

Recombinational DNA Repair: The RecF and RecR Proteins Limit the Extension of RecA Filaments beyond Single-Strand DNA Gaps

Brian L. Webb, Michael M. Cox,* and Ross B. Inman
Department of Biochemistry
University of Wisconsin at Madison
Madison, Wisconsin 53706

Summary

In the presence of both the RecF and RecR proteins, RecA filament extension from a single strand gap into adjoining duplex DNA is attenuated. RecR protein alone has no effect, and RecF protein alone has a reduced activity. The RecFR complexes bind randomly, primarily to the duplex regions of the DNA, and the extension of the RecA filament is halted at the first complex encountered. A very slow lengthening of RecA filaments observed in the presence of RecFR is virtually eliminated when RecF is replaced with an RecF mutant protein that does not hydrolyze ATP. These observations are incorporated into an expanded model for the functions of RecF, RecO, and RecR proteins in the early stages of postreplication DNA repair.

Introduction

When a replication fork is stalled at the site of an unrepaired DNA lesion, the lesion is left in a single-strand gap in the DNA. Recombinational DNA repair (postreplication repair) of such lesions relies upon RecA protein as well as a number of proteins generally assigned to the RecF recombination pathway. A model for postreplication repair originally proposed by Howard-Flanders and colleagues (West et al., 1981) has survived virtually unchanged for over 15 years (Figure 1). RecA protein forms a filament in the exposed DNA gap. The bound ssDNA¹ is then paired with homologous dsDNA from the opposite side of the replication fork. Unidirectional DNA strand exchange ensues, converting the lesion-containing strand into duplex DNA. Recombination is terminated by resolving the DNA crossover, the lesion is repaired, and replication is restarted. This pathway represents a unique confluence of DNA metabolic processes, with major elements of the DNA replication, recombination, and repair enzymatic machinery presumably interacting in a highly regulated sequence. Few of the molecular implications of the pathway of Figure 1 have been explored *in vitro*.

At the center of the proposed postreplication repair pathway is the RecA protein. The activities of RecA have been extensively reviewed (Kowalczykowski and Eggleston, 1994; Kowalczykowski et al., 1994; Kubista et al., 1996; Roca and Cox, 1997). *In vitro*, RecA will bind to ssDNA, pair it with a homologous dsDNA, and promote DNA strand exchange between them. The active form of RecA protein is a nucleoprotein filament. During postreplication repair, formation of this filament

specifically and reliably at the exposed DNA gap can arguably be viewed as the key enabling process.

On gapped DNA, RecA filament formation is nucleated in the single-strand gap. Filament extension proceeds 5' to 3' along the single-stranded DNA (Register and Griffith, 1985; Shan et al., 1997) and continues into the adjoining duplex DNA until the contiguous DNA is entirely enveloped or the available free RecA protein is exhausted. Since nucleation occurs very slowly on dsDNA at neutral pH (Pugh and Cox, 1988), the presence of an ssDNA gap may seem sufficient to assure targeting of RecA filaments to sites where repair is required.

However, there are several problems with this scenario. First, the ssDNA gap may be coated with the single-strand DNA binding-protein (SSB), which greatly inhibits the nucleation step in RecA filament assembly (Kowalczykowski and Krupp, 1987; Shan et al., 1997). Second, RecA filaments undergo an end-dependent disassembly reaction in the presence of SSB, proceeding from the end opposite to that at which RecA monomers are added in filament extension (Lindsley and Cox, 1990a; Shan et al., 1997), a process that could eliminate RecA filaments before they could initiate DNA strand exchange. Third, extension of RecA filaments into the adjoining dsDNA has no apparent limit other than the availability of free RecA protein. These considerations strongly suggest a need for the regulation of RecA filament assembly and disassembly.

Attractive candidates for this regulatory function are the RecF, RecO, and RecR proteins. Identified in three separate laboratories (Horii and Clark, 1973; Kolodner et al., 1985; Mahdi and Lloyd, 1989), these proteins are often discussed as components of a secondary pathway for recombination during conjugation (Smith, 1989a; Clark, 1991; Clark and Sandler, 1994; Kowalczykowski et al., 1994). However, the sensitivity of strains with *recF*, *recO*, and *recR* mutations to DNA damaging agents, even when the mutations are present in an otherwise wild-type background (Horii and Clark, 1973; Sinden and Cole, 1978; Kolodner et al., 1985; Mahdi and Lloyd, 1989; Sargentini and Smith, 1986; Tseng et al., 1994), indicates that these proteins function in a primary pathway for DNA repair. The RecFOR proteins function at an early stage of recombination and recombinational repair (Clark and Sandler, 1994; Kowalczykowski et al., 1994; Roca and Cox, 1997). Recent genetic results suggest an interaction between RecF protein and PriA protein *in vivo* (Sandler, 1996) and implicate RecF and RecR proteins in replication restart at disrupted replication forks (Courcelle et al., 1997).

Individually, the purified RecF, RecO, and RecR proteins have few measurable activities. RecF protein binds to ssDNA (Griffin and Kolodner, 1990) and dsDNA (Madiraju and Clark, 1992; Webb et al., 1995) and exhibits a weak ATPase activity (Webb et al., 1995). RecO protein binds to both ssDNA and dsDNA. RecO also promotes an ATP-independent renaturation of complementary DNA strands and a weak D-loop formation activity (Luisi-DeLuca and Kolodner, 1994; Luisi-DeLuca, 1995). The

*To whom correspondence should be addressed.

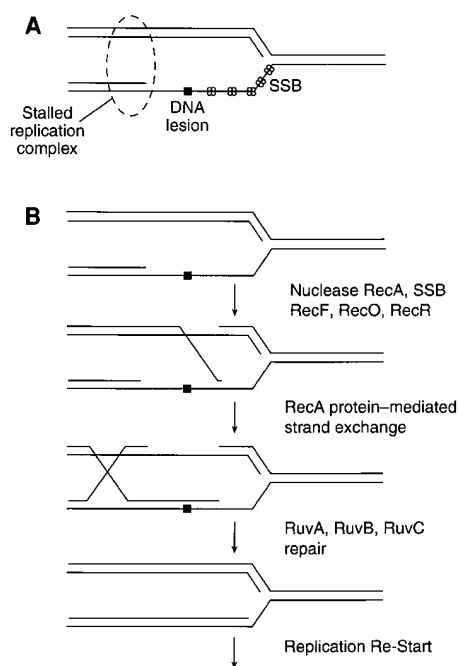


Figure 1. Recombinational DNA Repair at Stalled Replication Forks (A) A DNA lesion left in a single strand gap created by a stalled replication complex. (B) A model for postreplication DNA repair, based on that of West et al. (1981).

RecR protein from *Bacillus subtilis* binds to dsDNA (Alonso et al., 1993), although the *Escherichia coli* protein exhibits no DNA binding activity *in vitro* (Webb et al., 1995). There is clear evidence for interactions between the RecO and RecR proteins (Umezue et al., 1993; Umezue and Kolodner, 1994; Shan et al., 1997) and between the RecF and RecR proteins (Webb et al., 1995).

No positive effects of RecF protein on RecA activities *in vitro* have been reported. At sufficiently high concentrations, RecF protein alone inhibits RecA binding to DNA and RecA protein-mediated DNA strand exchange (Madiraju and Clark, 1991; Umezue et al., 1993). In contrast, RecO protein interacts with SSB, and the RecO and RecR proteins stimulate RecA protein binding to ssDNA coated with SSB (Umezue and Kolodner, 1994). This RecO and RecR complex has the additional effect of preventing the end-dependent disassembly of filaments (Shan et al., 1997). Thus, the RecO and RecR proteins overcome the inhibition of RecA filament nucleation by SSB and the possible premature end-dependent disassembly of those same RecA filaments. In both cases, the RecO and RecR proteins act together, with no effects seen with either protein alone.

The third problem for RecA filament assembly in postreplication repair, unregulated extension of nucleated filaments as alluded to above, is not addressed by these results. In addition, the RecF protein is left without a clear molecular function. In this report, we demonstrate that the RecF and RecR proteins, acting together, modulate the extension of RecA filaments into the dsDNA adjoining a single-strand gap.

Results

Experimental Design

The objective of this study was to determine the effect of the RecF and RecR proteins on the binding of the RecA protein to gapped DNA (gDNA). Reactions were monitored by electron microscopy, and the DNA-dependent dATPase activity of RecA was used as an independent (albeit indirect) method to assess RecA binding to DNA. Throughout, specific circular gDNA molecules are identified by the abbreviation GD, and the lengths of the single-strand gaps in total nucleotide residues are denoted by a subscript (e.g., GD₁₀₃₇).

RecF and RecR proteins interact to form complexes that bind to dsDNA in a random and noncooperative fashion in the presence of ATP (Webb et al., 1995). To facilitate discussion, we use the term RecFR when describing the combined effects of these proteins when both are present. At low RecFR/base pair ratios, these complexes are manifested as discrete dot-like structures distributed along the dsDNA. At higher RecFR/base pair ratios, RecFR forms a uniform coat on the DNA. In successfully prepared EM samples, such RecFR coats appear rather amorphous, while RecA filaments contain striations. The two are thus readily distinguished. The oligomeric state of RecFR complexes is unknown. Here, reported concentrations of RecFR complexes are equivalent to the concentration of RecF protein monomers in an experiment. In all cases, RecR is present in 2-fold excess relative to RecF (e.g., where the RecFR complex concentration is given as 58 nM, the reaction contains 58 nM RecF protein and 116 nM RecR protein).

The experiments focus on the extension of RecA filaments nucleated in a single strand gap. End-dependent disassembly (from the opposite end) is prevented simply by substituting dATP for ATP in the reaction mixtures (Shan et al., 1997) except as noted. The concentration of RecA protein in each experiment is sufficient to bind all DNA present. Hence, when filament extension is attenuated, the reaction mixture contains significant amounts of unbound RecA protein. To stabilize the filaments for procedures such as spreading for the EM, ATP γ S is added to halt the reactions. Although RecA protein binding to DNA can occur in the presence of ATP γ S, recent studies have shown that the effects of ATP γ S' addition on the result obtained are minimal when the procedure described in Experimental Procedures is used (Shan et al., 1997).

RecFR Limits RecA Filament Extension beyond DNA Gaps

In the presence of dATP and SSB, RecA protein forms a contiguous filament on circular GD₁₀₃₇, which envelops the entire DNA molecule (Figure 2A). These nucleoprotein filaments exhibited few imperfections or discontinuities. Typically, greater than 94% of the DNAs were found to be fully coated with RecA. The length of these filaments corresponds to about 50 of the arbitrarily defined length units used in our EM judgments (see Experimental Procedures).

To determine the effect of RecFR proteins on RecA

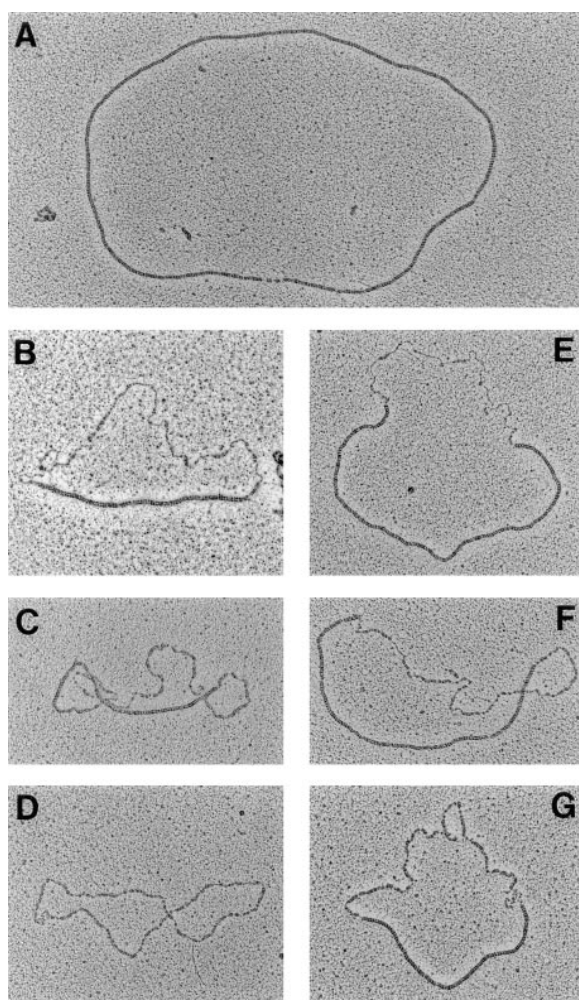


Figure 2. Effect of RecFR on RecA Binding to Gapped DNA

Reactions were carried out as described in Experimental Procedures and contained 3 mM gapped DNA, 0.75 μ M RecA, SSB at a ratio of 1:10 relative to nucleotides within the single-stranded region of the gap (20 nM for GD₁₀₃₇, 76 nM for GD₃₃₂₉), 3 mM dATP, and the indicated concentrations of RecFR complexes. (A–D) Reactions with GD₁₀₃₇: (A) No RecFR, (B) 58 nM RecFR or 300/DNA, (C) 700 RecFR/DNA, (D) 900 RecFR/DNA. (E–G) Reactions with GD₃₃₂₉: (E) 47 nM RecFR or 222/DNA, (F) 521 RecFR/DNA, (G) 670 RecFR/DNA. The DNA regions bound with RecA protein (e.g., [A]) are relatively thick, stiff, and feature discernible striations. DNA regions with bound RecFR complexes (e.g., [D]) feature a more amorphous coating with irregular blobs. As the concentration of RecFR increases, the observed lengths of RecA filaments decrease. The molecules shown in the micrographs were selected as representative of their respective sample on the basis of the length of the RecA filament corresponding to the peak length of that sample's histogram (Figure 3). RecA protein greatly extends the DNA to which it is bound; hence, a filament that binds to only part of the DNA circle appears to occupy a greater fraction of the total length of the DNA than it actually does. Consequently, the overall contour length of the protein–DNA complexes decreases as the RecA filament length decreases.

binding to gDNA, GD₁₀₃₇ was preincubated with various concentrations of RecFR in the presence of dATP. At 5 min intervals, RecA protein was added, and then SSB. The reaction was then incubated an additional 5 min before stopping the reaction with ATP γ S. Typical filaments observed by electron microscopy are presented

in Figures 2B–2D. Histograms detailing the results of filament length judgments are presented in Figure 3. The addition of relatively small amounts of RecFR (19 nM, or 100 per gDNA molecule) resulted in RecA filaments that were stopped short of completely coating the gDNA (Figure 3A). On a given gDNA molecule, the typical protein–DNA complex consisted of single linear RecA filament (of variable length) connected by naked dsDNA. At this low RecFR concentration, only a small number of RecFR dots were seen on the dsDNA. At somewhat higher levels of RecFR (300 per gDNA molecule, Figures 2B and 3C), the distribution of RecA filament lengths became considerably sharper, with an average of 8–12 relative length units. The length of filament expected if RecA binding was limited to the single-stranded region of GD₁₀₃₇ is approximately 6 units on this arbitrary unit scale. At this higher concentration of RecFR, the probability of a RecFR complex randomly binding to the dsDNA near the ss–ds junction of the gap is greater, increasing the likelihood that RecA filament extension would be halted near the ss–ds junction. Less than 4% of the circular DNA molecules in these samples had multiple RecA filament tracts.

At even higher RecFR concentrations (700 per gDNA molecule), the double-stranded region of the gDNA began to take on the appearance of DNA coated with RecFR (Figure 2C). The judged lengths of the RecA filaments did not change significantly, though they became even more homogeneous in length (Figure 3D). With 900 RecFR per gapped DNA molecule (174 nM RecFR), the RecFR complexes began to inhibit RecA protein binding to the single-strand gaps. About 60% of the GD₁₀₃₇ molecules had no bound RecA filament at all (Figure 2D), and the DNA that did have RecA bound had very short filaments (between 2 and 6 length units) (Figure 3E).

To substantiate the relationship between gap length and RecA filament length in the presence of RecFR, the same set of experiments was carried out with GD₃₃₂₉. The histograms for this series of experiments is shown in Figures 3F–3J. As with GD₁₀₃₇, the increase in RecFR concentration resulted in a progression from a situation where filaments were halted at random sites within the dsDNA portion of the molecule to one where there was a more uniform distribution of filament lengths close to the length of the longer single-strand gap (approximately 20 U) (Figures 2E–2G). Both sets of experiments in Figures 2 and 3 are consistent with RecA filaments being attenuated when they encounter a RecFR complex bound at random locations in the dsDNA. At low RecFR concentrations, the first RecFR complex encountered by the growing RecA filament is usually beyond the gap. At sufficiently high RecFR concentrations, RecFR inhibits RecA binding even in the single-stranded region.

As direct evidence that the RecA filaments were halted along the DNA as a result of a physical encounter with a bound RecFR complex, an immunogold EM-labeling procedure was used to determine the location of RecF and RecR in relationship to the RecA filament. Figure 4 shows representative RecA filaments halted on GD₁₀₃₇ by RecFR (300/DNA), which were then probed with either RecF antibody or RecR antibody complexed with gold particles. We assume the immunogold-labeling procedure is not 100% efficient. Nevertheless, gold label was

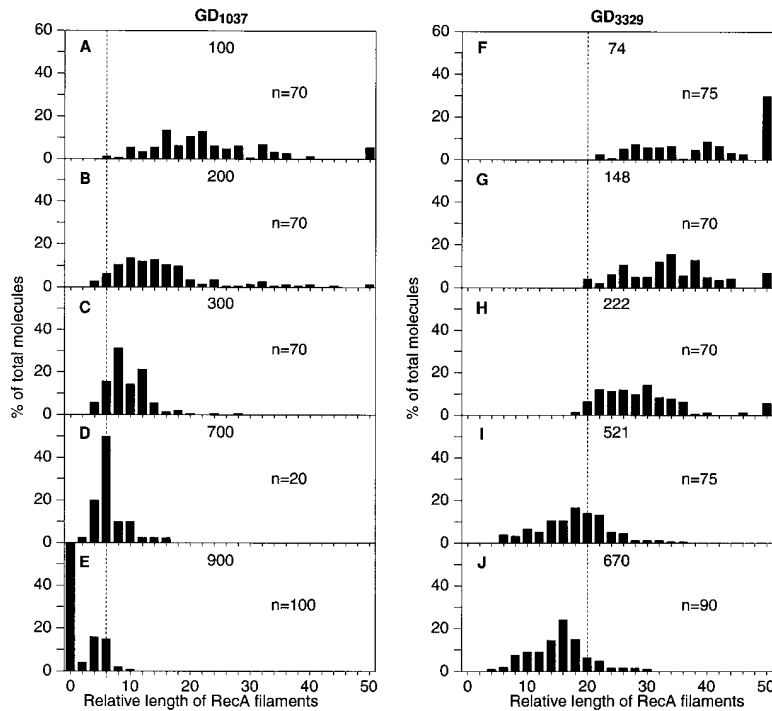


Figure 3. Histograms Showing the Distribution of RecA Filament Lengths on Gapped DNA in the Presence of Various Concentrations of RecFR

The reactions were performed as described in Figure 2, and after spreading for microscopy, the RecA filament lengths were judged and reported in arbitrary length units as described in Experimental Procedures. A DNA molecule completely coated with RecA protein would have a filament length of approximately 50 length units in these histograms. Reactions with GD₁₀₃₇ are shown in (A)–(E): (A) 19 nM RecFR or 100/DNA, (B) 200 RecFR/DNA, (C) 300 RecFR/DNA, (D) 700/DNA, (E) 900 RecFR/DNA. Parallel reactions with GD₃₃₂₉ are shown in (F)–(J): (F) 15 nM RecFR or 74/DNA, (G) 148 RecFR/DNA, (H) 222 RecFR/DNA, (I) 521 RecFR/DNA, (J) 670 RecFR/DNA. The number of RecA filaments judged to generate a histogram (n) is indicated in each panel. Vertical dotted lines represent the filament length expected if RecA bound only the single-stranded region of the gDNA.

observed along the dsDNA when either antibody was used. This demonstrates that the dots observed along the non-RecA-coated regions shown in Figure 2 contain both RecF and RecR proteins. Additionally, in duplicate experiments probed with either RecF antibody or RecR antibody, we found 66% and 60% of the molecules, respectively, to have a gold label present at one end of the RecA filaments. The average number of gold particles seen per DNA molecule in these experiments was 7 and 9 for RecF and RecR, respectively. If there were no relationship between the position of the RecFR complexes and the end of the RecA filaments, and random binding to the duplex DNA was assumed, a gold particle should appear at the end of a RecA filament only 10 or 13% of the time for RecF and RecR, respectively. Since a gold particle appears at the end of the RecA filament at 5–6 times this frequency, these results are consistent with the termination of RecA filaments at a position determined by the prior random binding of RecFR to dsDNA.

To determine if RecA filament extension could displace RecFR from the dsDNA, a time course was carried out using the GD₁₀₃₇ substrate and 300 RecFR complexes per gDNA molecule. A portion of the reaction was stopped by ATP γ S at 10 and 65 min after the addition of RecA, and the lengths of the RecA filaments were judged. Slow increases in the length of the RecA filaments were observed (data not shown). This suggests that bound RecFR is quite stable, but given sufficient time it is eventually displaced by RecA.

To confirm the EM results, RecA's DNA-dependent dATPase activity was used as an indirect measure of RecA binding to gDNA in the presence of RecFR. The results indicated that RecFR (300/DNA) significantly inhibited the RecA ATPase activity (Figure 5A). However,

the rate of dATP hydrolysis increased slowly with time, consistent with the slow increase in filament length observed in the EM. Secondary filament nucleation events to produce separate filaments on a single DNA molecule could, in principle, explain the increase in dATPase activity but were generally not evident in the EM.

One explanation for the apparent bypass of RecFR could be a slow hydrolysis of dATP by the RecF protein, leading to the dissociation of the RecFR complexes. Therefore, similar experiments were carried out combining RecR with the RecF K36R mutant protein, which binds to, but does not hydrolyze, dATP (Webb et al., 1995; B. L. W., unpublished data). As shown in Figure 5, the use of this mutant protein substantially reduced the observed increase in RecA-mediated dATP hydrolysis. In the EM, the length of the RecA filaments was essentially unchanged in the interval between 10 and 65 min when RecF K36R replaced RecF protein (data not shown). Hence, the capacity of RecFR complexes to block RecA filament extension is enhanced if RecF protein cannot hydrolyze dATP.

To determine if RecF and RecR are both required to halt RecA filament extension on gDNA, RecF and RecR were each tested alone for this activity. RecR protein alone had no detectable effect on RecA binding to GD₁₀₃₇. Complete RecA filaments were observed on GD₁₀₃₇ when preincubated with RecR at concentrations ranging from 38 to 350 nM (200–1800 RecR/DNA; data not shown). RecF protein alone produced some truncation of RecA filament extension on gDNA, but the effect was much reduced relative to that observed with RecF and RecR together. For example, with 58 nM RecF protein (300 RecFs per GD₁₀₃₇), 80% of the GD₁₀₃₇ was completely coated with RecA, and many of the remaining 20% had only small regions not coated by RecA. In the presence of RecR protein, this concentration of RecF

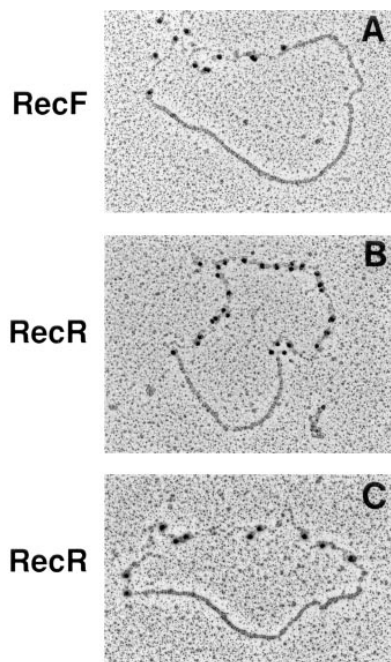


Figure 4. Localization of RecF and RecR with Respect to the RecA Filament on GD₁₀₃₇ Using an Immunoaffinity Gold-Labeling Procedure

The reaction was carried out as described in Experimental Procedures, using 3 μM GD₁₀₃₇, 58 nM RecFR or 300/DNA, 0.75 μM RecA, 76 nM SSB, and 3 mM dATP. Grids of the same sample were probed with either RecF antibody or RecR antibody as indicated. Gold particles complexed with the antibodies appear as small dense circles.

produces much shorter filaments (Figure 3C). A 3-fold increase in RecF protein (174 nM) left virtually all RecA filaments with at least one-third of their normal length, and 7% of the filaments were still full-length. In the presence of RecR protein, the same concentration of RecF precludes RecA protein binding to a majority of the gDNA molecules (Figure 3E).

A recent report (Hegde et al., 1996) indicates that in the presence of ATP γ S, RecF protein has a higher affinity for gDNA than for either dsDNA or ssDNA, implying it might bind tightly to the ss-ds junction. Our experiments provide no indication that RecFR complexes bind specifically to the ss-ds junction. Further experiments carried out with RecF protein alone in the presence of ATP γ S also produced no evidence for preferential binding to gap junctions (B. L. W., unpublished data).

RecFR Complexes Primarily Affect RecA Filament Extension on dsDNA

The apparent limitation of RecA filament extension on gDNA by RecFR complexes bound primarily to dsDNA regions (Figures 2–5) could be explained by preferential binding of RecFR to the dsDNA regions of the gDNA (leaving the ssDNA relatively free for RecA binding), by an increased capacity of RecA to displace RecFR bound to ssDNA, or by a capacity of RecA protein to form short filaments between RecFR complexes on ssDNA but not dsDNA. We therefore explored the effects of RecFR

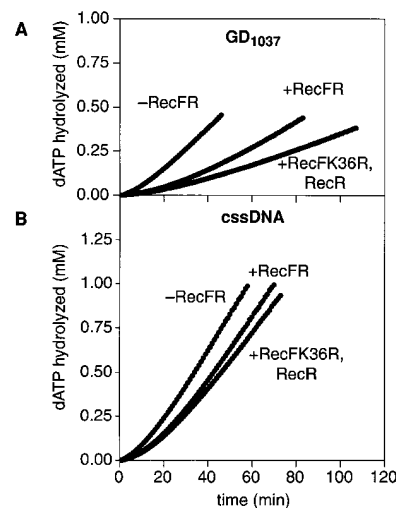


Figure 5. Effect of RecF and RecR on the dATPase Activity of RecA
Reactions were carried out as described in Experimental Procedures. Hydrolysis of dATP by RecA was monitored by the coupled spectrophotometric assay.

(A) Experiments with gapped DNA. Reactions contained 3 μM GD₁₀₃₇, 0.75 μM RecA, 20 nM SSB, and 3 mM dATP. GD₁₀₃₇ was preincubated with either no RecF and RecR, 300 RecFR complexes per DNA, or 300 RecF K36R /RecR complexes per DNA, as indicated, prior to RecA/SSB addition. The final dATPase rates of the reactions were 12.1, 7.63, and 4.78 $\mu\text{M min}^{-1}$, respectively.

(B) Experiments with circular ssDNA. Reactions contained 1.5 μM M13mp8.1037 circular ssDNA, 0.75 μM RecA, 0.15 μM SSB, 3 mM dATP, and 300 RecFR complexes (with wild-type or mutant K36R RecF protein as indicated) per ssDNA molecule. Final dATPase rates of the reactions from left to right were 20.3, 18.3, and 16.3 $\mu\text{M min}^{-1}$.

complexes on RecA filament extension on an ssDNA substrate, and the results were compared to those obtained on the gDNA. Identical concentrations of RecFR complexes had demonstrably reduced effects on RecA filament formation when ssDNA replaced gDNA as the DNA cofactor (Figure 5B). Increasing the concentration of RecFR complexes increased the lag times observed for the RecA ATPase activity but had only modest effects on the final rates observed (data not shown), suggesting that nearly complete RecA filament formed over the course of the experiment. Replacing RecF with the RecF K36R mutant protein in these experiments produces only a modest increase in the effects of the RecFR complexes on ssDNA (Figure 5B).

If RecA filaments could lengthen by independent nucleation on ssDNA between bound RecFR complexes, multiple RecA filaments should be evident in the electron microscope on individual ssDNA molecules prebound with RecFR. With 300 RecFR complexes per DNA molecule and at both 5 and 30 min time points, the majority of DNA molecules had a single RecA filament bound, typically nearly full-length with a single short structural irregularity we attributed to a region of bound RecFR (data not shown). At 600 RecFR per DNA molecule, the RecA filaments were variable in length but shorter. However, of 162 protein–DNA complexes examined at the 5 and 30 min time points, 129 (nearly 80%) had only a single RecA filament bound. Also, the immunogold-labeling procedure generally did not detect RecF or

RecR in the RecA-bound regions of gapped DNA (Figure 4). Overall, the results suggest that RecA protein usually did not bind to ssDNA gaps by filling in around bound RecFR complexes. Instead, RecA may have a greater capacity to displace RecFR complexes on ssDNA than on the duplex portion of gDNA, or the RecFR may bind preferentially to the duplex regions of gDNA.

RecFR Complexes Do Not Prevent End-Dependent Disassembly of RecA Filaments

The RecA filaments formed on gDNA in the presence of RecFR do not envelop the entire DNA molecule and thus possess a disassembly-competent filament end within the single-stranded DNA in the gap. We carried out a series of experiments with ATP rather than dATP to determine the effects of end-dependent filament disassembly on gDNA in the presence of RecFR. As was the case for linear ssDNA (Shan et al., 1997), we found that SSB displaces RecFR-capped RecA filaments on gDNA over time in the presence of ATP. To quantitate this displacement process, we used electron microscopy and counted the number of DNA molecules that had no RecA filament associated with them as a function of time. GD₃₃₂₉ was preincubated with RecFR (77 nM or 367/DNA). RecA protein and SSB were then added in succession, 5 min apart. The number of DNA molecules having no RecA filament associated with them increased from 2% 3 min after SSB addition to 56% after 60 min. Similar results were seen with GD₁₀₃₇. The presence of SSB on the RecA-free gDNA was verified by immunogold labeling of the SSB (data not shown). The RecFR-attenuated RecA filaments did not undergo significant disassembly and were not replaced with SSB when dATP was used in the reaction instead of ATP (Figures 2–5 and data not shown).

Attenuated RecA Filaments Promote DNA Pairing

DNA strand exchange experiments were carried out between GD₃₃₂₉ and a complementary linear duplex DNA (Figure 6). DNA strand exchange products were seen in the absence of RecFR. Addition of RecFR at a level of 222 RecFR complexes per gDNA molecule allowed DNA pairing but decreased the level of pairing intermediates observed and eliminated product formation. Relatively high levels of DNA pairing intermediates (but not products) were restored when RecF K36R replaced RecF protein in this experiment. These experiments indicate that RecA filaments attenuated by RecFR will promote DNA pairing reactions, but that strand exchange probably does not extend beyond the filament. Parameters that might affect DNA pairing by attenuated filaments have not yet been investigated in detail.

Discussion

Our primary conclusion is that the RecF and RecR proteins, acting together, bind randomly to dsDNA and readily halt RecA filament extension. After nucleation in the single-stranded region of a gapped DNA molecule, the extension of RecA filaments into the adjoining duplex DNA is thereby limited by RecFR in vitro. This work provides a potential molecular function for RecF protein,

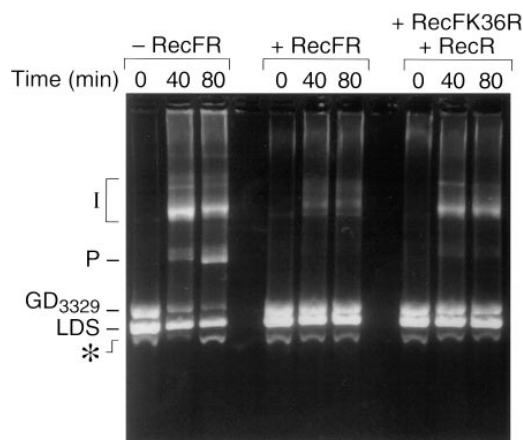


Figure 6. DNA Pairing Promoted by Attenuated RecA Filaments

Reactions were identical to those in Figure 5, except that GD₃₃₂₉ replaced GD₁₀₃₇, the creatine phosphokinase/phosphocreatine dATP regeneration system was used, and linear M13mp8.1037 (cleaved with NcoI) was added to the reaction 5 min after the RecA and SSB to initiate DNA pairing. RecFR or RecF K36R/RecR was added where indicated at a level of 222 FR complexes per gDNA molecule. Symbols are: I, DNA pairing intermediates; P, the nicked duplex product of a complete DNA strand exchange; GD₃₃₂₉, the gDNA substrate; LDS, linear duplex DNA substrate; asterisk, broken gapped DNA molecules (<10%) present in the gDNA preparation. The linear product of DNA strand exchange also migrates at the asterisk position.

as well as a function for the RecFR complexes characterized in an earlier study (Webb et al., 1995). We observe no specificity for RecFR or RecF protein binding to the ss-ds junction in gapped DNA under any conditions tested to date.

The importance of recombinational DNA repair has recently been highlighted by the discovery that two genes associated with an inherited predisposition to breast cancer, *BRCA1* and *BRCA2*, function through an interaction with the eukaryotic RecA homolog Rad51 (Scully et al., 1997; Sharan et al., 1997). Targeted disruption of Rad51 in mouse produces an embryonic lethal phenotype (Lim and Hasty, 1996; Tsuzuki et al., 1996). Targeted mutation of either *BRCA1* or *BRCA2* also results in a lethal phenotype (Ludwig et al., 1997; Suzuki et al., 1997). Interactions of Rad51 with the p53 (Lim and Hasty, 1996; Sturzbecher et al., 1996), Dmc1 (Bishop, 1994), Rad52 (Shen et al., 1996), Rad54 (Jiang et al., 1996), Rad55 (Sung, 1997), and Rad57 (Sung, 1997) proteins have also been reported. It is becoming clear that the function of Rad51 in eukaryotic recombination and recombinational DNA repair is modulated by a wide range of other proteins. The molecular function of most of these potential auxiliary or complementary functions is obscure. However, the Rad55 and Rad57 proteins exhibit activities that suggest they are functional homologs of the bacterial RecO and RecR proteins (Sung, 1997).

The RecF, RecO, and RecR proteins are likely to provide useful paradigms for elucidating the functions of eukaryotic proteins that interact with Rad51. In vitro studies to date indicate that the RecF, O, and R proteins have an important function in modulating the assembly and disassembly of RecA filaments (Umezumi and Kolodner, 1994; Shan et al., 1997; this work). However, this

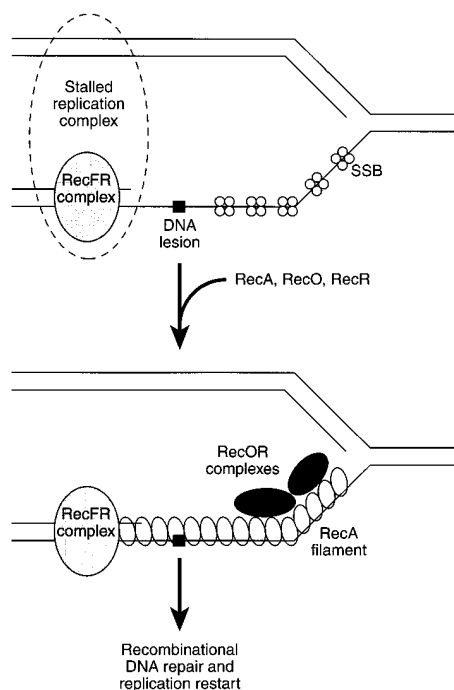


Figure 7. Model for the Early Stages of Postreplication DNA Repair. Important features are described in the text. A replication complex is represented stalled at a lesion on the leading strand, with completed Okazaki fragments shown on the lagging strand for simplicity. RecA filament formation is nucleated and stabilized with the aid of RecOR complexes, and RecA filament extension is attenuated by a RecFR complex positioned by the replication complex. No assumptions are made about the timing of an RecFR complex interaction with the replication complex, which proteins within the replication complex the interaction might occur with, or other functions the interaction might have.

is unlikely to be the only role of these proteins in a bacterial cell. Work *in vivo* suggests that at least the RecF and RecR proteins have an additional role in modulating the interface between recombination and replication in recombinational DNA repair (Sandler, 1996; Courcelle et al., 1997; Kogoma, 1997).

Random binding of a limited number of RecFR complexes to the 4.6 million base pair *E. coli* genome is likely to be of little use to recombinational DNA repair. The absence of a measurable specificity for the binding of ss-ds junctions at DNA gaps seems to leave no way to target the RecFR complexes to the locations where they are needed. However, the apparent interaction of RecF protein with the replication apparatus, as defined *in vivo* (Sandler, 1996; Courcelle et al., 1997), suggests that protein-protein interactions may provide the mechanism for the targeting of RecFR to regions requiring repair. This is outlined in the model for the early stages of postreplication DNA repair in Figure 7. The replication complex is first halted when it encounters an unrepaired DNA lesion (shown here on the leading strand). The DNA gap on the other side of the lesion is coated with SSB. RecA protein nucleates filament formation in this gap with the aid of the RecO and RecR proteins (Umezumi and Kolodner, 1994). The RecOR complexes prevent end-dependent disassembly of the RecA filament (Shan et

al., 1997). In this scenario, we suppose that the stalled replication complex also deposits an RecFR complex on the side of the DNA lesion distal to the growing end of the RecA filament, eventually halting its extension and thus conserving RecA protein. In this location, the RecFR complexes may also function in the disassembly of the replication complex, and/or its reassembly either downstream or in the same location after recombination and repair is complete. Either function of RecFR could, in principle, explain the requirement of RecF and RecR in replication restart after DNA damage (Courcelle et al., 1997).

A number of genetic studies have suggested that the RecF, O, and R proteins act at the same stage of recombinational processes, perhaps as a single complex (Smith, 1989b; Wang et al., 1993; Sawitzke and Stahl, 1994). Our *in vitro* studies to date and those of Kolodner and colleagues (Umezumi and Kolodner, 1994), along with recent *in vivo* work (Courcelle et al., 1997), have instead focused us on RecFR and RecOR as alternative protein pairings with potentially complementary rather than cooperative functions. No positive effects of RecF protein have been observed in the activity of RecOR to stimulate the nucleation of RecA filament formation and prevent end-dependent disassembly of those filaments (Umezumi and Kolodner, 1994; Shan et al., 1997). Similarly, we have found no evidence for a contribution of RecO protein in the attenuation of RecA filament extension (B. L. W., unpublished data). These functional pairings are reflected in the model of Figure 7.

Experimental Procedures

Enzymes and Reagents

E. coli RecF and RecF K36R (Webb et al., 1995), RecR (Webb et al., 1995), RecA (Shan et al., 1996), and SSB (Lohman et al., 1986) proteins were purified as described. The concentration of each protein was determined by absorbance at 280 nm using their extinction coefficients: $\epsilon_{280} = 3.87 \times 10^4 \text{ M}^{-1}\text{cm}^{-1}$ for RecF and RecF K36R (B. L. W., unpublished data), $\epsilon_{280} = 5.6 \times 10^3 \text{ M}^{-1}\text{cm}^{-1}$ for RecR (Shan et al., 1997), $\epsilon_{280} = 2.23 \times 10^4 \text{ M}^{-1}\text{cm}^{-1}$ for RecA (Craig and Roberts, 1981), and $\epsilon_{280} = 2.83 \times 10^4 \text{ M}^{-1}\text{cm}^{-1}$ for SSB (Lohman and Overman, 1985). RecF and RecR polyclonal antibodies were produced in rabbit using the purified proteins as antigens. SSB antibody was a gift from Tim Lohman. Restriction endonucleases were purchased from New England Biolabs. Tris buffer was from Fisher Scientific. ATP, dATP, phosphocreatine, and creatine phosphokinase were from Sigma. ATP γ S was purchased from Boehringer-Mannheim Biochemicals. SeaPlaque GTG low-melting agarose was from FMC.

DNA

Supercoiled circular duplex DNA and circular ssDNA from bacteriophages M13mp8 and M13mp8.1037 were prepared as described (Messing, 1983; Neuendorf and Cox, 1986). Bacteriophage M13mp8.1037 is derived from M13mp8, having a 1037 bp insertion (EcoRV-EcoRV fragment from the *E. coli galT* gene) in the SmaI site (Lindsley and Cox, 1990b). Linear dsDNA was prepared by complete digestion of supercoiled M13mp8.1037 by PstI. ϕ X174 FII DNA was purchased from New England Biolabs. The concentrations of dsDNA and ssDNA stock solutions were measured by absorbance at 260 nm, using 50 and 36 $\mu\text{g ml}^{-1} A_{260-1}$, respectively, as the conversion factors. The conversion factors used to calculate the concentrations of gapped DNA were based on the fractions of ssDNA and dsDNA in the gapped DNA molecules. DNA concentrations are expressed in terms of total nucleotides.

Gapped DNA with a precisely defined gap length was prepared essentially as described (Lindsley and Cox, 1989). Gapped DNA

having a 1037-base single-stranded gap (GD₁₀₃₇) was prepared by annealing NaOH-denatured M13mp8 linearized by SmaI digestion to M13mp8.1037 circular ssDNA. GD₃₃₂₉ was prepared by annealing the 4937 bp fragment of a SmaI-BspHI digest of M13mp8 to M13mp8.1037 circular ssDNA. Thus, GD₁₀₃₇ and GD₃₃₂₉ have a common 8266-base M13mp8.1037 inner circle and have different length linear DNA annealed to it (7229 and 4937, respectively). As an additional purification step, to remove contaminating M13mp8.1037 circular ssDNA and linear M13mp8 dsDNA after hydroxylapatite chromatography, the gapped DNA was electrophoresed on a 0.8% SeaPlaque GTG agarose gel, and the DNA band corresponding to GD₁₀₃₇ or GD₃₃₂₉ was excised. The gel slice was melted at 65°C and extracted 1:1 with phenol/chloroform/isoamyl alcohol (25:24:1) and chloroform/isoamyl alcohol (24:1). The final preparation of DNA was precipitated twice with ethanol.

Electron Microscopy

Reactions were performed in 25 mM Tris acetate (80% cation), 10 mM Mg(OAc)₂, 5% (w/v) glycerol, 3 mM potassium glutamate, 1 mM dithiothreitol. ATP or dATP at 3 mM was included as indicated. An ATP/dATP regenerating system (10 U ml⁻¹ creatine phosphokinase and 12 mM phosphocreatine) was also included. All incubations prior to spreading for microscopy were done at 37°C. The final pH after addition of all reaction components (substituting storage buffers for DNA and protein additions) was 7.15 at 25°C.

RecF and RecR proteins, at concentrations specified in the figure legends, were first incubated with gapped DNA (2.8 or 3 μM as indicated in figure legends) for 5 min. RecA protein (0.75 μM) and SSB (20 nM for GD₁₀₃₇, 76 nM for GD₃₃₂₉) were then added, separated by 5 min. The reaction was stopped 5 min after SSB addition by adding ATP-γS to 1 mM and incubating for an additional 5 min. Prior to spreading for microscopy by a modified adsorption procedure (Webb et al., 1995), the reaction was diluted 20-fold into 50 mM ammonium acetate (pH 7.5) containing 10% glycerol. The diluted reaction mixture was absorbed onto carbon films on copper grids for 3 min. Grids were washed by floating on two 1 ml drops of 10 mM HEPES (pH 7.5), 50 mM ammonium acetate, and 10% (w/v) glycerol. The grids were stained with uranyl acetate, washed with water, and dried under a heating lamp 10 min prior to shadowing.

Because of the large number of samples and the wide distribution of filament lengths within each sample, detailed length measurements were impractical, and we have judged rather than measured lengths. Filament length was judged by comparison with a small scratch on the EM screen, and reported lengths are given in arbitrary units, which are multiples of this reference length. At the magnification used, a "scratch unit" corresponds to approximately 80 nm. In the description given below, all judged lengths are the average of two judgments on each filament. Length distributions in the 10 experiments shown in Figure 3 are plotted as histograms in which the bin size is meant to represent roughly the reproducibility of judgments on single filaments. Judgment errors were a function of filament length: for filaments of 10 units, the repeatability was not more than ±2 units; filaments shorter than this were more accurately judged, but judgments became progressively less accurate for longer filaments. The data shown in Figure 3 was accumulated in a way designed to minimize judgment bias. First, each sample was judged in a preliminary survey, which involved a total of 205 judgments on the 10 samples (each of the 205 being the average of two repeated judgments). Next, samples were labeled with a nondescriptive identifier by BLW, put in a random order, and judged blind by RBI in 505 further judgments (again, each is based on the average of two estimates). Both sets of data compared well and were pooled to yield the histograms shown in Figure 3. The one exception to the above was with the data in histogram D, which comprised data from the initial survey only; unfortunately, the grid was mislaid before blind judgments could be made. Apart from the latter accident, no data was omitted from the histograms shown in Figure 3.

In a previous report (Jain et al., 1994) we also used judgments to obtain data on samples with wide length distributions. In this case a subset of the molecules were also actually measured and a comparison with the judgment data showed no significant difference when compared at the level of accuracy of the judgment data.

Immunoaffinity Gold-Labeling Procedure

To verify the location of specific proteins on the DNA, a gold-labeling procedure was performed on reaction mixtures already deposited and dried on activated carbon films. Reactions were stopped by the addition of ATP-γS as described above. Before spreading, the samples were dialyzed 1 hr at 4°C into 10 mM HEPES (pH 7.5), 20 mM NaCl, 5 mM EDTA. The dialyzed samples were then cross-linked with 0.2% glutaraldehyde 20 min at 25°C followed by 20 min on ice. To remove unreacted glutaraldehyde, the samples were again dialyzed. The samples were then diluted 10-fold into a solution containing 10 mM HEPES (pH 7.5), 50 mM ammonium acetate, 10% (w/v) glycerol and absorbed to an activated carbon grid. These grids were floated and incubated successively on the following solutions, all at room temperature: 0.1% BSA (10 min), primary antibody (anti-RecF, RecR, or SSB; 20 min); 0.1% BSA (3 min); protein A conjugated with gold (10 nm, Sigma Co.; 20 min); 0.1% BSA (3 min); water (1 min). Grids were then stained with uranyl acetate and shadowed. Additional experimental details can be obtained from R. B. I. upon request.

To calculate the probability that a gold particle would appear randomly at the very end of an RecA filament, we first estimated the diameter of a gold particle to be 0.2 scratch units. This is equivalent to about 16 nm. The gold particles used are 10 nm prior to shadowing. At 300 RecFR per DNA, the RecA filaments average 9.5 scratch units in length (full-length = 50). Since the RecA-bound DNA is extended, the RecA-free DNA should be 40.5/1.5 scratch units or 27 scratch units in length, providing 135 intervals of 0.2 scratch unit. The probability is then the average number of gold particles per molecule divided by 135. This was multiplied by 2 to account for both filament ends (we cannot distinguish the growing end in these experiments).

dATPase Assays

The DNA-dependent dATP hydrolysis activity of RecA protein was measured spectrophotometrically by a coupled enzyme assay described elsewhere (Morrical et al., 1986; Lindsley and Cox, 1990a; Shan et al., 1997). The reaction conditions were identical to the reactions conditions used for EM except that pyruvate kinase (4.5 U ml⁻¹) and phosphoenolpyruvate (1.5 mM) were used as the dATP regenerating system, and NADH (0.675 mM) and lactate dehydrogenase (4.5 U ml⁻¹) were included. The contribution of the very weak RecF dATPase (Webb et al., 1995) to the dATPase signal generated by RecA protein was insignificant. Further, experiments in which RecF was combined with the mutant RecA K72R (Rehrauer and Kowalczykowski, 1993; Shan et al., 1996) did not produce a detectable increase in the RecF dATPase (B. L. W., unpublished data), suggesting that the RecA protein does not stimulate the RecF dATPase.

Acknowledgments

This work was supported by grants from the National Institutes of Health, GM14711 (to R. B. I.) and GM52725 (to M. M. C.). B. L. W. was supported in part by National Institutes of Health predoctoral training grant GM07215. We dedicate this report to Tokio Kogoma, in memoriam.

Received June 30, 1997; revised September 26, 1997.

References

- Alonso, J.C., Stiege, A.C., Dobrinski, B., and Lurz, R. (1993). Purification and properties of the RecR protein from *Bacillus subtilis* 168. *J. Biol. Chem.* 268, 1424–1429.
- Bishop, D.K. (1994). RecA homologs Dmc1 and Rad51 interact to form multiple nuclear complexes prior to meiotic chromosome synapsis. *Cell* 79, 1081–1092.
- Clark, A.J. (1991). *rec* genes and homologous recombination proteins in *Escherichia coli*. *Biochimie* 73, 523–532.
- Clark, A.J., and Sandler, S.J. (1994). Homologous genetic recombination: the pieces begin to fall into place. *Crit. Rev. Microbiol.* 20, 125–142.

- Courcelle, J., Carswell-Crumpton, C., and Hanawalt, P. (1997). *recF* and *recR* are required for the resumption of replication at DNA replication forks in *Escherichia coli*. *Proc. Natl. Acad. Sci. USA*. *94*, 3714–3719.
- Craig, N.L., and Roberts, J.W. (1981). Function of nucleoside triphosphate and polynucleotide in *Escherichia coli* recA protein-directed cleavage of phage lambda repressor. *J. Biol. Chem.* *256*, 8039–8044.
- Griffin, T.T., and Kolodner, R.D. (1990). Purification and preliminary characterization of the *Escherichia coli* K-12 recF protein. *J. Bacteriol.* *172*, 6291–6299.
- Hegde, S.P., Rajagopalan, M., and Madiraju, M.V. (1996). Preferential binding of *Escherichia coli* RecF protein to gapped DNA in the presence of adenosine (γ -thio) triphosphate. *J. Bacteriol.* *178*, 184–190.
- Horii, Z., and Clark, A.J. (1973). Genetic analysis of the RecF pathway to genetic recombination in *Escherichia coli* K12: isolation and characterization of mutants. *J. Mol. Biol.* *80*, 327–344.
- Jain, S.K., Cox, M.M., and Inman, R.B. (1994). On the role of ATP hydrolysis in RecA protein-mediated DNA strand exchange III. unidirectional branch migration and extensive hybrid DNA formation. *J. Biol. Chem.* *269*, 20653–20661.
- Jiang, H., Xie, Y., Houston, P., Stemke, H.K., Mortensen, U.H., Rothstein, R., and Kodadek, T. (1996). Direct association between the yeast Rad51 and Rad54 recombination proteins. *J. Biol. Chem.* *271*, 33181–33186.
- Kogoma, T. (1997). Is RecF a DNA replication protein? *Proc. Natl. Acad. Sci. USA* *94*, 3483–3484.
- Kolodner, R., Fishel, R.A., and Howard, M. (1985). Genetic recombination of bacterial plasmid DNA: effect of RecF pathway mutations on plasmid recombination in *Escherichia coli*. *J. Bact.* *163*, 1060–1066.
- Kowalczykowski, S.C., and Krupp, R.A. (1987). Effects of *Escherichia coli* SSB protein on the single-stranded DNA-dependent ATPase activity of *Escherichia coli* RecA protein. Evidence that SSB protein facilitates the binding of RecA protein to regions of secondary structure within single-stranded DNA. *J. Mol. Biol.* *193*, 97–113.
- Kowalczykowski, S.C., and Eggleston, A.K. (1994). Homologous pairing and DNA strand-exchange proteins. *Annu. Rev. Biochem.* *63*, 991–1043.
- Kowalczykowski, S.C., Dixon, D.A., Eggleston, A.K., Lauder, S.D., and Rehauer, W.M. (1994). Biochemistry of homologous recombination in *Escherichia coli*. *Microbiol. Rev.* *58*, 401–465.
- Kubista, M., Simonson, T., Sjöback, R., Widlund, H., and Johansson, A. (1996). Towards an understanding of the mechanism of DNA strand exchange promoted by RecA protein. In *Biological Structure and Function: Proceedings of the Ninth Conversation*, the State University of New York, R.H. Sarma and M.H. Sarma, eds. (New York: Adenine Press), pp. 49–59.
- Lim, D.S., and Hasty, P. (1996). A mutation in mouse rad51 results in an early embryonic lethal that is suppressed by a mutation in p53. *Molec. Cell. Biol.* *16*, 7133–7143.
- Lindsley, J.E., and Cox, M.M. (1989). Dissociation pathway for RecA nucleoprotein filaments formed on linear duplex DNA. *J. Mol. Biol.* *205*, 695–711.
- Lindsley, J.E., and Cox, M.M. (1990a). Assembly and disassembly of RecA protein filaments occurs at opposite filament ends: relationship to DNA strand exchange. *J. Biol. Chem.* *265*, 9043–9054.
- Lindsley, J.E., and Cox, M.M. (1990b). On RecA protein-mediated homologous alignment of 2 DNA molecules—3 strands versus 4 strands. *J. Biol. Chem.* *265*, 10164–10171.
- Lohman, T.M., and Overman, L.B. (1985). Two binding modes in *Escherichia coli* single-strand binding protein—single-stranded DNA complexes. Modulation by NaCl concentration. *J. Biol. Chem.* *260*, 3594–3603.
- Lohman, T.M., Green, J.M., and Beyer, R.S. (1986). Large-scale overproduction and rapid purification of the *Escherichia coli* *ssb* gene product. Expression of the *ssb* gene under λ P_L control. *Biochemistry* *25*, 21–25.
- Ludwig, T., Chapman, D.L., Papiouannou, V.E., and Efstratiadis, A. (1997). Targeted mutations of breast cancer susceptibility gene homologs in mice: lethal phenotypes of *Brca1*, *Brca2*, *Brca1/Brca2*, *Brca1/p53*, and *Brca2/p53* nullizygous phenotypes. *Genes Dev.* *11*, 1226–1241.
- Luisi-DeLuca, C. (1995). Homologous pairing of single-stranded DNA and superhelical double-stranded DNA catalyzed by RecO protein from *Escherichia coli*. *J. Bact.* *177*, 566–572.
- Luisi-DeLuca, C., and Kolodner, R. (1994). Purification and characterization of the *Escherichia coli* RecO protein. Renaturation of complementary single-stranded DNA molecules catalyzed by the RecO protein. *J. Mol. Biol.* *236*, 124–138.
- Madiraju, M.V., and Clark, A.J. (1992). Evidence for ATP binding and double-stranded DNA binding by *Escherichia coli* RecF protein. *J. Bact.* *174*, 7705–7710.
- Madiraju, M.V., V.S., and Clark, A.J. (1991). Effect of RecF protein on reactions catalyzed by RecA protein. *Nucleic Acids Res.* *19*, 6295–6300.
- Mahdi, A.A., and Lloyd, R.G. (1989). Identification of the recR locus of *Escherichia coli* K-12 and analysis of its role in recombination and DNA repair. *Molec. Gen. Genet.* *216*, 503–510.
- Messing, J. (1983). New M13 vectors for cloning. *Methods Enzymol.* *101*, 20–78.
- Morriscal, S.W., Lee, J., and Cox, M.M. (1986). Continuous association of *Escherichia coli* single-stranded DNA binding protein with stable complexes of RecA protein and single-stranded DNA. *Biochemistry* *25*, 1482–1494.
- Neuendorf, S.K., and Cox, M.M. (1986). Exchange of RecA protein between adjacent RecA protein—single-stranded DNA complexes. *J. Biol. Chem.* *261*, 8276–8282.
- Pugh, B.F., and Cox, M.M. (1988). General mechanism for RecA protein binding to duplex DNA. *J. Mol. Biol.* *203*, 479–493.
- Register, J.C., III, and Griffith, J. (1985). The direction of RecA protein assembly onto single strand DNA is the same as the direction of strand assimilation during strand exchange. *J. Biol. Chem.* *260*, 12308–12312.
- Rehauer, W.M., and Kowalczykowski, S.C. (1993). Alteration of the nucleoside triphosphate (NTP) catalytic domain within *Escherichia coli* recA protein attenuates NTP hydrolysis but not joint molecule formation. *J. Biol. Chem.* *268*, 1292–1297.
- Roca, A.I., and Cox, M.M. (1997). RecA protein: structure, function, and role in recombinational DNA repair. *Prog. Nucl. Acids Res. Mol. Biol.* *56*, 129–223.
- Sandler, S.J. (1996). Overlapping functions for *recF* and *priA* in cell viability and UV-inducible SOS expression are distinguished by *dnaC809* in *Escherichia coli* K-12. *Molec. Microbiol.* *19*, 871–880.
- Sargentini, N.J., and Smith, K.C. (1986). Quantitation of the involvement of the *recA*, *recB*, *recC*, *recF*, *recJ*, *recN*, *lexA*, *radA*, *radB*, *uvrD*, and *umuC* genes in the repair of X-ray-induced DNA double-strand breaks in *Escherichia coli*. *Radiat. Res.* *107*, 58–72.
- Sawitzke, J.A., and Stahl, F.W. (1994). The phage lambda *orf* gene encodes a trans-acting factor that suppresses *Escherichia coli* *recO*, *recR*, and *recF* mutations for recombination of lambda but not of *E. coli*. *J. Bact.* *176*, 6730–6737.
- Scully, R., Chen, J., Plug, A., Xiao, Y., Weaver, D., Feunteun, J., Ashley, T., and Livingston, D.M. (1997). Association of BRCA1 with Rad51 in mitotic and meiotic cells. *Cell* *88*, 265–275.
- Shan, Q., Cox, M.M., and Inman, R.B. (1996). DNA strand exchange promoted by RecA K72R. Two reaction phases with different Mg²⁺ requirements. *J. Biol. Chem.* *271*, 5712–5724.
- Shan, Q., Bork, J.M., Webb, B.L., Inman, R.B., and Cox, M.M. (1997). RecA protein filaments: end-dependent dissociation from ssDNA, and stabilization by RecO and RecR proteins. *J. Mol. Biol.* *265*, 519–540.
- Sharan, S.K., Morimatsu, M., Albrecht, U., Lim, D.-S., Regel, E., Dinh, C., Sands, A., Eichele, G., Hasty, P., and Bradley, A. (1997). Embryonic lethality and radiation hypersensitivity mediated by Rad51 in mice lacking Brca2. *Nature* *386*, 804–810.
- Shen, Z., Cloud, K.G., Chen, D.J., and Park, M.S. (1996). Specific interactions between the human RAD51 and RAD52 proteins. *J. Biol. Chem.* *271*, 148–152.
- Sinden, R.R., and Cole, R.S. (1978). Repair of cross-linked DNA and

- survival of *Escherichia coli* treated with psoralen and light: effects of mutations influencing genetic recombination and DNA metabolism. *J. Bacteriol.* **136**, 538–547.
- Smith, G.R. (1989a). Homologous recombination in *E. coli*: multiple pathways for multiple reasons. *Cell* **58**, 807–809.
- Smith, G.R. (1989b). Homologous recombination in prokaryotes: enzymes and controlling sites. *Genome* **31**, 520–527.
- Sturzbecher, H.W., Donzelmann, B., Henning, W., Knippschild, U., and Buchhop, S. (1996). p53 is linked directly to homologous recombination processes via RAD51/RecA protein interaction. *EMBO J.* **15**, 1992–2002.
- Sung, P. (1997). Yeast Rad55 and Rad57 proteins form a heterodimer that functions with replication protein A to promote DNA strand exchange by Rad51 recombinase. *Genes Dev.* **11**, 1111–1121.
- Suzuki, A., de la Pompa, J.L., Hakem, R., Elia, A., Yoshida, R., Mo, R., Nishina, H., Chuang, T., Wakeham, A., Itie, A., et al. (1997). *Brca2* is required for embryonic cellular proliferation in the mouse. *Genes Dev.* **11**, 1242–1252.
- Tseng, Y.C., Hung, J.L., and Wang, T.C. (1994). Involvement of RecF pathway recombination genes in postreplication repair in UV-irradiated *Escherichia coli* cells. *Mutat. Res.* **315**, 1–9.
- Tsuzuki, T., Fujii, Y., Sakumi, K., Tominaga, Y., Nakao, K., Sekiguchi, M., Matsushiro, A., Yoshimura, Y., and Morita, T. (1996). Targeted disruption of the Rad51 gene leads to lethality in embryonic mice. *Proc. Natl. Acad. Sci. USA.* **93**, 6236–6240.
- Umezū, K., and Kolodner, R.D. (1994). Protein interactions in genetic recombination in *Escherichia coli*. Interactions involving RecO and RecR overcome the inhibition of RecA by single-stranded DNA-binding protein. *J. Biol. Chem.* **269**, 30005–30013.
- Umezū, K., Chi, N.W., and Kolodner, R.D. (1993). Biochemical interaction of the *Escherichia coli* RecF, RecO, and RecR proteins with RecA protein and single-stranded DNA binding protein. *Proc. Natl. Acad. Sci. USA* **90**, 3875–3879.
- Wang, T.C., Chang, H.Y., and Hung, J.L. (1993). Cosuppression of recF, recR and recO mutations by mutant recA alleles in *Escherichia coli* cells. *Mutat. Res.* **294**, 157–166.
- Webb, B.L., Cox, M.M., and Inman, R.B. (1995). An interaction between the *Escherichia coli* RecF and RecR proteins dependent on ATP and double-stranded DNA. *J. Biol. Chem.* **270**, 31397–31404.
- West, S.C., Cassuto, E., and Howard-Flanders, P. (1981). Mechanism of *E. coli* RecA protein-directed strand exchanges in postreplication repair of DNA. *Nature* **294**, 659–662.