THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 279, No. 5, Issue of January 30, pp. 3492–3496, 2004

Printed in U.S.A.

RecO Acts with RecF and RecR to Protect and Maintain Replication Forks Blocked by UV-induced DNA Damage in *Escherichia coli**

Received for publication, October 7, 2003, and in revised form, November 14, 2003
Published, JBC Papers in Press, November 18, 2003, DOI 10.1074/jbc.M311012200

Kin-Hoe Chow‡ and Justin Courcelle

From the Department of Biological Science, Mississippi State University, Mississippi State, Mississippi 39762

In *Escherichia coli*, *recF* and *recR* are required to stabilize and maintain replication forks arrested by UV-induced DNA damage. In the absence of RecF, replication fails to recover, and the nascent lagging strand of the arrested replication fork is extensively degraded by the RecQ helicase and RecJ nuclease. *recO* mutants are epistatic with *recF* and *recR* with respect to recombination and survival assays after DNA damage. In this study, we show that RecO functions with RecF and RecR to protect the nascent lagging strand of arrested replication forks after UV-irradiation. In the absence of RecO, the nascent DNA at arrested replication forks is extensively degraded and replication fails to recover. The extent of nascent DNA degradation is equivalent in single, double, or triple mutants of *recF*, *recO*, or *recR*, and the degradation is dependent upon RecJ and RecQ functions. Because RecF has been shown to protect the nascent lagging strand from degradation, these observations indicate that RecR and RecO function with RecF to protect the same nascent strand of the arrested replication fork and are likely to act at a common point during the recovery process. We discuss these results in relation to the biochemical and cellular properties of RecF, RecO, and RecR and their potential role in loading RecA filaments to maintain the replication fork structure after the arrest of replication by UV-induced DNA damage.

The failure to accurately replicate the genomic template in the presence of DNA damage is believed to be a primary cause of mutagenesis, genomic rearrangements, and lethality in all cells. Irradiation with near-UV (254 nm) light induces DNA lesions that block replication (1, 2). In wild-type *Escherichia coli*, replication is inhibited after a moderate dose of UV irradiation, but it efficiently recovers at a time correlating with the removal of the lesions by the nucleotide excision repair proteins (2–4).

The recovery of replication in *E. coli* requires RecA to stabilize and maintain the integrity of replication forks after arrest by DNA lesions. Mutants lacking RecA fail to recover replication after UV-irradiation and exhibit a rapid and eventually complete degradation of the chromosome (5, 6). The degradation initiates at the blocked replication forks and regresses back from these points (6). *In vitro*, RecA proteins will bind to form a filament on single-strand DNA, and they pair the single-strand region with homologous duplex DNA (7–9), an activity which would be consistent with maintaining the DNA at blocked replication forks (10, 11).

Similar to *recA*, *recF*, and *recR* mutants also fail to maintain replication forks that are blocked by DNA damage and do not recover replication (4, 5). In contrast to *recA* mutants, however, the DNA degradation is less extensive and is limited to ~50% of the nascent DNA localized at the blocked replication fork (4, 5). *In vitro*, RecF, RecO, and RecR interact with and stabilize RecA filaments bound to DNA (12), a role that would be consistent with the *in vivo* observation of limiting DNA degradation at the replication fork. Mutants lacking RecF and RecR also exhibit a delayed induction of the SOS response, consistent with the idea that these genes may help stabilize the RecA filaments which are required for SOS induction (7, 13–15).

The nascent DNA degradation that occurs in *recF* mutants has been shown to result from the combined action of RecQ, a 3′-5′ helicase, and RecJ, a 5′-3′ exonuclease (16, 17). Based upon the extent of nascent DNA degradation in *recF* mutants (5), the polarity of the helicase and nuclease *in vitro* (18, 19), and the preferential loss of the nascent lagging strand DNA at the fork (16), RecJ and RecQ are thought to degrade the nascent lagging strand of blocked replication forks prior to the recovery of replication, as depicted in the model shown in Fig. 6. In the absence of either gene product, the nascent DNA degradation does not occur irrespective of whether RecF is present to protect the lagging strand DNA (16), and the frequency of illegitimate recombination is altered (20, 21), suggesting that these enzymes may affect the frequency with which replication resumes accurately when it is disrupted.

recO is classified with *recF*, *recR*, *recJ*, and *recQ* as genes belonging to the *recF* pathway. Like *recF* and *recR*, *recO* mutants exhibit a similar delay in SOS induction, hypersensitivity to UV irradiation, reduced plasmid recombination, reduced conjugational recombination in a *recBCsbcBC* background, and the persistence of daughter-strand gaps in the nascent DNA of UV-irradiated *uvrA* mutants (22–24). Based upon these phenotypes, *recF*, *recR*, and *recO* have been suggested to form an epistatic group, RecFOR. *In vitro*, RecO physically interacts with both RecF and RecR to form either RecFO, RecRO, or RecFRO complexes, a role which is believed to be important for RecA stabilization (7, 12, 25, 26). These observations, taken together, suggest that RecO may be required with RecF and RecR to maintain arrested replication forks and promote the resumption of DNA synthesis after arrest. However, to date, the structures that RecO and RecR act upon at DNA damage-blocked replication forks *in vivo* have not been examined. To identify the relationship between RecF, RecO, and RecR at arrested replication fork structures *in vivo*, we have characterized the role that RecO plays during the recovery of replication after UV-induced DNA damage to determine when and where this protein functions during the recovery process.

* This work was supported by National Science Foundation Grant MCB0130486. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

‡ To whom correspondence should be addressed: Mississippi State Univ., P.O. Box GY, Mississippi State, MS 39762. Tel.: 662-325-4583; Fax: 662-325-7939; E-mail: kc10@msstate.edu.

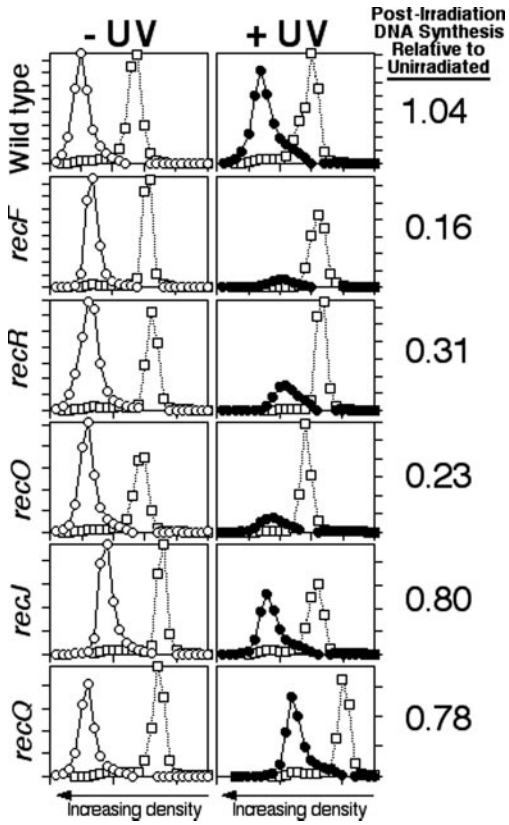

FIG. 1. ***recO* fails to recover replication after UV irradiation.** [^{14}C]Thymine-prelabeled cultures were split and either UV-irradiated (27 J/m^2) (*right panel*) or mock-irradiated (*left panel*) and allowed to recover for 1 h in media containing 5-bromouracil with a trace amount of [^{3}H]thymine prior to isopycnic CsCl gradient analysis. □, ^{14}C-pretreatment DNA; ○, ^{3}H-post-treatment DNA synthesized in unirradiated cultures; ●, ^{3}H-replicated DNA in irradiated cultures.

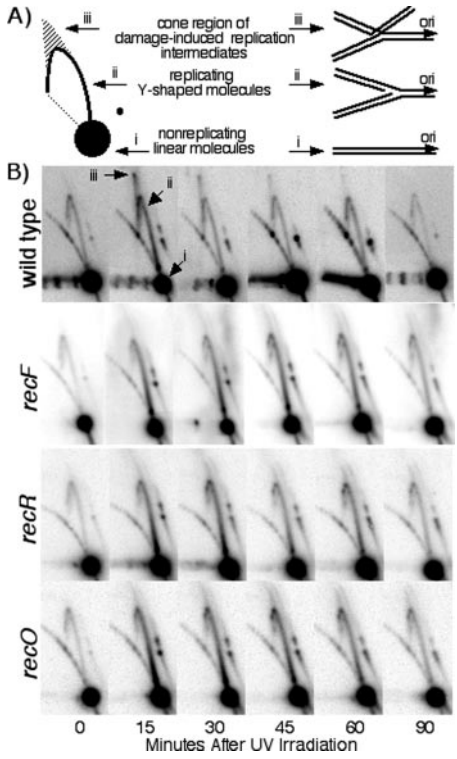

FIG. 2. ***recO* fails to maintain arrested replication forks after arrest by UV-induced DNA damage.** *A*, diagram of the migration pattern of PvuII-digested pBR322 during two-dimensional agarose gel analysis. Nonreplicating plasmids run as a linear 4.4-kb fragment (*i*). Normal replicating fragments form Y-shaped structures and migrate more slowly because of their larger size and nonlinear shape, forming an arc that extends out from the linear fragment (*ii*). Double Y- or X-shaped molecules migrate in the cone region (*iii*). *B*, blocked replication forks and cone region intermediates are not maintained in the absence of *recF*, *recR*, or *recO* after UV irradiation. Cells containing the plasmid pBR322 were UV-irradiated with 50 J/m^2, and genomic DNA was purified, digested with PvuII, and analyzed by two-dimensional agarose gels at the times indicated.

EXPERIMENTAL PROCEDURES

Bacterial Strains—SR108 is a *thyA36 deoC2* derivative of W3110 (27). HL946 (SR108 *recF332*::Tn*3*), CL544 (SR108 *recR6212*::cat*883*), and HL973 (SR108 *recF332*::Tn*3*; *recJ284*::Tn*10*) have been reported previously (4, 16, 17). CL584 (SR108 *recO1504*::Tn*5*) was made by P1 transduction of the *recO1504*::Tn*5* alleles from RDK1541 (23) into SR108. CL546 (SR108 *recF332*::Tn*3*; *recR6212*::cat*883*) and CL588 (SR108 *recF332*::Tn*3*; *recO1504*::Tn*5*) were made by P1 transduction of the *recR6212*::cat*883* and *recO1504*::Tn*5* alleles from strains TP647 (28) and RDK1541 (23), into HL946. CL592 (SR108 *recR6212*::cat*883*; *recO1504*::Tn*5*) was made by P1 transduction of the *recO1504*::Tn*5* allele from RDK1541 (23) into CL544. CL628 (SR108 *recF332*::Tn*3*; *recQ6215*::cat*883*) was made by P1 transduction of the *recF332*::Tn*3* allele from HL946 (4) into CL581 (17). CL691 (SR108 *recR6212*::cat*883*; *recQ1803*::Tn*3*) was made by P1 transduction of the *recR6212*::cat*883* allele from strain TP647 (28) into HL944 (16). CL684 (SR108 *recR6212*::cat*883*; *recJ284*::Tn*10*) was made by P1 transduction of the *recR6212*::cat*883* allele from strain TP647 (28) into HL942 (16). CL666 (SR108 *recO1504*::Tn*5*; *recJ284*::Tn*10*) and CL668 (SR108 *recO1504*::Tn*5*; *recQ1803*::Tn*3*) were made by P1 transduction of the *recJ284*::Tn*10* and *recQ1803*::Tn*3* alleles from HL924 (16) and HL944 (16), respectively, into CL584. CL590 (SR108 *recF332*::Tn*3*; *recO1504*::Tn*5*; *recR6212*::cat*883*) was made by P1 transduction of the *recO1504*::Tn*5* allele from CL544 into CL546.

UV Irradiation—Cultures were UV-irradiated in DGCthy media in Petri dishes on a rotating orbital shaker using a Sylvania 15-W germicidal light bulb (254 nm; 0.9 J/m^2/s).

Density Labeling of Replicated DNA—A fresh overnight culture was diluted 1:100 and grown in Davis minimal media with 0.4% glucose, 0.2% casamino acids, and 10 μg/ml thymine (DGCthy media) supplemented with [^{14}C]thymine (0.1 μCi/ml) to an A_{600} of 0.45 in a 37 °C shaking water bath. The culture was then split into two halves and either mock UV-irradiated or UV-irradiated with 27 J/m^2, before the cells were filtered on general filtration 0.45-μm membranes (Fisherbrand) and resuspended in DGC media containing 20 μg/ml 5-bromouracil in place of thymine and 0.5 μCi/ml of [^{3}H]thymine (60.5 Ci/mmol). Cultures were allowed to recover for 1 h at 37 °C in a shaking water bath. Then, two volumes of ice-cold NET buffer (100 mM NaCl, 10 mM EDTA, 10 mM Tris, pH 8.0) were added. Cells were pelleted, resuspended in 150 μl of TE buffer (10 mM Tris, 1 mM EDTA, pH 8.0) and lysed by the addition of 150 μl K_2HPO_4/KOH (pH 12.5) and 20 μl of 20% sarcosyl. The lysate was then subjected to isopycnic alkaline CsCl gradient centrifugation by combining 0.3 g lysate, 3.31 g 0.1 M K_2HPO_4/KOH (pH 12.5), and 2.23 g CsCl (refractive index = 1.4055) in a 5-ml polyallomer tube and centrifuged to equilibrium. Gradient fractions were collected on Whatman No. 17 paper, precipitated in 5% trichloroacetic acid, washed in 95% ethanol, and the amount of ^{14}C and ^{3}H in each fraction was quantitated in a scintillation counter.

Degradation Assay—A fresh overnight culture was diluted 1:100 and grown in DGCthy media supplemented with [^{14}C]thymine (0.1 μCi/ml) to an A_{600} of 0.4 in a 37 °C shaking incubator. Cultures were then pulse-labeled with 1 μCi/ml [^{3}H]thymidine for 10 s (to label the nascent DNA at the replication fork) before being filtered on 0.45-μm membranes (Fisherbrand General Filtration), washed with 2–5 ml of NET buffer, resuspended in prewarmed non-radioactive DGCthy media, UV-irradiated with 27 J/m^2, and then incubated in a 37 °C shaking incubator. Duplicate 200-μl aliquots of the culture (triplicate at time 0) were collected at 20-min intervals and lysed/precipitated in cold 5% trichloroacetic acid before being filtered on Millipore glass fiber prefilters. The amount of radioactivity in each filter was determined in a scintillation counter.

Two-dimensional Gel and Southern Analysis—Fresh overnight cultures of cells that contain the plasmid pBR322 were grown in the presence of 100 μg/ml ampicillin. The overnight cultures were diluted 1:100 and grown without ampicillin selection in a shaking incubator at 37 °C to an A_{600} of 0.5 (~5 × 10^8 cells/ml) and UV-irradiated with 50 J/m^2. At the indicated time points, 0.75-ml samples were placed into 0.75 ml of cold 2× NET (100 mM NaCl, 10 mM Tris, pH 8.0, 10 mM

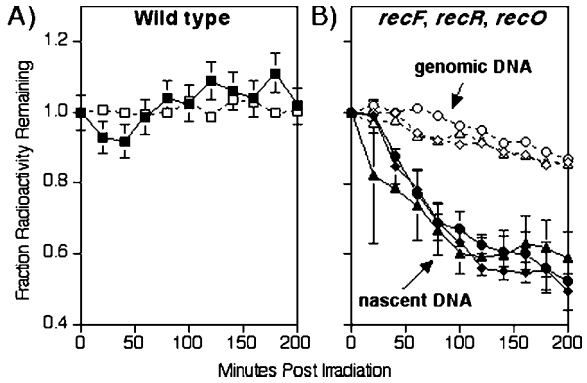

FIG. 3. **Extensive nascent DNA degradation occurs in *recO* after UV irradiation.** [^{14}C]Thymine-prelabeled cultures were pulse-labeled with [^{3}H]thymidine for 10 s immediately before being filtered and irradiated with 27 J/m^2 in nonlabeled medium. The relative amount of radioactivity remaining in DNA is plotted over time. Degradation of the ^{14}C genomic DNA (white symbols) can be compared with the degradation of the ^{3}H-labeled nascent DNA (black symbols) synthesized at the growing fork just before irradiation. *A*, (■), parental cells. *B*, (♦) *recF*; (●), *recR*; (▲), *recO*.

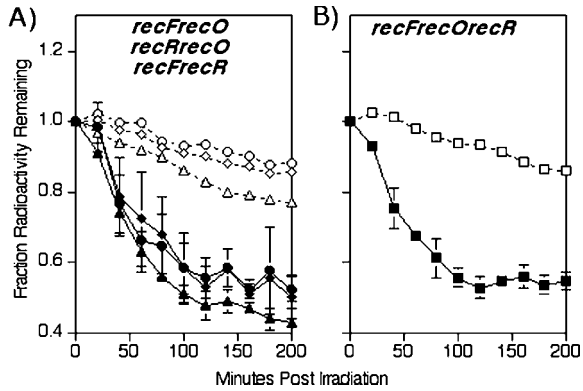

FIG. 4. **The absence of two or more of the *recF*, *recR*, and *recO* products does not increase the extent of nascent DNA degradation.** The assay was performed as in Fig. 3. *A*, (♦), *recFrecO*; (●), *recRrecO*; (▲), *recFrecR*. *B*, the triple mutant of *recF*, *recR*, and *recO*. (■), *recFrecRrecO*. White symbols, ^{14}C genomic DNA; black symbols, ^{3}H nascent DNA.

EDTA). Each sample was pelleted, resuspended in 150 μl of 1 mg/ml lysozyme and 0.2 mg/ml RNaseA in TE buffer, and lysed at 37 °C for 20 min. At this time, proteinase K (10 μl, 10 mg/mg) and sarcosyl (10 μl, 20%) were added and incubated at 50 °C for 1 h. Samples were then extracted, twice, with four volumes of phenol/chloroform/isoamyl alcohol (25/24/1), once with four volumes of chloroform/isoamyl alcohol (24/1), and dialyzed for 3 h on 47-mm Whatman 0.05-μm pore disks (VMWP04700, Whatman Bioscience) floating on a 250-ml beaker of TE. Samples were then digested with PvuII (New England Biolabs), extracted with chloroform/isoamyl alcohol (24/1), and equal volumes were loaded onto the gel. Restricted genomic DNA samples were run in the first dimension in 0.4% agarose, 1× Tris-borate-EDTA at 1 volume/cm. Gel lanes were cut out, recast, and run in the second dimension in 1.0% agarose, 1× Tris-borate-EDTA at 6.5 volumes/cm. Gels were transferred to Hybond N+ nylon membranes and probed with pBR322 that had been labeled with ^{32}P by nick translation according to the protocol supplied by Promega using [α-^{32}P]dCTP (ICN Biomedicals). Radioactivity was visualized and quantitated with a Storm 820 PhosphorImager and its associated ImageQuant Software (Molecular Dynamics).

RESULTS

RecO Is Required to Resume Replication After UV-induced DNA Damage—Both *recF* and *recR* are required for the recovery of replication after disruption by UV-induced DNA damage (5). The ability of *recO* to recover replication after UV irradiation was compared with that of *recF* and *recR* and visualized by density-labeling the post-irradiation DNA synthesis with 5-bromouracil. Cultures that were either UV-irradiated with 27 J/m^2 or mock-irradiated were allowed to recover for a period of one hour in media containing 5-bromouracil in place of thymine, so that any DNA replicated during this period would be of a greater density than the DNA synthesized before irradiation. The denser, replicated DNA in each culture was then separated from the rest of the DNA by centrifugation in isopycnic alkaline CsCl gradients and quantitated. As shown in Fig. 1, one hour after irradiation, UV-irradiated wild-type cells had replicated nearly as much DNA as their unirradiated counterparts, demonstrating that replication had fully recovered within this time frame. However, the amount of DNA synthesized in either *recF, recR,* or *recO* mutants was inhibited to a similar extent after this dose of UV irradiation. By contrast, although both *recJ* and *recQ* process the nascent DNA after UV irradiation (16) and affect the time that replication recovers,[1] these genes are not essential for replication to resume, and significant amounts of DNA synthesis are observed in the absence of these gene products. It is of interest to note that, in an otherwise wild-type background, mutations in *recJ* or *recQ* do not render cells sensitive to UV irradiation (29, 30).

The lack of recovery in *recF* and *recR* mutants is associated with a failure to maintain replication forks blocked by DNA damage. This can be visualized by examining the structural intermediates that are associated with arrested replication forks on plasmids such as pBR322 using two-dimensional agarose gel analysis (17, 31). To determine whether *recO* mutants also fail to maintain the replication fork, we characterized the structural intermediates that occurred on replicating plasmid molecules of pBR322 after UV irradiation with 50 J/m^2 in growing *E. coli* cultures. Previous studies by our group (17) have shown that this dose produces 0.5 lesions per plasmid strand and that 90% of the parental cells survive the irradiation to form colonies. Cells containing the plasmid pBR322 were UV-irradiated, and the genomic DNA was purified, digested with PvuII (which cuts the plasmid just downstream of the unidirectional origin of replication), and analyzed by two-dimensional agarose gels at the times indicated. In this technique, nonreplicating plasmids migrate as linear 4.4-kb fragments, whereas replicating fragments form Y-shaped structures and migrate more slowly because of their larger size and nonlinear shape. These replicating fragments form an arc that extends out from the linear fragment (Fig. 2*A*). In wild-type cells, a transient reversal of the replication fork has been shown to occur on plasmids after UV irradiation (17). The regressed fork intermediate is maintained by the RecF, RecR, and RecA proteins until a time that correlates with the removal of the lesions by nucleotide excision repair and the recovery of replication. The extrusion of the nascent DNA converts the three-arm replication fork structure into a four-arm, replication intermediate that further retards its mobility in the gel. These four-arm structures migrate in a cone region beyond the normal replication arc (Fig. 2*A*). Consistent with our previous studies, UV-irradiated *recF* and *recR* mutants did not accumulate the cone region intermediates to any significant extent (17). When we examined the replicating plasmids in UV-irradiated *recO* mutants, the replication fork intermediate also failed to accumulate (Fig. 2*B*), indicating that RecO, like RecF and RecR, is required to maintain the replication fork after arrest by UV-induced DNA damage.

RecO Is Required with RecF and RecR to Protect the Nascent DNA at Blocked Replication Forks from Degradation by RecQ and RecJ—The failure to recover replication in UV-irradiated *recF* or *recR* mutants is associated with the extensive loss of nascent DNA made just prior to irradiation (5). The failure of

[1] K.-H. Chow and J. Courcelle, unpublished observations.

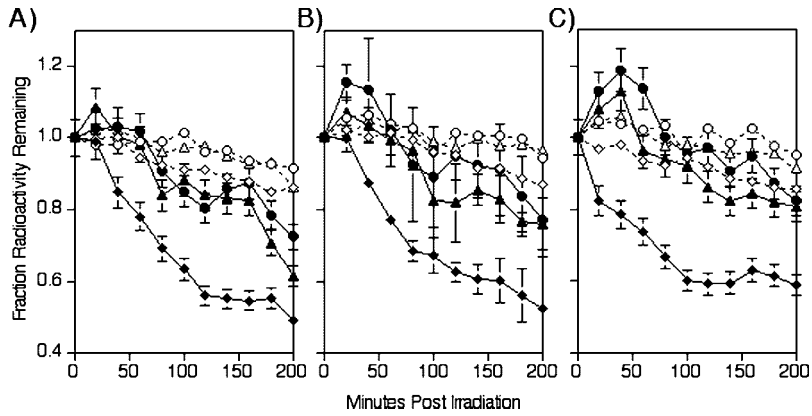

FIG. 5. **Nascent DNA degradation in *recO* mutants is mediated by the RecJ/RecQ, nuclease/helicase.** The assay was performed as in Fig. 3. *A*, (♦) *recF*; (●), *recFrecJ*; (▲), *recFrecQ*. *B*, (♦) *recR*; (●), *recRrecJ*; (▲), *recRrecQ*. *C*, (♦), *recO*; (●), *recOrecJ*; (▲), *recOrecQ*. White symbols, ^{14}C genomic DNA; black symbols, ^{3}H nascent DNA.

recO mutants to recover replication could indicate that RecO is required at a step similar to RecF and RecR and is needed to limit the nascent DNA degradation. Alternatively, RecO could be required at a subsequent step in the recovery process once the nascent DNA strands have been stabilized or protected. To differentiate between these possibilities, we examined the nascent degradation pattern in *recO* mutants. To this end, exponentially growing, [^{14}C]thymine-prelabeled cultures were pulse-labeled with [^{3}H]thymidine for 10 s to label the DNA at replication forks. Then, the culture was transferred to nonradioactive medium and immediately UV-irradiated with 27 J/m^2. The ^{14}C prelabel allowed us to compare the degradation occurring in the overall genome to that in the ^{3}H-labeled DNA made at replication forks just prior to UV irradiation. As shown previously, UV-irradiated wild-type cells degrade very little of their overall genomic DNA after irradiation. However, some limited degradation of the nascent DNA was detected at times prior to the recovery of replication (Fig. 3*A*; Refs. 4, 5, 32). In contrast to the limited degradation in wild-type cells, *recF* or *recR* mutants degraded approximately half of the nascent DNA made just prior to UV irradiation (Fig. 3*B*). When the degradation pattern was examined in *recO* mutants, we found that the nascent DNA was extensively degraded, and that the extent of degradation was similar to that occurring in *recF* and *recR* mutants (Fig. 3*B*). The result indicates that, like RecF and RecR, RecO contributes to protecting the DNA at blocked replication forks in UV-irradiated cells.

Although extensive degradation occurs in all three *recF*, *recR*, and *recO* single mutants, it is possible that these gene products protect different strands of the blocked replication fork *in vivo*. If this is the case, then we would predict that the nascent DNA degradation would increase when more than one of these gene products is absent. To examine this possibility, we examined the nascent DNA degradation in double mutants of *recF recR*, *recF recO*, and *recR recO*, as well as the corresponding *recF recO recR* triple mutant. All double mutants exhibited nascent degradation patterns that were similar in extent to the *recF* or *recR* single mutants (Fig. 4*A*). Furthermore, the nascent DNA degradation in the triple mutant was also limited to approximately half of the nascent DNA (Fig. 4*B*), suggesting that RecO functions together with RecF and RecR to protect the same strand (or structural aspect) of the blocked replication fork.

If RecO acts together with RecF and RecR to protect the nascent lagging strand of the arrested replication fork, then the observed nascent DNA degradation should be dependent upon RecJ and RecQ (16). To test this idea, we examined the nascent DNA degradation in *recO* mutants that also lacked either the RecQ helicase or RecJ nuclease (Fig. 3*B*). Consistent with this idea, the nascent DNA degradation was reduced to a similar extent in *recF, recR*, or *recO* mutants when either RecJ or RecQ was inactivated (Fig. 5). These observations indicate that the nascent DNA degradation in *recO*, *recF*, and *recR* mutants results from the same enzymatic degradation of the lagging strand by RecJ and RecQ.

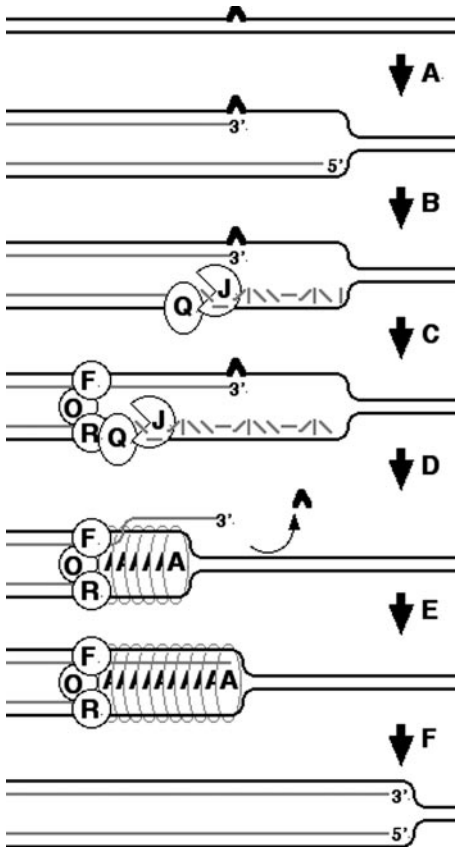

FIG. 6. **Model for RecF, RecO, and RecR function during the recovery of replication after UV-induced DNA damage.** *A*, replication is blocked by DNA lesions. *B*, the nascent DNA is degraded by RecQ and RecJ. RecF, RecO, and RecR limit the degradation by RecJ and RecQ (*C*), and promote the loading and stabilization of a RecA filament to maintain the integrity of the replication fork DNA until the lesion can be repaired or bypassed (*D*) and replication can resume (*E*), thereby maintaining the processive replication of the genomic template (*F*).

DISCUSSION

RecF, RecO, and RecR are proposed to form an epistatic group based upon several shared biochemical and genetic characteristics. All three mutations render cells equally hypersensitive to UV irradiation, reduce the frequency of recombinant progeny in conjugation or transduction assays in *recBC* or *recD* backgrounds, and delay the induction of the SOS response after

DNA damage (14, 15, 23, 24). A recent study suggested that the UV hypersensitivity of *recO* is associated with a delayed recovery of replication similar to *recF* and *recR* (33). The results presented here indicate that RecF, RecR, and RecO function together to maintain replication forks arrested by UV-induced DNA damage. In addition, all three proteins are required together to limit the degradation of the nascent lagging strand by RecJ and RecQ after UV irradiation.

In vitro, all three proteins bind DNA, and RecO has been shown to promote annealing between homologous DNA strands (34). RecO and RecR promote RecA protein-mediated D-loop formation at the 5′ end of linear ssDNA and stabilize RecA filaments to prevent their disassembly (7, 25). Complexes of RecF and RecR bind double-strand DNA and gapped DNA substrates and limit how far RecA filaments are able to extend into double-strand regions (35). Furthermore, the RecFOR proteins in combination have been shown to facilitate RecA loading onto gapped DNA substrates (12, 36). These *in vitro* characterizations are consistent with the *in vivo* observations that RecF, RecO, and RecR may recognize and bind to nascent DNA at blocked replication forks and serve to stabilize the RecA filaments at these regions, as proposed previously (5, 37). The data presented here and in previous work indicate that binding by these proteins limits the nascent degradation on the lagging strand by the RecQ-RecJ helicase-nuclease *in vivo* (Fig. 5). However, to date, the binding and activity of these gene products has not been examined on replication fork-like structures *in vitro*.

A role for RecF, RecO, and RecR in stabilizing RecA filaments at blocked replication forks is also consistent with several genetic observations. *recF*, *recO,* and *recR* mutants exhibit a delayed SOS induction (14, 15). Because RecA filaments bound to single-strand DNA function as the inducing signal for up-regulation of the SOS genes, the delay in up-regulation of SOS genes may reflect the reduced ability of RecA to bind to the replication fork substrates in the absence of RecFOR. Additionally, certain mutant alleles of RecA that increase its affinity to bind DNA are able to partially bypass the requirement for RecFOR and partially suppress the UV sensitivity of *recF*, *recR*, and *recO* mutants (38–40). To incorporate these observations, we have placed RecFOR at the nascent lagging strand junction of the block replication fork (Fig. 6). However, the precise arrangement and stoichiometry of this complex will require further investigation, and it remains possible that RecF, RecO, and RecR independently bind and recognize different portions of the replication fork structure to achieve its task of limiting the nascent lagging strand degradation and of stabilizing RecA filaments at arrested replication fork structures. This possibility is supported by genetic studies that show overexpression of RecR or RecO alone can partially suppress the UV sensitivity of *recF* mutants (41, 42). However, the stoichiometry and constitution of the functional complex (or complexes) on the nascent lagging strand remains an important aspect that has not yet been identified.

It is clear from this and previous studies that these genes are required for maintenance of replication forks blocked at DNA lesions until a time corresponding to when the lesions are repaired and replication can resume. In the absence of DNA damage when replication is not frequently disrupted, inactivation of RecF, RecO, or RecR does not seem to affect the growth rate or viability of growing *E. coli* cultures. However, in the event that replication arrests before the duplication of the genome has been completed, RecF, RecO, and RecR play a critical role in recognizing arrested fork structures as a proper substrate and in facilitating the stabilization of the RecA filament to protect and promote the resumption of replication, thereby allowing the processive duplication of the chromosome to be completed.

Acknowledgments—We thank Anthony Poteete and Susan Rosenberg for providing bacterial strains used in this study.

REFERENCES

1. Smith, K. C. (1969) *Mutat. Res.* **8,** 481–495
2. Setlow, R. B., Swenson, P. A., and Carrier, W. L. (1963) *Science* **142,** 1464–1466
3. Setlow, R. B., and Carrier, W. L. (1963) *Proc. Natl. Acad. Sci. U. S. A.* **51,** 226
4. Courcelle, J., Crowley, D. J., and Hanawalt, P. C. (1999) *J. Bacteriol.* **181,** 916–922
5. Courcelle, J., Carswell-Crumpton, C., and Hanawalt, P. C. (1997) *Proc. Natl. Acad. Sci. U. S. A.* **94,** 3714–3719
6. Horii, Z. I., and Suzuki, K. (1968) *Photochem. Photobiol.* **8,** 93–105
7. Shan, Q., Bork, J. M., Webb, B. L., Inman, R. B., and Cox, M. M. (1997) *J. Mol. Biol.* **265,** 519–540
8. Konforti, B. B., and Davis, R. W. (1987) *Proc. Natl. Acad. Sci. U. S. A.* **84,** 690–694
9. Cox, M. M., and Lehman, I. R. (1982) *J. Biol. Chem.* **257,** 8523–8532
10. Kowalczykowski, S. C., Dixon, D. A., Eggleston, A. K., Lauder, S. D., and Rehrauer, W. M. (1994) *Microbiol. Rev.* **58,** 401–465
11. Roca, A. I., and Cox, M. M. (1997) *Prog. Nucleic Acid Res. Mol. Biol.* **56,** 129–223
12. Umezu, K., Chi, N. W., and Kolodner, R. D. (1993) *Proc. Natl. Acad. Sci. U. S. A.* **90,** 3875–3879
13. Thoms, B., and Wackernagel, W. (1987) *J. Bacteriol.* **169,** 1731–1736
14. Hegde, S., Sandler, S. J., Clark, A. J., and Madiraju, M. V. (1995) *Mol. Gen. Genet.* **246,** 254–258
15. Whitby, M. C., and Lloyd, R. G. (1995) *Mol. Gen. Gent.* **246,** 174–179
16. Courcelle, J., and Hanawalt, P. C. (1999) *Mol. Gen. Gent.* **262,** 543–551
17. Courcelle, J., Donaldson, J. R., Chow, K. H., and Courcelle, C. T. (2003) *Science* **299,** 1064–1067
18. Lovett, S. T., and Kolodner, R. D. (1989) *Proc. Natl. Acad. Sci. U. S. A.* **86,** 2627–2631
19. Umezu, K., Nakayama, K., and Nakayama, H. (1990) *Proc. Natl. Acad. Sci. U. S. A.* **87,** 5363–5367
20. Hanada, K., Ukita, T., Kohno, Y., Saito, K., Kato, J., and Ikeda, H. (1997) *Proc. Natl. Acad. Sci. U. S. A.* **94,** 3860–3865
21. Ukita, T., and Ikeda, H. (1996) *J. Bacteriol.* **178,** 2362–2367
22. Horii, Z., and Clark, A. J. (1973) *J. Mol. Biol.* **80,** 327–344
23. Kolodner, R., Fishel, R. A., and Howard, M. (1985) *J. Bacteriol.* **163,** 1060–1066
24. Mahdi, A. A., and Lloyd, R. G. (1989) *Mol. Gen. Genet.* **216,** 503–510
25. Umezu, K., and Kolodner, R. D. (1994) *J. Biol. Chem.* **269,** 30005–30013
26. Hedge, S. P., Qin, M. H., Li, X. H., Atkinson, M. A. L., Clark, A. J., Rajagopalan, M., and Madiraju, M. V. V. S. (1996) *Proc. Natl. Acad. Sci. U. S. A.* **93,** 14468–14473
27. De-Lucia, P., and Cairns, J. (1969) *Nature* **224,** 1164–1166
28. Murphy, K. C., Campellone, K. G., and Poteete, A. R. (2000) *Gene* **246,** 321–330
29. Lovett, S. T., and Clark, A. J. (1984) *J. Bacteriol.* **157,** 190–196
30. Nakayama, H., Nakayama, K., Nakayama, R., Irino, N., Nakayama, Y., and Hanawalt, P. C. (1984) *Mol. Gen. Genet.* **195,** 474–480
31. Friedman, K. L., and Brewer, B. J. (1995) *Methods Enzymol.* **262,** 613–627
32. Hanawalt, P., and Brempelis, I. (1967) *Proceedings of the 7th International Congress of Biochemistry, August, 1967*, p. 650, Japanese Biochemical Society, Tokyo, Japan
33. Rangarajan, S., Woodgate, R., and Goodman, M. F. (2002) *Mol. Microbiol.* **43,** 617–628
34. Luisi-Deluca, C. (1994) *J. Mol. Biol.* **236,** 124–138
35. Webb, B. L., Cox, M. M., and Inman, R. B. (1997) *Cell* **91,** 347–356
36. Morimatsu, K., and Kowalczykowski, S. C. (2003) *Mol. Cell* **11,** 1337–1347
37. Courcelle, J., and Hanawalt, P. C. (2001) *Proc. Natl. Acad. Sci. U. S. A.* **98,** 8196–8202
38. Madiraju, M. V., Lavery, P. E., Kowalczykowski, S. C., and Clark, A. J. (1992) *Biochemistry* **31,** 10529–10535
39. Madiraju, M. V., Templin, A., and Clark, A. J. (1988) *Proc. Natl. Acad. Sci. U. S. A.* **85,** 6592–6596
40. Wang, T. C., Chang, H. Y., and Hung, J. L. (1993) *Mutat. Res.* **294,** 157–166
41. Sandler, S. J. (1994) *Mol. Gen. Genet.* **245,** 741–749
42. Sandler, S. J., and Clark, A. J. (1994) *J. Bacteriol.* **176,** 3661–3672