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Roles of *ruvA*, *ruvC* and *recG* Gene Functions in Normal and DNA Damage-Inducible Replication of the *Escherichia coli* Chromosome

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ABSTRACT

Induction of the SOS response in *Escherichia coli* activates normally repressed DNA replication which is termed inducible stable DNA replication (iSDR). We previously demonstrated that initiation of iSDR requires the products of genes, such as *recA*, *recB* and *recC*, that are involved in the early stages of homologous recombination. By measuring the copy number increase of the origin (*oriM1*) region on the chromosome, we show, in this study, that initiation of iSDR is stimulated by mutations in the *ruvA*, *ruvC* and *recG* genes which are involved in the late stages of homologous recombination. Continuation of iSDR, on the other hand, is inhibited by these mutations. The results suggest that Holliday recombination intermediates, left on the chromosome due to abortive recombination, arrest replication fork movement. Low levels of iSDR and *sfiA* (*sulA*) gene expression were also observed in exponentially growing *ruvA*, *ruvC* and *recG* mutants, suggesting that the SOS response is chronically induced in these mutants. We propose that replication forks are arrested in these mutants, albeit at a low frequency, even under the normal (uninduced) conditions.

DNA replication and recombination occur concurrently in *Escherichia coli* cells and are tightly interrelated. The relationship becomes evident especially in SOS-induced cells. Blockade of an active replication fork by DNA-damaging treatments or thymine deprivation induces the SOS response [for a review, see Walker (1984)]. The response profoundly alters several aspects of DNA replication [see Witkin (1991) for a review]. Initiation of chromosome replication usually occurs at *oriC* and requires DnaA protein, transcription and translation [for a review see McMacken *et al.* (1987)]. After induction of the SOS response, however, the chromosome can be replicated in the absence of these factors (Kogoma and Lark 1970, 1975; Ciesla and Jonczyk 1980; Magee *et al.* 1992) [for a review, see Asai and Kogoma (1994)]. This type of DNA replication, which is termed inducible stable DNA replication (iSDR), thus occurs in the presence of rifampicin (RIF) and chloramphenicol (CAM), inhibitors of transcription and translation, respectively. Two origins (*oriM1A* and *oriM1B*) for iSDR are mapped in two separable regions within the minimal *oriC* (Asai *et al.* 1994). The *terC* region also contains another origin (*oriM2*) for iSDR (Magee *et al.* 1992). The replication originating from these origins proceeds bidirectionally (Magee *et al.* 1992).

Initiation of iSDR requires gene functions that are involved in the early stages of homologous recombination. iSDR is not induced in *recA428* mutant cells which are defective in homologous recombination but proficient in SOS induction (Asai *et al.* 1993), indicating that the recombinase activity of RecA is necessary for iSDR initiation. The helicase activity of RecBC or RecBCD enzyme, which unwinds linear double-stranded (ds) DNA from the ends, is also essential for iSDR induction (Magee and Kogoma 1990; Asai *et al.* 1993). This was concluded from the observation that *recD*(Null) mutants are proficient in iSDR induction whereas *recB* and *recC* mutations block the induction completely. The only enzymatic activity thought to remain in the RecBC enzyme prepared from *recD*(Null) mutant cells is the helicase activity (Palas and Kushner 1990) [for a review, see Rosenberg and Hastings (1991)]. Recently, *in vivo* experiments have also demonstrated that RecBC enzyme in *recD* mutant cells is capable of DNA unwinding, but not DNA degradation (Rinken *et al.* 1992).

Defects in the RecBCD pathway of homologous recombination in *recB* and *recC* mutants are suppressed by a mutation in the *sbcA* gene or by mutations in the *sbcB* and *sbcC* genes, which activate the RecE or RecF alternative pathways, respectively [for reviews, see Smith (1988) and Clark (1991)]. Inactivation of the *sbcA* gene leads to the expression of exonuclease VIII (Exo VIII), the *recE* gene product, and also the *recT* gene product, both of which are essential for the RecE pathway (Clark *et al.* 1993). Exo VIII digests one strand of dsDNA processively from the 5′ end (Joseph and Kolodner 1993). We have previously demonstrated that defects of *recBC* mutants in iSDR induction are also suppressed by an *sbcA* mutation and that the suppression is abolished by a mutation in the *recE* gene (Asai *et al.* 1993). These

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results strongly support the conclusion that homologous recombination is involved in iSDR initiation and indicate that the RecE pathway can mediate the initiation reaction. Similarly, iSDR is induced in *recBC sbcB sbcC* mutant cells and the inducibility is eliminated by a mutation in the *recJ* gene, which is involved in the RecF pathway of homologous recombination (T. ASAI and T. KOGOMA, manuscript in preparation). This indicates that the RecF pathway can also catalyze the initiation reaction of iSDR.

On the basis of the above and other observations, we previously proposed a model (the D-loop model) (ASAI *et al.* 1993) that, upon induction of the SOS response, a double-strand break is introduced into the chromosome within or near the origin of iSDR. Single-stranded (ss) DNA generated from the break by RecBC(D) helicase or Exo VIII would then be assimilated into an uncut homolog by the action of RecA recombinase. This reaction, *i.e.*, D-loop formation [for reviews see ROSENBERG and HASTINGS (1991), CLARK (1991), KOWALCZYKOWSKI (1991) and WEST (1992)], accomplishes the initial duplex opening essential for initiation of chromosome replication. Actual synthesis of daughter DNA molecules would then be primed by a PriA-mediated mechanism [for a review, see ASAI and KOGOMA (1994)]. The D-loop model is supported by our recent observation that an artificial double-strand break introduced into plasmid DNA induces, in the presence of an intact homolog, extensive DNA replication that is independent of the plasmid replication origin, transcription and translation (T. AKAI, D. B. BATES and T. KOGOMA, submitted for publication). The replication is completely dependent on homologous recombination functions.

The model implies that both homologous recombination and iSDR initiation share D-loop structures as a common intermediate, and suggests that the number of D-loops available for initiation determines the level of iSDR. In support of this contention, introduction of additional copies of *oriM1* into a cell, which would result in an increase of D-loops in the cell, stimulates iSDR (ASAI *et al.* 1994). Mutations in the *ruvA*, *ruvB*, *ruvC* and *recG* genes also enhance iSDR activity. The products of these genes are involved in the stages in homologous recombination that follow D-loop formation. RuvA and RuvB constitute the RuvAB helicase which catalyzes bidirectional branch migration (TSANEVA *et al.* 1992). RuvC, a resolvase, catalyzes resolution of Holliday junction intermediates (CONNOLLY *et al.* 1991; DUNDERDALE *et al.* 1991; IWASAKI *et al.* 1991). RecG is another helicase which promotes reversible branch migration of Holliday junctions (WHITBY *et al.* 1993). Recently, a mutation, designated *rus*, has been identified which activates an additional factor, most likely a resolvase, that works with RecG to resolve Holliday junctions independently of RuvAB and RuvC proteins (MANDAL *et al.* 1993). The observation that inactivation of these genes stimulates iSDR (ASAI *et al.* 1993) strongly suggests that the failure to process Holliday junctions also results in an increase in the number of D-loops available for iSDR initiation.

To obtain further insight into the roles of homologous recombination in DNA replication in SOS-induced cells, we have analyzed effects of various *rec* mutations on the elongation stage of DNA replication. The results suggest that activities of RuvA, RuvC and RecG are necessary for replication fork movement in SOS-induced cells. Moreover, we have found that the SOS response is chronically induced in exponentially growing *ruvA*, *ruvC* and *recG* mutants, suggesting that the activities of these genes are important for uninterrupted replication fork movement even in uninduced cells.

MATERIALS AND METHODS

Media and growth conditions: Unless otherwise stated, cells were grown at 37° in M9 salts-glucose medium (MILLER 1992) supplemented with casamino acids (0.2%; Difco Laboratories, Detroit, Michigan), required amino acids (50 μg/ml), thymine (8 μg/ml) and thiamine hydrochloride (2 μg/ml).

Chemicals and radioisotopes: Chemicals were purchased from Sigma Chemical (St. Louis, Missouri). [*methyl*-^{3}H]Thymine and [α-^{32}P]deoxycytidine-5′-triphosphate were from New England Nuclear Corp. (Boston, Massachusetts).

***E. coli* strains:** *E. coli* strains used in this study are listed in Table 1. Strains were constructed by phage P1-mediated transduction. The presence of the *recD1903*::mini-*tet* mutation was confirmed by the sensitivity to phage T4 gene 2 mutants (CHAUDHURY and SMITH 1984). The presence of the *recJ284*::Tn*10*, *ruvC51*, *recG258*::Tn*10kan* and *ruvA60*::Tn*10* mutations was confirmed by sensitivity to UV irradiation. The *ruvC* mutation was transduced by virtue of its linkage to the *eda-51*::Tn*10* mutation. Tetracycline-sensitive derivatives from Tn*10*-carrying strains were isolated by the method of BOCHNER *et al.* (1980).

Induction of the SOS response and determination of the iSDR activity: These were described previously (ASAI *et al.* 1993). Cells were grown to exponential phase in the presence of [^{3}H]thymine (10 μCi/8 μg/ml) for three generations. Radioactive thymine was then removed by filtration and the cells were resuspended in medium lacking required thymine. Immediately, a sample (90 μl) was withdrawn and the radioactivity (the initial radioactivity) of the trichloroacetic acid (TCA)-insoluble fraction was determined by liquid scintillation counting as described previously (KOGOMA *et al.* 1979). The cell suspension was split into two halves and a mixture of [^{3}H]thymine (10 μCi/8 μg/ml), RIF (200 μg/ml) and CAM (150 μg/ml) was immediately added to one half of the suspension (uninduced control). The other half of the suspension was starved of thymine for a duration approximately equivalent to two doublings (shown in the legend to Figure 1) and then the mixture was added. Samples (90 μl) were taken at the indicated times and the radioactivity of the TCA-insoluble fraction of each sample was determined as described above. The relative increase of DNA during iSDR was determined by dividing the radioactivity of each sample taken after the addition of RIF and CAM by the initial radioactivity. The average of standard error of means of relative DNA increases obtained by this method was calculated from a number of independent experiments. It was 4.3%.

Extraction of chromosomal DNA and Southern blot hybridization: These were described previously (MAGEE *et al.* 1992). Cells were grown to exponential phase in the presence of [^{3}H]thymine (10 μCi/8 μg/ml). Radioactive thymine was then removed by filtration and the cells were resuspended in me-

TABLE 1

E. coli strains

Strain	Genotype	Source, reference or construction
AQ634	F⁻ *ilv metB his-29 trpA9605 pro thyA deoB* (or *C*)	OGAWA *et al.* (1984)
AQ3362	As AQ634 but *recJ284*::Tn*10*	AQ634 × P1.AQ3023 select Tc^r, UV^s
AQ7960	As AQ634 but *ruvC51 eda-51*::Tn*10*	AQ634 × P1.TNM554 select Tc^r, UV^s
AQ8022	As AQ634 but *recJ284*::dTn*10* (Tc^s)	AQ3362 select Tc^s on Bochner plates.
AQ8070	As AQ634 but *recJ284*::dTn*10 recD1903*::mini-*tet*	AQ8022 × P1.DPB271 select Tc^r, T4.2^s
AQ8107	F⁻ D(*pro lac*) *pro rpsL* lp[*sfiA*::*lacZ* cI(Ind⁻)]	HUISMAN and D'ARI (1981)
AQ8130	As AQ634 but *recG258*::Tn*10kan*	AQ634 × P1.N2731 select Km^r, UV^s
AQ8134	As AQ634 but *recD1903*::mini-*tet*	AQ634 × P1.AQ8070 select Tc^r, T4.2^s
AQ8146	As AQ634 but *ruvC51 eda-51*::Tn*10 recG258*::Tn*10kan*	AQ7960 × P1.N2731 select Km^r
AQ8194	As AQ634 but *recJ284*::Tn*10 recG258*::Tn*10kan*	AQ8022 × P1.N2731 select Km^r
AQ8198	As AQ8107 but *ruvC51 eda-51*::Tn*10*	AQ8107 × P1.TNM554 select Tc^r, UV^s
AQ8208	As AQ634 but *recD1903*::mini-*tet recG258*::Tn*10kan*	AQ8134 × P1.N2731 select Km^r, UV^s
AQ8353	As AQ8107 but *recG258*::Tn*10kan*	AQ8107 × P1.N2731 select Km^r, UV^s
AQ8368	As AQ8107 but *ruvC51 eda-51*::Tn*10 recG258*::Tn*10kan*	AQ8198 × P1.N2731 select Km^r
AQ9317	As AQ634 but *ruvA60*::Tn*10*	AQ634 × P1.N2057 select Tc^r, UV^s
AQ9336	As AQ634 but *ruvA60*::Tn*10 recG258*::Tn*10kan*	AQ8130 × P1.N2057 select Tc^r
AQ9436	As AQ8107 but *ruvA60*::Tn*10*	AQ8107 × P1.N2057 select Tc^r, UV^s
AQ9438	As AQ8107 but *ruvA60*::Tn*10 recG258*::Tn*10kan*	AQ8353 × P1.N2057 select Tc^r
DPB271	*recD1903*::mini-*tet*	BIEK and COHEN (1986)
N2057	*ruvA60*::Tn*10*	SHARPLES *et al.* (1990)
N2731	*recG258*::Tn*10kan*	LLOYD and BUCKMAN (1991)
TNM554	*ruvC51 eda-51*::Tn*10*	G. J. SHARPLES

UV^s, sensitivity to UV; T4.2^s, sensitivity to phage T4 gene 2 mutants.

dium lacking required thymine. The suspension was starved of thymine as described above. A mixture of RIF (200 μg/ml) and CAM (150 μg/ml) was then added and samples (500 μl) were taken at the indicated times. Chromosomal DNA was extracted from each sample according to AUSUBEL *et al.* (1987) except that the cells were treated with lysozyme at a final concentration of 1 mg/ml for 60 min at 37° before the addition of SDS and proteinase K. Amounts of DNA samples normalized to give equal ^{3}H counts were digested with restriction enzymes and loaded on a 1% agarose gel. Southern blot hybridization was carried out as described by SAMBROOK *et al.* (1989) with nylon membrane (Hybond-N$^+$; Amersham, Arlington Heights, Illinois). The *Pst*I- (coordinate −1560 (MEIJER *et al.* 1979; SUGIMOTO *et al.* 1979) *Bam*HI (−100) fragment carrying the *gidA* sequence (WALKER *et al.* 1984) and the *Eco*RI-*Hin*dIII fragment (1.3 kb) of pMJR1560 (Amersham) containing the *lacI^q* gene were used for the preparation of probes to determine the copy number of the *oriM1* and *lac* regions, respectively. Probes were prepared by using Random Primers DNA Labeling System (BRL, Gaithersburg, Maryland) and [α-^{32}P]deoxycytidine-5′-triphosphate. The copy number of each region was determined by cutting out the corresponding bands from Southern blots and counting radioactivity by liquid scintillation. The average of standard error of means of copy number determinations was calculated from a number of independent experiments. It was 9.3%.

Determination of *sfiA::lacZ* expression:: β-Galactosidase activity was measured as described by MILLER (1992).

RESULTS

Effects of *rec* mutations on the initiation of iSDR: By monitoring the incorporation of [^{3}H]thymine into the acid-insoluble fraction, we previously demonstrated that the initial rate of iSDR was stimulated by mutations in the *recD, recJ, ruvA, ruvB, ruvC* and *recG* genes (ASAI *et al.* 1993). Similar results were obtained in the present study (Figure 1). To test whether or not the stimulation by these mutations is due to an increase in the initiation frequency of iSDR, we measured the copy number increase of the *oriM1* region on the chromosome in the presence of RIF and CAM after SOS induction (Figure 2A). The result shows that the copy number of the *oriM1* region in *ruvC* and *recG* mutants is about twice as high as that in wild type (wt). The *ruvA60*::Tn*10* mutation had a larger effect on the stimulation of iSDR initiation than the *ruvC51* or *recG258*::Tn*10kan* mutation. The effects of the *ruvC* and *recG* mutations were additive, though introduction of the *recG* mutation into the *ruvA* mutant did not show appreciable effects. *recD* mutations also stimulate the copy number increase of the *oriM1* region (ASAI *et al.* 1993). These results support our postulate that the enhanced iSDR in these *rec* mutants is a result of the elevated frequency of initiation.

In the wt and *ruvC* and *recG* single mutant cells, the relative increase of chromosomal DNA measured by [^{3}H]thymine incorporation (Figure 1B) is about threefold higher than the relative copy number increase at *oriM1* (Figure 2A). This is because the DNA synthesis measured by [^{3}H]thymine incorporation reflects DNA replication that is originated not only from *oriM1* but also from *oriM2* and other minor origins (MAGEE *et al.* 1992). The difference in relative increase of DNA between the two assays for other mutants could be explained by the inhibition of iSDR elongation (see below).

Effects of *rec* mutations on the elongation of iSDR: In *recD1903*::mini-*tet* mutant cells, the rate of DNA synthesis decreased gradually, but iSDR continued at a relatively high level until the end of an 8-hour incubation (Figure 1A). A very high level of incorporation of [^{3}H]thymine during the first few hours is due most likely to the increased number of D-loops accumulated during thymine

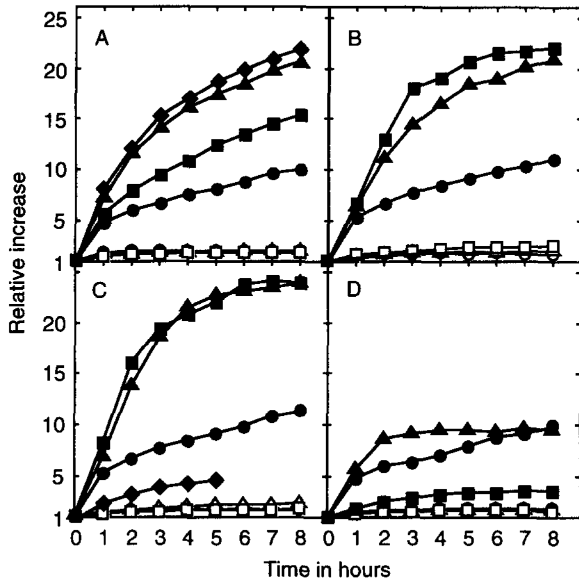

FIGURE 1.—Effects of *recD*, *recJ*, *ruvC*, *recG* and *ruvA* mutations on iSDR. Cells were grown to 2×10^8 cells/ml in the presence of [^{3}H]thymine and starved of thymine for 80 min, except for AQ8146 and AQ9336, which were starved for 100 min to obtain a maximum induction of iSDR. A mixture of RIF, CAM and [^{3}H]thymine was then added at time 0. iSDR was monitored by measuring the incorporation of [^{3}H]thymine into the acid-insoluble fraction, and the relative increase of chromosomal DNA was determined as described in MATERIALS AND METHODS. Controls are unstarved cultures. Filled symbols, starved; open symbols, unstarved. (A) AQ634 (wt, ● ○); AQ8134 (*recD*, ▲ △); AQ3362 (*recJ*, ■ □); and AQ8070 (*recD recJ*, ◆ ◇). (B) AQ634 (wt, ● ○); AQ7960 (*ruvC*, ▲ △); and AQ8130 (*recG*, ■ □). (C) AQ634 (wt, ● ○); AQ8208 (*recD recG*, ▲ △); AQ8194 (*recJ recG*, ■ □); and AQ8146 (*ruvC recG*, ◆ ◇). (D) AQ634 (wt, ● ○); AQ9317 (*ruvA*, ▲ △); and AQ9336 (*ruvA recG*, ■ □). The points up to five hour samples obtained with AQ8208 and AQ8194 (panel C) were previously reported (ASAI *et al.* 1993).

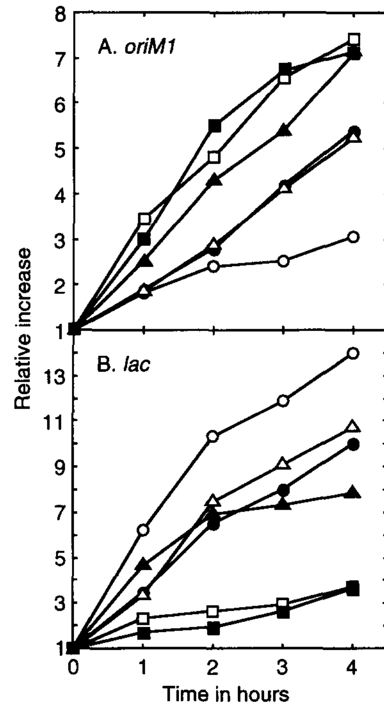

FIGURE 2.—Relative copy number increase of the *oriM1* and *lac* regions on the chromosome during iSDR. Cultures were made as described in Figure 1 and MATERIALS AND METHODS, and samples (500 µl) were withdrawn from thymine-starved cultures after the addition of RIF and CAM at time 0. Chromosomal DNA was prepared from each sample and amounts of DNA that gave the following ^{3}H counts (cpm) were digested with *Hin*dIII (A) or *Eco*RI and *Pst*I (B). AQ634, 7.5×10^3; AQ7960, 8.4×10^3; AQ8130, 8.7×10^3; AQ9317, 7.7×10^3; AQ8146, 7.8×10^3; AQ9336, 7.6×10^3. The copy number of each region was measured by Southern blot hybridization as described in MATERIALS AND METHODS. AQ634 (wt, ○); AQ7960 (*ruvC*, ●); AQ8130 (*recG*, △); AQ9317 (*ruvA*, ▲); AQ8146 (*ruvC recG*, □); AQ9336 (*ruvA recG*, ■).

starvation (see Introduction). The *recJ284*::Tn*10* mutant also showed a similar incorporation profile (Figure 1A).

In contrast, *ruvC* and *recG* mutants lost iSDR activity rapidly after two hours of steady increase at high rates and ceased completely by the end of the incubation (Figure 1B). Similar results were obtained with *recD recG* and *recJ recG* double mutants (Figure 1C). The inhibition of iSDR was particularly severe in *ruvA* mutant cells (Figure 1D). Introduction of the *recG258*::Tn*10kan* mutation into the *ruvC* and *ruvA* mutants aggravated the effect (Figures 2C and D). Since the frequency of initiation of iSDR is elevated in these mutants (Figure 2A), these results strongly suggested that the activities of the *ruvA*, *ruvC* and *recG* genes are necessary for elongation of iSDR. This idea was examined by measuring the copy number increase of the *lac* region (located at 8 min on the chromosome) (Figure 2B). In contrast to the *oriM1* region (84 min), the copy number increase of the *lac* region in these mutants was lower than that of wt. The levels of the copy number increase were in good agreement with the rate of [^{3}H]thymine incorporation seen in Figure 1. This indicates that the progress of replication forks started from *oriM1* was arrested in these mutants before they reached the *lac* region. Similar results were obtained with a *galK* probe (data not shown; for a result obtained with *ruvC recG* double mutant cells, see ASAI *et al.* 1994), suggesting that replication forks started from *oriM2* (~31 min) as well as *oriM1* were also arrested before they reached the *galK* region (17 min).

The relative copy number increase of the *lac* region in wt cells is about four times as high as that of the *oriM1* region after 4 hr of incubation (Figure 2). There are at least two reasons for this. One is that the copy number of the *lac* region is lower than that of the *oriM1* (*oriC*) region in exponentially growing cells. Thus, the same number of copies produced should contribute more to the relative copy number increase in the *lac* region than in the *oriM1* region. The second reason is that the *lac* region is replicated by replication forks originated not only from *oriM1* but also from *oriM2*.

Chronic induction of the SOS response in *ruvA*, *ruvC* and *recG* mutants: In *ruvA*, *ruvC* and *recG* mutants,

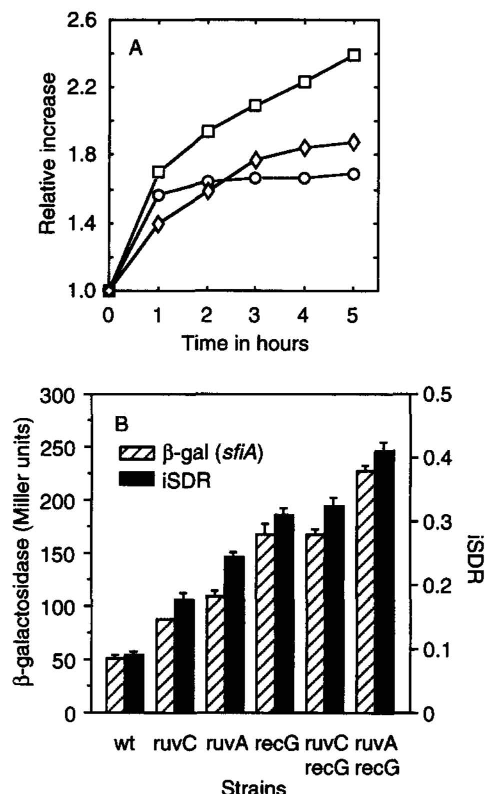

FIGURE 3.—Chronic induction of iSDR and the *sfiA* gene in exponentially growing *rec* mutants. (A) Relative increase of chromosomal DNA after the addition of RIF and CAM in uninduced cells (see Figure 1) is shown in an expanded ordinate. AQ634 (wt, ○); AQ8130 (*recG*, □); and AQ8146 (*ruvC recG*, ◇). (B) The iSDR activity (filled bars) in uninduced cells is represented as a difference of DNA increase between the first and third hours of incubation. wt, AQ634; *ruvC*, AQ7960; *ruvA*, AQ9317; *recG*, AQ8130; *ruvC recG*, AQ8146; *ruvA recG*, AQ9336. The induction level of the *sfiA* gene (hatched bars) in exponentially growing (uninduced) cells was determined by measuring the β-galactosidase activity resulted from the expression of an *sfiA::lacZ* operon fusion carried by a lysogenizing λ phage (Table 1). wt, AQ8107; *ruvC*, AQ8198; *ruvA*, AQ9436; *recG*, AQ8353; *ruvC recG*, AQ8368; *ruvA recG*, AQ9438.

DNA synthesis continued, albeit at a slow rate, after the addition of RIF and CAM without SOS-inducing treatment (open symbols in Figure 1). This can be clearly seen in Figure 3A which depicts the incorporation of [^{3}H]thymine in uninduced wt, *recG* and *ruvC recG* cells in an expanded ordinate. The level of drug-resistant DNA replication observed in several *rec* mutants without SOS-inducing treatments is summarized in Figure 3B. The result suggests that signals for iSDR induction are constitutively generated in these mutants even when they grow exponentially. We further found that the level of *sfiA* expression is significantly high in *ruvA*, *ruvC* and *recG* mutants compared with wt (Figure 3B). Elevated expression of the *sfiA* gene in exponentially growing *recG* mutants was previously reported (LLOYD and BUCKMAN 1991). These observations indicate that the SOS response is chronically expressed in these mutants.

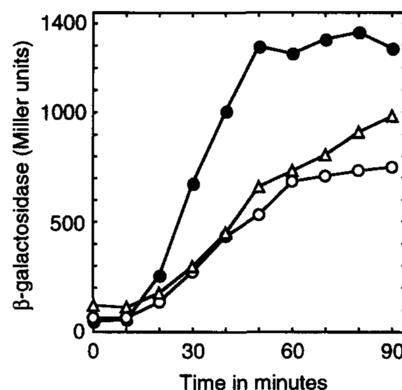

FIGURE 4.—SOS inducibility in *ruvC* and *recG* mutants. AQ8107 (wt, ●), AQ8198 (*ruvC*, ○) and AQ8353 (*recG*, △) were grown to 1.5 × 10^8 cells/ml, and mitomycin C was added at time 0 to a final concentration of 2.5 μg/ml. Samples were taken at the indicated time, and β-galactosidase activity was measured as described (MILLER 1992).

We considered the possibility that the condition that leads to the chronic SOS expression and constitutive iSDR activity in uninduced cells (Figure 3) might also give rise to propensity of the mutant cells to express higher levels of the SOS response than the wt level of induction. This could simply be a reason for the elevated iSDR activity observed in induced cells of these mutants (see Figure 1). The possibility was tested by analyzing the expression of the *sfiA* gene in *ruvC* and *recG* mutants after treating the cells with mitomycin C (MMC). The result (Figure 4) shows that the SOS inducibility in these mutants was in fact lower than that in wt. The reduced induction of the SOS response in MMC-treated *recG258*::Tn*10kan* mutant cells was previously observed with a different genetic background (LLOYD and BUCKMAN 1991). These results make the above possibility very unlikely.

DISCUSSION

The major finding in this study is that the continuation of iSDR is severely inhibited in *ruvA*, *ruvC*, and *recG* mutants which have defects in late stages of homologous recombination (Figure 1). This effect is striking particularly in light of the fact that iSDR initiation is actually stimulated by these mutations and can continue for at least 4 hr in most mutants (Figure 2A). Therefore, it seems as if replication forks that are repeatedly formed at *oriMs* would continue to travel to a blockade at which they might pile up. Since DNA increase comes to a complete halt at later times, for example, after 6 hours in *recG recD* and *recG recJ* double mutants (Figure 2C), it is likely either that initiation eventually completely ceases or that replication forks travel so short a distance at later times that the replication does not significantly contribute to the overall DNA increase.

Several lines of evidence suggest that in *E. coli* the rate-limiting step in homologous recombination is often

formation of appropriately activated DNA substrate (recombinogenic DNA) (Konrad 1977; Zieg *et al.* 1978; Hays and Boehmer 1978; Korba and Hays 1980; Zagursky and Hays 1983). In thymine-starved cells, ssDNA, which is a substrate of RecA-mediated strand exchange reaction (Kowalczykowski 1991; West 1992), could be generated at an elevated frequency due to the stalling of replication fork movement. The frequency of homologous recombination is thus thought to be enhanced in such cells. Whole recombination reactions, however, cannot be completed in *ruvC* and *recG* mutant cells, and the intermediates such as Holliday junctions would remain unresolved. The helicases associated with replication forks (*e.g.*, DnaB helicase) could not overcome the obstacle. Consequently, the replication fork originated from *oriMs* upon restoration of thymine would be arrested at these sites. *In vitro* experiments (Pugh and Cox 1987) suggest that, even if Holliday junctions are resolved by RuvC, RecA filaments may still hold two DNA duplexes together *in vivo*. This could also result in the inhibition of fork movement. It has recently been suggested that the helicase activity of RuvAB could be employed to remove these "used" RecA filaments from DNA (Kuzminov 1993). This hypothesis accounts for our observation that elongation of iSDR is inhibited in *ruvA* mutant cells even in the presence of active RuvC. In fact, arrest of fork movement appears to be much more severe in a *ruvA* single mutant than in *ruvC* and *recG* single mutants. Since expression of the *ruvA* and *ruvB* genes is derepressed by SOS induction (Shurvinton and Lloyd 1982; Shinagawa *et al.* 1988), the result suggests that the increased amount of RuvAB helicase may be very important for replication fork movement in SOS-induced cells.

The first *ruv* mutants were isolated by Otsuji *et al.* (1974) some 20 years ago. The initial characterization revealed that despite the sensitivity to UV radiation, the mutants appeared to be proficient in repair of UV damage. DNA synthesis after UV irradiation resumed at near normal rates in these mutants as in wt. After low doses of UV irradiation, the mutants formed multinucleate filaments. It was suggested that the mutants were UV sensitive because the mutations might cause some defect in cell division. The subsequent revelation that RuvAB is a helicase which functions in branch migration (Shiba *et al.* 1991; Tsaneva *et al.* 1992, 1993) offered no obvious further insight into the phenotype. Our observation described here provides an explanation. UV irradiation at a low dose creates DNA damage sites at which homologous recombination repair could be initiated. Unresolved intermediates of the repair events in the mutants could continuously block replication fork movement, inducing SfiA and causing filamentation. The rate of resumed DNA replication would appear normal, because inhibition of replication forks would be compensated for by the additional initiation at *oriMs*. This line of consideration leads us to a generalized contention that the UV sensitivity of *ruvAB*, *ruvC* and *recG* mutants (Sharples *et al.* 1990; Lloyd and Buckman 1991) can be accounted for to a large extent, if not solely, by the blockade of replication fork movement due to the unresolved recombination intermediates in these mutants.

Mutations in *ruv* genes have been shown to reduce the recovery of F-prime transconjugants (Benson *et al.* 1991). Since the reduction depends on $recA^+$ and homologous sequences between conjugative plasmids and the chromosome, it has been proposed that abortive recombination in *ruv* mutants results in loss of viability. Our observations which suggest that replication forks are arrested in *ruv* mutants could account for the loss of viability.

The activities of the *ruvA*, *ruvC* and *recG* genes must be important not only for iSDR but also for normal DNA replication started from *oriC* in SOS-induced cells. We wish to propose, further, that the same activities are necessary for uninterrupted replication fork movement even under the normal (uninduced) conditions. The procedure employed in Figure 1 was unsuccessful in detecting replication fork arrest in uninduced cells. This is probably due to low frequency of recombination in uninduced cells and high frequency of replication initiation at *oriC* compared with the frequencies at *oriMs*. However, we observed chronic induction of the SOS response (induction of the *sfiA* gene and iSDR) in exponentially growing *ruvA*, *ruvC* and *recG* mutant cells. The chromosomes of aerobically growing cells suffer a constant insult inflicted by active oxygen species (Cox 1993). Homologous recombination initiated, albeit at a low frequency, from these damage sites in the mutants would leave some intermediates unresolved. These intermediates could arrest replication forks and induce the SOS response. In keeping with this proposal, exponentially growing cell populations of *ruvA*, *ruvC* and *recG* mutants contain a significant portion of filamented cells (Shurvinton and Lloyd 1982; our unpublished observation).

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