RecA protein promotes the regression of stalled replication forks in vitro

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Replication forks are halted by many types of DNA damage. At the site of a leading-strand DNA lesion, forks may stall and leave the lesion in a single-strand gap. Fork regression is the first step in several proposed pathways that permit repair without generating a double-strand break. Using model DNA substrates designed to mimic one of the known structures of a fork stalled at a leading-strand lesion, we show here that RecA protein of *Escherichia coli* will promote a fork regression reaction in vitro. The regression process exhibits an absolute requirement for ATP hydrolysis and is enhanced when dATP replaces ATP. The reaction is not affected by the inclusion of the RecO and R proteins. We present this reaction as one of several potential RecA protein roles in the repair of stalled and/or collapsed replication forks in bacteria.

Under normal growth conditions, bacterial replication forks are halted by DNA damage in virtually every cell and every cell generation (1–4). Replication forks can stall for a variety of reasons. They may encounter a DNA lesion or a single-stranded (ss)DNA nick, leading to collapse of the fork and formation of a DNA gap or double-strand break, respectively. They might encounter a region of secondary structure in DNA that constitutes a pause site. They might also collide with another protein or protein complex on DNA, such as an RNA polymerase itself stalled at a DNA lesion (5). Proteins bound on DNA can temporarily block a replication fork by inhibiting the progression of the replicative helicase.

Genetic and structural evidence suggests that a first step in the repair of a stalled fork is fork regression (Fig. 1A) (5–7). The products of fork regression have been observed by electron microscopy (EM) (6, 8). The resulting recombination intermediates can subsequently be processed to function as substrates for replication restart in an origin-independent manner (4). This process does not repair the lesion itself but instead establishes the necessary conditions for accurate repair by supplying an undamaged complementary strand opposite the lesion. The lesion can then be removed via a process like excision repair. Another key outcome of fork repair pathways is the nonmutagenic restoration of the replication fork.

In principle, a lesion on the lagging strand presents fewer problems to the replication machinery, because another Okazaki fragment can be initiated downstream (9). However, a lesion on the leading strand may block replication and lead to the formation of a ssDNA gap on this strand, whereas synthesis can become uncoupled and continue for some distance on the lagging strand under at least some circumstances (9, 10). Thus a fork with a leading-strand ssDNA gap at the branch is generated (Fig. 1A). Recombination is required to generate a DNA structure on which replication can resume and to allow for repair and/or bypass of the lesion. As part of recombinitational DNA repair, *Escherichia coli* RecA protein is thought to promote DNA pairing, strand exchange, and branch migration beyond the lesion. These activities allow the lesion-containing strand to pair with an intact complementary strand so that repair can ensue (11). In some early models, a nuclease was proposed to generate a free end in the lagging-strand template for the RecA-catalyzed strand invasion (12). However, the required nuclease has not been found. The alternative fork regression pathway (Fig. 1A) accomplishes the same purpose with no break in the parental strands (5–7, 11). A similar process of fork regression may occur spontaneously at stalled forks that do not have a ssDNA gap at the branch, facilitated by the superhelical stress generated during replication (13).

Fork regression leads to the formation of a Holliday intermediate (Fig. 1A). In principle, this intermediate could be processed in two ways. The tendency of stalled forks to generate double-strand breaks provides evidence that some intermediates are cleaved by the RuvABC system (7, 14). The resulting double-strand break would then be funneled into the RecBCD pathway for subsequent repair. The alternative path would involve a reverse branch migration to regenerate a fork-like structure, perhaps propelled by the RecG protein (5).

The regression of replication forks could be promoted by enzymes known to process branched DNAs, such as the RecG helicase (5) or the RuvAB system (14, 15). The RecA protein is also known to play an important role in replication fork repair. A role in the strand invasion step of double-strand break repair has extensive experimental support (16–20). Recent evidence suggests that a major pathway leading to the formation of cleavable Holliday intermediates at stalled forks requires the RecA protein (21), suggesting a role for RecA protein in fork regression. This would be a novel activity of RecA protein. At a stalled fork with a structure like that shown in Fig. 1A, RecA filaments would nucleate within the gap and extend away from the branch (22, 23). To promote fork regression, the RecA protein would thus act to move a DNA branch that was initially beyond one end of the filament. This study was initiated to investigate whether a replication fork with a gap at the branch could regress through the action of RecA protein. We report here that RecA protein does indeed promote fork regression in vitro.

Materials and Methods

**Enzymes and Reagents.** *E. coli* RecA (24), RecO (23), RecR (25), RecF (25), and single-strand DNA-binding protein (26) proteins were purified as described. The concentration of each protein was determined by absorbance at 280 nm by using their extinction coefficients: $e_{280} = 2.23 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$ for RecA (27), $e_{280} = 5.6 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$ for RecR (23), $e_{280} = 3.87 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$ for RecF (28), and $e_{280} = 2.83 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$ for SSB (29), with the exception of RecO protein, for which the concent-
DNA was purified from infected JM101 E. coli et al. according to Maniatis. The restriction endonuclease Pst was used to cut M13mp8 dsDNA and cloned I-cut M13mp8 dsDNA and cloned supercoiled M13mp8.32 with EcoRI and BsrG1 enzymes. The fragment was gel purified by using 1% SeaPlaque GTG agarose gel and was extracted from gel pieces by using QIAquick Gel Extraction Kit from Qiagen. The product of the RecA reaction was GD2051, which has a 2,051-nt ssDNA gap. GD2051 was gel purified as described above.

Linear dsDNA (7.3 kb) was prepared by complete digestion of supercoiled M13mp8.32 DNA with SapI and SmaI enzymes, which leave a nonpalindromic ssDNA overhang and a blunt end, respectively. The MM1 DNA was assembled by using a completely homologous linker prepared by annealing two synthetic oligonucleotides: oligo1 5’-CACCAGAAACGCGACAGGTG-CCACGTGCTGCAGGTCGACGGATCCCCGGG-3’ and oligo2 5’-CCTGCAGCACGTTGCAGCTGGGCGCTTCTTG-3’. The MM2 DNA was assembled by using a linker that has six mismatches at the branch point (with respect to M13mp8.32) to prevent spontaneous branch migration. The linker was prepared by annealing two synthetic oligonucleotides: oligo1 M 5’-CACCAGAAACGCGACAGGTG-CCACGTGCTGCAGGTCGACGGATCCCCGGG-3’ and oligo2 M 5’-GGCGCCCGCGCGGCGACCGTGCAGCTGGGCGCTTCTTG-3’. The nucleotides in bold correspond to the mismatches. Either one of the linkers was ligated with the nonpalindromic ssDNA overhang of linear dsDNA (7.3 kb). The ligated products were purified from excess oligonucleotides by gel filtration on a Sephacryl 300 column. Thus two-tailed linear dsDNA were generated: lds [1–2] and lds [1 M-2 M], respectively (Fig. 1 C).

For some experiments, the MM2 DNA was linearized (before final purification) by complete digestion with MscI enzyme and purified from MscI by using Microcon 100 columns. This linear MM2 was mixed with linear GD2051 and 6.2-kb linear dsDNA generated by MscI digestion of unreacted GD2051 and lds [1 M-2 M]. Some of these linear MM2 DNA mixtures were used directly in reactions with RecA protein, for some routine trials, and others were purified further. The mixtures were gel purified by using 1% SeaPlaque GTG agarose gel, and the linear MM2 DNA was extracted from gel pieces by electroelution using a GeneCapsule kit.

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RecA Protein Reactions. Reactions were carried out in 25 mM Tris acetate (80% cation)/10 mM Mg(OAc)/2.5% (wt/vol) glycerol/3 mM potassium glutamate/1 mM DTT in a final volume of 10 μl. ATP, dATP, or ATPγS at 3 mM was included, as indicated. An ATP or dATP regenerating system (10 units/ml creatine phosphokinase and 12 mM phosphocreatine) was also included. RecA and DNA concentrations are provided in the text and figure legends. Typically, RecA protein was incubated with DNA in reaction buffer and regenerating system for 10 min. Then, SSB protein (to a final ratio of 1 monomer per 10 single-stranded nucleotides) and the nucleotide cofactor were added as indicated. Reaction mixtures were incubated for different amounts of time, as indicated. When RecORF proteins were used, they were added as part of a preincubation mixture with the nucleotide cofactor and SSB. All incubations were carried out at 37°C. Reactions were stopped and deproteinized by adding SDS to 1%, EDTA to 12 mM, and proteinase K to 1 mg/ml (all final concentrations). Proteinase K was incubated for 30 min at 37°C before addition for predigestion. After addition, incubation was carried out for an additional 30 min at 37°C. Control reactions lacking one or more components were carried out as indicated in the text and legends. Reaction mixtures were electrophoresed on 1.4% agarose gels. The gels were run at 25–30 mV, at 4°C, typically for 16 h. DNA was stained with ethidium bromide and visualized with a charge-coupled device camera on a transilluminator.

EM. Samples for EM were obtained by spreading the reaction mixtures or DNA solutions as noted with a cytochrome spreading technique to visualize the DNA (25). Reaction mixtures were first deproteinized as described above and dialyzed against 20 mM NaCl and 5 mM EDTA for at least 4 h at 25°C on Millipore type VM (0.05 mm) filters. The samples were then diluted to a final concentration of 0.004 μg/μl and spread, as described previously (34). Photography and measurements of the DNA molecules were performed as described (35).

Results

Experimental Design. The purpose of this study was to investigate the action of RecA protein on a stalled replication fork. We therefore designed and constructed a DNA molecule that mimics one of the few stalled replication fork structures observed and characterized to date (Fig. 1A) (10). This branched model molecule was designated MM1 (Fig. 1B). It consists of a gapped DNA circle with a homologous dsDNA tail attached at the 5’ region of the gap. RecA protein can bind the ssDNA region from the gap starting at the branching point in the 5’ to 3’ direction (36). The molecule was assembled from a gapped DNA circle and a homologous linear dsDNA joined by a synthetic linker (Fig. 1C).

To prevent or diminish spontaneous branch migration at the branch, we changed six base pairs in the linear dsDNA tail in positions 1, 2, 3, 6, 9, and 12 from the branch. These mismatches with the ssDNA in the gap were designed to prevent both end fraying of the linear dsDNA tail and spontaneous branch migration. The molecule constructed in this way was designated MM2. Except for the mismatches at the branch point, the overall structure of MM2 is the same as the structure of MM1 (Fig. 1A).

On a gapped DNA, RecA protein nucleates on the ssDNA region and extends the filament 5’ to 3’. For the MM1 or MM2 branched molecules, filament extension within the gap will progress away from the branch, into the adjacent duplex DNA. Given enough protein, the filament can extend all the way around the circle, straddling the branch. Under some conditions, some binding might also occur on the linear dsDNA tail if enough protein were available. If a cellular replication fork stalled so as to leave a leading-strand gap as in Fig. 1A, the 5’ to 3’ direction of filament extension in the gap (away from the branch) would ensure that the RecA filament appeared only on the gap side of the branch. To better mimic this situation, we linearized MM2 with a restriction enzyme MscI that generates blunt ends (Fig. 1C). This molecule will be called LMM2. On LMM2, RecA filaments nucleated in the gap would be extended to the end of the DNA but would not be expected to straddle the branch itself.

The potential regression reactions and expected products of a RecA reaction with MM1 or MM2 and LMM2 are shown in Fig. 2. In the reactions, we used either unpurified branched model molecules.
molecules (that also contained some unreacted substrates: GD2051 and one dsDNA ligated with the appropriate linker) or purified branched model molecules, as indicated. In cases where the unpurified DNA was used, the branched model molecules made up at least 40% of the total DNA molecules present. The background of unreacted DNA substrate molecules provided useful standards for recognition in experiments involving EM.

RecA Protein Reactions with MM1 DNA. Reaction mixtures were incubated either with (Fig. 3, lane 5) or without (Fig. 3, lane 4) RecA protein, in the presence of dATP and SSB protein for 2 h. The dATP is used for the standard reaction conditions to minimize end-dependent RecA filament disassembly (23). The reactions were deproteinized and divided in two for analysis by gel electrophoresis (Fig. 3) or by EM (Fig. 4).

In the absence of RecA protein, spontaneous branch migration occurs to a limited degree, as evidenced by the appearance of the minor upper bands in lane 4, Fig. 3. Counts of molecules observed in the electron microscope revealed that ~60% of the branched MM1 molecules had undergone no branch migration (Fig. 4A), and about 21% showed branch migration occurring over less than 1/3 of the ssDNA gap, which is 2,051 nt long. Approximately 18% of the model molecules showed branch migration occurring over more than 1/3 of the ssDNA gap and less than 2,051 nt (Fig. 4B). None of the molecules had undergone branch migration beyond the ssDNA gap.

In the presence of RecA protein, essentially all model molecules reacted to generate the final product after 2 h, represented by the intense upper band in lane 5, Fig. 3. This is the product expected for a complete RecA protein-mediated regression reaction of the model fork in the MM1 DNA, as confirmed by EM analysis. The second band from the top represents the nicked circles, one of the products of strand exchange between the excess of linear dsDNA and gapped circles still present in the reaction mixture. EM counts revealed that almost 100% of the model molecules had the structure of the final product (with the ssDNA region at the end of the tail) (Fig. 4C).

RecA Protein Reactions with MM2 DNA. To diminish the background of spontaneous branch migration of the MM1 DNA, we introduced six mismatches at the branch of MM1 to generate MM2, as described above. MM2 did not exhibit spontaneous branch migration, as shown in Fig. 5A, lane 4. Control experiments quantified by EM confirmed that no spontaneous branch migration occurred when MM2 DNA was incubated in the absence of RecA protein. In the presence of RecA protein, after 2 h, a very pronounced fork regression reaction was observed. EM counts revealed that ~81% of the purified MM2 reacted to generate the final product. Another 5% was in an intermediate stage of branch migration, with the remainder unreacted. Four different experiments analyzed by gel electrophoresis with both unpurified and purified MM2 confirmed that the reaction is both very efficient and completely dependent on RecA protein.

Nucleotide Cofactor and Protein Requirements for RecA Protein-Promoted Fork Regression in Vitro. We wished to determine whether ATP hydrolysis was required for the in vitro fork regression reaction. We also wanted to explore the nucleotide cofactor requirements for the reaction. On ssDNA, dATP tends to prevent end-dependent filament disassembly (23), which would remove RecA from the filament end nearest the branch.
The preparation of ATP

Reactions of RecA protein as indicated. (A) Gel electrophoresis of reactions to test nucleotide cofactor requirements for RecA protein reaction with purified MM2 DNA. The reaction contained MM2 DNA at a concentration of 17.7 μM total nucleotides, corresponding to 1.42 μM single-stranded nucleotides (in the specified conditions. Lane 1, GD2051 marker; lane 2, ldsDNA[1 M-2 M] marker; lane 3, MM2 mixture; lanes 4–12, RecA reactions with MM2 DNA as indicated; lane 13, supercoiled M13mp8.32 DNA marker. (B) RecA protein titration in reactions with MM2 DNA. Purified MM2 DNA at a concentration of 10 μM total nucleotides, corresponding to 0.57 μM single-stranded nucleotides in the gaps, was incubated with increasing concentrations of RecA protein. 1× RecA represents 0.19 μM RecA, or enough to bind all of the available single-stranded gap DNA. Lanes: 1, linear dsDNA [1 M-2 M] marker; 2, λ DNA–BsIIEI DNA marker (from top: 8,654, 7,242, 6,369, 5,686, 4,822, 4,324, 3,675 bp); 3, GD2051 marker; 4, purified MM2, 5–10, reactions with increasing concentrations of RecA protein as indicated.

Therefore, additional RecA reactions with MM2 were carried out in the presence of dATP, ATP, or ATPγS, and in the presence or absence of SSB. These reactions contained unpurified MM2 (40% MM2 molecules) and were analyzed by gel electrophoresis. The RecA protein-mediated fork regression reaction is most efficient in the presence of dATP (Fig. 5A, lanes 5 and 6). ATP makes the reaction less efficient than that observed with dATP (Fig. 5A, lane 7), perhaps because RecA filaments are less stable in the presence of ATP (23). ATPγS (an ATP analog that is not significantly hydrolyzed by RecA on this time scale) abolishes the reaction completely (Fig. 5A, lane 8). The preparation of ATPγS used in this experiment was also used with consistent success in several dozen other RecA protein-promoted DNA-pairing reactions in the laboratory (data not shown), so that the lack of detectable regression in this experiment was not because of an inactive or degraded ATPγS reagent. Also in the absence of any nucleotide cofactor, the reaction is blocked (Fig. 5A, lane 12). Thus ATP hydrolysis is required for RecA-mediated fork regression.

Reactions in the absence of SSB protein were less efficient than those that contained it (Fig. 5A, lanes 9–11, as compared with lanes 6–8, respectively). A RecA titration revealed that an optimal reaction also required sufficient RecA protein to saturate the circular part of the MM2 DNA. A concentration of 1× RecA represents the amount of RecA protein required to saturate only the single-stranded gap in MM2 (and any other ssDNA in a particular reaction) at one RecA per three nucleotides. A concentration of ~3.5× represents enough RecA protein to coat the circular part of MM2. Approximately 9.5× RecA protein represents enough RecA protein to coat all of the DNA in the reaction mixture. No product is formed in the absence of RecA protein (Fig. 5B, lane 4). With increasing concentrations of RecA protein, more product is formed (upper band) as the MM2 substrate is consumed (second band from the top, Fig. 5B, lanes 5–10). A concentration of 4× RecA protein provided an essentially complete reaction with MM2.

To examine the kinetics of RecA-mediated in vitro fork regression, purified MM2 DNA was incubated with RecA protein at 4× concentration for up to 3 h. At different time points, aliquots were removed from the reaction mixture and were analyzed by gel electrophoresis (data not shown). The reaction approaches maximum efficiency at 40 min and is essentially complete after 60 min.

RecA Protein Reactions with LMM2 DNA. To better mimic the DNA structures that could be present in vivo and to better define the requirements for RecA protein, we carried out a series of similar experiments with the linearized version of MM2, LMM2. Purified LMM2 molecules were spread by using the cytochrome spreading technique and analyzed with EM. A representative molecule is shown in Fig. 6A. Purified LMM2 DNA was incubated with or without RecA protein for 150 min, and the reaction mixtures were analyzed by EM after cytochrome spreading. More than 30% of the LMM2 reacted to generate final products (linear dsDNA 7.3 kb and linear gDNA 6.2 kb with a 2-kb single-strand extension at one end; Fig. 2). This reaction was carried out under standard reaction conditions in the presence of dATP.

Purified LMM2 DNA was incubated with increasing amounts of RecA protein for 2 h, and reaction mixtures were analyzed by gel electrophoresis (Fig. 6B). As in the previous set of experiments, a concentration of 1× RecA protein represents the amount of RecA protein needed to saturate the available DNA-binding sites in the single-stranded gaps of the DNA molecules at one RecA per three nucleotides. Under these conditions, the RecA may not all be bound to the gaps, with some bound in filaments extending beyond the gaps into adjacent duplex DNA and some gap DNA unbound.

A concentration of ~2.7× represents enough RecA protein to coat the arm of the forked MM2 that contains the ssDNA gap. Approximately 6× RecA protein represents enough RecA protein to coat all DNA in the mixture. The reaction with LMM2 depends completely on RecA protein, as no product is formed in its absence (Fig. 6B, lane 3). With increasing concentrations of RecA protein, more products are formed [linear dsDNA 7.3 kb (second band from the top) and linear gDNA 6.2 kb with the 2-kb gap at one end (bottom band)] as the LMM2 substrate is consumed (Fig. 6B, lanes 4–8). The efficiency of the reaction starts to level off at 3× RecA protein.

The kinetics of the reaction are shown in Fig. 6C. Purified LMM2 DNA was incubated with RecA protein at 4× concentration for up to 3 h. At different times, aliquots were removed as indicated and were analyzed by gel electrophoresis. The reaction approached an endpoint of about 64% after ~150 min and increased little more over the 3 h time point.
Reaction requirements were again explored and found to be similar to those observed for the MM2 substrate. Various RecA reactions with LMM2 were carried out with dATP, ATP, or ATPγS (Fig. 6D). As with circular model molecules, the reaction with dATP is more efficient than the one with ATP (Fig. 6D, lanes 5 and 6). Also, ATP hydrolysis is required for the reaction, as ATPγS abolishes the RecA protein reaction (Fig. 6D, lane 7). At a low concentration, RecOR proteins do not exhibit a significant effect on the RecA reaction under our conditions (Fig. 6D, lane 8). When added at a higher concentration, RecOR proteins inhibited the reaction, probably by binding nonspecifically to DNA and preventing RecA-catalyzed fork strand exchange (Fig. 6D, lane 9). We have...
tried using different concentrations of RecOR proteins, sometimes in combination with RecF protein, using MM2 instead of LMM2 and with different orders of addition. In three different experiments with both unpurified and purified LMM2, we did not observe any stimulation of the reaction by RecOR in the presence of ATP (data not shown). RecF protein was evidently important in the fork regression reaction. However, we note that there is no lesion or barrier in the model DNA substrates used in this study, other than the six mismatches present near the branch in MM2 and LMM2. ATP hydrolysis is not needed simply to bypass these mismatches in our model system, because MM1 (which has no mismatches) also exhibited no reaction with ATP by (although spontaneous branch migration could obscure a minimal reaction in this case; data not shown).

There are two major models for the mechanism by which the coupling between ATP hydrolysis and DNA strand exchange occurs. In one, ATP hydrolysis is coupled only to the dissociation of RecA filaments from the DNA (40, 43, 44). ATP hydrolysis can result in the disassembly of RecA filaments from one end (23). However, filament disassembly appears to be deleterious in the fork regression reaction we have observed in this study, and it has been noted elsewhere that much ATP hydrolysis takes place in the filament interior that does not result in RecA dissociation from DNA (11, 45). The alternative has been called the facilitated DNA rotation model. In this scenario, RecA protein is a motor protein, actively promoting DNA strand exchange and branch migration by the rotation of a DNA duplex around the homologous DNA molecule coated with the RecA filament (11), coupled to the hydrolysis of ATP. Several kinetic tests have provided evidence for this activity [(24, 33); T. A. Arenson and M. M. C., unpublished results], although the postulated motor has lacked an obvious in vivo function. We propose that this motor activity of RecA protein might be important for DNA pairing and branch migration to promote regression of a stalled replication fork. Regression of the fork requires a repairing of template DNA strands, and one must be wound around the other to recreate the helical duplex. A facilitated DNA rotation promoted by RecA protein, coupled to ATP hydrolysis, would provide the energy required to bring about fork regression in a cellular environment where the DNA had limited freedom of movement. An extensive regression reaction would also require the assistance of topoisomerases, but this would be true regardless of the mechanism by which regression occurred.

ATP hydrolysis would also be required to promote the four-strand exchange step in the in vitro reaction, leading to the formation of a Holliday intermediate. In the cell, this intermediate could then be processed by other enzymes such as the RuvABC system (15, 21, 46). ATP hydrolysis is clearly required for the four-strand DNA strand-exchange reactions promoted by RecA protein in vitro (24, 38). The facilitated DNA rotation model provides a mechanism to effect a four-strand exchange (11, 45). No attempt has been made to explain the requirement for ATP hydrolysis in four-strand exchange reactions within the context of the alternative RecA dissociation model.

If RecA protein is to promote fork regression, RecA filaments must be positioned in the DNA gap of a leading strand and maintained there. Some other factor or protein might be needed in the cell to prevent RecA filament disassembly and/or ensure RecA filament nucleation near the branch. Substitution of dATP for ATP seems unlikely to be a significant option in vivo. Complexes of the RecO and RecR proteins have been shown to stabilize the RecA filament in the presence of ATP by preventing a net end-dependent dissociation of RecA monomers (23). The RecF and RecR protein complexes attenuate in vitro extension of RecA filaments formed on gapped DNA molecules beyond the ssDNA gap (47). Modulation of RecA filament dynamics by the RecOR and RecFR complexes is thought to be important in the recombinational repair process by targeting and confining the RecA protein to the ssDNA gap that needs to be repaired
(47). However, the RecO and RecR proteins do not appear to be good candidates for such a regression-stimulating factor, because they had no effect on the fork regression reaction in vitro. Moreover, high concentrations of RecOR proteins inhibited the reaction, probably by binding nonspecifically to DNA and impeding RecA-catalyzed fork regression. When RecF protein was added with RecOR proteins, under conditions where RecOR did not have any effect on RecA-protein reaction, RecF inhibited the reaction (Fig. 6D). This is consistent with recent results of Michel et al., who demonstrated that RecA protein, but not RecO or RecF, is required for certain in vivo reactions that lead up to formation of an intermediate that can be processed by the RuvABC system (46). Although many questions remain about the protein transactions that might take place in replication fork repair, it is now clear that RecA protein can promote the potentially critical fork regression process in at least some situations.

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