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Biochemical interaction of the *Escherichia coli* RecF, RecO, and RecR proteins with RecA protein and single-stranded DNA binding protein

(genetic recombination/RecF pathway/strand transfer/DNA repair)

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ABSTRACT **The *Escherichia coli* RecF, RecO, and RecR proteins were analyzed for their effect on RecA-mediated pairing of single-stranded circular DNA and homologous linear duplex DNA substrates. As shown by other workers, joint molecule formation by RecA was inhibited by *E. coli* single-stranded DNA binding protein (SSB) when it was added to single-stranded DNA before RecA. This inhibitory effect was overcome by the addition of RecO and RecR or RecF, RecO, and RecR. Both the rate and extent of joint molecule formation were restored to the maximal level observed when SSB was added after RecA. RecF, RecO, and RecR proteins had no effect on the conversion of joint molecules to final products and only appeared to stimulate an early step in the pairing reaction. The stimulatory effect of RecF, RecO, and RecR was not seen without SSB or when SSB was added after RecA. RecF protein by itself inhibited reactions in mixtures containing RecA and SSB, and this inhibition was overcome by the addition of RecO and RecR. These data suggest that RecO and RecR, and possibly RecF, help RecA overcome inhibition by SSB and utilize SSB–single-stranded-DNA complexes as substrates.**

Genetic analysis has identified multiple pathways for recombination in *Escherichia coli*. In wild-type *E. coli* strains, plasmid recombination and recombinational repair of UV damage are dependent on some RecF pathway genes (1, 2). Some experiments suggest that RecF pathway gene products play a role in conjugational recombination in wild-type *E. coli* strains, a recombinational event usually thought to be promoted by the RecBCD pathway (3). In the absence of RecBCD function in *E. coli recBC sbcBC* mutants, the RecF pathway is the major recombination pathway and is capable of promoting all homologous recombination events known to occur in *E. coli* (1, 2, 4, 5). Thus far, 10 genes have been identified in the RecF pathway, including *recA, recF, recJ, recN, recO, recQ, recR*, and *ruvABC* (1, 2, 5, 6). In some cases, activities have been identified for individual proteins encoded by these genes: RecA catalyzes homologous pairing and strand exchange (7, 8); RecF binds to single-stranded DNA (ssDNA) and double-stranded DNA (dsDNA) (9, 10); RecJ is a ssDNA-specific 5′ → 3′ exonuclease (11); RecO promotes renaturation of complementary ssDNA (C. Luisi-DeLuca and R.D.K., unpublished results); RecQ is a DNA helicase (12); RuvA and RuvB promote branch migration of Holliday junctions at about the same rate as RecA (13, 14); and RuvC cleaves Holliday junctions at low rates (15, 16). No activity has been reported for RecN or RecR.

Genetic studies suggest the *recF, recO*, and *recR* gene products function at the same step of recombination and interact with RecA. (*i*) Recombination of λ *red* mutant phage in *E. coli recBC sbcBC* mutants does not require the *recF, recO*, and *recR* genes. However, if the λ phage has a mutated *ninB* gene, then *recF, recO*, and *recR* are required (17). (*ii*) Genetic analysis has indicated that *recF, recO*, and *recR* belong to the same epistasis group (18). (*iii*) The *recA803* mutation suppresses the defect of *recF, recO*, and *recR* mutants in recombination and UV repair (5). This suppression does not appear to be due to suppression of a defect in SOS regulation (19). These results and the biochemical analysis of RecA803 (19, 20) suggest the defects caused by *recF, recO*, and *recR* mutations are directly suppressed by the RecA803 protein.

Genetic and biochemical studies have indicated that single-stranded DNA binding protein (SSB) acts in genetic recombination (1, 7, 8, 21, 22). SSB stimulates joint molecule formation promoted by RecA *in vitro*, presumably by removing secondary structure from ssDNA (7, 8, 23). This enhancement is dependent on the concentration and order of addition of RecA and SSB (24, 25). Apparently the binding of RecA and SSB to ssDNA can be competitive (25–27). RecA must be added to the ssDNA prior to the addition of saturating amounts of SSB to see the enhancement by SSB, otherwise SSB interferes with the binding of RecA (24, 25). This inhibition of RecA by SSB appears to be significant *in vivo*. The observation that overproduction of SSB causes a defect in recombination of UV-damaged DNA and this defect is similar to the phenotype of *recF* mutants (28) could be explained by increased levels of SSB competing with RecA for regions of ssDNA. The RecF pathway has been suggested to act on ssDNA gaps left by discontinuous DNA synthesis during conjugation or postreplicational repair, and RecA and SSB could compete for these regions of ssDNA (1, 2, 5). Consequently, the RecF pathway could require a specific mechanism to overcome the negative effects of SSB on RecA.

Biochemical analysis of RecA803 protein indicates this mutant RecA protein has a higher association rate with ssDNA than the wild-type protein and has an enhanced ability to compete with SSB for joint molecule formation (19, 20). One interpretation of these results is that RecF, RecO, and RecR proteins normally modify the ability of RecA protein to interact with ssDNA in the presence of SSB so that wild-type RecA protein in the presence of RecF, RecO, and RecR proteins has biochemical properties similar to those of the RecA803 protein. Consistent with this idea, the results presented here suggest that RecO and RecR, and possibly RecF, act together to allow RecA to utilize SSB–ssDNA complexes as substrates.

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Abbreviations: ssDNA, single-stranded DNA; dsDNA, double-stranded DNA; SSB, single-stranded DNA binding protein.

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MATERIALS AND METHODS

Strains and Plasmids. *E. coli* RDK2032 (*his-4, argE3, leuB6, proA2, thr-1, thi-1, rpsL31, galK2, lacY1, ara-14, xyl-5, mtl-1, kdgK51, supE44, tsx-33, thyA, deo,* F′::Tn*10-Km*) and phage M13mp19 were from our stocks. *E. coli* HMS174 (*hsdR, recA1, rif*), pTTG20, and pRG2 (pACYC184 containing the cloned *E. coli lacI*^{q} gene) were from C. C. Richardson (Harvard Medical School), J. Walker (University of Texas, Austin), and R. Garcea (Dana–Farber Cancer Institute), respectively. pKK223-3 was from Pharmacia.

DNAs. Unlabeled M13mp19 phage viral and replicative form I DNAs were purified as described (29). To purify ^{3}H-labeled DNAs, *E. coli thyA* RDK2032 was used as the host and was grown in Fraser's medium containing thymine (2 μg/ml), tetracycline (15 μg/ml), and [^{3}H]thymidine (1.25 mCi/500 ml; 1 Ci = 37 GBq). The specific activity of the form I DNA used was 8.7×10^3 cpm/nmol. DNA concentrations are given as moles of nucleotide residues per liter.

Enzymes and Proteins. RecO protein was purified by the method of C. Luisi-DeLuca and R.D.K. (unpublished data, available from authors upon request). RecA (30) and RecF (9) proteins were kindly supplied by C. Laski and T. Griffin of this laboratory, respectively. RecR was purified as follows. pRDK260, a RecR-overproducing plasmid, contains the *recR* gene fused to a synthetic ribosome binding site and the *tac* promoter on the expression vector pKK223-3 (31). pRDK260 contains four DNA fragments joined in the following order: (*i*) The larger *Sca* I–*Hin*dIII fragment of pKK223-3, which contains the C-terminal portion of the ampicillin-resistance gene, the origin of replication, and the *tac* promoter; (*ii*) a linker (5′-AAGCTTAAGGAGGTACAAGC**ATGCAGAC-CAGCCCGCTGTT**-3′) containing a *Hin*dIII site, a ribosome binding site (underlined), and the first 20 bp of the *recR* coding region (in boldface type) [the second codon was changed from CAA to CAG to provide a more frequently used codon (32)]; (*iii*) the 1011-bp *Hpa* I–*Eco*RI fragment of pTTG20 containing the *Hpa* I–*Xmn* I fragment of *recR* gene (from +21 to +1015 bp relative to the first codon) joined to the *Sma* I–*Eco*RI fragment of the pUC18 polylinker; and (*iv*) the smaller *Eco*RI–*Sca* I fragment of pUC19 that contained the N-terminal portion of the ampicillin-resistance gene. Nine liters of a derivative of *E. coli* HMS174 containing pRDK260 and pRG2 was grown in LB broth supplemented with ampicillin (50 μg/ml) and tetracycline (10 μg/ml) at 37°C with aeration until the culture reached an A_{590} value of 0.6. Isopropyl β-D-thiogalactopyranoside was added to 1 mM, and incubation continued for 3 hr. The cells were harvested by centrifugation, resuspended in 210 ml of buffer L (10% sucrose/50 mM Tris·HCl, pH 7.5), and frozen in liquid nitrogen in 30- to 40-ml aliquots. The cells were thawed on ice and lysed by adding 5 M NaCl/0.5 M spermidine/lysozyme (10 mg/ml) in buffer L to 100 mM, 10 mM, and 0.2 mg/ml, respectively (final concentrations). The cells were incubated on ice for 45 min, heated to 20°C in a 37°C water bath and then incubated on ice until the temperature was <10°C. All subsequent steps were carried out at 0–4°C and purification of RecR protein (a M_r 21,000 species) was monitored using SDS/PAGE. The cell lysate was clarified by centrifugation at 19,000 × *g* for 35 min and the supernatant was saved (fraction I, 190 ml and 945 mg of protein). Ammonium sulfate (47.5 g) was added to fraction I with stirring over a 45-min period. After an additional 45 min, the precipitated proteins were collected by centrifugation at 20,000 × *g* for 20 min and suspended in 15 ml of buffer A [20 mM Tris·HCl, pH 7.5/10% (wt/vol) glycerol/0.1 mM EDTA/10 mM 2-mercaptoethanol] containing 0.75 M ammonium sulfate (fraction II, 15 ml and 160 mg of protein). Fraction II was applied at 43 ml/hr to a 12.3 cm × 2.9 cm^2 phenyl-Sepharose CL-4B column (Pharmacia) equilibrated in buffer A containing 0.75 M ammonium sulfate. The column was then washed with 100 ml of equilibration buffer and the proteins were eluted with a 960-ml linear gradient from 0.75 to 0 M ammonium sulfate in buffer A. Fractions containing RecR protein, which was eluted at ≈50 mM ammonium sulfate, were pooled (fraction III, 57 ml and 25 mg of protein). Fraction III was diluted with 66 ml of buffer A and applied at 30 ml/hr to a 9 cm × 2 cm^2 cibacron blue 3GA-agarose type 100 column (Sigma) equilibrated in buffer A containing 100 mM NaCl, and then the column was washed with equilibration buffer. The flow-through fractions containing protein were applied at 30 ml/hr to a 19 cm × 1.8 cm^2 PBE94 column (Pharmacia) equilibrated in buffer A containing 100 mM NaCl. After washing the column with 170 ml of equilibration buffer, the proteins were eluted with a 950-ml linear gradient from 0.1 to 1 M NaCl in buffer A. Fractions containing the RecR protein (fraction IV, 10 ml and 14 mg of protein) that eluted at ≈350 mM NaCl, were pooled, dialyzed against buffer A containing 60% (wt/vol) glycerol and 100 mM NaCl, and stored at −20°C. The purified protein was confirmed to be RecR by sequencing 23 residues from its N terminus.

E. coli SSB was from United States Biochemical. Analysis of these preparations by SDS/PAGE indicated that they only contained a single detectable protein species (Fig. 1). All restriction endonucleases were from New England Biolabs. Creatine phosphokinase was from Calbiochem, lysozyme was from Worthington, crystalline bovine serum albumin was from ICN, and proteinase K was from Beckman. SDS/PAGE was performed as described (9). Protein concentrations were determined by the Bradford method (Bio-Rad) using bovine serum albumin as a standard.

Joint Molecule Formation. The basic reaction mixture used in these studies (20 μl) contained 35 mM Tris·HCl (pH 7.5), 10 mM $MgCl_2$, 1.8 mM dithiothreitol, bovine serum albumin (88 μg/ml), 1.3 mM ATP, creatine phosphokinase (10 units/ml), 10 mM phosphocreatine, and as the substrates, 6 μM circular ssDNA (viral M13mp19 DNA) and either 6 μM ^{3}H-labeled or 6 μM unlabeled *Sma* I-cleaved linear dsDNA (M13mp19 replicative form DNA) as appropriate. Standard reaction mixtures contained 3.5 μM RecA (1 molecule per 1.7 nt of ssDNA), 0.6 μM SSB (1 molecule per 10 nt of ssDNA), and 80 or 120 nM RecF, 80 or 120 nM RecO, and 80 or 120 nM RecR (1 molecule per 75 or 50 nt of ssDNA, respectively) as indicated. Reactions were incubated at 37°C. The order of addition of proteins and incubation times are described in individual experiments. The filter binding method of Shibata *et al.* (33) was used for detecting joint molecules formed in 20-μl basic reaction mixtures. Agarose gel electrophoresis assays were performed as described by Lavery and Kowalczykowski (34) except that the concentration of the DNA substrates and RecA, RecF, RecO, RecR, and SSB proteins present in 20-μl basic reaction mixtures was increased 2-fold.

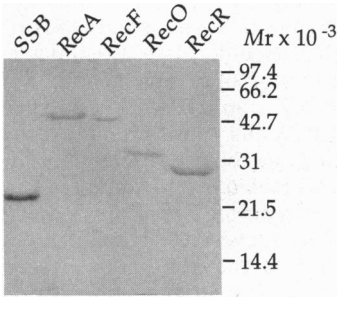

FIG. 1. SDS/PAGE analysis of the proteins used in these studies. Each lane contains 2 μg of the indicated protein. Electrophoresis was performed using a 7-cm-long 0.75-mm-thick 15% polyacrylamide gel followed by staining with Coomassie blue as described (9).

The resulting gels were photographed and quantitated by scanning with an LKB Ultroscan XL laser densitometer. The percentage of joint molecules formed was defined as the percentage of linear dsDNA substrate converted to joint molecules (intermediates plus open circles). The percentage of final products formed was defined as the percentage of linear dsDNA converted to open circular dsDNA molecules.

RESULTS

Effect of RecF, RecO, and RecR on RecA-Catalyzed Joint Molecule Formation. To test the hypothesis that RecF, RecO, and RecR might enhance the ability of RecA to use SSB–ssDNA complexes as substrates, we studied the effects of RecF, RecO, and RecR on RecA-mediated strand-exchange reactions using circular ssDNA and homologous linear dsDNA as substrates. Three types of reactions have been performed: (*i*) SSB was added to ssDNA prior to RecA to determine whether RecF, RecO, and RecR could overcome the inhibition by SSB; (*ii*) RecA was added to ssDNA prior to SSB to determine whether RecF, RecO, and RecR affected strand exchange under these conditions; and (*iii*) reactions were performed without SSB to determine whether RecF, RecO, and RecR could stimulate RecA.

Table 1 summarizes the effects of RecF, RecO, and RecR on joint molecule formation detected using filter-binding assays. Joint molecule formation by RecA was enhanced if SSB was added to ssDNA after RecA, whereas it was inhibited if SSB was added prior to RecA as described (24). This inhibitory effect of SSB was overcome by the addition of RecO and RecR or RecF, RecO, and RecR. No other combination of RecF, RecO, and RecR had a stimulatory effect. The stimulatory effect was not seen without SSB or when SSB was added after RecA. Joint molecule formation was not detected without RecA.

Fig. 2 compares the time course of joint molecule formation assayed by filter binding in the presence and absence of RecF, RecO, and RecR. When SSB was added first, the inhibitory effect of SSB on the extent of joint molecule formation was overcome by the addition of RecF, RecO, and RecR. After an initial lag of <5 min, the rate and extent of joint molecule formation were restored to the maximal level observed when SSB was added after RecA. RecF, RecO, and RecR showed the same effect when they were added at the same time as RecA or were preincubated with SSB and ssDNA prior to the addition of RecA. RecF, RecO, and RecR could overcome the inhibitory effect of SSB present at concentrations of up to 1.8 μM, which was the highest concentration tested (1 SSB per 3.3 nt of ssDNA; 3 times saturation). No effect of RecF, RecO, and RecR was detected when RecA was added before SSB. When RecF was omitted and only RecO and RecR were present, we often observed a slight decrease in the reaction rate compared to that observed when RecF, RecO, and RecR were all present. However, essentially all the effects observed were due to RecO and RecR.

Table 1. Effect of RecF, RecO, and RecR on joint molecule formation

Other proteins added	% joint molecules formed		
	SSB first	RecA first	No SSB
—	27 (1)	66 (1)	33 (1)
RecF	17 (0.63)	68 (1.03)	29 (0.88)
RecO	23 (0.85)	77 (1.17)	25 (0.76)
RecR	27 (1.00)	69 (1.05)	35 (1.06)
RecF + O	14 (0.52)	77 (1.17)	21 (0.64)
RecF + R	25 (0.93)	70 (1.06)	35 (1.06)
RecO + R	50 (1.85)	64 (0.97)	28 (0.85)
RecF + O + R	55 (2.04)	76 (1.16)	28 (0.85)

Joint molecule formation was determined by filter binding. For SSB added first, SSB was added to ssDNA followed by the indicated proteins. After preincubation for 5 min, RecA and dsDNA were added and incubation continued for 30 min. The percent of DNA converted to joint molecules is given. Numbers in parentheses are the proportion of joint molecules formed relative to that formed in the absence of RecF, RecO, and RecR. Similar results were obtained with a 15-min incubation. For RecA added first, reactions were performed as for SSB added first except the order of addition of RecA and SSB was reversed. When no SSB was present, reactions were performed as for RecA added first except SSB was omitted.

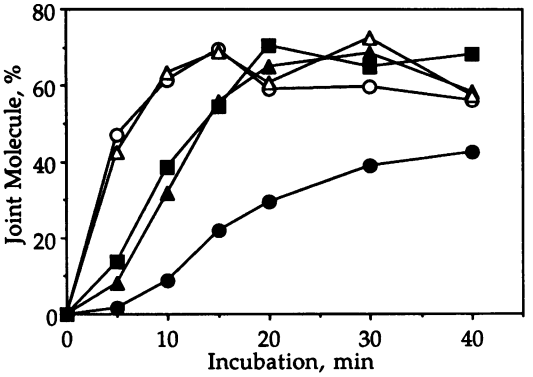

FIG. 2. Effect of RecF, RecO, and RecR on joint molecule formation. Joint molecule formation was quantitated using filter binding assays. In the reactions where SSB was added prior to RecA, SSB was added to ssDNA in the reaction mixture, preincubated for 5 min, and the reaction was initiated by the addition of dsDNA and RecA. In the reactions in which RecA was added before SSB, the order of the addition of RecA and SSB was reversed but the incubation times remained the same. ○, RecA added before SSB with no RecF, RecO, or RecR present; △, RecA added before SSB with RecF, RecO, and RecR added at the same time as RecA; ▲, SSB added prior to RecA with RecF, RecO, and RecR added at the same time as SSB; ■, SSB added prior to RecA with RecF, RecO, and RecR added at the same time as RecA; ●, SSB added prior to RecA with no RecF, RecO, or RecR present.

RecO and RecR Stimulate the Initiation of Joint Molecule Formation. Agarose gel electrophoresis assays were used to analyze the effect of RecF, RecO, and RecR on the formation of both joint molecules and open circular dsDNA molecules, the final products of strand-exchange reactions (Fig. 3). Fig. 3 shows experiments analyzing four types of strand-exchange reactions: (*i*) SSB added to ssDNA before RecA; (*ii*) SSB added to ssDNA after RecA; (*iii*) SSB, RecO, and RecR added to ssDNA before RecA; and (*iv*) SSB, RecF, RecO, and RecR added to ssDNA before RecA. The results were similar to those obtained using filter binding assays (Fig. 2). The addition of RecF, RecO, and RecR or RecO and RecR overcame the inhibitory effect of SSB added prior to RecA on the extent of joint molecule formation (intermediates plus final products); the rate and final level of joint molecules obtained were almost the same as observed in reactions where SSB was added after RecA (Fig. 3*A*). We detected little effect of RecF in addition to that observed with RecO and RecR. Independent of the order of addition of RecA and SSB and the presence of RecF, RecO, and RecR, open circular dsDNA end products began to appear 15 min after the appearance of joint molecules (Fig. 3*B*). More than 90% of the joint molecules were converted to final products after 60 min. This is consistent with results that SSB inhibits formation of presynaptic filaments but does not inhibit strand exchange (7, 8, 24, 25). Since RecF, RecO, and RecR overcame the inhibitory effect of SSB on the rate of formation of joint molecules and had no effect on conversion of joint molecules to final products, this suggests that RecO and

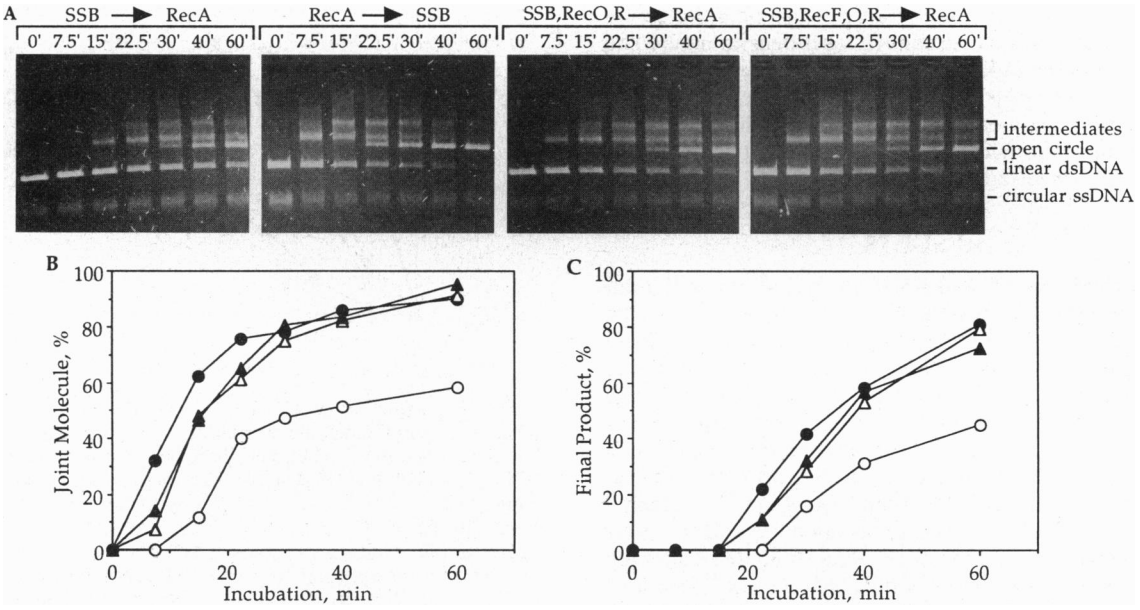

FIG. 3. Joint molecule formation assayed by agarose gel electrophoresis. (*A*) Reactions were performed as described in Fig. 2. The preincubation step contained the proteins listed before the arrow above each gel. Then the proteins listed after the arrow were added along with the dsDNA to initiate the reactions. After the indicated incubation times, the reactions were terminated by the addition of EDTA to 50 mM, deproteinized by incubation at 37°C for 10 min with proteinase K (0.3 mg/ml) and 0.15% SDS, and then electrophoresed through an 0.8% agarose gel followed by staining with ethidium bromide (34). Joint molecules (intermediates plus final products) (*B*) and open circular products (*C*) were quantitated by densitometric scanning of photographic negatives of the agarose gels. ○, SSB added before RecA; ●, RecA added before SSB; △, SSB, RecO, and RecR added before RecA; ▲, SSB, RecF, RecO, and RecR added before RecA.

RecR, and possibly RecF, act to help RecA at early steps in the reaction.

Titration of RecF, RecO, and RecR. To determine the amount of RecF, RecO, and RecR required, we measured joint molecule formation as a function of the concentration of one protein in the presence of saturating amounts of the other two proteins (Fig. 4). Joint molecule formation increased as a function of the RecO concentration up to ≈120 nM (Fig. 4*A*). RecO slightly inhibited the reaction in the absence of RecF and RecR. For RecR (Fig. 4*B*), the addition of RecR in the presence of RecF and RecO stimulated the reaction up to a concentration of 100 nM. RecR had no effect on the reaction in the absence of RecF and RecO. These data indicate that RecO and RecR are required in a 1:1 molar ratio and that maximal stimulation occurs at ≈1 RecO and RecR molecule per 50 bases of ssDNA (120 nM RecO and RecR). Titration of RecO and RecR in the absence of RecF gave similar results (data not shown). Addition of RecF inhibited joint molecule formation in the absence of RecO and RecR (Fig. 4*C*). The presence of RecO and RecR prevented this inhibition by RecF, suggesting that RecF somehow interacts with RecA, RecO, RecR, and SSB.

DISCUSSION

Our results indicate that RecO and RecR can act in a 1:1 molar ratio to overcome the inhibitory effect of SSB on RecA-mediated homologous pairing when SSB is added to ssDNA substrates before RecA. RecO and RecR appear to act by stimulating the rate of initiation and do not appear to affect the rate of strand exchange once homologous pairing has initiated. RecF interacts in some way with RecA, RecO, RecR, and SSB; however, RecF plays little, if any, role in helping RecA overcome inhibition by SSB. These results are consistent with genetic experiments suggesting that the RecF, RecO, and RecR proteins function at the same step in recombination and play a role in some aspect of RecA-mediated pairing reactions (5, 17, 18). Prior to our studies, the most compelling experiments suggesting that RecF, RecO, and RecR interact with RecA and SSB were a combination of

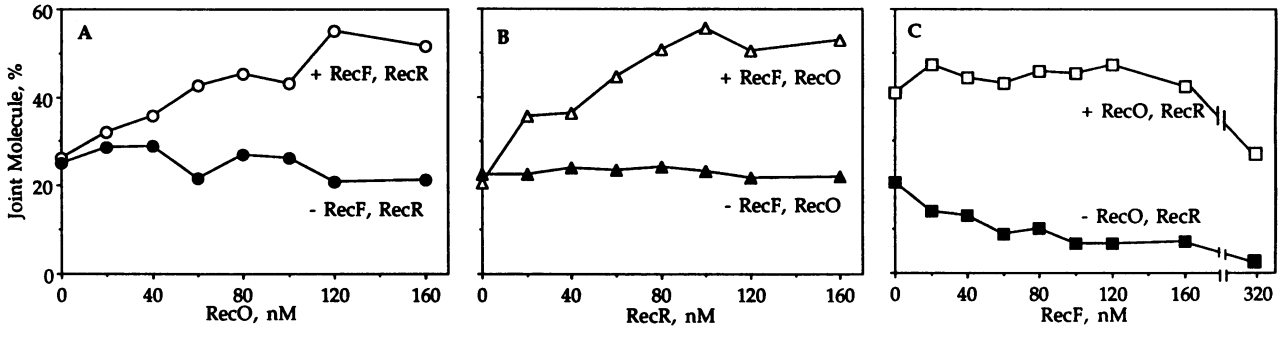

FIG. 4. Determination of the required amounts of RecF, RecO, and RecR. Joint molecule formation was measured using filter binding assays. SSB was preincubated for 5 min with ssDNA and the indicated amount of each one of RecO (*A*), RecR (*B*), or RecF (*C*) in the presence or absence of 120 nM each of the other two proteins (RecF, RecO, and RecR, as appropriate). The reactions were then initiated by the addition of RecA and dsDNA and incubated for 15 min.

the genetic and biochemical experiments characterizing the *recA803* gene product (5, 19, 20). These studies suggested that the mutant RecA803 protein suppresses the defect of *recF*, *recO*, and *recR* mutations because it competes more efficiently with SSB for binding to ssDNA than wild-type RecA (19, 20). The results presented here show that RecA in the presence of RecO and RecR or RecF, RecO, and RecR has an activity similar to the RecA803 protein alone (19, 20). Combined with the results of previous studies (5, 17–20), our results suggest that RecF, RecO, and RecR proteins normally affect the interaction of RecA, SSB, and ssDNA.

Genetic studies (5, 17, 18) also suggest the involvement of RecF at the same step that RecO and RecR act. We have not been able to consistently demonstrate a significant stimulation of homologous pairing reactions by RecF like that observed with RecO and RecR. RecF protein inhibited homologous pairing promoted by RecA and SSB and this inhibition was overcome by the addition of RecO and RecR. This suggests there is some type of interaction involving RecF, RecO, and RecR. There are at least four possible reasons why we did not see significant stimulation of homologous pairing by RecF. (*i*) It is possible we have not yet discovered appropriate reaction conditions to allow demonstration of an effect of RecF. (*ii*) The substrates used, circular ssDNA and homologous linear dsDNA, may not reflect the substrates RecF acts on *in vivo*. (*iii*) It is possible that RecF acts at a step that is not rate limiting *in vitro* in the overall pairing reaction we have analyzed. (*iv*) RecF may act on some protein other than SSB that interacts with RecA, RecO, RecR, SSB, and ssDNA such as HU protein or another DNA binding protein. Additional analysis will be required to elucidate the role of RecF.

SSB is directly involved in recombination and stimulates strand-exchange reactions promoted by RecA (1, 7, 8, 22). When RecA protein is incubated with ssDNA substrates under optimal conditions for homologous pairing reactions, RecA cannot form complete presynaptic filaments that initiate homologous pairing because the ssDNA contains secondary structure that prevents optimal binding of RecA (23, 35). Addition of SSB to such reaction mixtures stimulates homologous pairing by disrupting secondary structure in ssDNA and this allows the optimal formation of RecA–ssDNA presynaptic filaments (7, 8, 23, 25). Since the binding of RecA and SSB to ssDNA is competitive under some conditions (25–27), assembly of RecA presynaptic filaments is highly dependent on the order of addition of RecA and SSB to the reaction and the concentrations of each protein. When SSB is added to ssDNA prior to RecA or a high concentration of SSB is included, assembly of the RecA–ssDNA nucleoprotein filament is decreased and the reaction is inhibited (24, 25). The binding of RecA to ssDNA to form a nucleation site appears to be the rate-limiting step in the formation of presynaptic filaments and it is this step that is inhibited when the ssDNA substrate is saturated with SSB prior to the addition of RecA (36). Once the RecA nucleation site forms on the ssDNA, RecA cooperatively polymerizes onto the ssDNA and displaces the SSB (36). Our observation that RecO and RecR, and possibly RecF, help overcome the SSB inhibition of RecA by stimulating the rate of initiation of joint molecule formation is consistent with this view of the interplay between RecA and SSB.

At present, it is unclear how RecO and RecR, and possibly RecF, help overcome the SSB inhibition of RecA. Given that these proteins stimulate the rate of initiation of joint molecule formation, it seems likely that they act by helping the RecA nucleation sites to form on SSB–ssDNA complexes. There are a number of ways in which this could occur. (*i*) RecO and RecR, and possibly RecF, could displace SSB from ssDNA allowing RecA to form a nucleation site. The ability of RecF (9) and RecO (C. Luisi-DeLuca and R.D.K., unpublished results) to bind to ssDNA could allow these proteins to displace SSB from ssDNA. (*ii*) These proteins could interact directly with RecA and transfer it onto SSB–ssDNA complexes. Alternatively, RecO and RecR, and possibly RecF, could promote the initiation of homologous pairing independently of RecA and then RecA could function at later step in the homologous pairing process. The observation that RecO can promote renaturation of complementary ssDNA (C. Luisi-DeLuca and R.D.K., unpublished results) is consistent with this latter idea.

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