RuvAB and RecG Are Not Essential for the Recovery of DNA Synthesis Following UV-Induced DNA Damage in *Escherichia coli*

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ABSTRACT

Ultraviolet light induces DNA lesions that block the progression of the replication machinery. Several models speculate that the resumption of replication following disruption by UV-induced DNA damage requires regression of the nascent DNA or migration of the replication machinery away from the blocking lesion to allow repair or bypass of the lesion to occur. Both RuvAB and RecG catalyze branch migration of three- and four-stranded DNA junctions *in vitro* and are proposed to catalyze fork regression *in vivo*. To examine this possibility, we characterized the recovery of DNA synthesis in *ruvAB* and *recG* mutants. We found that in the absence of either RecG or RuvAB, arrested replication forks are maintained and DNA synthesis is resumed with kinetics that are similar to those in wild-type cells. The data presented here indicate that RecG- or RuvAB-catalyzed fork regression is not essential for DNA synthesis to resume following arrest by UV-induced DNA damage *in vivo*.

ALL cells must accurately replicate their entire genome each time they reproduce. Although the replication machinery is extremely processive, DNA damage such as that induced by near-ultraviolet light (254 nm) can block the progression of the DNA replication machinery and prevent it from completing its task (Setlow *et al.* 1963; Howard-Flanders *et al.* 1968). The failure to accurately resume replication following disruption by DNA damage can result in mutation if an incorrect nucleotide is incorporated, rearrangement if replication resumes from the wrong site, or lethality if the blocking lesions cannot be overcome. In *Escherichia coli*, the recovery of replication following UV irradiation correlates with the time at which the lesions have been repaired by nucleotide excision repair (Courcelle *et al.* 1999, 2003). Cells deficient in nucleotide excision repair are unable to remove UV-induced DNA lesions, fail to recover replication, and exhibit elevated levels of mutagenesis, rearrangements, and cell lethality (Howard-Flanders 1968; Howard-Flanders *et al.* 1968; Rothman and Clark 1977; Courcelle and Hanawalt 2001; Hanawalt 2002).

The recovery of replication also depends on RecA and several gene products of the RecF pathway (Rothman and Clark 1977; Courcelle *et al.* 1997, 1999; Courcelle and Hanawalt 2001). In the absence of RecA, RecF, RecO, or RecR, the blocked replication fork is not maintained, replication fails to recover, and extensive degradation of the nascent DNA at the replication fork occurs (Courcelle *et al.* 1997, 1999, 2003; Chow and Courcelle 2004). The RecQ helicase and RecJ nuclease also belong to the RecF pathway and partially degrade the nascent lagging strand of the arrested replication fork prior to the resumption of replication (Courcelle and Hanawalt 1999; Courcelle *et al.* 2003). These observations have led to the general model that RecA and these RecF pathway gene products function to maintain and process blocked replication forks until the blocking lesion can be repaired by nucleotide excision repair or bypassed by translesion DNA polymerases (Courcelle *et al.* 1997, 1999, 2003; Rangarajan *et al.* 2002). It is proposed that RecF, RecO, and RecR help stabilize activated RecA filaments at the arrested replication fork structure, thereby maintaining the replication fork DNA and limiting the degradation of the nascent DNA by RecJ and RecQ (Courcelle *et al.* 1997, 1999, 2003; Chow and Courcelle 2004). The genetic observation that mutations in *recF*, *recO*, or *recR* delay the induction of LexA-regulated gene expression following DNA damage is consistent with the idea that there is less activated RecA present at early times when RecF-O-R is absent (Thoms and Wackernagel 1987; Hegde *et al.* 1995).

On the basis of this model, it has been proposed that the repair of the DNA lesions in this situation may require displacement of the arrested replication machinery and nascent DNA to allow repair enzymes to gain access to the damaged region (Courcelle *et al.* 1997, 1999, 2001). The displacement of the nascent DNA would allow the parental template strands in that region to reanneal, in effect reversing the branch point of the replication fork to generate a four-arm regressed intermediate (Figure 1). Other models have speculated that replication fork regression could facilitate a recombina-

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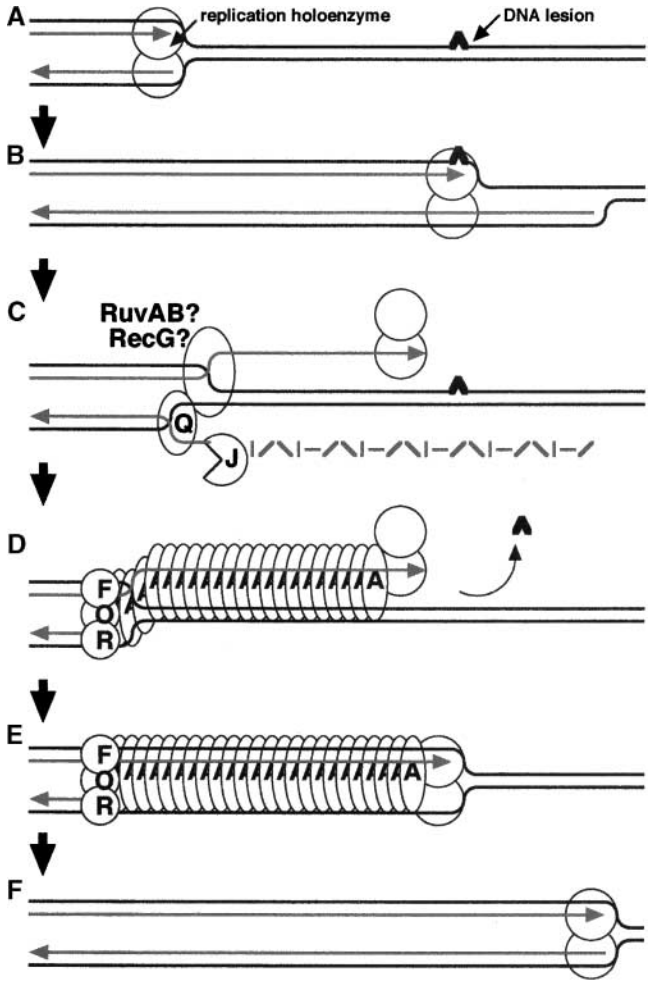

FIGURE 1.—Proposed function of RecG or RuvAB during the recovery of replication forks arrested at UV-induced lesions. (A) Replication is normally extremely processive (B) but is arrested by DNA lesions in the leading strand template (HIGUCHI *et al.* 2003; PAGES and FUCHS 2003). (C) RecQ displaces the nascent lagging strand for degradation by RecJ. On the basis of *in vitro* characterizations, it has been suggested that RecG or RuvAB catalyze fork regression as part of the process required for the recovery of replication. (D) RecFOR and RecA limit nascent DNA degradation and maintain the replication fork until the blocking lesions can be repaired or bypassed. (E) Then the replication fork can be reestablished and (F) processive replication can resume.

tion-mediated template switch that allows synthesis to occur past the blocking lesion (HIGGINS *et al.* 1976; KUZMINOV 2001; MICHEL *et al.* 2001; COX 2002; LUSETTI and COX 2002; JAKTAJI and LLOYD 2003; WEST 2003).

Direct evidence for regressed replication fork intermediates has been observed following replication arrest on plasmids. Plasmid replication forks blocked by the DNA-binding protein Tus form a reversed intermediate both *in vivo* and *in vitro* (POSTOW *et al.* 2001; OLAVARRIETA *et al.* 2002). In this case, replication fork regression occurs spontaneously following arrest due to the unwinding of positive supercoils ahead of the replication fork (POSTOW *et al.* 2001). A transient regression of the replication fork also occurs following arrest by UV-induced DNA damage on plasmids *in vivo* (COURCELLE *et al.* 2003). The regressed replication fork persists until a time correlating with lesion removal and the resumption of DNA replication. Similar to arrested replication forks on the chromosome, the arrested replication fork intermediates on the plasmid are maintained by RecA, RecF, RecO, and RecR and are processed by RecQ and RecJ (COURCELLE *et al.* 2003).

Although UV-induced replication fork reversal occurs on plasmids, it is not known whether fork regression also occurs on the bacterial chromosome or whether fork regression is required for replication to resume following disruption. Both RecG and RuvAB have been proposed to catalyze fork reversal *in vivo* on the basis of their *in vitro* activities (COURCELLE *et al.* 2001; MCGLYNN and LLOYD 2001a,b). The *ruv* locus, consisting of *ruvA*, *ruvB*, and *ruvC*, was originally identified in a genetic screen for UV-sensitive mutants (OTSUJI *et al.* 1974). In addition to their hypersensitivity to UV, *ruv* mutants also exhibit lower recombination frequencies during conjugation and transduction and abnormalities in cell division following UV irradiation as seen by the accumulation of long filamentous cells that fail to undergo septation (OTSUJI *et al.* 1974; LLOYD *et al.* 1984). Purified RuvA and RuvB form a complex that binds to Holliday junctions and promotes ATP-dependent branch migration (PARSONS *et al.* 1992; PARSONS and WEST 1993). RuvC interacts with RuvAB at Holliday junctions and produces symmetric endonucleolytic incisions at the crossover point to resolve joint molecules (CONNOLLY *et al.* 1991). *In vitro*, RuvAB can promote branch migration on synthetic replication fork structures to form Holliday junctions (MCGLYNN and LLOYD 2001a). However, the enzyme complex preferentially catalyzes the reverse reaction, converting a four-arm Holliday junction into a three-arm, replication fork-like structure (MCGLYNN and LLOYD 2001a).

Mutations that inactivate RecG also render cells moderately sensitive to UV and reduce the frequency of conjugational recombination (STORM *et al.* 1971). Purified RecG is a helicase that is also capable of promoting branch migration of Holliday junctions (LLOYD and SHARPLES 1993). In addition, RecG catalyzes the conversion of synthetic three-arm replication fork substrates into four-arm molecules in a manner that preferentially displaces what would represent the nascent lagging strand (MCGLYNN and LLOYD 1999, 2001b).

These biochemical characterizations have led to the general view that RecG and potentially RuvAB are required for the recovery of replication following UV-induced DNA damage. We examined this possibility directly and observed that following replication disruption by UV-induced DNA damage, the replication fork is maintained and DNA synthesis resumes at a time comparable to that of wild type when either RecG or RuvAB is absent.

MATERIALS AND METHODS

Bacterial strains and UV irradiation: Our parental strain, SR108, is a *thyA36 deoC2* derivative of W3110 (De Lucia and Cairns 1969). The strains HL946 (SR108 *recF332::*Tn*3*) and HL921 (SR108 *recA306::*Tn*10*) have been described previously (Courcelle *et al.* 1997, 1999). The strains CL008 (SR108 *recG258::*Tn*5*), CL532 (SR108 *ruvA59::*Tn*10*), and CL578 (SR108 *ruvAB6204::kan*) were constructed by P1 transduction of the *recG258::*Tn*5*, *ruvA59::*Tn*10*, and *ruvAB6204::kan* alleles from JC19245 (gift from S. Sandler), RDK2641 (Lombardo and Rosenberg 2000), and TP541 (Murphy *et al.* 2000), respectively, into SR108. The strains CL628 (SR108 *recQ6215::cam recF332::*Tn*3*), CL011 (SR108 *recG258::*Tn*5 recF332::*Tn*3*), and CL744 (SR108 *ruvAB6204::kan recF332::Tn3*) were constructed by P1 transduction of the *recQ6215::cam*, *recG258::*Tn*5*, and *ruv AB6204::kan* alleles from TP648 (Murphy *et al.* 2000), CL008, and CL578, respectively, into HL946. The strain CL561 (SR108 *recG258::*Tn*5 ruvA59::*Tn*10*) was constructed by P1 transduction of the *recG258::*Tn*5* allele into CL532. *ruvA59::*Tn*10* is reported to be a polar mutation affecting both *ruvA* and *ruvB* (Sharples *et al.* 1990). Phenotypes were confirmed by antibiotic resistance and, when appropriate, UV hypersensitivity or nascent DNA degradation. UV irradiation for all experiments was performed using a Sylvania 15-W germicidal lamp (254 nm) at an incident dose of 0.9 J/m^2/sec.

UV survival studies: Fresh overnight cultures were diluted 1:100 in 10 ml of Davis medium (2.0 g KH_2PO_4, 7.0 g K_2HPO_4, 0.5 g $Na_3C_6H_5O_7$, 0.1 g $MgSO_4$, 1.0 g $(NH_4)_2SO_4$ per liter, pH 7.0) supplemented with 0.4% glucose, 0.2% casamino acids, and 10 μg/ml thymine (DGCthy medium) and grown to an OD_{600} of 0.5 in a 37° shaking incubator. Serial dilutions of each culture were plated in triplicate on Luria-Bertani plates supplemented with 10 μg/ml thymine and UV irradiated at the indicated doses. Plates were incubated overnight at 37° and colonies were counted the next day.

Growth rates: Fresh overnight cultures were diluted 1:1000 in DGCthy medium and 200-μl aliquots were plated on a 96-well microtiter plate. The OD_{600} for each culture was measured with Molecular Devices (Menlo Park, CA) SPECTRAmax Plus and analyzed with SOFTmax Pro 4.0.

Total DNA accumulation: Fresh overnight cultures were diluted 1:100 in 40 ml DGCthy medium supplemented with 0.1 μCi/ml [^{3}H]thymine (60.5 Ci/mmol) and grown to an OD_{600} of 0.4 in a 37° shaking incubator. At this time, half the culture was UV irradiated with 27 J/m^2 and the other half was mock irradiated. At 5 min intervals, duplicate 200-μl aliquots were precipitated in 5 ml of 5% trichloroacetic acid (TCA) and filtered onto Millipore glass fiber prefilters. The amount of ^{3}H-labeled DNA on each filter was determined by liquid scintillation counting (Courcelle *et al.* 1999).

Density labeling and CsCl analysis: Fresh overnight cultures were diluted 1:100 in 20 ml of DGCthy medium supplemented with 0.1 μCi/ml of [^{14}C]thymine (53 mCi/mmol) and were grown to an OD_{600} of 0.5 (~10^8 cells/ml) in a 37° shaking incubator. At this time, half the culture was UV irradiated with 27 J/m^2 and the other half was mock irradiated. Cultures were then filtered onto FisherBrand general filtration 0.45-μm membranes, washed with NET buffer (10 mm NaCl, 10 mm Tris, pH 8.0, 10 mm EDTA, pH 8.0), resuspended in 10 ml DGC medium supplemented with 20 μg/ml 5-bromouracil in place of thymine and 0.5 μCi/ml [^{3}H]thymine (60.5 Ci/mmol), and allowed to recover for a period of 1 hr in a 37° shaking incubator. Two volumes of ice-cold NET buffer were added to the 10-ml cultures, and the cells were then pelleted, resuspended in 150 μl TE (10 mm Tris, 1 mm EDTA, pH 8.0), and lysed in 170 μl of 0.5 m H_2KPO_4/KOH, pH 12.5, and 1.25% Sarkosyl. Isopycnic alkali CsCl gradients composed of 0.3 g of a DNA lysate solution, 2.23 g CsCl, and 3.31 g of a 0.1 m H_2KPO_4/KOH, pH 12.5, solution (refractive index 1.4055) were centrifuged to equilibrium at 80,000 × *g* for 96 hr at 20°. Gradients were collected in ~30 fractions onto Whatman no. 17 paper, washed in 5% TCA, and then washed in 95% ethanol. The quantity of ^{3}H and ^{14}C in each fraction was determined by liquid scintillation counting (Courcelle *et al.* 1997).

Rate of DNA synthesis: The assay to measure the rate of DNA synthesis was modified from previous studies (Khidhir *et al.* 1985; Rangarajan *et al.* 2002). Fresh overnight cultures were diluted 1:100 in 50 ml of DGCthy medium supplemented with 0.1 μCi/ml of [^{14}C]thymine (53 mCi/mmol) and grown to an OD_{600} of precisely 0.3 in a 37° shaking incubator. At this time, half the culture was UV irradiated with 27 J/m^2 and the other half was mock irradiated. At the indicated times, 1 μCi/ml [^{3}H]thymidine (77.8 Ci/mmol) was added to duplicate 0.5-ml aliquots for 2 min at 37°, before the cells were lysed and DNA precipitated in 5 ml of 5% TCA and filtered onto Millipore glass fiber prefilters. The amount of ^{3}H and ^{14}C on each filter was determined by liquid scintillation counting.

Degradation of nascent and genomic DNA: Fresh overnight cultures were diluted 1:100 in 10 ml DGCthy medium supplemented with 0.1 μCi/ml [^{14}C]thymine (53 mCi/mmol) and grown to an OD_{600} of 0.4 in a 37° shaking incubator. Cultures were labeled for 5 sec with 1 μCi/ml [^{3}H]thymidine (77.8 Ci/mmol), filtered onto FisherBrand general filtration 0.45-μm membranes, washed with NET buffer, and resuspended in nonradioactive DGCthy medium. Cultures were immediately irradiated with a UV dose of 27 J/m^2. At the indicated times, duplicate 0.2-ml aliquots (triplicate for the 0 time point) were precipitated in 5 ml of 5% cold TCA and filtered on Millipore glass fiber prefilters. The amount of ^{3}H and ^{14}C on each filter was determined by liquid scintillation counting (Courcelle *et al.* 1997).

RESULTS

RuvAB and RecG are not required for the recovery of DNA synthesis following UV-induced DNA damage: Isogenic strains lacking RecG, RuvAB, or both gene products were constructed by standard P1 transduction. As previously reported, the *recG* and *ruvAB* mutants were moderately hypersensitive to UV irradiation (Figure 2A; Ryder *et al.* 1994; Bolt and Lloyd 2002; Gregg *et al.* 2002). Hypersensitivity was more severe in the *ruvAB recG* double mutant than in either single mutant and was comparable to that of *recA* (Figure 2A). However, unlike *recA*, the *ruvAB recG* double mutant grew poorly even in the absence of exogenous DNA damage (Figure 2B). Previous studies have documented that RecA is absolutely required for the recovery of replication following inhibition of DNA synthesis (Howard-Flanders 1968), yet *recA* mutants grow at rates comparable to wild-type cells in the absence of DNA damage. This observation then suggests that replication is not frequently inhibited in the absence of DNA damage. Furthermore, the poor growth of the *ruvAB recG* mutant relative to that of *recA* under these same conditions indicates that RuvAB or RecG is required to process DNA structures other than disrupted replication forks that arise during the normal replication cycle. However, this observation alone does not preclude the possibility

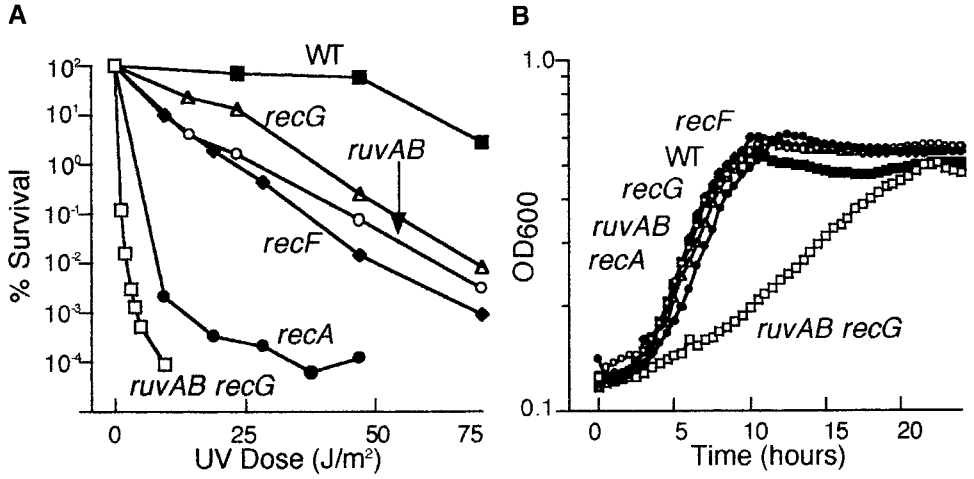

FIGURE 2.—Survival following UV irradiation and growth in the absence of DNA damage of wild-type, *recF*, *recG*, *ruvAB*, and *ruvAB recG* strains of *E. coli*. (A) The percentage of cells surviving the indicated dose of UV irradiation is plotted for each strain. Survival curves represent an average of at least two independent experiments. (B) The OD_{600} of each strain is plotted over time. ■, wild-type; ◆, *recF*; △, *recG*; ○, *ruvAB*; ●, *recA*; and □, *ruvAB recG*.

that, in addition to these alternative roles, they may also be required to process replication forks prior to their recovery.

To determine whether the hypersensitivity of *ruvAB* or *recG* mutants results directly from a failure to resume DNA synthesis following disruption by UV irradiation, we monitored DNA synthesis after UV irradiation in these mutants by [^{3}H]thymine incorporation. Following a UV dose of 27 J/m^2, wild-type cultures exhibited a transient arrest of replication before synthesis resumed at a rate comparable to that in unirradiated cultures (Figure 3). In contrast, *recF* mutants, which are deficient in the resumption of disrupted replication forks (COURCELLE *et al.* 1997, 1999), exhibited no further increase in the amount of ^{3}H-labeled DNA following UV irradiation. When we examined UV-irradiated cultures of *ruv AB* or *recG*, we observed that both mutants resumed replication at a time comparable to that in wild-type cultures (Figure 3).

We also examined *ruvAB recG* double mutants to determine if the absence of both gene products prevented the recovery of replication following UV-induced DNA damage. In these mutants, the rate of DNA synthesis recovered to an extent that was comparable to unirradiated *ruvAB recG* cultures. However, the slow growth that occurs in unirradiated *ruvAB recG* cultures makes it inappropriate to compare the recovery observed in this mutant directly to wild-type cells.

The recovery of replication in *ruvAB* and *recG* mutants was also monitored by density labeling the DNA synthesized during the first hour following UV irradiation. Irradiated or mock-irradiated cultures were incubated in medium containing 5-bromouracil in place of thymine for 1 hr such that the density of the DNA made during this period was greater than that of the DNA synthesized before treatment. DNA synthesized during the recovery period was then isolated and quantitated in isopycnic alkali CsCl gradients. By this measure, wild-type cultures had almost completely recovered replication 1 hr after UV irradiation, as seen by the nearly equivalent amounts of DNA synthesis in the irradiated and unirradiated cultures (Figure 4). By contrast, very little DNA synthesis occurred following UV treatment in *recF* mutants. When we examined postirradiation DNA synthesis in *ruvAB* and *recG* mutants, we observed an amount of DNA synthesis that was comparable to the unirradiated controls, indicating that DNA synthesis was resuming similar to that in wild-type cultures (Figure 4).

In *ruvAB recG* double mutants, we observed an intermediate amount of DNA synthesis in the irradiated culture relative to the unirradiated culture. However, both irradiated and nonirradiated cultures exhibited abnormal patterns of replication, with a significant amount of the DNA synthesis migrating at densities in the intermediate and light regions of the gradient. DNA migrating in these regions may indicate elevated levels of recombination or repair synthesis. The detection of this type of synthesis in unirradiated *ruvAB recG* mutants may be due in part to the toxicity associated with the 5-bromouracil that is used to density label the DNA in this assay. The toxicity of 5-bromouracil is thought to be due in part to the lower incorporation efficiency of this base analogue compared to thymine and also because the bromine group on the analogue is labile, leading to elevated levels of uracil and uracil glycolyase-induced nicks in the DNA. Incubation in media containing 5-bromouracil results in elevated levels of sister chromatid exchanges and cell death within approximately two rounds of replication (HACKETT and HANAWALT 1966; LITTLE 1976; KRASIN and HUTCHINSON 1978). Thus, similar to the previous assay, a direct comparison between wild-type and *ruvAB recG* mutants should be interpreted with caution. However, some DNA synthesis occurs in the UV-irradiated *ruvAB recG* mutants, although the viability of these cells is clearly compromised and abnormal relative to wild-type cells even in the absence of UV irradiation.

The previous two assays indicate that replication recovers in the absence of either *recG* or *ruvAB*. However, it remains possible that although robust replication re-

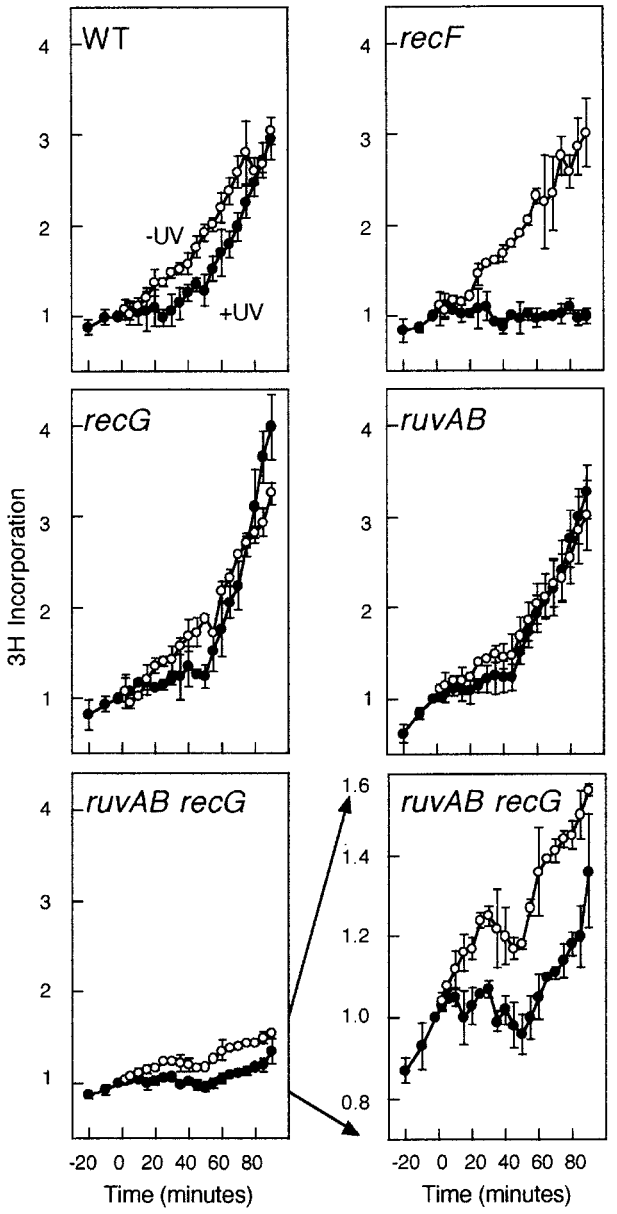

FIGURE 3.—*recG* and *ruvAB* recover robust replication at a time similar to that in wild-type cells. Cultures grown in the presence of [^{3}H]thymine were either UV irradiated with 27 J/m^2 (●) or mock irradiated (○). The amount of ^{3}H incorporated over time is plotted. Cultures were irradiated at time 0. Each graph represents an average of three independent experiments. Error bars represent 1 SD.

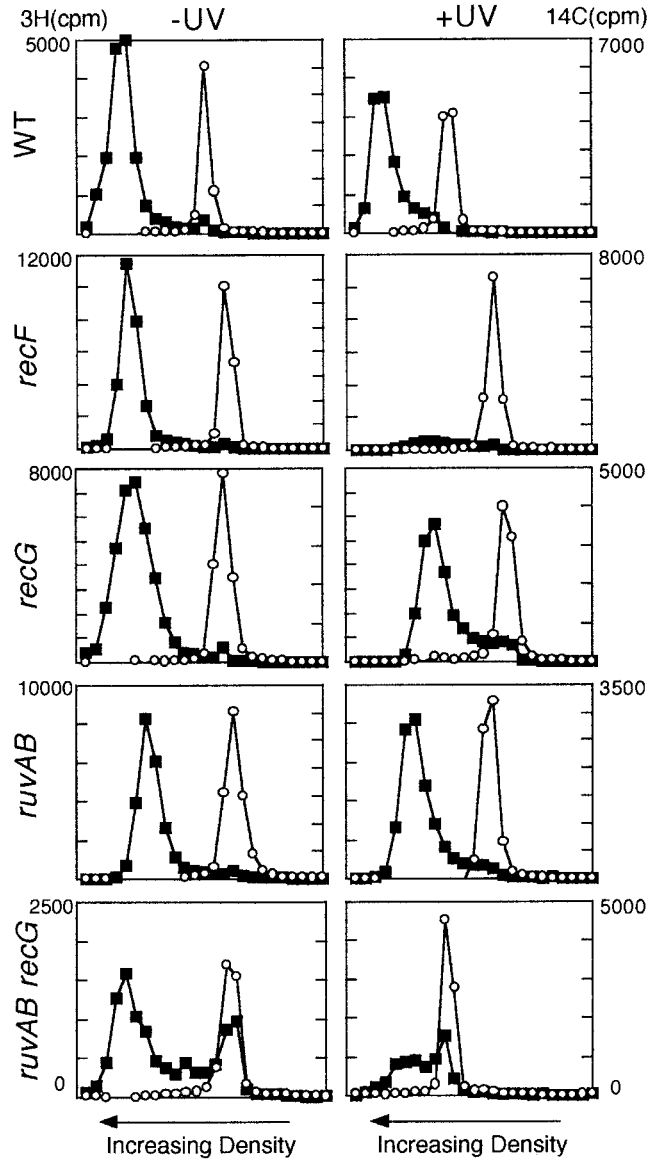

FIGURE 4.—*recG* and *ruvAB* cultures synthesize an amount of DNA similar to that of wild-type cultures during the first hour after UV irradiation. The amount of DNA synthesized in UV-irradiated (25 J/m^2) or mock-irradiated cultures was determined by density labeling the DNA with 5-bromouracil and subsequent isolation in alkali CsCl gradients. ○, DNA synthesized before treatment (^{14}C); ■, DNA synthesized following treatment (^{3}H). Each graph represents one of at least two independent experiments.

sumes in *ruvAB* or *recG* mutants, the time at which DNA synthesis recovers may be delayed relative to that of wild type. To examine this possibility in *recG* and *ruvAB* mutants, we measured the rate of DNA synthesis following UV irradiation by incubating [^{14}C]thymine-labeled cultures for 2 min with [^{3}H]thymidine at various times after treatment. The rate of DNA synthesis (^{3}H incorporation/min) could then be determined relative to the total amount of DNA present (^{14}C incorporation) at specific times following treatment. Using this assay, we observed that the rate of DNA synthesis was reduced by ~90% in wild-type cells at early times following UV irradiation (Figure 5). Within 20 min, the rate of DNA synthesis began to recover, and by 40 min, the rate of replication was nearly restored to preirradiation levels and there was a detectable increase in total DNA accumulation. In UV-irradiated *recF* mutants, the reduction in DNA synthesis was more severe and, consistent with our previous assays, the rate of synthesis did not recover. However, following UV irradiation of *recG* or *ruvAB* mu-

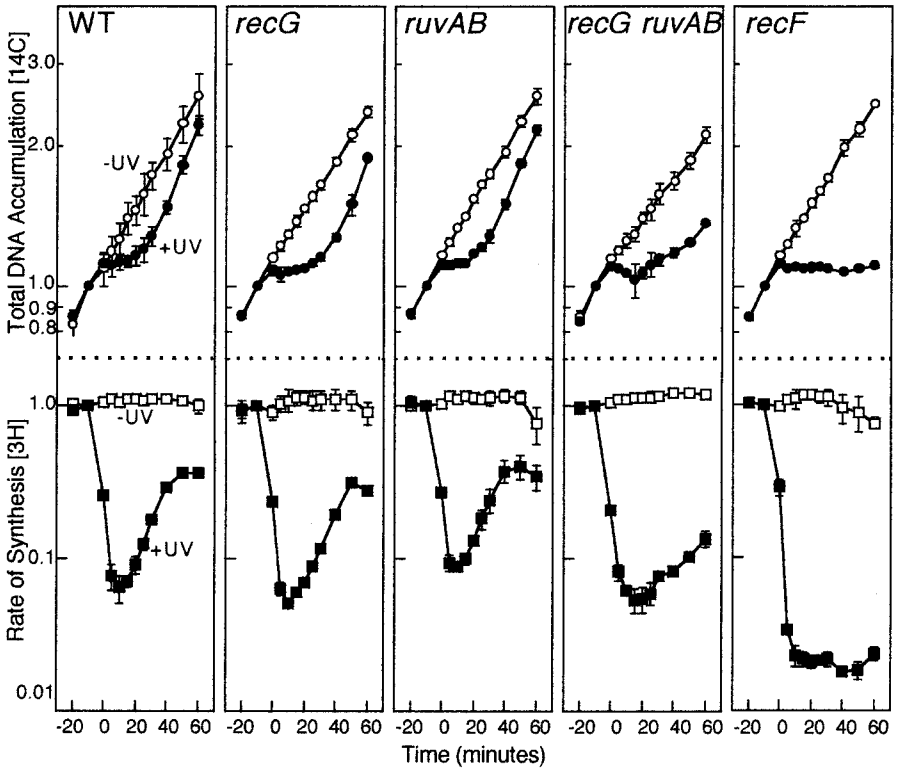

FIGURE 5.—*recG* and *ruvAB* resume DNA synthesis with kinetics similar to that of wild-type following UV irradiation. Cultures grown in the presence of [^{14}C]thymine were pulse-labeled with [^{3}H]thymidine for 2 min at the indicated times following either 27 J/m^2 of UV irradiation or mock irradiation. The relative amount of ^{14}C and ^{3}H incorporated into the DNA is plotted over time. Cultures were irradiated at time 0. Graphs represent an average of three independent experiments. Error bars represent 1 SD. ○, total DNA in mock-irradiated cultures; ●, total DNA in irradiated cultures; □, rate of DNA synthesis in mock-irradiated cultures; ■, rate of DNA synthesis in irradiated cultures.

tants, we observed that the time and efficiency with which DNA synthesis recovered were similar to those in wild type. These observations indicate that RuvAB or RecG function is not essential for replication to resume following disruption by UV-induced DNA damage. In the *ruvAB recG* double mutants, the rate of DNA synthesis recovered to a significant extent and approximated the recovery observed in wild-type cultures much more closely than that observed in *recF* mutants. Although direct comparisons between these strains should be made with caution, the observation that DNA synthesis is inhibited to a greater extent in *recF* mutants than in *ruvAB recG* double mutants suggests that the recovery of DNA synthesis in the single mutants is not due to the simple interpretation that RecG and RuvAB serve redundant functions in this respect. The double mutant recovers to a greater extent than the *recF* mutant despite the fact that it is much more sensitive to DNA damage and grows more poorly than the *recF* mutant (Figure 2).

RuvAB and RecG are not required to maintain the replication fork after UV irradiation: Strains lacking RecF, RecO, or RecR fail to maintain disrupted replication forks, resulting in extensive degradation of the nascent DNA at the replication fork (COURCELLE *et al.* 1997, 2003). Both RecG and RuvAB have also been proposed to act on arrested replication fork structures *in vivo* (MCGLYNN and LLOYD 2001a,b). To determine whether RuvAB or RecG are required to maintain replication forks arrested at UV-induced DNA damage *in vivo*, we measured the amount of degradation that occurred in the nascent DNA at the replication fork. To this end, ^{14}C-labeled cultures were pulse labeled with [^{3}H]thymidine for 5 sec, transferred to nonradioactive media, and immediately UV irradiated. Then, the amount of radioactivity remaining in the DNA was followed over time. This assay allowed us to compare the amount of degradation that occurred in the nascent strands of the replication fork directly to the total DNA in the cell. In UV-irradiated wild-type cells, we observed a limited amount of nascent DNA degradation at times prior to the recovery of replication, consistent with our previous studies (Figure 6; COURCELLE and HANAWALT 1999). In *recF* mutants, the replication fork was not maintained and approximately half of the nascent DNA was degraded. By comparison, in *ruvAB* or *recG* mutants, the nascent DNA was not extensively degraded following UV irradiation. In addition, *ruvAB recG* double mutants did not exhibit extensive degradation of DNA following UV irradiation, indicating that these gene products are not required to maintain or protect the nascent DNA at replication forks.

The nascent DNA degradation that occurs prior to the resumption of replication is dependent on RecQ helicase and RecJ nuclease (COURCELLE and HANAWALT 1999). RecQ helicase is required to displace the nascent lagging strand for degradation by RecJ nuclease *in vivo*. On the basis of *in vitro* characterizations, it has been proposed that RecG and potentially RuvAB also displace the nascent lagging strand of arrested replication forks (MCGLYNN *et al.* 2001; MICHEL *et al.* 2001). If true, then we would predict that inactivation of RecG or RuvAB should also prevent nascent DNA degradation from oc-

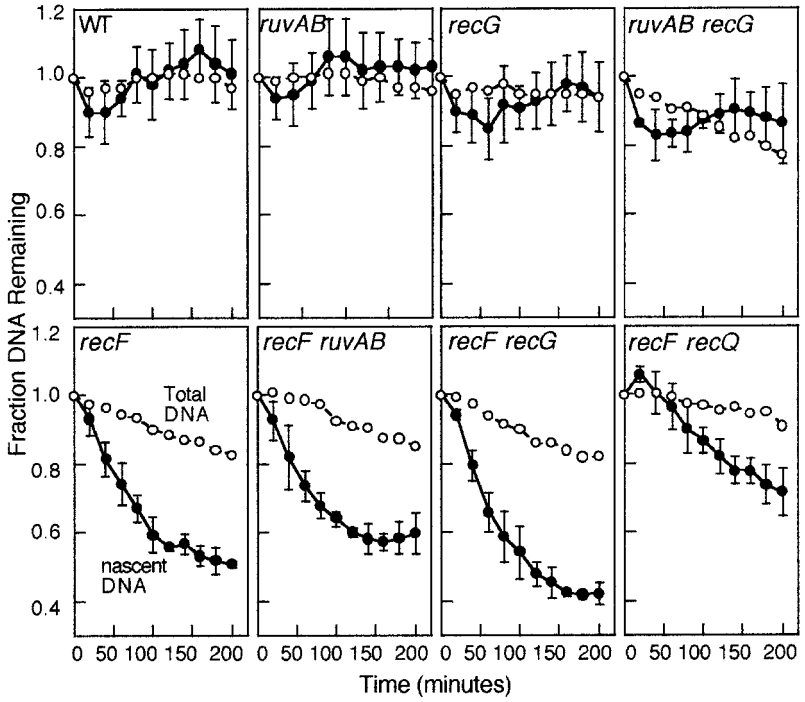

FIGURE 6.—RuvAB or RecG is not required to maintain arrested replication forks or displace the nascent DNA prior to degradation following UV irradiation. [^{3}H]Thymidine was added to [^{14}C]thymine prelabeled cells for 5 sec, washed, and UV irradiated in nonlabeled medium. The relative amount of ^{3}H and ^{14}C remaining in the DNA is plotted over time. Graphs represent an average of at least three independent experiments for each strain. Error bars represent 1 SD. ○, total DNA (^{14}C); ●, nascent DNA (^{3}H).

curring similar to when RecQ is inactivated. To test this possibility, we examined the degradation that occurred in UV-irradiated *recF* mutants that were also deficient in either RuvAB or RecG. As shown in Figure 6, the absence of RuvAB or RecG did not prevent the extensive degradation of the nascent DNA in *recF* mutants. In contrast, in *recF* mutants that also lacked RecQ, the degradation of nascent DNA was significantly reduced. The lack of nascent DNA processing in the *recF recQ* mutant was most evident during the first hour following UV irradiation (Figure 6). This result indicates that RuvAB and RecG are not required to displace the nascent lagging strand prior to degradation *in vivo*.

Since this assay specifically measures nascent DNA degradation, and previous studies have shown this degradation occurs preferentially on the nascent lagging strand (COURCELLE and HANAWALT 1999), it remains possible that RuvAB or RecG facilitates the displacement of the nascent leading strand or portions of the lagging strand that are not subject to degradation and therefore are not detected in this assay.

DISCUSSION

On the basis of biochemical data, several studies have speculated that either RecG or RuvAB catalyze replication fork regression *in vivo* and play a critical role in promoting the recovery of replication when it is blocked by DNA damage (MCGLYNN and LLOYD 1999, 2001a,b; BOLT and LLOYD 2002; GREGG *et al.* 2002). Using a number of cellular assays, we examined the contribution of RuvAB and RecG to the ability of cells to recover replication following UV irradiation *in vivo*. We found that the absence of either RecG or RuvAB does not affect the cell's ability to resume DNA synthesis. In addition, unlike RecF, RecO, or RecR, we observe that RuvAB or RecG is not required to maintain replication forks following arrest by DNA damage and that neither protein prevents the extensive nascent DNA degradation that occurs in the absence of RecF.

Although these results cannot exclude the possibility that RuvAB or RecG proteins catalyze fork regression *in vivo*, they demonstrate that their function is not required for DNA synthesis to resume following UV-induced DNA damage. It remains possible that RuvAB- or RecG-catalyzed replication fork regression increases the accuracy or fidelity of replication recovery, but that the regression is not essential for the resumption to occur. By analogy, both RecJ and RecQ process or partially degrade the nascent DNA at arrested replication forks in a manner that is believed to increase the frequency that replication resumes from the proper location (COURCELLE and HANAWALT 1999). However, the absence of either RecJ or RecQ does not prevent replication from resuming following UV irradiation, although it does affect the time at which DNA synthesis resumes (COURCELLE and HANAWALT 1999). A second possibility is that fork regression catalyzed by RecG and RuvAB is required for recovery in only a small subset of the total arrested-fork substrates and therefore is below our limit of detection in these cellular assays. Another possibility is that alternative or redundant activities may allow replication to recover in the absence of RecG or RuvAB. Along this line of reasoning, RadA was recently proposed to offer a third potentially redundant activity for replication fork processing on the basis of survival stud-

ies (Beam *et al.* 2002). However, if either RecG- or RuvAB-catalyzed fork regression is a predominant mechanism by which arrested replication forks normally resume, it seems reasonable to expect that we would have observed a delay in the timing, or a reduction in the efficiency, of the resumption of DNA synthesis. Even allowing for potential redundancies, one might expect that the secondary activity would promote recovery with different (or reduced) kinetics when the primary activity is absent. Our observations show that even though *ruvAB* and *recG* mutants are more sensitive to UV irradiation than wild-type cells are, *ruvAB* and *recG* mutants are able to recover DNA synthesis as efficiently as wild-type cells, arguing against a *requirement* for either of these enzymes in a prominent pathway that allows DNA synthesis to resume.

The poor growth of *ruvAB recG* double mutants is often interpreted to suggest that replication is frequently disrupted by DNA damage or other impediments during replication, which then requires processing by branch migration enzymes to resume (Mandal *et al.* 1993; Ryder *et al.* 1994). However, cell viability is an extremely broad criterion by which to measure a specific question such as the ability for DNA synthesis to resume. The survival of a cell could also be compromised by any of a large number of alternative DNA processing events such as chromosome partitioning, replication termination, or resolution of joint dimer chromosomes, among others. The observation that wild-type cells recover from UV doses that reduce the viability of *recG* or *ruvAB* mutants by >99% highlights the observation that these enzymes are essential for some DNA processing event that arises in these cells following moderate levels of DNA damage. If any of the several processing events mentioned above were to function as the preferred targets for these branch migration enzymes, the normal resumption of DNA synthesis would not be expected to be impaired, but could result in elevated levels of lethality in the presence of DNA damage. A second possibility is that the DNA synthesis that occurs in *recG* or *ruvAB* mutants represents an aberrant form of DNA synthesis, potentially resuming from the wrong template, and leads to lethality in the absence of RecG or RuvAB processing. However, it is clear from the observations presented in this study that the lethality is not the result of a failure to resume DNA synthesis, such as occurs in *recF* or *recA* mutants.

Other genetic studies have previously been interpreted to support a role for RuvAB or RecG at arrested replication forks. Following prolonged incubation of a thermosensitive *dnaB* mutant at the restrictive temperature, elevated levels of double-strand breaks accumulate in the genome of *recBC* mutants as observed by pulsed-field gel electrophoresis (Michel *et al.* 1997). The accumulation of double-strand breaks in *dnaB recB* mutants requires RuvABC function (Seigneur *et al.* 1998). On the basis of these observations, it has been speculated by others that inactivation of replication proteins such as DnaB may mimic the disruption that occurs following replication fork encounters with DNA damage (Gregg *et al.* 2002; Jaktaji and Lloyd 2003). It was further speculated that if this interpretation were true, then the RuvAB-dependent double-strand breaks could be explained if RuvAB catalyzed the formation of Holliday junctions at stalled replication forks, which are then cleaved by RuvC endonuclease or degraded by RecBCD (Cox *et al.* 2000; Bolt and Lloyd 2002; Gregg *et al.* 2002). On the basis of these observations in thermosensitive replication mutants, it has been inferred from several subsequent studies that RuvABC and RecBCD are required to resume replication following arrest by DNA damage. However, our observations indicate that the resumption of DNA synthesis following UV-induced DNA damage does not require RuvAB, and several previous studies have shown that replication resumes normally in *recBC* and *recD* mutants following UV-induced DNA damage (Khidhir *et al.* 1985; Courcelle *et al.* 1997; Courcelle and Hanawalt 1999). Furthermore, although RecJ and RecQ process the nascent DNA at lesion-arrested replication forks, RecBCD does not degrade the nascent DNA at arrested replication forks (Khidhir *et al.* 1985; Courcelle *et al.* 1997; Courcelle and Hanawalt 1999). Therefore, we believe these observations indicate that the events and enzymes operating at lesion-blocked replication forks are different from those that occur following the removal of specific proteins of the replication machinery.

The basis for the proposal that RecG may promote the rescue of arrested replication forks *in vivo* comes primarily from survival studies following UV irradiation (Gregg *et al.* 2002; Jaktaji and Lloyd 2003). By examining the survival of *recG* mutants following UV irradiation in various genetic backgrounds, it has been widely speculated that RecG promotes the rescue of stalled replication forks through a number of different recovery pathways (McGlynn and Lloyd 2000, 2002; Dillingham and Kowalczykowski 2001; Gregg *et al.* 2002; Jaktaji and Lloyd 2003). However, if this were true, one would predict that the absence of RecG would have an effect on the cell's ability to recover DNA synthesis following UV irradiation. Our observation that *recG* recovers DNA synthesis with kinetics similar to that in wild-type cells argues against the interpretation that RecG has an essential role in promoting the rescue of arrested replication forks following UV-induced DNA damage. However, this does not necessarily exclude the possibility that RecG participates in the recovery process or possibly enhances strand displacement at arrested replication forks.

Although many gene products have been intensely studied for how they affect recombinational processes over the years, the conceptual realization that many of the "*rec*" gene products function to maintain the strands of genetic information rather than rearrange them during chromosome replication has been suggested previously and investigated recently (Campbell 1984; Cour-

celle *et al.* 1997). This perspective has generated significant amounts of discussion and renewed enthusiasm concerning how replication recovers following disruption. As a result, in some cases, it has been speculated that genes are required for this process primarily on the basis of the observation that their respective mutants grow poorly or render cells hypersensitive to UV. RuvAB and RecG have both been characterized for their recombinational roles and only recently were proposed to promote the recovery of replication on the basis of the observation that the mutants are moderately hypersensitive to UV irradiation (McGlynn and Lloyd 2001a,b; McGlynn *et al.* 2001; Bolt and Lloyd 2002). Although this represents a good starting point for investigation, the recovery of replication is not the only process required for successful reproduction, and UV hypersensitivity does not always correlate with a defect in the recovery of replication. For example, both *recF* and *recBC* mutants are equally hypersensitive to UV irradiation, but while *recF* mutants fail to recover replication following UV irradiation, *recBC* mutants recover replication normally (Courcelle *et al.* 1997; Courcelle and Hanawalt 1999). By comparison, *recJ* and *recQ* mutants are not sensitive to UV irradiation, yet these gene products participate in the processing of nascent DNA at replication forks prior to the recovery of replication (Courcelle and Hanawalt 1999). Although the results presented here cannot exclude the possibility that RuvAB or RecG participate in the regression of the arrested replication forks, we can conclude that neither gene product is essential for DNA synthesis to resume. This implies that the hypersensitivity of these mutants arises from an event(s) that potentially occurs after replication resumes or because replication resumes from the wrong template or the wrong place in their absence. It will be important to develop novel cellular assays to determine whether replication fork regression is required for the resumption of replication on the bacterial chromosome. In light of these observations, it may also be useful to consider potential alternative roles for RecG and RuvAB function following UV-induced DNA damage.

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LITERATURE CITED

Beam, C. E., C. J. Saveson and S. T. Lovett, 2002 Role for *radA/sms* in recombination intermediate processing in *Escherichia coli*. J. Bacteriol. **184:** 6836–6844.

Bolt, E. L., and R. G. Lloyd, 2002 Substrate specificity of RusA resolvase reveals the DNA structures targeted by RuvAB and RecG *in vivo*. Mol. Cell **10:** 187–198.

Campbell, A., 1984 Types of recombination: common problems and common strategies. Cold Spring Harbor Symp. Quant. Biol. **49:** 839–844.

Chow, K. H., and J. Courcelle, 2004 RecO acts with RecF and RecR to protect and maintain replication forks blocked by UV-induced DNA damage in *Escherichia coli*. J. Biol. Chem. **279:** 3492–3496.

Connolly, B., C. A. Parsons, F. E. Benson, H. J. Dunderdale, G. J. Sharples *et al.*, 1991 Resolution of Holliday junctions *in vitro* requires the Escherichia coli *ruvC* gene product. Proc. Natl. Acad. Sci. USA **88:** 6063–6067.

Courcelle, J., and P. C. Hanawalt, 1999 RecQ and RecJ process blocked replication forks prior to the resumption of replication in UV-irradiated *Escherichia coli*. Mol. Gen. Genet. **262:** 543–551.

Courcelle, J., and P. C. Hanawalt, 2001 Participation of recombination proteins in rescue of arrested replication forks in UV-irradiated *Escherichia coli* need not involve recombination. Proc. Natl. Acad. Sci. USA **98:** 8196–8202.

Courcelle, J., C. Carswell-Crumpton and P. C. Hanawalt, 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.

Courcelle, J., D. J. Crowley and P. C. Hanawalt, 1999 Recovery of DNA replication in UV-irradiated Escherichia coli requires both excision repair and recF protein function. J. Bacteriol. **181:** 916–922.

Courcelle, J., A. K. Ganesan and P. C. Hanawalt, 2001 Therefore, what are recombination proteins there for? BioEssays **23:** 463–470.

Courcelle, J., J. R. Donaldson, K. H. Chow and C. T. Courcelle, 2003 DNA damage-induced replication fork regression and processing in *Escherichia coli*. Science **299:** 1064–1067.

Cox, M. M., 2002 The nonmutagenic repair of broken replication forks via recombination. Mutat. Res. **510:** 107–120.

Cox, M. M., M. F. Goodman, K. N. Kreuzer, D. J. Sherratt, S. J. Sandler *et al.*, 2000 The importance of repairing stalled replication forks. Nature **404:** 37–41.

De Lucia, P., and J. Cairns, 1969 Isolation of an *E. coli* strain with a mutation affecting DNA polymerase. Nature **224:** 1164–1166.

Dillingham, M. S., and S. C. Kowalczykowski, 2001 A step backward in advancing DNA replication: rescue of stalled replication forks by RecG. Mol. Cell **8:** 734–736.

Gregg, A. V., P. McGlynn, R. P. Jaktaji and R. G. Lloyd, 2002 Direct rescue of stalled DNA replication forks via the combined action of PriA and RecG helicase activities. Mol. Cell **9:** 241–251.

Hackett, P., Jr., and P. Hanawalt, 1966 Selectivity for thymine over 5-bromouracil by a thymine-requiring bacterium. Biochim. Biophys. Acta **123:** 356–363.

Hanawalt, P. C., 2002 Subpathways of nucleotide excision repair and their regulation. Oncogene **21:** 8949–8956.

Hegde, S., S. J. Sandler, A. J. Clark and M. V. Madiraju, 1995 *recO* and *recR* mutations delay induction of the SOS response in *Escherichia coli*. Mol. Gen. Genet. **246:** 254–258.

Higgins, N. P., K. Kato and B. Strauss, 1976 A model for replication repair in mammalian cells. J. Mol. Biol. **101:** 417–425.

Higuchi, K., T. Katayama, S. Iwai, M. Hidaka, T. Horiuchi *et al.*, 2003 Fate of DNA replication fork encountering a single DNA lesion during *oriC* plasmid DNA replication *in vitro*. Genes Cells **8:** 437–449.

Howard-Flanders, P., 1968 Some properties of excision-defective recombination-deficient mutants of *Escherichia coli* K-12. J. Bacteriol. **97:** 1134–1141.

Howard-Flanders, P., W. D. Rupp, B. M. Wilkins and R. S. Cole, 1968 DNA replication and recombination after UV irradiation. Cold Spring Harbor Symp. Quant. Biol. **33:** 195–207.

Jaktaji, R. P., and R. G. Lloyd, 2003 PriA supports two distinct pathways for replication restart in UV-irradiated *Escherichia coli* cells. Mol. Microbiol. **47:** 1091–1100.

Khidhir, M. A., S. Casaregola and I. B. Holland, 1985 Mechanism of transient inhibition of DNA synthesis in ultraviolet-irradiated *E. coli*: inhibition is independent of *recA* whilst recovery requires RecA protein itself and an additional, inducible SOS function. Mol. Gen. Genet. **199:** 133–140.

Krasin, F., and F. Hutchinson, 1978 Strand breaks and alkali-labile bonds induced by ultraviolet light in DNA with 5-bromouracil in vivo. Biophys. J. **24:** 657–664.

Kuzminov, A., 2001 DNA replication meets genetic exchange: chromosomal damage and its repair by homologous recombination. Proc. Natl. Acad. Sci. USA **98:** 8461–8468.

Little, J. W., 1976 The effect of 5-bromouracil on recombination of phage lambda. Virology **72:** 530–535.

Lloyd, R. G., and G. J. Sharples, 1993 Processing of recombination intermediates by the RecG and RuvAB proteins of *Escherichia coli*. Nucleic Acids Res. **21:** 1719–1725.

Lloyd, R. G., F. E. Benson and C. E. Shurvinton, 1984 Effect of *ruv* mutations on recombination and DNA repair in *Escherichia coli* K12. Mol. Gen. Genet. **194:** 303–309.

Lombardo, M. J., and S. M. Rosenberg, 2000 *radC102* of *Escherichia coli* is an allele of *recG*. J. Bacteriol. **182:** 6287–6291.

Lusetti, S. L., and M. M. Cox, 2002 The bacterial RecA protein and the recombinational DNA repair of stalled replication forks. Annu. Rev. Biochem. **71:** 71–100.

Mandal, T. N., A. A. Mahdi, G. J. Sharples and R. G. Lloyd, 1993 Resolution of Holliday intermediates in recombination and DNA repair: indirect suppression of *ruvA*, *ruvB*, and *ruvC* mutations. J. Bacteriol. **175:** 4325–4334.

McGlynn, P., and R. G. Lloyd, 1999 RecG helicase activity at three- and four-strand DNA structures. Nucleic Acids Res. **27:** 3049–3056.

McGlynn, P., and R. G. Lloyd, 2000 Modulation of RNA polymerase by (p)ppGpp reveals a RecG-dependent mechanism for replication fork progression. Cell **101:** 35–45.

McGlynn, P., and R. G. Lloyd, 2001a Action of RuvAB at replication fork structures. J. Biol. Chem. **276:** 41938–41944.

McGlynn, P., and R. G. Lloyd, 2001b Rescue of stalled replication forks by RecG: simultaneous translocation on the leading and lagging strand templates supports an active DNA unwinding model of fork reversal and Holliday junction formation. Proc. Natl. Acad. Sci. USA **98:** 8227–8234.

McGlynn, P., and R. G. Lloyd, 2002 Genome stability and the processing of damaged replication forks by RecG. Trends Genet. **18:** 413–419.

McGlynn, P., R. G. Lloyd and K. J. Marians, 2001 Formation of Holliday junctions by regression of nascent DNA in intermediates containing stalled replication forks: RecG stimulates regression even when the DNA is negatively supercoiled. Proc. Natl. Acad. Sci. USA **98:** 8235–8240.

Michel, B., S. D. Ehrlich and M. Uzest, 1997 DNA double-strand breaks caused by replication arrest. EMBO J. **16:** 430–438.

Michel, B., M. J. Flores, E. Viguera, G. Grompone, M. Seigneur *et al.*, 2001 Rescue of arrested replication forks by homologous recombination. Proc. Natl. Acad. Sci. USA **98:** 8181–8188.

Murphy, K. C., K. G. Campellone and A. R. Poteete, 2000 PCR-mediated gene replacement in *Escherichia coli*. Gene **246:** 321–330.

Olavarrieta, L., M. L. Martinez-Robles, J. M. Sogo, A. Stasiak, P. Hernandez *et al.*, 2002 Supercoiling, knotting and replication fork reversal in partially replicated plasmids. Nucleic Acids Res. **30:** 656–666.

Otsuji, N., H. Iyehara and Y. Hideshima, 1974 Isolation and characterization of an *Escherichia coli ruv* mutant which forms nonseptate filaments after low doses of ultraviolet light irradiation. J. Bacteriol. **117:** 337–344.

Pages, V., and R. P. Fuchs, 2003 Uncoupling of leading- and lagging-strand DNA replication during lesion bypass *in vivo*. Science **300:** 1300–1303.

Parsons, C. A., and S. C. West, 1993 Formation of a RuvAB-Holliday junction complex *in vitro*. J. Mol. Biol. **232:** 397–405.

Parsons, C. A., I. Tsaneva, R. G. Lloyd and S. C. West, 1992 Interaction of *Escherichia coli* RuvA and RuvB proteins with synthetic Holliday junctions. Proc. Natl. Acad. Sci. USA **89:** 5452–5456.

Postow, L., C. Ullsperger, R. W. Keller, C. Bustamante, A. V. Vologodskii *et al.*, 2001 Positive torsional strain causes the formation of a four-way junction at replication forks. J. Biol. Chem. **276:** 2790–2796.

Rangarajan, S., R. Woodgate and M. F. Goodman, 2002 Replication restart in UV-irradiated *Escherichia coli* involving pols II, III, V, PriA, RecA and RecFOR proteins. Mol. Microbiol. **43:** 617–628.

Rothman, R. H., and A. J. Clark, 1977 The dependence of postreplication repair on *uvrB* in a *recF* mutant of *Escherichia coli* K-12. Mol. Gen. Genet. **155:** 279–286.

Ryder, L., M. C. Whitby and R. G. Lloyd, 1994 Mutation of *recF*, *recJ*, *recO*, *recQ*, or *recR* improves Hfr recombination in resolvase-deficient *ruv recG* strains of *Escherichia coli*. J. Bacteriol. **176:** 1570–1577.

Seigneur, M., V. Bidnenko, S. D. Ehrlich and B. Michel, 1998 RuvAB acts at arrested replication forks. Cell **95:** 419–430.

Setlow, R. B., P. A. Swenson and W. L. Carrier, 1963 Thymine dimers and inhibition of DNA synthesis by ultraviolet irradiation of cells. Science **142:** 1464–1466.

Sharples, G. J., F. E. Benson, G. T. Illing and R. G. Lloyd, 1990 Molecular and functional analysis of the *ruv* region of *Escherichia coli* K-12 reveals three genes involved in DNA repair and recombination. Mol. Gen. Genet. **221:** 219–226.

Storm, P. K., W. P. Hoekstra, P. G. deHaan and C. Verhoef, 1971 Genetic recombination in *Escherichia coli*. IV. Isolation and characterization of recombination-deficiency mutants of *Escherichia coli* K12. Mutat. Res. **13:** 9–17.

Thoms, B., and W. Wackernagel, 1987 Regulatory role of *recF* in the SOS response of *Escherichia coli*: impaired induction of SOS genes by UV irradiation and nalidixic acid in a *recF* mutant. J. Bacteriol. **169:** 1731–1736.

West, S. C., 2003 Molecular views of recombination proteins and their control. Nat. Rev. Mol. Cell. Biol. **4:** 435–445.

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