex formation (Fig. 5 and Table I). However, slightly lower concentrations (70 mM) of NaCl had very little effect (5% inhibition) on the extent of strand exchange (data not shown).

*ATP Hydrolysis during Strand Exchange*—The rate of ATP hydrolysis during strand exchange varied depending on whether SSB and/or NaCl were added (Table I). In the presence of SSB, the rate of ATP hydrolysis was nearly the same as that with added NaCl. When NaCl was added to a reaction containing SSB, the rate of ATP hydrolysis was reduced 40 to 60%. A similar reduction in the rate of ATP hydrolysis was observed when NaCl was omitted from a reaction lacking SSB. The rate of ATP hydrolysis under all four conditions was constant throughout the entire reaction. Omission of the linear duplex had no effect on the rate of ATP hydrolysis (data not shown). These results indicate that the rate of ATP hydrolysis is proportional to the amount of recA protein bound to the ssDNA except when both SSB and

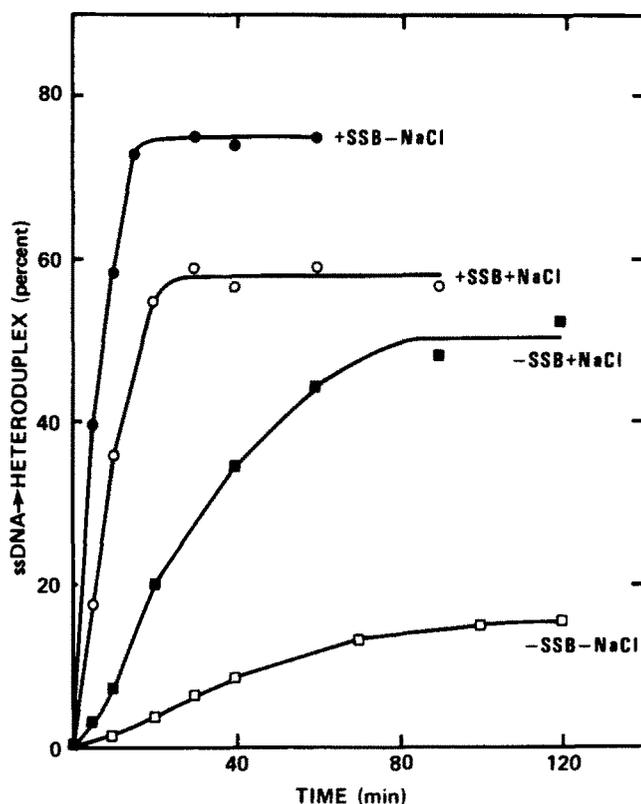


FIG. 5. Effect of added NaCl on heteroduplex formation. The reactions were performed and heteroduplex formation was measured as described under "Experimental Procedures." All reaction mixtures contained 2.2  $\mu$ M recA protein, 4.5  $\mu$ M  $^3$ H-labeled  $\phi$ X ssDNA, 9.1  $\mu$ M linear duplex  $\phi$ X DNA, and where indicated 0.56  $\mu$ M SSB and 90 mM NaCl. For reactions performed without SSB, 4 mM phosphocreatine and 20  $\mu$ g/ml creatine phosphokinase were added. All reactions also contained 4 mM ATP except for the reaction performed with 90 mM NaCl and no SSB to which 12 mM ATP was added.

TABLE I  
Effect of ionic strength and SSB on parameters of DNA strand exchange

Reactions were performed as described under "Experimental Procedures." To determine the rate of heteroduplex formation, reaction mixtures contained 2.2  $\mu$ M recA protein, 4.5  $\mu$ M  $^3$ H-labeled  $\phi$ X ssDNA, 9.1  $\mu$ M linear duplex  $\phi$ X DNA, and where indicated, 0.56  $\mu$ M SSB and 90 mM NaCl. All reactions also contained 4.0 mM ATP except for the reaction performed with NaCl but no SSB, in which 12 mM ATP was added. To determine rate of ATP hydrolysis, the reaction mixtures were the same except that the  $^3$ H-labeled  $\phi$ X ssDNA was replaced with unlabeled ssDNA and [ $^3$ H]ATP was added.

	Rate of heteroduplex formation <i>nmol base pairs/min/nmol recA</i>	Rate of ATP hydrolysis <i>nmol ADP formed/min/nmol recA</i>	ATP hydrolyzed per heteroduplex base pair formed <i>mol/mol</i>
+ SSB - NaCl	0.14	20	140
+ SSB + NaCl	0.071	13	180
- SSB + NaCl	0.026	23	870
- SSB - NaCl	0.0044	9	2000

NaCl are added. Under these conditions, most of the recA protein is associated with the ssDNA (data not shown); however, the rate of ATP hydrolysis was comparatively low, implying that factors other than the binding of recA protein to ssDNA affect the rate of ATP hydrolysis. These results also suggest that ATP hydrolysis is not tightly coupled to

heteroduplex formation since the rates of hydrolysis were the same whether or not the linear duplex DNA was present.

The apparent lack of tight coupling of ATP hydrolysis to heteroduplex formation provides an explanation for the inefficiency of strand exchange. As shown in Table I, approximately 2000 molecules of ATP were hydrolyzed per base pair of heteroduplex formed. When SSB, or SSB and NaCl were added, the amount of ATP hydrolyzed per heteroduplex base pair formed was reduced 10-fold. The addition of 90 mM NaCl resulted in only a 2-fold reduction in the number of ATP molecules hydrolyzed per base pair formed. Thus, SSB is significantly more effective in reducing the level of ATP hydrolysis during strand exchange than the addition of NaCl; however, these reactions still appear to be inefficient, and other factors including low levels of ADP (14) may be required to reduce ATP hydrolysis even further.

**Effect of *recA* Protein Concentration on Rate and Extent of Strand Exchange**—The dependence of the extent of heteroduplex formation on *recA* protein concentration is shown in Fig. 6. For the reactions performed with SSB, strand exchange

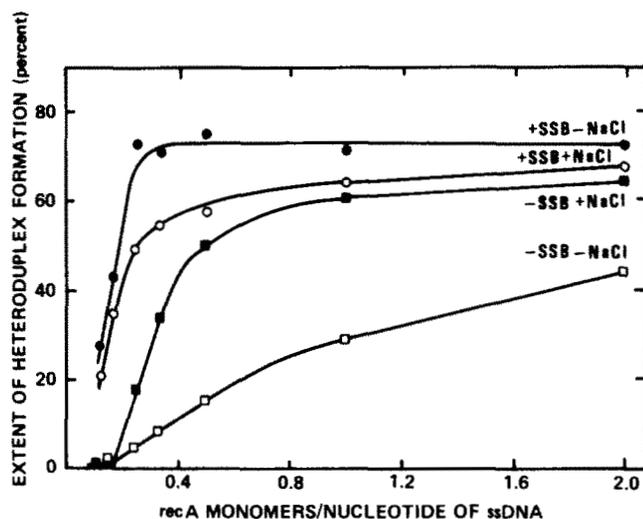


FIG. 6. Effect of *recA* protein concentration on the extent of DNA strand exchange. Reactions were carried out as described under "Experimental Procedures." Reaction mixtures were the same as those described in the legend to Fig. 5 except that the *recA* protein concentration was varied from 0.55 to 8.8  $\mu$ M.

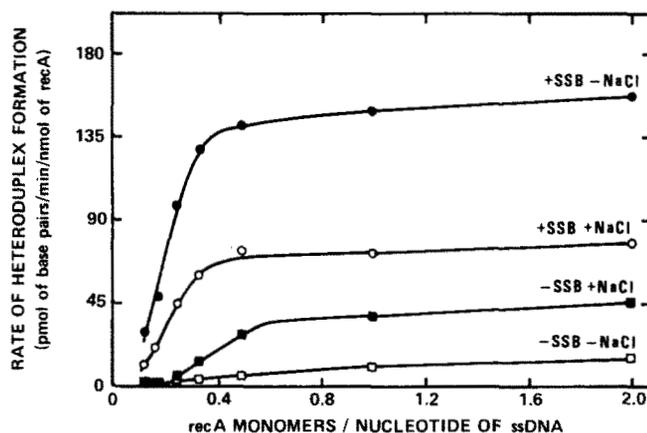


FIG. 7. Effect of *recA* protein concentration on the initial rate of DNA strand exchange. The reactions were performed as described under "Experimental Procedures." Reaction mixtures were the same as those described in the legend to Fig. 5 except that the *recA* protein concentration was varied from 0.55 to 8.8  $\mu$ M.

reached a maximal extent at approximately one *recA* protein monomer/three or four nucleotides of ssDNA. In the absence of SSB, but with 90 mM NaCl added, one *recA* protein monomer/one or two nucleotides was required. When neither SSB or NaCl was added, strand exchange failed to reach a limit even at concentrations of *recA* protein equivalent to two *recA* protein monomers/nucleotide of ssDNA. Thus, either the addition of SSB or an increase in the ionic strength reduces the amount of *recA* protein required to promote efficient strand exchange; however, SSB is significantly more effective than added NaCl.

The initial rates of DNA strand exchange showed a similar dependence on *recA* protein concentration (Fig. 7). Again, in the presence of SSB, the initial rate of strand exchange reached saturation at a lower ratio of *recA* protein to ssDNA than without SSB. In addition, the initial rates of the four different reactions ( $\pm$ SSB and NaCl) failed to converge at high *recA* protein to ssDNA ratios as was found in measurements of the extent of these reactions (Fig. 6). This finding implies that factors other than *recA* protein concentration are important in determining the initial rates of strand exchange. Such factors may include features of DNA structure and/or reaction conditions.

## DISCUSSION

Our major finding is that an increase in the ionic strength of the *recA* protein-promoted DNA strand-exchange reaction produces a stimulation in heteroduplex formation that is similar to, but less extensive than that found when SSB is added (4, 7). Stimulation results from the formation of a relatively stable complex between the *recA* protein and ssDNA as previously observed in the presence of SSB (8, 10). It would therefore appear that the efficiency of strand exchange is dependent on the stability of the *recA* protein-ssDNA complex. In the presence of either SSB or added NaCl, maximum rates and extents of reaction are achieved when the *recA* protein-ssDNA complex is preformed and then allowed to react with the linear duplex DNA. However, in the absence of SSB or added NaCl, a stable complex is not formed and the rate and extent of strand exchange are greatly diminished.

Although the addition of either SSB or NaCl will promote the formation of a stable *recA* protein-ssDNA complex, it is unclear how these complexes are stabilized. We have been unable to detect a specific interaction between SSB and the *recA* protein-ssDNA complex, suggesting that SSB may act indirectly (10, 15).

Although there is a marked enhancement of strand exchange by the addition of NaCl, there is an almost absolute requirement for an unpaired 3' terminus on the linear duplex DNA under these conditions. A similar requirement had been observed for the reaction performed in the presence of both SSB and NaCl and was attributed to an increase in the stability of the duplex DNA at the higher ionic strengths (12). In the absence of added NaCl, all three of the linear duplex DNAs (blunt end, unpaired 3' and unpaired 5' ends) were reactive (7), indicating that there is an equilibrium between paired and unpaired duplex termini. At higher ionic strengths, the duplex DNA termini are stabilized in a paired form, and only those with unpaired 3' ends are reactive. Thus, it would appear that an unpaired 3' terminus is required to initiate strand exchange and that more extensively unpaired 3' termini may increase even further the initial rate of strand exchange. Such termini might be generated *in vivo* by the *recBC* nuclease (16) and/or exonuclease VIII (17).

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