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

Tsuneaki Asai*,[1] and Tokio Kogoma*,†

Departments of *Cell Biology and Microbiology, and †Cancer Research and Treatment Center, University of New Mexico School of Medicine, Albuquerque, New Mexico 87131

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.

D NA 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 profi-

cient 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

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-³H*]Thymine and [α-³²P]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 [³H]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 [³H]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 [³H]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::Tn10	AQ634 × P1.AQ3023 select Tcʳ, UVˢ
AQ7960	As AQ634 but ruvC51 eda-51::Tn10	AQ634 × P1.TNM554 select Tcʳ, UVˢ
AQ8022	As AQ3362 but recJ284::Tn10 (Tcˢ)	AQ3362 select Tcˢ on Bochner plates.
AQ8070	As AQ634 but recJ284::dTn10 recD1903::mini-tet	AQ8022 × P1.DPB271 select Tcʳ, T4.2ˢ
AQ8107	F⁻ D(pro lac) pro rpsL lp[sfiA::lacZ cI(Ind⁻)]	HUISMAN and D'ARI (1981)
AQ8130	As AQ634 but recG258::Tn10kan	AQ634 × P1.N2731 select Kmʳ, UVˢ
AQ8134	As AQ634 but recD1903::mini-tet	AQ634 × P1.AQ8070 select Tcʳ, T4.2ˢ
AQ8146	As AQ7960 but ruvC51 eda-51::Tn10 recG258::Tn10kan	AQ7960 × P1.N2731 select Kmʳ
AQ8194	As AQ634 but recJ284::Tn10 recG258::Tn10kan	AQ8022 × P1.N2731 select Kmʳ
AQ8198	As AQ8107 but ruvC51 eda-51::Tn10	AQ8107 × P1.TNM554 select Tcʳ, UVˢ
AQ8208	As AQ634 but recD1903::mini-tet recG258::Tn10kan	AQ8134 × P1.N2731 select Kmʳ, UVˢ
AQ8353	As AQ8107 but recG258::Tn10kan	AQ8107 × P1.N2731 select Kmʳ, UVˢ
AQ8368	As AQ8107 but ruvC51 eda-51::Tn10 recG258::Tn10kan	AQ8198 × P1.N2731 select Kmʳ
AQ9317	As AQ634 but ruvA60::Tn10	AQ634 × P1.N2057 select Tcʳ, UVˢ
AQ9336	As AQ634 but ruvA60::Tn10 recG258::Tn10kan	AQ8130 × P1.N2057 select Tcʳ
AQ9436	As AQ8107 but ruvA60::Tn10	AQ8107 × P1.N2057 select Tcʳ, UVˢ
AQ9438	As AQ8107 but ruvA60::Tn10 recG258::Tn10kan	AQ8353 × P1.N2057 select Tcʳ
DPB271	recD1903::mini-tet	BIEK and COHEN (1986)
N2057	ruvA60::Tn10	SHARPLES et al. (1990)
N2731	recG258::Tn10kan	LLOYD and BUCKMAN (1991)
TNM554	ruvC51 eda-51::Tn10	G. J. SHARPLES

UVˢ, sensitivity to UV; T4.2ˢ, 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 ³H 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 PstI- (coordinate −1560 (MEIJER et al. 1979; SUGIMOTO et al. 1979) BamHI (−100) fragment carrying the gidA sequence (WALKER et al. 1984) and the EcoRI-HindIII fragment (1.3 kb) of pMJR1560 (Amersham) containing the lacIᵠ 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-³²P]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 [³H]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::Tn10 mutation had a larger effect on the stimulation of iSDR initiation than the ruvC51 or recG258::Tn10kan 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 [³H]thymine incorporation (Figure 1B) is about three-fold higher than the relative copy number increase at oriM1 (Figure 2A). This is because the DNA synthesis measured by [³H]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 [³H]thymine during the first few hours is due most likely to the increased number of D-loops accumulated during thymine
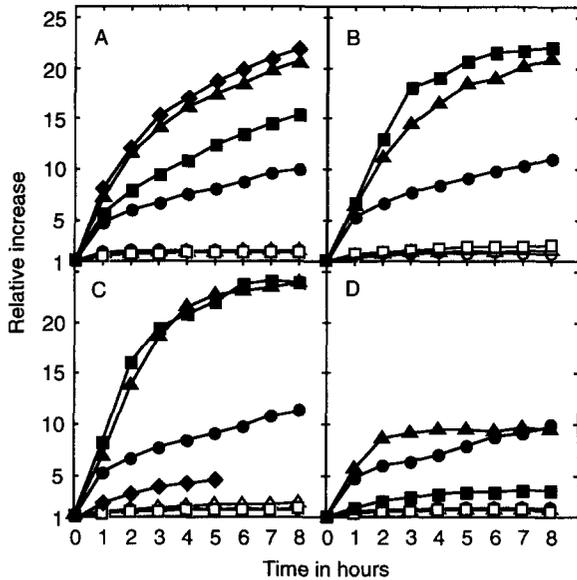
FIGURE 1.—Effects of *recD*, *recJ*, *ruvC*, *recG* and *ruvA* mutations on iSDR. Cells were grown to 2×10^8 cells/ml in the presence of [^{3}H]thymine and starved of thymine for 80 min, except for AQ8146 and AQ9336, which were starved for 100 min to obtain a maximum induction of iSDR. A mixture of RIF, 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).
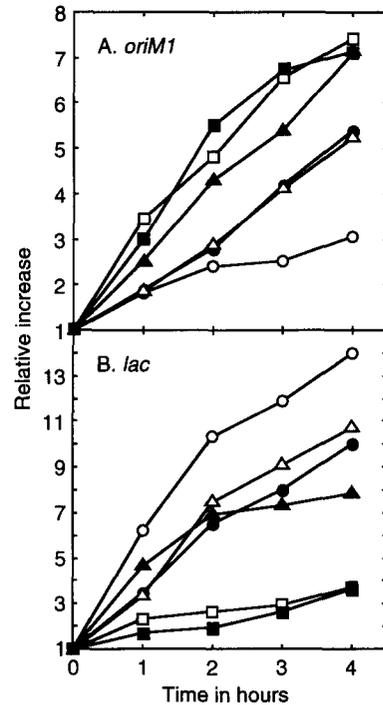

FIGURE 2.—Relative copy number increase of the *oriM1* and *lac* regions on the chromosome during iSDR. Cultures were made as described in Figure 1 and MATERIALS AND METHODS, and samples (500 μl) were withdrawn from thymine-starved cultures after the addition of RIF and CAM at time 0. Chromosomal DNA was prepared from each sample and amounts of DNA that gave the following ^{3}H counts (cpm) were digested with *Hind*III (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,
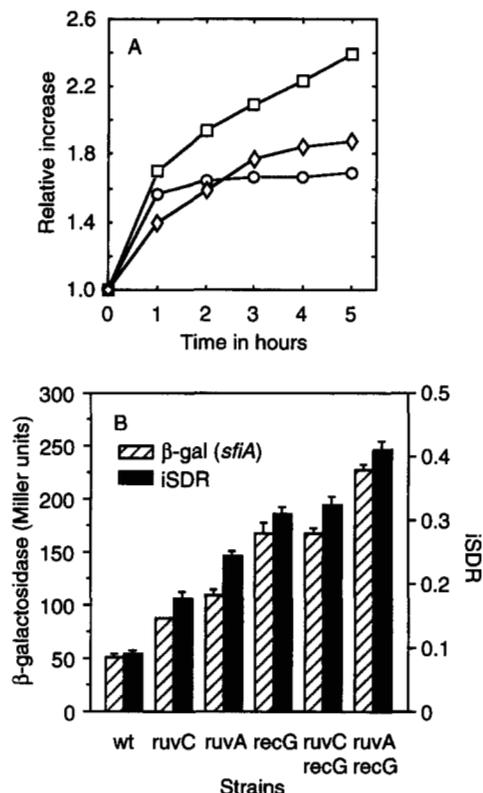
FIGURE 3.—Chronic induction of iSDR and the *sfiA* gene in exponentially growing *rec* mutants. (A) Relative increase of chromosomal DNA after the addition of RIF and CAM in un-induced cells (see Figure 1) is shown in an expanded ordinate. AQ634 (wt, ○); AQ8130 (*recG*, □); and AQ8146 (*ruvC recG*, ◇). (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.
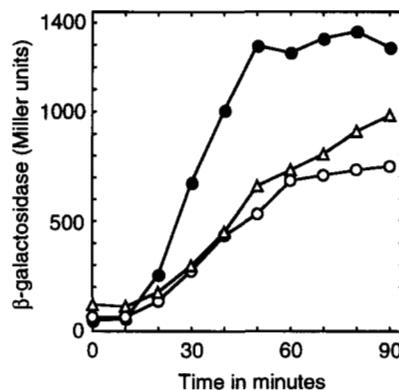

FIGURE 4.—SOS inducibility in *ruvC* and *recG* mutants. AQ8107 (wt, ●), AQ8198 (*ruvC*, ○) and AQ8353 (*recG*, △) were grown to 1.5×10^8 cells/ml, and mitomycin C was added at time 0 to a final concentration of 2.5 µg/ml. Samples were taken at the indicated time, and β-galactosidase activity was measured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

LITERATURE CITED

ASAI, T., and T. KOGOMA, 1994 D-loops and R-loops: alternative mechanisms for the initiation of chromosome replication in Escherichia coli. J. Bacteriol. 176: 1807–1812.

ASAI, T., S. SOMMER, A. BAILONE and T. KOGOMA, 1993 Homologous recombination-dependent initiation of DNA replication from DNA damage-inducible origins in Escherichia coli. EMBO J. 12: 3287–3295.

ASAI, T., M. IMAI and T. KOGOMA, 1994 DNA damage-inducible replication of the Escherichia coli chromosome is initiated at separable sites within the minimal oriC. J. Mol. Biol. 235: 1459–1469.

AUSUBEL, F. M., R. BRENT, R. E. KINGSTON, D. E. MOORE, J. G. SEIDMAN et al., 1987 pp 2.4.1–2.4.2 in *Current Protocols in Molecular Biology*. Wiley-Interscience, New York.

BENSON, F., S. COLLIER and R. G. LLOYD, 1991 Evidence of abortive recombination in *ruv* mutants of *Escherichia coli* K12. Mol. Gen. Genet. **225:** 266–272.

BIEK, D. P., and S. N. COHEN, 1986 Identification and characterization of *recD*, a gene affecting plasmid maintenance and recombination in *Escherichia coli*. J. Bacteriol. **167:** 594–603.

BOCHNER, B., H.-C. HUANG, G. SCHIEVEN and B. N. AMES, 1980 Positive selection for loss of tetracycline resistance. J. Bacteriol. **143:** 926–933.

CHAUDHURY, A. M., and G. R. SMITH, 1984 A new class of *Escherichia coli recBC* mutants: implications for the role of RecBC enzyme in homologous recombination. Proc. Natl. Acad. Sci. USA **81:** 7850–7854.

CIESLA, Z., and P. JONCZYK, 1980 The *dnaA* gene product is not required during stable DNA replication in *Escherichia coli*. Mol. Gen. Genet. **180:** 617–620.

CLARK, A. J., 1991 *rec* genes and homologous recombination proteins in *Escherichia coli*. Biochimie **73:** 523–532.

CLARK, A. J., V. SHARMA, S. BRENOWITZ, C. C. HU, S. SANDLER et al., 1993 Genetic and molecular analysis of the C-terminal region of the *recE* gene from the Rac prophage of *Escherichia coli* K-12 reveal the *recT* gene. J. Bacteriol. **175:** 7673–7682.

CONNOLLY, B., C. A. PARSONS, F. E. BENSON, H. J. DUNDERDALE, G. J. SHARPLES et al., 1991 Resolution of Holliday junctions *in vitro* requires *Escherichia coli ruvC* gene product. Proc. Natl. Acad. Sci. USA **88:** 6063–6067.

COX, M. M., 1993 Relating biochemistry to biology: how the recombinational repair function of RecA protein is manifested in its molecular properties. Bioessays **15:** 617–623.

DUNDERDALE, H. J., F. E. BENSON, C. A. PARSONS, G. J. SHARPLES, R. G. LLOYD et al. 1991 Formation and resolution of recombination intermediates by *E. coli* RecA and RuvC proteins. Nature **354:** 506–510.

HAYS, J. B., and S. BOEHMER, 1978 Antagonists of DNA gyrase inhibit repair and recombination of UV-irradiated phage l. Proc. Natl. Acad. Sci. USA **75:** 4125–4129.

HUISMAN, O., and R. D'ARI, 1981 An inducible DNA replication-cell division coupling mechanism of *E. coli*. Nature **290:** 797–799.

IWASAKI, H., M. TAKAHAGI, T. SHIBA, A. NAKATA and H. SHINAGAWA, 1991 *Escherichia coli* RuvC protein is an endonuclease that resolves the Holliday structure. EMBO J. **10:** 4381–4389.

JOSEPH, J. W., and R. KOLODNER, 1983 Exonuclease VIII of Escherichia coli. II. Mechanism of action. J. Biol. Chem. **258:** 10418–10424.

KOGOMA, T., and K. G. LARK, 1970 DNA replication in *Escherichia coli*: replication in absence of protein synthesis after replication inhibition. J. Mol. Biol. **52:** 143–164.

KOGOMA, T., and K. G. LARK, 1975 Characterization of the replication of *Escherichia coli* DNA in the absence of protein synthesis: stable DNA replication. J. Mol. Biol. **94:** 243–256.

KOGOMA, T., T. A. TORREY and M. J. CONNAUGHTON, 1979 Induction of UV-resistant DNA replication in *Escherichia coli*: induced stable DNA replication as an SOS function. Mol. Gen. Genet. **176:** 1–9.

KONRAD, E. B., 1977 Method for the isolation of *Escherichia coli* mutants with enhanced recombination between chromosomal duplications. J. Bacteriol. **130:** 167–172.

KORBA, B. E., and J. B. HAYS, 1980 Novel mutants of *Escherichia coli* that produce recombinogenic lesions in DNA. II. Properties of recombinogenic l phages grown on bacteria carrying *arl* mutations. J. Mol. Biol. **139:** 473–489.

KOWALCZYKOWSKI, S. C., 1991 Biochemical and biological function of *Escherichia coli* RecA protein: behavior of mutant RecA proteins. Biochimie **73:** 289–304.

KUZMINOV, A., 1993 RuvA, RuvB and RuvC proteins: cleaning-up after recombinational repairs in *E. coli*. Bioessays **15:** 355–358.

LLOYD, R. G., and C. BUCKMAN, 1991 Genetic analysis of the *recG* locus of *Escherichia coli* K-12 and of its role in recombination and DNA repair. J. Bacteriol. **173:** 1004–1011.

MAGEE, T. R., and T. KOGOMA, 1990 Requirement of RecBC enzyme and an elevated level of activated RecA for induced stable DNA replication in *Escherichia coli*. J. Bacteriol. **172:** 1834–1839.

MAGEE, T. R., T. ASAI, D. MALKA and T. KOGOMA, 1992 DNA damage-inducible origins of DNA replication in *Escherichia coli*. EMBO J. **11:** 4219–4225.

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.

MCMACKEN, R., L. SILVER and C. GEORGOPOULOS, 1987 DNA replication, pp. 564–612 in *Escherichia coli and Salmonella typhimurium: Cellular and Molecular Biology*, edited by J. L. INGRAHAM et al. American Society for Microbiology, Washington, D.C.

MEIJER, M., E. BECK, F. G. HANSEN, H. E. N. BERGMANS, W. MESSER et al., 1979 Nucleotide sequence of the origin of replication of the *Escherichia coli* K-12 chromosome. Proc. Natl. Acad. Sci. USA **76:** 580–584.

MILLER, J. H., 1992 *A Short Course in Bacterial Genetics*. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

OGAWA, T., G. G. PICKETT, T. KOGOMA and A. KORNBERG, 1984 RNase H confers specificity in the *dnaA*-dependent initiation of replication at the unique origin of the *Escherichia coli* chromosome *in vivo* and *in vitro*. Proc. Natl. Acad. Sci. USA **81:** 1040–1044.

OTSUJI, N., H. IYEHARA and Y. HIDESHIMA, 1974 Isolation and characterization of an *Escherichia coli ruv* mutant which forms nonseptable filaments after low doses of ultraviolet irradiation. J. Bacteriol. **117:** 337–344.

PALAS, K. M., and S. R. KUSHNER, 1990 Biochemical and physical characterization of exonuclease V from *Escherichia coli*. J. Biol. Chem. **265:** 3447–3454.

PUGH, B. F., and M. M. COX, 1987 RecA protein binding to the heteroduplex product of DNA strand exchange. J. Biol. Chem. **262:** 1337–1343.

RINKEN, R., B. THOMS and W. WACKERNAGEL, 1992 Evidence that *recBC*-dependent degradation of duplex DNA in *Escherichia coli recD* mutants involves DNA unwinding. J. Bacteriol. **174:** 5424–5429.

ROSENBERG, S. M., and P. J. HASTINGS, 1991 The split-end model for homologous recombination at double-strand breaks and at chi. Biochimie **73:** 385–397.

SAMBROOK, J., E. F. FRITSCH and T. MANIATIS, 1989 *Molecular Cloning: A Laboratory Manual*. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

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.

SHIBA, T., H. IWASAKI, A. NAKATA and H. SHINAGAWA, 1991 SOS-inducible DNA repair proteins, RuvA and RuvB, of *Escherichia coli*: functional interactions between RuvA and RuvB for ATP hydrolysis and renaturation of the cruciform structure in supercoiled DNA. Proc. Natl. Acad. Sci. USA **88:** 8445–8449.

SHINAGAWA, H., K. MAKINO, M. AMEMURA, S. KIMURA, H. IWASAKI and A. NAKATA, 1988 Structure and regulation of the *Escherichia coli ruv* operon involved in DNA repair and recombination. J. Bacteriol. **170:** 4322–4329.

SHURVINTON, C. E., and R. G. LLOYD, 1982 Damage to DNA induces expression of *ruv* gene of *Escherichia coli*. Mol. Gen. Genet. **185:** 352–355.

SMITH, G. R., 1988 Homologous recombination in procaryotes. Microbiol. Rev. **52:** 1–28.

SUGIMOTO, K., A. OKA, H. SUGISAKI, M. TAKANAMI, A. NISHIMURA et al., 1979 Nucleotide sequence of *Escherichia coli* K-12 replication origin. Proc. Natl. Acad. Sci. USA **76:** 575–579.

TSANEVA, I. R., B. MULLER and S. C. WEST, 1992 ATP-dependent branch migration of Holliday junctions promoted by the RuvA and RuvB proteins of *E. coli*. Cell **69:** 1171–1180.

TSANEVA, I. R., B. MULLER and S. C. WEST, 1993 RuvA and RuvB proteins of *Escherichia coli* exhibit DNA helicase activity *in vitro*. Proc. Natl. Acad. Sci. USA **90:** 1315–1319.

WALKER, G. C., 1984 Mutagenesis and inducible responses to deoxyribonucleic acid damage in *Escherichia coli*. Microbiol. Rev. **48:** 60–93.

WALKER, J. E., N. J. GAY, M. SARASTE and A. N. EBERLE, 1984 DNA

sequence around the *Escherichia coli unc* operon. Biochem. J. **224:** 799–815.

WEST, S. C., 1992 Enzymes and molecular mechanisms of genetic recombination. Annu. Rev. Biochem. **61:** 603–640.

WHITBY, M. C., L. RYDER and R. G. LLOYD, 1993 Reverse branch migration of Holliday junctions by RecG protein: a new mechanism for resolution of intermediates in recombination and DNA repair. Cell **75:** 341–350.

WITKIN, E. M., 1991 RecA protein in the SOS response: milestones and mysteries. Biochimie **73:** 133–141.

ZAGURSKY, R. J., and J. B. HAYS, 1983 Expression of the phage lambda recombination genes *exo* and *bet* under *lacOP* control on a multicopy plasmid. Gene **23:** 277–292.

ZIEG, J., V. F. MAPLES and S. R. KUSHNER, 1978 Recombination levels of *Escherichia coli* K-12 mutants deficient in various replication, recombination or repair genes. J. Bacteriol. **134:** 958–966.