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recF and *recR* are required for the resumption of replication at DNA replication forks in *Escherichia coli*

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ABSTRACT ***Escherichia coli* containing a mutation in *recF* are hypersensitive to UV. However, they exhibit normal levels of conjugational or transductional recombination unless the major pathway (*recBC*) is defective. This implies that the UV sensitivity of *recF* mutants is not due to a defect in recombination such as occurs during conjugation or transduction. Here, we show that when replication is disrupted, at least two genes in the *recF* pathway, *recF* and *recR*, are required for the resumption of replication at DNA replication forks, and that in their absence, localized degradation occurs at the replication forks. Our observations support a model in which *recF* and *recR* are required to reassemble a replication holoenzyme at the site of a DNA replication fork. These results, when taken together with previous literature, suggest that the UV hypersensitivity of *recF* cells is due to an inability to resume replication at disrupted replication forks rather than to a defect in recombination. Current biochemical and genetic data on the conditions under which *recF*-mediated recombination occurs suggest that the recombinational intermediate also may mimic the structure of a disrupted replication fork.**

In *Escherichia coli*, recombination is classically thought to occur through one of two pathways termed the *recBC* (major) pathway and the *recF* (minor) pathway (1, 2). *recBC* mutants originally were identified because of the 10^2- to 10^3-fold reduction in their recombination efficiency (3). Further characterization showed that *recBC* mutants were also hypersensitive to UV and x-rays and had a reduced plating efficiency (3, 4). Biochemical studies since have shown that RecBC forms a DNA helicase that associates with a nuclease, RecD, and is thought to unwind and process the DNA ends required for the initiation of strand invasion during recombination (1, 2).

The *recF* pathway of recombination is less well understood. *recF* was identified by screening mutagenized *recBC* cells to isolate the genes required for the 0.1 to 1 percent of recombination remaining in these cells (5). However, when the *recF* mutations were moved into a $recBC^+$ background, the recipient cells were found to have normal levels of recombination. Although not deficient in recombination, these cells were as sensitive to UV as were *recBC* cells, and they also displayed a low plating efficiency (6). Purified RecF protein has been shown to preferentially bind gapped DNA in the presence of ATP, but its function *in vivo* has not been determined (7–9). The RecR protein is also in the *recF* pathway and has been shown to interact with the RecO protein(8). Both RecO and RecR are epistatic with RecF and are thought to function at a common, yet unknown, step in the recombination process (1, 8).

Several studies have shown a link between DNA replication and the *recF* pathway of recombination. Early studies of phage lambda showed that recombination dependent on either the *recF* pathway or a *recF* homologous pathway required the presence of replication (10). Certain forms of aberrant replication, such as plasmid linear multimer formation and rifampicin resistant plasmid replication, also have been shown to be dependent upon *recF* (11, 12). Genes belonging to the *recF* pathway also are required for cells to undergo "thymineless death," a process by which cells rapidly die in the absence of thymine (13–15). Here, as well, aberrant replication has been shown to be occurring during the process (16, 17).

Another phenomenon linking the *recF* pathway to replication is long-patch excision repair (18, 19). Cooper and P. C. H. (18) found that the size distribution of DNA repair patches in UV-irradiated *E. coli* was bimodal. Short patches appeared at early times and were shown to be due to normal nucleotide excision repair. *recF*-dependent long patches were observed at the time the cells recovered DNA replication, and these were primarily localized at DNA replication forks (20). The long patches were found to be either 1500 bp or greater than 9000 bp in size, corresponding to those expected for Okazaki fragments on the lagging strand and leading strand DNA synthesis, respectively.

At the level of genomic organization, both *recF* and *recR* also appear to be linked with replication. The *recF* gene is found in the same operon as the *dnaN* gene (the beta subunit of the replication holoenzyme), while the *recR* gene is found in the same operon as the *dnaXZ* gene (the tau and gamma subunits of the holoenzyme) (21–23).

While *recF* and *recR* mutants have relatively subtle phenotypes with respect to recombination, their UV sensitivities are comparatively dramatic. We have considered the possibility that recombination is not the primary function of *recF*. By studying why *recF* causes hypersensitivity to UV, we hoped to gain a better understanding of its function *in vivo*. We have found that the resumption of DNA replication from existing replication forks requires both the *recF* and *recR* genes.

MATERIALS AND METHODS

Bacterial Strains. SR108 is a *thyA36 deoC2* derivative of W3110 (24). HL919 (SR108 *recF349 tnaA300*::*tn10*) and HL920 (SR108 *recR252*:*tn10–9*) were made by P1 transduction of the *recF349 tnaA300*::*tn10* and *recR252*::*tn10–9* markers from strains JC15359 and AM207, respectively (25). HL921 (SR108 Δ(*srlR-recA*)*306*::*tn10*) was made by P1 transduction of Δ(*srlR-recA*)*306*::*tn10* from JC10289 (26). HL922 (SR-108 *recB21C22 argA81*::*tn10*) and HL923 (SR108*recD1011 argA81*::*tn10*) were made by P1 transduction of *recB21C22 argA81*::*tn10* from strain V1307 and *recD1011 argA81*::*tn10* from strain V220 (27, 28). The *recF*, *recR*, *recA*, and *recBC* phenotypes were checked by UV sensitivity. The *recBC* and *recD* phenotypes were checked by their ability to support growth of phage T4 gene 2^- mutants (29). SR1601 and

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CAG12156 carry a *uvrA*::*tn10* marker and *uvrC*::*tn10* marker, respectively (30, 31).

Survival Studies. UV irradiations used a 15-watt germicidal lamp (254 nm, 0.6 J/m^2 per sec at the sample position). Cells were grown in Davis medium supplemented with 0.4% glucose, 0.2% casa amino acids, and 10 μg/ml thymine (DGCthy media). Inoculated cultures were incubated for 2 to 3 days before irradiation to obtain stationary phase cells. Log phase cultures were typically inoculated from stationary phase cultures and grown to an OD_{600} between 0.2 and 0.3 (approximately 5×10^8 cells/ml). Chloramphenicol pretreatment (150 μg/ml) was for 3 h before the cultures were filtered on Millipore 0.45-μm HA filters and resuspended in fresh medium. All experiments were carried out at 37°C.

Time Course of Replication Recovery. Cells were grown in DGCthy media containing 0.5 μCi/ml [^{3}H]thymine to an OD_{600} of 0.2 at which point half the culture received an incident dose of 25 J/m^2. The incorporation of ^{3}H into the DNA was measured by averaging duplicate 0.2-ml samples precipitated in 5% cold trichloroacetic acid filtered onto Whatman glass fiber filters.

Density Labeling of Replicated DNA. Cells were grown in DGCthy media containing 0.2 μCi/ml [^{14}C]thymine to an OD_{600} between 0.2 and 0.3 before being harvested by filtration and resuspended in DGC media containing 10 μg/ml 5-bromodeoxyuridine. Half the culture received 25 J/m^2; each half received 0.5 μCi/ml [^{3}H]thymine, and was then incubated for 1 h. Cells were pelleted and lysed in 0.4 ml NET (100 mM NaCl/10 mM Tris, pH 8.0/1 mM EDTA) containing 1 mg/ml lysozyme and 100 μg/ml RNase A at 37°C for 30 min. Ten microliters of 10 mg/ml proteinase K and 10 μl of 10% sarcosyl was added, and incubation continued for 1 h at 65°C. The solution then was subjected to isopycnic alkaline CsCl gradient sedimentation as described previously (32). Thirty fractions were collected from each gradient on Whatman no. 17 paper. ^{14}C and ^{3}H were determined by scintillation counting.

DNA Degradation After UV Irradiation. Cells were grown in DGCthy media containing 0.2 μCi/ml [^{14}C]thymine to an OD_{600} between 0.2 and 0.3. Thirty seconds before harvesting by filtration, 1 μCi/ml [^{3}H]thymidine was added to the culture. Cells were washed with 1× Davis medium, resuspended in nonradioactive DGCthy medium, and given a dose of 25 J/m^2. ^{14}C and ^{3}H remaining in the DNA were measured as before.

Completion of Ongoing Rounds of DNA Replication and FACS Analysis. Cells were grown in DGCthy media containing 0.5 μCi/ml [^{3}H]thymine to an OD_{600} of 0.2 at which point chloramphenicol or rifampicin was added (150 μg/ml). ^{3}H incorporation into the DNA was measured as before. For FACS analysis, the same procedure was used except 10 μg/ml cephalexin (inhibitor of septation) also was added (33). After 3 h, cells were fixed in 70% ethanol. Staining was done in 50 mM Tris, pH 7.5/1.5 mM $MgCl_2$/100 mM NaCl/20 μg/ml chromomycin A3. FACS analysis was performed using a EPIC753 flow cytometer (Coulter) at 457 nm excitation. Elite software (Coulter) was used for analysis (33).

RESULTS

The UV Sensitivity of *recF* and *recR* Mutants Correlates with DNA Replication. We found that the UV sensitivity of *recF* and *recR* cells was dependent upon the replication state of the cells. When wild-type, *recF*, and *recR* cells were grown to stationary phase before irradiation they were more resistant to UV than were exponentially growing cultures (Fig. 1*A*). Similarly, when the cells were pretreated for 3 h with chloramphenicol, a protein synthesis inhibitor, they were also more resistant to UV irradiation (Fig. 1*B*). Inhibition of protein synthesis has been shown to prevent the initiation of new rounds of DNA replication (14, 34). Treatment with chloramphenicol for 3 h allows the ongoing rounds of replication to be completed (35, 36). By contrast, the UV hypersensitivity of excision repair mutants *uvrA* and *uvrC* was unaffected by the chloramphenicol pretreatment (Fig. 1*C*). This is consistent with the early results of P. C. H. (36), demonstrating that after a period of amino acid starvation, wild-type, but not repair-deficient cells, were more resistant to UV. Similarly, Tang and Smith (37) found that liquid holding recovery was blocked in *uvr*$^-$ mutants, but not recF cells. Common to both stationary-phase cultures and chloramphenicol-treated cultures, among other things, is that replication has been eliminated or greatly reduced. Therefore, one interpretation of these results is that the UV hypersensitivity associated with *recF* and *recR* cells is due to ongoing replication at the time DNA damage is introduced, rather than to a problem with the repair of the damage. Consistent with this, it has been shown that UV-induced lesions are removed from DNA in *recF* cells with an efficiency comparable to that in wild-type cells (38).

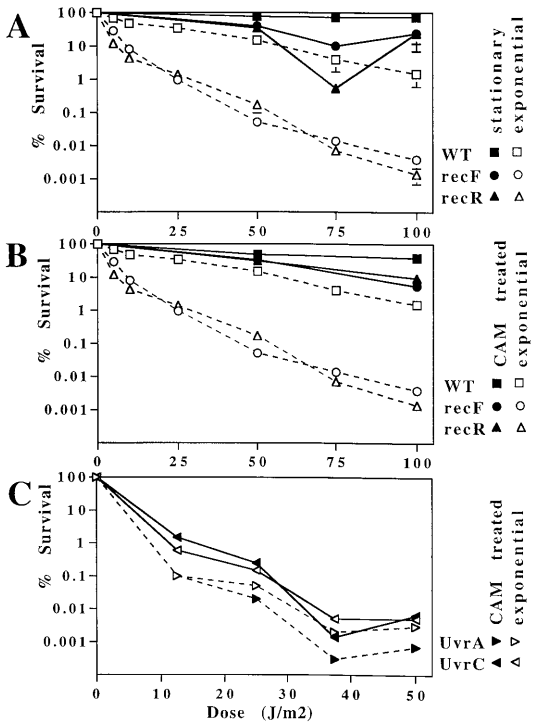

FIG. 1. The UV hypersensitivity of *recF* and *recR* is dependent upon the replication state of the cell. (*A*) The survival of parental (■, □) SR108, *recF* (●, ○) HL219, and *recR* (▲, △) HL220 is shown after UV irradiation at the indicated dose for both stationary phase (filled symbols) and exponentially growing cultures (open symbols). *recF* and *recR* cells are less UV sensitive in stationary phase cultures. (*B*) The survival of parental (■, □) SR108, *recF* (●, ○) SR108F, and *recR* cells (▲, △) SR108R, is shown after UV irradiation at the indicated dose for both cultures pretreated for 3 h with chloramphenicol (filled symbols) and untreated exponentially growing cultures (open symbols). (*C*) Cells were treated as in (*B*) for *uvrA* (▶, ▷) HL758 and *uvrC* cells (◀, ◁) HL763. Survival curves represent the average from at least 3 independent experiments, except for *uvrA* and *uvrC* that were carried out once and twice, respectively.

***recF* and *recR* Show a Delay in Recovery of Replication.** To investigate how replication may be affected in *recF* cells after UV irradiation, we examined the recovery of replication after UV irradiation in these cells. Using the incorporation of [^{3}H]thymine to monitor replication, we found that after a dose of 25 J/m^2, wild-type cells exhibited a brief arrest before replication resumed at a rate comparable to that of unirradiated cultures. For wild-type cells, replication had fully recovered within 1 h after irradiation (Fig. 2*A*). In contrast, replication in *recF* and *recR* cells recovered more slowly, and a significant lag was observed before any replication resumed.

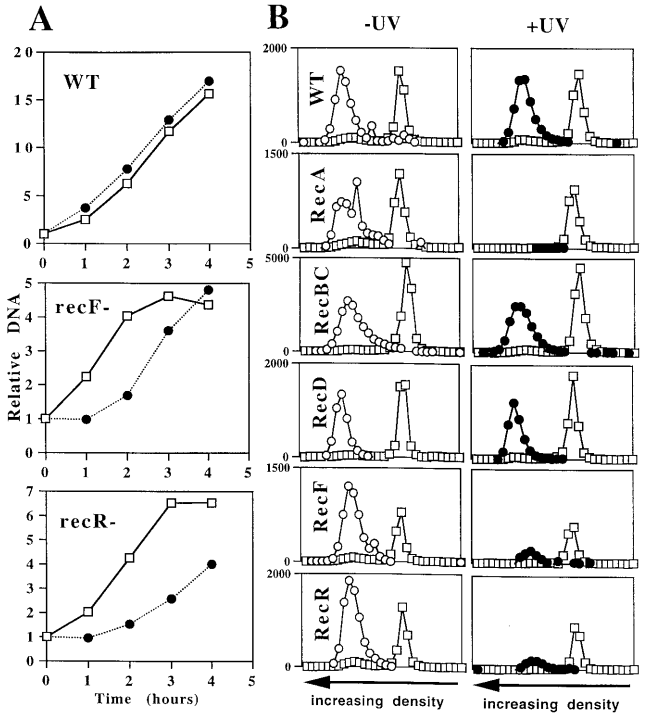

FIG. 2. *recF* and *recR* cells show a delay in the recovery of DNA synthesis after UV irradiation. (*A*) Cells were prelabeled with [^{3}H]thymine. At time 0, half the culture was removed and given a dose of 25 J/m^2 (●), while the other half was left unirradiated (□). The relative increase of DNA as measured by ^{3}H incorporation is plotted. (*B*) The amount of replication occurring within 1 h postirradiation was analyzed by alkaline CsCl density gradients. Cells prelabeled with [^{14}C]thymine were irradiated or not at time 0. Cells then were filtered and grown in media containing 5-bromodeoxyuridine and [^{3}H]thymine for 1 h to density label replication occurring after time 0. □, ^{14}C prelabeled DNA; ○, ^{3}H replicated DNA in unirradiated cultures; and ●, ^{3}H replicated DNA in irradiated cultures.

This effect also was observed by density-labeling the DNA with 5-bromodeoxyuridine to quantitate the amount of DNA replicated during the first hour after UV irradiation (Fig. 2*B*). Cultures receiving either 25 J/m^2 or no irradiation were incubated in medium containing 5-bromodeoxyuridine for 1 h, so that any DNA synthesized during this period would be of a greater density than that of DNA synthesized before the irradiation. The denser, replicated DNA in each culture was separated from the rest of the DNA in an isopycnic alkaline CsCl gradient and quantitated. By this measure, the wild-type cells had fully recovered within 1 h. Thus, the amount of replicated DNA in the irradiated culture was nearly equivalent to the amount occurring in an unirradiated culture. Neither *recF* nor *recR* cells, however, appeared to recover replication within this period. Interestingly, both *recBC* and *recD* cells appeared to recover fully within this time frame, suggesting that the defect in recovering replication is specific to the *recF* pathway and not likely to be due to increased cell death occurring in these populations. However *recA*, which is known to be required for replication recovery, also showed a complete lack of replication after UV (39).

Increased Degradation Occurs at Replication Forks in *recF* and *recR* Mutants. Because ongoing replication affected the UV survival of *recF* cells, we speculated that the replication defect after UV may occur at existing replication forks rather than at new origins of replication initiation. We therefore examined the DNA at replication forks after UV. Exponentially growing [^{14}C]thymine prelabeled cultures were pulse-labeled with [^{3}H]thymidine for 15 sec to label the DNA at replication forks, and then transferred to nonradioactive medium just before irradiation. This facilitated the comparison of the amount of degradation occurring in the nascent strands of DNA at replication forks and in the genome overall.

In wild-type cells, while very little degradation of the overall genomic DNA occurred, measurable degradation of the [^{3}H]-DNA was seen before replication recovered (Fig. 3). The evident accessibility of the DNA ends at replication forks to nuclease(s) suggests that the holoenzyme is often at least partially disrupted by DNA lesions, consistent with previous studies both *in vivo* and *in vitro* (40, 41). That complete degradation of the pulse-labeled DNA does not occur before replication recovers is also consistent with the conclusions of others that cells have a mechanism to recover replication at existing forks rather than to abandon or degrade the partially replicated chromosomes (39, 42).

In principle, the precipitable ^{3}H pulse label should remain constant or decrease over time. However, in wild-type cells we consistently saw an increase in precipitable ^{3}H label over time after replication had recovered. A comparison of the total ^{3}H within the cells with the acid-precipitable ^{3}H label suggested that the effect is likely to be due to remaining intracellular pools of labeled nucleotides (data not shown). Excessive washing and chasing with nonradioactive nucleotides did not significantly reduce this phenomenon.

In contrast to the limited degradation seen in wild-type cells, the DNA at replication forks in *recF* and *recR* mutants was observed to undergo significantly more degradation for a greater extent of time. That degradation also appeared to be largely specific to growing fork regions (Fig. 3). Genomic DNA was not extensively degraded in these cells. We interpret this to be consistent with the failure of *recF* and *recR* cells to recover DNA replication at the sites of existing DNA forks.

Unlike *recF* and *recR* cells, degradation occurring in *recA* cells was not specific to the growing fork DNA (Fig. 3). This is consistent with Skarstad and Boye (43) who reported that degradation of individual chromosomes occurs in *recA* cells in a *recD*-dependent manner. In contrast to the *recA* degradation, we found that the *recF* degradation occurring at the growing forks was dependent upon *recJ*, a 5′-3′ exonuclease belonging to the *recF* pathway, and was not dependent upon *recD* (unpublished results).

Decreased Ability to Complete Ongoing Replication in *recF* and *recR* Mutants. The preferential degradation of the growing fork DNA and the lag in recovery of replicative synthesis

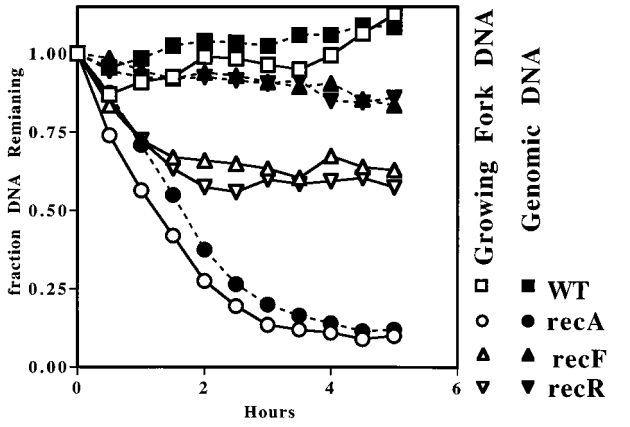

FIG. 3. Increased degradation occurs at the growing fork after irradiation in *recF* and *recR* cells. [^{3}H]Thymine was added to [^{14}C]thymine-prelabeled cells for 30 sec immediately before the cells were filtered and irradiated with 25 J/m^2 in nonlabeled medium. The fraction of the radioactive nucleotides remaining in the DNA is plotted over time. (■, □) Parental cells, (●, ○) *recF*, (▲, △) *recR*. Loss of ^{14}C genomic DNA (open symbols) can be compared with the loss of the [^{3}H] DNA synthesized at the growing fork just before irradiation (filled symbols).

in *recF* and *recR* mutants imply that *recF* and *recR* function is required after replication disruption for the reassembly of a replication holoenzyme at a DNA fork. This suggests that DNA lesions create problems for *recF* mutants not because of a repair deficiency but rather because the lesions disrupt replication. If replication could be disrupted in the absence of DNA damage, a prediction would be that *recF* and *recR* cells, but not wild-type cells, would show defects in the resumption of replication. For this reason, we looked at genomic replication in exponentially growing cells to see whether replication abnormalities could be detected in the absence of exogenous DNA damage. In our first approach, we used chloramphenicol or rifampicin to inhibit new rounds of DNA replication initiation and examined the cells' ability to complete the ongoing rounds of DNA synthesis as measured by incorporation of [^{3}H]thymine. By this criterion, both *recF* and *recR* cells were seen to accumulate less DNA than wild-type cells (Fig. 4*A*), suggesting that *recF* and *recR* cells are less able to complete ongoing rounds of DNA replication.

In an alternative approach, the chloramphenicol-treated cells were analyzed by FACS after replication had ceased (3 h after chloramphenicol treatment) to determine the chromosomal DNA content in individual cells. The *E. coli* chromosome contains one bidirectional origin of replication, and because all origins within a cell initiate synchronously, normal cells complete replication with 2^n chromosomes per cell (43). Typically when exponentially growing cultures were treated, wild-type cells completed replication with a distribution of 2, 4, or 8 chromosomes per cell (Fig. 4*B*). However, when *recF* or *recR* cells were grown under the same conditions, significant

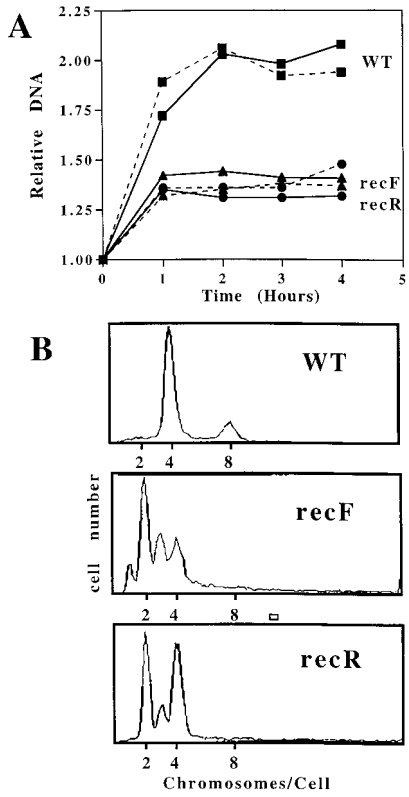

FIG. 4. RecF and RecR cells are less able to complete ongoing rounds of DNA replication. (*A*) Chloramphenicol or rifampicin was added to cells prelabeled with [^{3}H]thymine, and their ability to complete ongoing rounds of DNA synthesis as measured by net increase in DNA was analyzed. ■, Wild type; ●, *recF*; ▲, *recR*. Chloramphenicol (solid line); rifampicin (broken line). (*B*) Chloramphenicol and cephalexin were added to cells prelabeled with [^{3}H]thymine, and incubation continued for 3 h. The cells then were analyzed by FACS to determine the DNA content per cell.

deviations from this pattern were observed. In general, both mutants had fewer chromosomes per cell. In addition, however, cells containing odd numbers of chromosomes were seen. The irregular chromosome number is consistent with the idea that a portion of the replication attempts failed to go to completion. It is likely that the disrupted replication forks, upon failing to restart eventually are degraded as has been reported to occur in *recA* cells (44). Genomic replication requires that two holoenzymes each replicate approximately 2.5 megabases of DNA to then meet at the other side of the genome. If the processivity of the polymerase were less than this extent, successful replication of the chromosome would require the restart of the replication fork. These results are consistent with a defect of *recF* and *recR* cells in restarting disrupted replication forks. Disruption in the absence of DNA damage may occur because DNA-bound proteins or DNA secondary structures block replication, a lack of DNA precursors required for the elongation inhibits progression, or perhaps the inherent processivity of the holoenzyme sometimes is exceeded during genomic replication.

It is important to point out that isogenic *thy*$^-$ strains were used in these studies to facilitate efficient radioactive labeling of the DNA. When we tested thy$^+$ strains of *recF*, *recR*, and *recO*, all were found to have normal chromosome profiles by FACS analysis (data not shown), suggesting that replication is disrupted more frequently in the *thy*$^-$ background. Consistent with this, the *thy*$^-$ mutation has been shown to affect the elongation rate of replication forks, presumably because the efficiency of processing and delivery of thymine nucleotides to the replication machinery is compromised in the auxotrophs, causing replication to stall more frequently (45, 46). By itself, the *thy*$^-$ mutation presumably does not introduce any exogenous damage. We postulate that by hindering the forward elongation reaction in thy$^-$ cells, the disassembly of a replication fork is favored by the slowed or stalled replication complex. Recently, it also has been shown that thy$^-$ strains of *E. coli* and *Bacillus subtilis* are slightly more UV sensitive than wild-type cells (47). Whether this is a replication-dependent phenomenon has not been examined.

DISCUSSION

We have shown that the UV hypersensitivity of *recF* and *recR* cells correlates with ongoing replication at the time of UV irradiation. The lag in replication recovery, in addition to the preferential degradation at existing growing forks, suggests that the hypersensitivity arises from a failure to resume replication from the DNA replication forks disrupted after irradiation. Our results support the idea that *recF* and *recR* gene functions are required for the reassembly of a holoenzyme at the site of a DNA replication fork. In the absence of *recF* or *recR*, reassembly does not occur, and as a consequence, the DNA ends at the growing fork are accessible to more extensive degradation. These failed replication attempts can result in odd chromosome numbers and/or lethality when the cell is unable to recover or degrade the partially completed chromosomes.

While the problem of how replication deals with DNA damage has obvious relevance to both cell survival and mutagenesis, extremely little is known about it. Perhaps the simplest way one can imagine for a cell to deal with premature replication disruption would be to reassemble and reinitiate from the point of disruption. Presumably, in the case where a DNA lesion disrupts the replication holoenzyme, the disassembly and reassembly process would allow both the accessibility and time required for normal DNA repair processes to occur. Thus, the lack of any lesion blocking the resumption of DNA synthesis implies that there is no requirement for strand switching or recombination to occur during *recF*-dependent reinitiation. Mechanistically, the reassembly reaction could be

rather straightforward. RecF and RecR may be involved in recognizing the replication fork structure and assembling a holoenzyme at this site much as the DnaA protein recognizes the structure created at *oriC* (Fig. 5).

The well documented complexity of initiation from *oriC* demonstrates how critical the proper initiation of replication is for the cell. Through tight regulation of the time and location at which a replication fork is initiated, the cell ensures that upon division, each daughter cell will receive an equal and precise copy of the chromosome. Replication initiated either at the wrong time or at the wrong site(s) could be disastrous for the cell. Both thymineless death and stable DNA replication are phenomena that demonstrate the deleterious effects on viability caused by the loss of regulation in replication initiation (16, 48). Should a replication fork fall apart before reaching the terminus, the cell is faced with a dilemma. Simply abandoning the fork likely would create a lethal situation in which subsequent rounds of replication would only amplify the

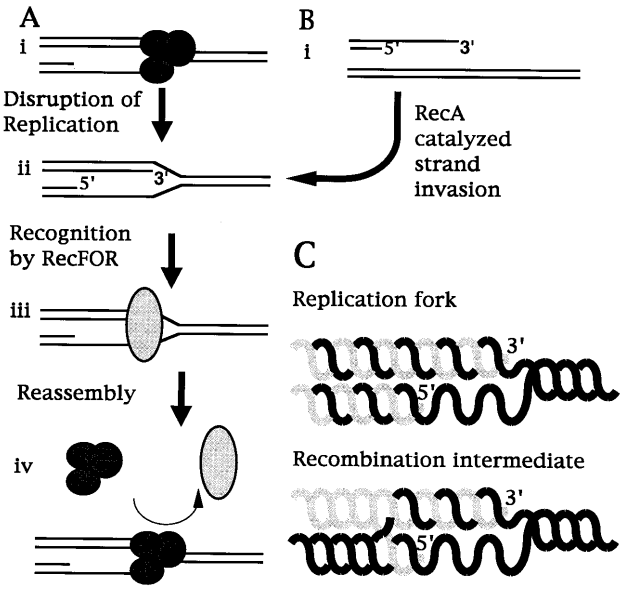

FIG. 5. Model of *recF* function *in vivo*. DNA synthesis occurs in a 5′-3′ direction on both strands of duplex DNA. Thus, during semiconservative replication there exists a single-stranded region near the replication fork on the lagging strand template, which will vary in length depending upon where the last lagging strand primer exists (*A*, *i*). During genomic replication, if the holoenzyme were to fall off before the completion of replication, the replication fork would be expected to have a structure similar to that shown (*ii*). Polymerization of the leading strand will terminate with a 3′ end inserted into the homologous double-stranded template DNA. In the simplest model consistent with our results, RecF, RecO, and RecR would recognize this structure as a disrupted replication fork (*iii*) and facilitate the reassembly of a replication holoenzyme at this structure such that semiconservative DNA synthesis could resume (*iv*). Such a function for the RecF proteins also could result in recombination when DNA ends are introduced into the system. DNA ends may be present when excessive damage has created strand breaks, when phage DNA has infected the cell, or when DNA has been transfected in artificially. In this situation, *recF*-dependent recombination is observed to occur when exonucleases process the DNA ends to leave 3′ overhangs (*B*, *i*). RecA, which is also required for *recF* recombination, is known to catalyze the strand invasion of 3′ single-stranded DNA into homologous duplex DNA. If this occurs, the structure created again would be a DNA strand terminating with a 3′ end inserted into homologous duplex DNA as shown (*ii*). Comparing the resulting structures one finds that they are very similar (*C*), suggesting that the *recF* pathway proteins also would recognize this structure. Replication initiated from these DNA ends would incorporate the foreign DNA into the host and result in a recombination event. Such a role for *recA* in recombination suggests that *in vivo* it may help maintain the replication fork after holoenzyme disruption.

partial genomes and create problems of hyperrecombinagenic ends, gene dosage problems, and problems for chromosomal segregation. Survival likely will require that the cell either degrade these aborted attempts or ensure that they are completed. Under conditions in which no exogenous source of DNA ends is introduced or generated within the cell, the structure shown in Fig. 5*C* should arise only when the holoenzyme is disrupted. If so, this would represent a legitimate substrate from which to initiate replication and still maintain the proper symmetry of replication forks per chromosome per cell.

Such a model implies that the UV sensitivity of *recF* is not due to a recombination defect. However, there exist abundant genetic and biochemical data demonstrating that recombination occurs in a *recF*-dependent fashion. The literature suggests that recombination initiated via the *recF* pathway requires the presence of a 3′ overhanging DNA end and the participation of the RecA protein. These requirements for recombination also may provide insight as to how RecF may be functioning at replication forks *in vivo*.

The requirement for a 3′ single-stranded DNA end is inferred from the properties of the exonucleases that are associated with the *recF* pathway of recombination. RecJ is a 5′-3′ exonuclease belonging to the *recF* pathway (49). Supressors that restore recombination to *recBC* mutants through activation of the *recF* pathway thus far have been shown to map to genes for other DNA exonucleases. SbcA and sbcB are generally thought to activate exoVIII, a 5′-3′ exonuclease, and to inactivate exoI, a 3′-5′ exonuclease, respectively (50, 51). SbcC and sbcD suppressors also are thought to encode nuclease activities, but their substrate specificities have not been defined (52). Thus, exonucleases involved in the *recF* pathway appear to process DNA ends so as to generate a 3′ overhang.

A second requirement for *recF* recombination is the RecA protein. Biochemically, RecA is known to use an invasive 3′ single-stranded end to initiate the pairing of homologous DNA sequences (53, 54). In general, the biochemical and genetic evidence suggests that *recF*-mediated recombination is initiated by RecA using a 3′ overhang to invade a homologous double-stranded target sequence as shown (Fig. 5*B*). When one compares the structure generated by the RecA strand invasion to that of a disrupted replication fork, there is a striking similarity (Fig. 5*C*). Just as RecA is thought to catalyze the strand invasion of a 3′ single-stranded end into homologous duplex DNA, the holoenzyme, through the polymerization of the leading strand, catalyzes the invasion of a 3′ single-stranded end into homologous duplex DNA. In essence, the disrupted replication fork is the product of the reaction catalyzed by RecA. Based upon the same biochemical reaction, the RecA protein should promote the maintenance of this structure should it begin to disassemble. Thus the substrate from which the cell reinitiates replication and the substrate believed to initiate *recF* recombination are structurally identical. It is then tempting to speculate that because *recF* and *recR* are required to reinitiate replication from this substrate *in vivo*, as we have shown, the recombination that results from the *recF* pathway may occur when foreign DNA ends present in the cell are processed to mimic those of a disrupted replication fork. In the case of recombination, the invading DNA has "fooled" the cell into believing that this is a legitimate substrate upon which to initiate replication.

The idea that a recombination deficiency is not responsible for the UV hypersensitivity in *recF* and *recR* mutants also can be inferred from genetic data on other genes in the recF pathway. *recJ* and *recQ* are also genes belonging to the *recF* pathway. Similar to *recF* and *recR*, these genes are required for recombination when the major pathway (*recBC*) is defective (55, 56). However, neither *recJ* nor *recQ* is hypersensitive to UV (55, 56). Thus, cells that should lack the ability to carry out

recombination via the *recF* pathway are not necessarily hypersensitive to UV.

Replication recently has been shown to be required for other forms of recombination as well. The primosomal mutant, *priA*, has been shown to be defective in both conjugational and transductional recombination. Interestingly, suppressor mutations that restore recombination in this background map to the *dnaC* gene (6, 57). The absolute requirement of replication for recombination to occur in these systems has led Kogoma (58) to suggest that all recombination and double-strand break repair in *E. coli* is carried out via replication. We believe that at the level of the chromosome it is worthwhile to consider the possibility that many of the classically defined recombination proteins may function to maintain the chromosome without DNA strand exchange.

Our *in vivo* observations of *recF* and *recR* suggest that their association with the replication machinery goes beyond the level of genomic organization. We see that RecF and RecR are required to resume replication from a replication fork, and that this can account for the UV hypersensitivity of these mutants. A general model is proposed for *recF* and *recR* function in which they participate to recognize a replication fork structure and reassemble a replication holoenzyme at this site. The model implies that the UV hypersensitivity is not due to a recombination defect, but is still consistent with many of the recombinational phenotypes associated with the *recF* pathway.

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1. Kowalczykowski, S. C., Dixon, D. A., Eggleston, A. K., Lauder, S. D. & Rehrauer, W. M. (1994) *Microbiol. Rev.* **58,** 401–465.
2. Smith, G. R. (1988) *Microbiol. Rev.* **52,** 1–28.
3. Emmerson, P. T. (1968) *Genetics* **60,** 19–30.
4. Capaldo-Kimball, F. & Barbour, S. D. (1971) *J. Bacteriol.* **106,** 204–212.
5. Horii, Z. & Clark, A. J. (1973) *J. Mol. Biol.* **80,** 327–344.
6. Sandler, S. J. (1996) *Mol. Microbiol.* **19,** 871–880.
7. Hegde, S. P., Rajagopalan, M. & Madiraju, M. V. (1996) *J. Bacteriol.* **178,** 184–190.
8. Webb, B. L., Cox, M. M. & Inman, R. B. (1995) *J. Biol. Chem.* **270,** 31397–31404.
9. Madiraju, M. V. & Clark, A. J. (1992) *J. Bacteriol.* **174,** 7705–7710.
10. Stahl, F. W., McMilin, K. D., Stahl, M. M. & Nozu, Y. (1972) *Proc. Natl. Acad. Sci. USA* **69,** 3598–3601.
11. Kusano, K., Nakayama, K. & Nakayama, H. (1989) *J. Mol. Biol.* **209,** 623–634.
12. Magee, T. R. & Kogoma, T. (1991) *J. Bacteriol.* **173,** 4736–4741.
13. Cohen, S. S. & Barner, H. D. (1954) *Proc. Natl. Acad. Sci. USA* **40,** 885–893.
14. Maaløe, O. & Hanawalt, P. C. (1961) *J. Mol. Biol.* **3,** 144–155.
15. Nakayama, K., Shiota, S. & Nakayama, H. (1988) *Can. J. Microbiol.* **34,** 905–907.
16. Kogoma, T. & Lark, K. G. (1970) *J. Mol. Biol.* **52,** 143–164.
17. Magee, T. R. & Kogoma, T. (1990) *J. Bacteriol.* **172,** 1834–1839.
18. Cooper, P. K. & Hanawalt, P. C. (1972) *J. Mol. Biol.* **67,** 1–10.
19. Cooper, P. K. (1982) *Mol. Gen. Genet.* **185,** 189–197.
20. Cooper, P. K. (1990) *Carcinogenic DNA Damage; Department of Energy Project Summary* (University of California Lawrence Berkeley Laboratory, Berkeley).
21. Flower, A. M. & McHenry, C. S. (1991) *J. Mol. Biol.* **220,** 649–658.
22. Perez-Roger, I., Garcia-Sogo, M., Navarro-Avino, J. P., Lopez-Acedo, C., Macian, F. & Armengod, M. E. (1991) *Biochimie* **73,** 329–334.
23. Ream, L. W., Margossian, L., Clark, A. J., Hansen, F. G. & von Meyenburg, K. (1980) *Mol. Gen. Genet.* **180,** 115–121.
24. Mellon, I. & Hanawalt, P. C. (1989) *Nature (London)* **342,** 95–98.
25. Sandler, S. J. & Clark, A. J. (1994) *J. Bacteriol.* **176,** 3661–3672.
26. Willis, D. K., Uhlin, B. E., Amini, K. S. & Clark, A. J. (1981) *Mol. Gen. Genet.* **183,** 497–504.
27. Amundsen, S. K., Neiman, A. M., Thibodeaux, S. M. & Smith, G. R. (1990) *Genetics* **126,** 25–40.
28. Chaudhury, A. M. & Smith, G. R. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 7850–7854.
29. Schultz, D. W., Taylor, A. F. & Smith, G. R. (1983) *J. Bacteriol.* **155,** 664–680.
30. Bridges, B. A. (1984) *Mutat. Res.* **132,** 153–160.
31. Singer, M., Baker, T. A., Schnitzler, G., Deischel, S. M., Goel, M., Dove, W., Jaacks, K. J., Grossman, A. D., Erickson, J. W. & Gross, C. A. (1989) *Microbiol. Rev.* **53,** 1–24.
32. Smith, C. A., Cooper, P. K. & Hanawalt, P. C. (1981) in *DNA Repair: A Manual of Research Proceedures*, eds. Friedberg, E. C. & Hanawalt, P. C. (Dekker, New York), Vol. 1B, pp. 289–305.
33. Boye, E. & Lobner-Olesen, A. (1991) *Res. Microbiol.* **142,** 131–135.
34. Hanawalt, P. C., Maaløe, O., Cummings, O. & Schaecter, M. (1961) *J. Mol. Biol.* **3,** 156–165.
35. Lark, K. G. (1973) *J. Bacteriol.* **113,** 1066–1069.
36. Hanawalt, P. C. (1966) *J. Photochem. Photobiol. B* **5,** 1–12.
37. Tang, M. S. & Smith, K. C. (1981) *Mutat. Res.* **80,** 15–25.
38. Rothman, R. H. & Clark, A. J. (1977) *Mol. Gen. Genet.* **155,** 279–86.
39. Khidhir, M. A., Casaregola, S. & Holland, I. B. (1985) *Mol. Gen. Genet.* **199,** 133–140.
40. Hanawalt, P. & Brempelis, I. (1967) *Proceedings of the 7th International Congress of Biochemistry, Japan,* p. 650 (abstr.).
41. Livneh, Z. (1986) *J. Biol. Chem.* **261,** 9526–9533.
42. Rupp, W. D. & Howard-Flanders, P. (1968) *J. Mol. Biol.* **31,** 291–304.
43. Skarstad, K., Bernander, R. & Boye, E. (1995) *Methods Enzymol.* **262,** 604–613.
44. Skarstad, K. & Boye, E. (1993) *J. Bacteriol.* **175,** 5505–5509.
45. Zaritsky, A. (1970) *J. Gen. Microbiol.* **63,** vi-vii.
46. Pritchard, R. H. & Zaritsky, A. (1970) *Nature (London)* **226,** 126–131.
47. Lojo, M. M. (1995) *Mutat. Res.* **347,** 25–30.
48. Nakayama, K., Kusano, K., Irino, N. & Nakayama, H. (1994) *J. Mol. Biol.* **243,** 611–620.
49. Lovett, S. T. & Kolodner, R. D. (1989) *Proc. Natl. Acad. Sci. USA* **86,** 2627–2631.
50. Phillips, G. J., Prasher, D. C. & Kushner, S. R. (1988) *J. Bacteriol.* **170,** 2089–2094.
51. Joseph, J. W. & Kolodner, R. (1983) *J. Biol. Chem.* **258,** 10411–10417.
52. Leach, D. R., Lloyd, R. G. & Coulson, A. F. (1992) *Genetica* **87,** 95–100.
53. Konforti, B. B. & Davis, R. W. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 690–694.
54. Konforti, B. B. & Davis, R. W. (1990) *J. Biol. Chem.* **265,** 6916–6920.
55. Nakayama, H., Nakayama, K., Nakayama, R., Irino, N., Nakayama, Y. & Hanawalt, P. C. (1984) *Mol. Gen. Genet.* **195,** 474–480.
56. Lovett, S. T. & Clark, A. J. (1984) *J. Bacteriol.* **157,** 190–196.
57. Kogoma, T., Cadwell, G. W., Barnard, K. G. & Asai, T. (1996) *J. Bacteriol.* **178,** 1258–1264.
58. Kogoma, T. (1996) *Cell* **85,** 625–628.