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Requirement of RecBC Enzyme and an Elevated Level of Activated RecA for Induced Stable DNA Replication in *Escherichia coli*

THOMAS R. MAGEE[1] AND TOKIO KOGOMA[1,2]*

Departments of Cell Biology and Microbiology, University of New Mexico Medical Center,[2] and Department of Biology,[1] University of New Mexico, Albuquerque, New Mexico 87131

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During SOS induction, *Escherichia coli* cells acquire the ability to replicate DNA in the absence of protein synthesis, i.e., induced stable DNA replication (iSDR). Initiation of iSDR can occur in the absence of transcription and DnaA protein activity, which are both required for initiation of normal DNA replication at the origin of replication, *oriC*. In this study we examined the requirement of *recB*, *recC*, and *recA* for the induction and maintenance of iSDR. We found that *recB* and *recC* mutations blocked the induction of iSDR by UV irradiation and nalidixic acid treatment. In *recB*(Ts) strains, iSDR activity induced at 30°C was inhibited by subsequent incubation at 42°C. In addition, iSDR that was induced after heat activation of the RecA441 protein was abolished by the *recB21* mutation. These results indicated that the RecBC enzyme was essential not only for SOS signal generation but also for the reinitiation of DNA synthesis following DNA damage. *recAo*(Con) *lexA3*(Ind$^-$) strains were found to be capable of iSDR after nalidixic acid treatment, indicating that the derepression of the *recA* gene and the activation of the elevated level of RecA protein were the necessary and sufficient conditions for the induction of iSDR.

Chromosome replication is one of the most crucial events in the cell cycle of *Escherichia coli* and is regulated at the initiation step (for reviews, see references 28 and 34). Initiation of chromosome replication occurs at a unique site on the chromosome, termed *oriC* (8). The process involves several proteins, including DnaA protein encoded by the *dnaA* gene. In vivo, initiation requires transcription in the *oriC* region and therefore is blocked by the addition of rifampin (RIF), an RNA polymerase inhibitor (22, 30). Repeated initiation also requires protein synthesis; thus, chloramphenicol (CAM), a protein synthesis inhibitor, prevents initiation while allowing completion of the ongoing round of replication (24, 26).

Under certain circumstances, *E. coli* exhibits altered modes of DNA replication. These are induced stable DNA replication (iSDR) and constitutive SDR. Both are characterized as DNA replication that can occur in the absence of protein synthesis (for a review, see reference 12). Constitutive SDR, which occurs in *rnh* mutants lacking RNase H, requires transcription for initiation (11, 33). Initiation of constitutive SDR occurs at several origins other than *oriC*, which are termed *oriK*s (5). It is independent of DnaA protein (16). On the other hand, iSDR is induced as part of the SOS response, for example, after UV irradiation, incubation with nalidixic acid (NAL; a gyrase inhibitor), or thymine starvation of thymine-requiring cells (13, 15). iSDR replicates UV-damaged DNA more efficiently than normal DNA replication (15) and appears to be error prone (23). iSDR can occur, however, in *umuC* mutants which are defective in UV mutagenesis (36). In addition, the mode of iSDR substantially differs from normal replication. Initiation can occur in the presence of not only CAM but also RIF (22). DnaA protein is not required for initiation (3).

The induction of iSDR is blocked by the *lexA3*(Ind$^-$) mutation, indicating that activation of this mode of DNA replication requires derepression of one or more genes of the LexA regulon (15). However, the derepression of the LexA regulon genes is not sufficient for the induction; it requires the activation of RecA protein. This conclusion was based on the observation that *lexA71*::Tn*5*(Def) *recA441* (encoding a heat-activatable RecA) double mutants did not exhibit iSDR, despite the derepression of the LexA regulon, unless the strains were incubated at 42°C in the presence of adenine, a condition that activates RecA441 protein (36). The role of the activated RecA (RecA*) is not known. Since the induction of iSDR by the activation of RecA protein in *recA441 lexA*(Def) mutants could occur in the complete absence of protein synthesis, it was concluded that the unknown role of RecA* is not the derepression of any genes outside the LexA regulon (36). Thus, the minimum requirements for the induction of iSDR are (i) derepression of one or more of the LexA regulon genes and (ii) activation of RecA protein. In this report, we show that derepression of the *recA* gene and the activation of the elevated level of RecA protein are the necessary and sufficient conditions for the induction of iSDR. We also show that RecBC enzyme is required not only for the induction of iSDR but also for maintenance of iSDR.

MATERIALS AND METHODS

Chemicals and radioisotopes. NAL was from Aldrich Chemical Co., Inc., Milwaukee, Wis. RIF, CAM, 2′-deoxyadenosine, thymine, thymidine, and adenine were from Sigma Chemical Co., St. Louis, Mo. [*methyl*-^{3}H]thymidine (20 Ci/mmol) was obtained from New England Nuclear Corp., Boston, Mass.

Bacteria and plasmids. The bacterial strains used are described in Table 1. *zga*::Tn*10* (AQ2561), which is 48% linked to *recB recC* (33), was used to construct *recB21* strains by P1 transduction. All *recB21* mutants were tested for UV sensitivity as well as recombination deficiency after conjugation with an Hfr strain. pCDK3 is a derivative of pBR322 which carries cloned *recB* and *recC* genes. The plasmid has been shown to overproduce RecB and RecC proteins by 30-fold in a wild-type strain (6). Plasmid pUC18::*dnaT*$^+$ was a generous gift from W. Messer.

* Corresponding author.

TABLE 1. *E. coli* strains used

Strain	Relevant genotype	Source or reference
JC5519[a]	*recB21 recC22*	A. J. Clark
AQ634[b]	*recB*$^+$ *recC*$^+$ *thyA*	31
AQ2781[b]	*recB*$^+$ *recC*$^+$ *thyA*$^+$	P1(JC5519) × AQ634, select Thy$^+$
AQ2782[b]	*recB21 recC22 thyA*$^+$	P1(JC5519) × AQ634, select Thy$^+$, screen UV^{sc}
SK119[d]	*recB270*(Ts)	20
JC5412[e]	*recB21*	A. J. Clark
AQ2561[f]	*zga*::Tn*10* (44% linked to *thyA*, 48% linked to *recB recC*)	34
AQ5786[b]	*zga*::Tn*10 thyA*	P1(AQ2561) × AQ634, select Tet^r, screen Thy$^-$
AQ5931[b]	*recB21 zga*::Tn*10 thyA*$^+$	P1(JC5412) × AQ5786, select Thy$^+$, screen UV^s
JM441[g]	*recA441 lexA71*::Tn*5*	E. Witkin
AQ5937[g]	*recA441 lexA71*::Tn*5 recB21 zga*::Tn*10*	P1(AQ5931) × JM441, select Tet^r, screen UV^s
MV1138[h]	*recA281o*(Con) *lexA*$^+$	4
JC11867[h]	*recA281o*(Con) *lexA3*(Ind$^-$)	4
AQ6152[h]	*recA281o*(Con) *lexA3*(Ind$^-$)(pCDK3)	JC11867 transformed with pCDK3, select Amp^r Cam^r
AQ6226[h]	*recA281o*(Con) *lexA3*(Ind$^-$)(pUC18::*dnaT*$^+$)	JC11867 transformed with pUC18::*dnaT*$^+$, select Amp^r

[a] Remaining genotype: F$^-$ *thr-1 leu-6 thi-1 lacY galK2 ara-14 xyl-5 mtl-1 proA2 his-4 argE3 rpsL31 tsx-33 supE44* lambda^s.
[b] Remaining genotype: F$^-$ *argH ilv metB his-29 trpA9605 deoB* or *C proB rpoB*.
[c] UV^s, Sensitive to UV irradiation.
[d] Remaining genotype: F$^-$ *thr-1 thi-1 his-4 argE3 proA2 rpsL31*.
[e] Remaining genotype: HfrKL16 *polA1 sbcA8 thr-300 ilv-318 spc-300 thi-1 rel-1* lambda^s.
[f] Remaining genotype: HfrH *thi galE* Δ(*attB-bio*) *deoA103 deoC lysA cytR upp udp pyrD*::Tn*5 fuc relA1*.
[g] Remaining genotype: F$^-$ *thr-1 leuB6* Δ(*gpt-proA*)*62 his-4 argE3 thi-1 rpsL31 galK2 ara-14 xyl-5 mtl-1 tsx-33 supE44 ilv*(Ts) *sulA221*.
[h] Remaining genotype: F$^-$ *thr-1 leuB6 thi-1 argE3 his-4 proA2 lacY1 galK2 ara-14 xyl-5 mtl-1 rpsL31 tsx-33 supE44 srl-300*::Tn*10* lambda$^-$.

Media and growth conditions. Unless otherwise stated, cells were grown at 37°C in M9 salts-glucose medium (24) supplemented with Casamino Acids (0.2%; Difco Laboratories, Detroit, Mich.), specifically required amino acids (50 μg/ml), and thiamine hydrochloride (2 μg/ml). The cells were grown to exponential phase (1×10^8 to 2×10^8 cells per ml) before treatments.

Induction and measurement of iSDR. Cells grown to exponential phase were induced for iSDR by the following treatments. For induction with NAL, 50 μg of NAL per ml was added to the culture and incubation was continued for 70 min. The cells were then washed by filtration and suspended in prewarmed medium containing [^{3}H]thymidine (10 μCi/μg per ml), 2′-deoxyadenosine (300 μg/ml), and CAM (150 μg/ml). Immediately after the addition of NAL, a sample (control) was filtered, washed, and suspended, as were the treated cultures. For induction by UV irradiation, the cultures were centrifuged and suspended in M9 salts. The cells were then irradiated with UV at 30 J/m^2 and grown in pretreatment medium. After 70 min of growth, a mixture of [^{3}H]thymidine, 2′-deoxyadenosine, and CAM was added. The control culture consisted of an unirradiated portion of the culture which was centrifuged and resuspended as were the irradiated cultures. Samples (0.1 ml) were withdrawn every half hour, and radioactivity in acid-insoluble fractions was determined as described previously (15).

RESULTS

Requirement for *recB*$^+$ *recC*$^+$. The induction of the SOS response involves a signal generation step (see reference 35 for a review). The genetic requirement for this step differs, depending on the inducing treatments; whereas the induction by UV irradiation requires *recF*$^+$, the induction by NAL treatment depends on *recB*$^+$ *recC*$^+$ (9, 32). When *recB recC* mutants were either treated with NAL or irradiated by UV light, the subsequent DNA replication ceased in the presence of CAM, whereas wild-type strains exhibited continued DNA synthesis in the absence of protein synthesis after either treatment, i.e., iSDR (Fig. 1A and B). In contrast, *recF* mutations blocked induction of iSDR by UV irradiation but not by NAL treatment (data not shown). These results suggested to us that the *recB recC* gene products were essential not only in the signal generation step for induction but also in a subsequent step leading to the manifestation of iSDR activity. This possibility was further examined by use of a *recB*(Ts) mutation (Fig. 1C). When the *recB*(Ts) mutant was treated with NAL at 42°C and assayed for iSDR at 42°C, there was no induction, as expected. Treatment at 30°C induced iSDR, but iSDR was inhibited when the temperature was raised to 42°C during the subsequent incubation in CAM (Fig. 1C). We note that the plateau reached by the induced cells after the shift from 30 to 42°C was higher than that reached by untreated cells. It appears, therefore, that initiation of an extra round of DNA replication could occur at 42°C after the inducing treatment at 30°C. Since inactivation of RecB(Ts) protein at 42°C in vivo is relatively quick (20), it is probable that the process leading to the first round of initiation had proceeded beyond the step that required RecB activity during the incubation at 30°C, although subsequent initiation was inhibited because of inactivation of RecB protein at 42°C.

Coupled with the *lexA*::Tn*5*(Def) mutation, which derepresses the LexA regulon genes, the *recA441* mutation allows direct induction of iSDR by heat activation of RecA441 protein (see above). Thus, in *recA441 lexA*(Def) mutants, the signal generation step can be bypassed, allowing a direct test for the requirement for RecBC enzyme in the subsequent step in iSDR induction. The result shown in Fig. 2 indicates that direct induction of iSDR in the *recA441 lexA*(Def) strain was blocked by the *recB21* mutation. Taken together, these results (Fig. 1 and 2) indicate the requirement of RecBC enzyme not only for signal generation in the induction step but also for subsequent initiation of iSDR.

***recA* is the only LexA regulon gene that must be derepressed.** The *lexA3*(Ind$^-$) mutation blocks the induction of iSDR, indicating that one or more genes in the LexA regulon

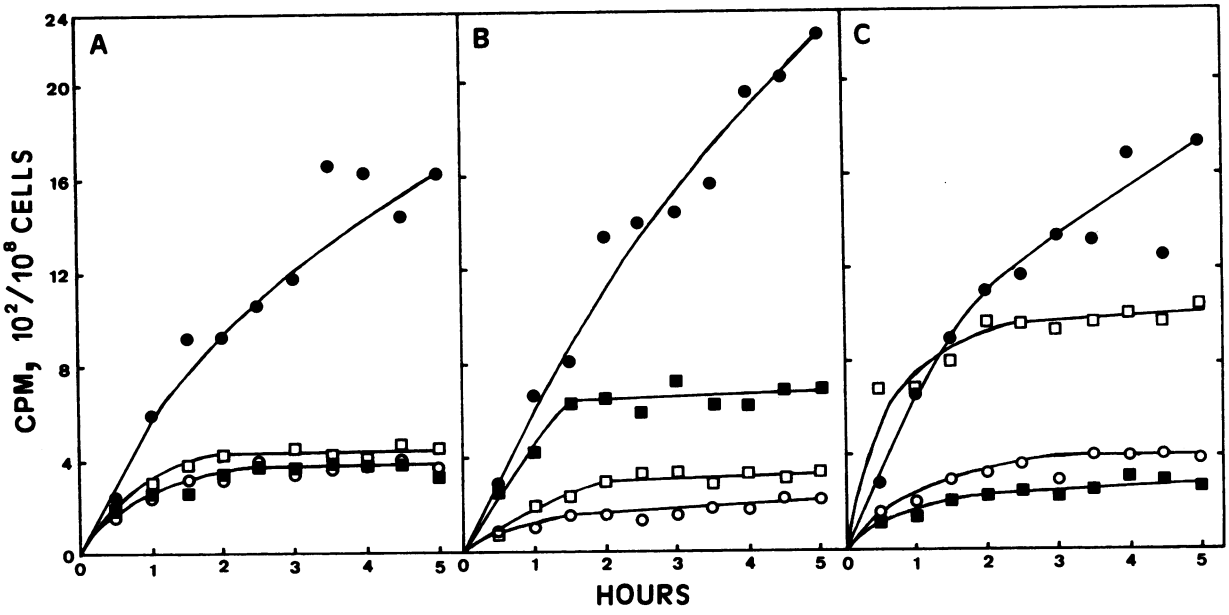

FIG. 1. DNA replication in the presence of CAM after treatment with NAL or UV irradiation. (A and B) Cultures of AQ2781 (*recB*$^+$ *recC*$^+$) and AQ2782 (*recB21 recC22*) were grown at 37°C to 2 × 10^8 cells per ml. The cultures were induced for iSDR by UV irradiation (A) or NAL treatment (B), and DNA replication was measured as described in Materials and Methods. Results are shown for uninduced (○) and induced (●) AQ2781 and uninduced (□) and induced (■) AQ2782. (C) Strain SK119 [*recB270*(Ts)] was grown at 30°C to 2 × 10^8 cells per ml, whereupon NAL was added. The culture was immediately split into two portions; one was incubated at 30°C, and the other was incubated at 42°C. A control sample of the 30°C culture was immediately filtered after NAL addition and suspended in medium containing a mixture of [^{3}H]thymidine, CAM, and 2-deoxyadenosine (○). After 80 min in NAL at 30 and 42°C, the treated cultures were filtered, suspended in prewarmed medium containing [^{3}H]thymidine, CAM, and 2-deoxyadenosine, and incubated at 30°C (●) and 42°C (■). A portion of the 30°C-treated culture was shifted to 42°C at 0 h (□).

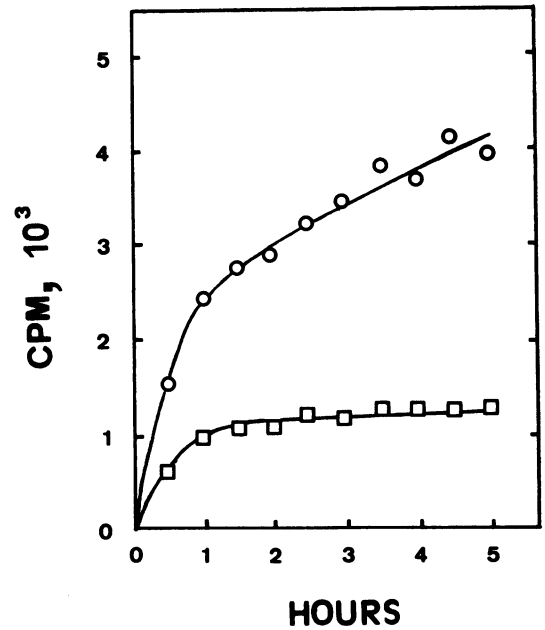

FIG. 2. DNA replication in the presence of CAM after heat induction in strains JM441 and AQ5937. Cultures of JM441 (*recA441 recB*$^+$) (○) and AQ5937 (*recA441 recB21*) (□) were grown at 30°C to 2 × 10^8 cells per ml. At 0 h, cultures were shifted to 42°C and [^{3}H]thymidine, 2′-deoxyadenosine, adenine (100 μg/ml), and CAM were added. Samples were measured for iSDR as described in Materials and Methods.

must be derepressed for the induction (15). To test the possibility that *recA* is the gene that must be derepressed, a *recAo*(Con) *lexA3*(Ind$^-$) mutant was tested for iSDR inducibility (Fig. 3). In the double mutant, the *recA* gene is derepressed because of the operator-constitutive mutation while all other LexA regulon genes remain repressed, even during inducing treatment, because of the *lexA3* mutation. Treatment of the double mutant with NAL induced iSDR to a level comparable to that seen in a *lexA*$^+$ counterpart after a similar treatment (Fig. 3). The result indicated that *recA* was the only LexA-regulated gene that must be derepressed during the induction of iSDR. It should be noted that mere derepression of *recA* is not sufficient; the overproduced RecA protein must be activated because the manifestation of iSDR required NAL treatment regardless of the presence or absence of the *lexA3* mutation (Fig. 3).

Requirement for an additional gene product(s) outside the LexA regulon. The results described above indicated that the minimum requirement for the induction of iSDR was the activation of overproduced RecA protein. In order to determine any further requirement for the synthesis of other gene products during induction, the effect of the inhibition of transcription with RIF during the induction period in the *recAo*(Con) *lexA3*(Ind$^-$) mutant was examined (Fig. 4). RIF was added to an exponentially growing culture of the double mutant 5 min before and 15, 30, and 60 min after the addition of NAL. After the 60-min induction period, NAL was removed and iSDR was assayed in the presence of RIF and CAM, which blocked RNA and protein synthesis, respectively. The cells that were allowed RNA and protein synthesis for more than 15 min at the beginning of NAL treatment exhibited increased DNA synthesis rates initially and increased amounts of persisting DNA synthesis (i.e., iSDR)

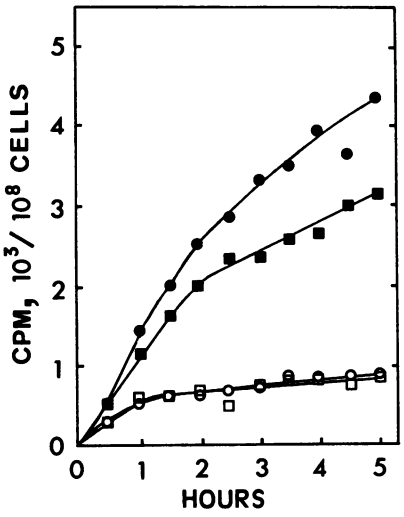

FIG. 3. DNA replication in the presence of CAM after treatment with NAL in strains MV1138 and JC11867. Cultures of strains MV1138 [*recA281o*(Con) *lexA*$^+$] and JC11867 [*recA281o*(Con) *lexA3*(Ind$^-$)] were grown at 37°C to 2 × 10^8 cells per ml. NAL treatment and measurement of DNA replication were done as described in Materials and Methods. Results are shown for uninduced (○) and induced (●) MV1138 and uninduced (□) and induced (■) JC11867.

subsequently, compared with the uninduced control culture, in which DNA synthesis ceased after 1 h in the absence of protein synthesis (Fig. 4A). Although the overall DNA synthesis rate was much less than that in the other induced cells, the cells that had RIF during the entire period of induction also acquired a capacity for persisting DNA synthesis. This is clearly shown in Fig. 4B, in which DNA synthesis is expressed as the increase relative to that in the 1-h sample after removal of NAL. Thus, the rate of iSDR in the cells that had no RNA synthesis during the induction was as high as that in the cells that had RNA synthesis for 15 min. We conclude that iSDR can be induced without derepression of any other inducible genes, provided that RecA protein is overproduced and activated.

Although iSDR could be induced without RNA or protein synthesis during NAL treatment in the *recAo*(Con) *lexA3* mutant, longer periods of protein synthesis during induction led to increased iSDR activity (Fig. 4). The observation suggested that some constitutive component of iSDR activity was limiting in exponentially growing cells and that accumulation of the component during NAL treatment led to increased iSDR activity. DnaT protein (27) and RecBC enzyme (see above) are essential for iSDR. To determine whether this component was DnaT protein or RecBC enzyme, a high-copy-number plasmid carrying *dnaT*$^+$ (pUC18::*dnaT*$^+$) or *recB*$^+$ and *recC*$^+$ (pCDK3) was introduced into the double mutant, and the experiment whose results are shown in Fig. 4 was repeated with the DnaT- or RecBC-overproducing strain. The result (not shown) indicated that there was no effect of the overproduction of DnaT or RecBC on the iSDR activity acquired by the cells.

DISCUSSION

Previous works indicated that derepression of one or more genes of the LexA regulon is essential for the induction of iSDR (15, 36). In this study, we have demonstrated that the gene that must be derepressed is *recA* and that no other genes need be derepressed. The overproduced RecA, however, must be activated, in keeping with the previous observation (36). Thus, we conclude that the derepression of *recA* and the activation of the elevated level of RecA are the necessary and sufficient conditions for the induction of iSDR. The present results, however, do not exclude the possibility that LexA-controlled gene products at a basal level may also be involved. In addition, the manifestation of iSDR requires the following gene products: DnaB, DnaC, DnaE, and DnaG (14); DnaT (21, 27); and RecB and RecC (this work). The levels of these gene products constitutively expressed during exponential growth appear to be sufficient for iSDR activity, but accumulation of some of the gene products during inducing treatments may lead to enhanced iSDR activities (Fig. 4).

UV irradiation effectively blocks DNA replication at the

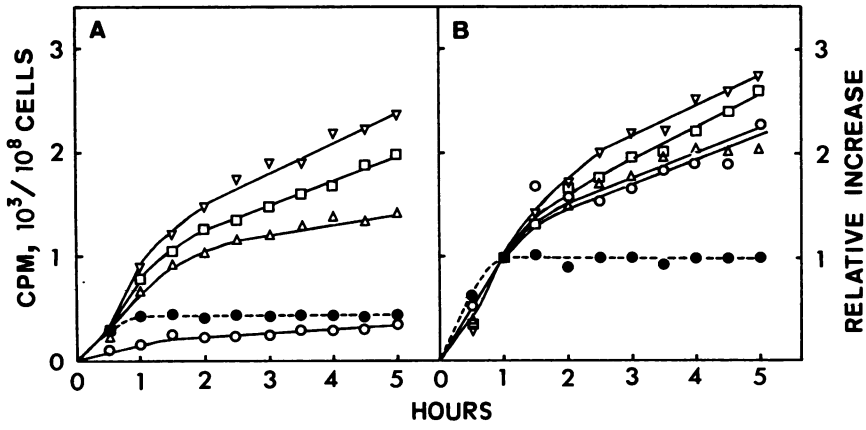

FIG. 4. Effect of RIF during NAL treatment on DNA replication in CAM in strain JC11867. (A) A culture of strain JC11867 [*recA281o*(Con) *lexA3*(Ind$^-$)] was grown at 37°C to 2 × 10^8 cells per ml. Five minutes before the addition of NAL to the culture, RIF (200 μg/ml) was added to a sample to prevent gene expression during NAL treatment (○). To the remaining culture, RIF was added to separate portions 15 (△), 30 (□), and 60 (▽) min after NAL addition. The 60-min portion had RIF only briefly. After 60 min in NAL, all culture samples were filtered, washed, and suspended in medium containing [^{3}H]thymidine, 2′-deoxyadenosine, CAM, and RIF. The control culture (●), which was filtered immediately after the addition of NAL, was processed identically. (B) The data obtained from the experiment whose results are shown in panel A are plotted as the ratio of the sample counts per minute divided by the 1-h sample counts per minute.

replication forks, and the recovery of the stalled replisomes (induced replisome reactivation [IRR]) has been demonstrated to be an SOS function (10). The requirement for IRR closely parallels that of iSDR; derepression of *recA* and at least one additional gene (IRR factor) is required (10). The IRR factor is not under LexA control, and in fact *recA* is the only gene controlled by LexA that must be amplified (37). The requirement of RecA* for IRR has not been rigorously tested. Casaregola et al. (2) hypothesized that the IRR factor may be an inhibitor of RNase H because a moderate recovery of UV-inhibited DNA replication was detected in the absence of protein synthesis in *rnh* mutants in which RecA protein was amplified. Unlike iSDR, however, amplified RecA is not sufficient for IRR, and IRR does not require the *recB* gene product (10). These considerations make it seem unlikely that reactivated replisomes are responsible for iSDR. The induction of iSDR seems to involve the assembly of a replisome at an origin which can initiate rounds of replication without concomitant protein synthesis. In support of this hypothesis, we have recently detected new origins of DNA replication that are activated after inducing treatments (T. Magee and T. Kogoma, unpublished data). These origins, termed *oriM*s, map near *oriC* and in the region of *terC*. The initiation from *oriM*s occurs in the absence of transcription or translation in a manner which is independent of DnaA protein. Despite the report implicating an RNase H inhibitor in the recovery process of inhibited replisomes (2), we previously detected little effect of overproduction of RNase H on the inducibility of iSDR (1). Thus, iSDR and IRR appear not to be identical. Since both iSDR and IRR contribute to the replication of damaged DNA, we suggest that there are at least two replication modes that operate after induction of the SOS response, one involving initiation at fixed sites on the chromosome and the other involving the reactivation of stalled replisomes.

It is clear from this work as well as the previous work (36) that RecA has a direct role in iSDR in addition to the regulatory role. However, what this role of RecA might be is not clear at present. Our finding that RecBC is essential for iSDR strongly suggests an involvement of the recombinase activity of RecA. Since initiation of iSDR occurs at fixed sites on the chromosome, some special sequence at these sites must be recognized by protein(s) involved. One such possible sequence is the *chi* sequence, which is recognized by RecBC enzyme (32). We have not tested *recD* mutations, which are known to abolish *chi*-dependent recombination enhancement (32). It has been reported that during the late stage of T4 bacteriophage infection DNA replication is initiated in a manner which is dependent on recombination proteins (7, 19, 25). In addition, T4 phage also engages in a mode of DNA replication which is independent of transcription and homologous recombination (17, 18, 29). Further insights into the mechanism of iSDR initiation and its relation, if any, to these modes of T4 DNA replication await characterization of the newly identified inducible origins of DNA replication in SOS-induced cells.

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LITERATURE CITED

1. **Bialy, H., and T. Kogoma.** 1986. RNase H is not involved in the induction of stable DNA replication in *Escherichia coli*. J. Bacteriol. **165**:321–323.
2. **Casaregola, S., M. Khidhir, and I. B. Holland.** 1987. Effects of modulation of RNase H production on the recovery of DNA synthesis following UV-irradiation in *Escherichia coli*. Mol. Gen. Genet. **209**:494–498.
3. **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.
4. **Clark, A. J.** 1982. *recA* operator mutations and their usefulness. Biochimie **64**:669–675.
5. **de Massy, B., O. Fayet, and T. Kogoma.** 1984. Multiple origin usage for DNA replication in *sdrA*(*rnh*) mutants of *Escherichia coli* K-12: initiation in the absence of *oriC*. J. Mol. Biol. **178**:227–236.
6. **Dykstra, C. C., D. Prasher, and S. R. Kushner.** 1984. Physical and biochemical analysis of the cloned *recB* and *recC* genes of *Escherichia coli* K-12. J. Bacteriol. **157**:21–27.
7. **Formosa, T., and B. M. Alberts.** 1986. DNA synthesis dependent on genetic recombination: characterization of a reaction catalyzed by purified bacteriophage T4 proteins. Cell **47**:793–806.
8. **Hiraga, S.** 1976. Novel F prime factors able to replicate in *Escherichia coli* Hfr strains. Proc. Natl. Acad. Sci. USA **73**:198–202.
9. **Karu, E. A., and E. D. Belk.** 1982. Induction of *E. coli recA* protein via *recBC* and alternative pathways: quantitation by enzyme-linked immunosorbent assay (ELISA). Mol. Gen. Genet. **185**:275–282.
10. **Khidhir, A. M., 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.
11. **Kogoma, T.** 1978. A novel *Escherichia coli* mutant capable of DNA replication in the absence of protein synthesis. J. Mol. Biol. **121**:55–69.
12. **Kogoma, T.** 1986. RNase H-defective mutants of *Escherichia coli*. J. Bacteriol. **166**:361–363.
13. **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.
14. **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.
15. **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.
16. **Kogoma, T., and K. von Meyenburg.** 1983. The origin of replication, *oriC*, and the *dnaA* protein are dispensable in stable DNA replication (*sdrA*) mutants of *Escherichia coli* K12. EMBO J. **2**:463–468.
17. **Kreuzer, K. N., and B. M. Alberts.** 1985. A defective phage system reveals bacteriophage T4 replication origins that coincide with recombination hotspots. Proc. Natl. Acad. Sci. USA **82**:3345–3349.
18. **Kreuzer, K. N., W. H. Engman, and W. P. Yap.** 1988. Tertiary initiation of replication in bacteriophage T4: deletion of the overlapping *uvsY* promoter/replication origin from the phage genome. J. Biol. Chem. **263**:11348–11357.
19. **Kreuzer, K. N., W. P. Yap, A. E. Menkens, and H. W. Engman.** 1988. Recombination-dependent replication of plasmids during bacteriophage T4 infection. J. Biol. Chem. **263**:11366–11373.
20. **Kushner, S. R.** 1974. In vivo studies of temperature-sensitive *recB* and *recC* mutants. J. Bacteriol. **120**:1213–1218.
21. **Lark, C. A., J. Riazi, and K. G. Lark.** 1978. *dnaT*, dominant conditional-lethal mutation affecting DNA replication in *Escherichia coli*. J. Bacteriol. **136**:1008–1017.
22. **Lark, K. G.** 1972. Evidence for the direct involvement of RNA in the initiation of DNA replication in *Escherichia coli* 15T$^-$. J.

Mol. Biol. **64**:47–60.
23. **Lark, K. G., and C. A. Lark.** 1979. *recA*-dependent DNA replication in the absence of protein synthesis: characteristics of a dominant lethal replication mutation, *dnaT*, and requirement for *recA*$^+$ function. Cold Spring Harbor Symp. Quant. Biol. **43**:537–549.
24. **Lark, K. G., T. Repco, and E. J. Hoffman.** 1963. The effect of amino acid deprivation on subsequent DNA replication. Biochim. Biophys. Acta **76**:9–24.
25. **Luder, A., and G. Mosig.** 1982. Two alternative mechanisms for initiation of DNA replication forks in bacteriophage T4: priming by RNA polymerase and by recombination. Proc. Natl. Acad. Sci. USA **79**:1101–1105.
26. **Maaloe, O., and P. C. Hanawalt.** 1961. Thymine deficiency and the normal DNA replication cycle. J. Mol. Biol. **3**:144–155.
27. **Masai, H., and K. Arai.** 1988. Operon structure of *dnaT* and *dnaC* genes essential for normal and stable DNA replication of *Escherichia coli* chromosome. J. Biol. Chem. **263**:15083–15093.
28. **McMacken, R., L. Silver, and C. Georgopoulos.** 1987. DNA replication, p. 564–612. *In* J. L. Ingraham, K. B. Low, B. Magasanik, F. C. Neidhardt, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella typhimurium*: cellular and molecular biology. American Society for Microbiology, Washington, D.C.
29. **Menkens, A. E., and K. N. Kreuzer.** 1988. Deletion analysis of bacteriophage T4 tertiary origins: a promoter sequence is required for a rifampicin-resistant replication origin. J. Biol. Chem. **263**:11358–11365.
30. **Messer, W.** 1972. Initiation of deoxyribonucleic acid replication in *Escherichia coli* B/r: chronology of events and transcriptional control of initiation. J. Bacteriol. **112**:7–12.
31. **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.
32. **Smith, G. R.** 1988. Homologous recombination in procaryotes. Microbiol. Rev. **52**:1–28.
33. **von Meyenburg, K., E. Boye, K. Skarstad, L. Koppes, and T. Kogoma.** 1987. Mode of initiation of constitutive stable DNA replication in RNase H-defective mutants of *Escherichia coli* K-12. J. Bacteriol. **169**:2650–2658.
34. **von Meyenburg, K., and F. G. Hansen.** 1987. Regulation of chromosome replication, p. 1555–1577. *In* J. L. Ingraham, K. B. Low, B. Magasanik, F. C. Neidhardt, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella typhimurium*: cellular and molecular biology. American Society for Microbiology, Washington, D.C.
35. **Walker, G. C.** 1984. Mutagenesis and inducible responses to deoxyribonucleic acid damage in *Escherichia coli*. Microbiol. Rev. **48**:60–93.
36. **Witkin, E. M., and T. Kogoma.** 1984. Involvement of the activated form of RecA protein in SOS mutagenesis and stable DNA replication in *Escherichia coli*. Proc. Natl. Acad. Sci. USA **81**:7539–7543.
37. **Witkin, E. M., V. Roegner-Maniscalo, J. B. Sweasy, and J. O. McCall.** 1987. Recovery from ultraviolet light-induced inhibition of DNA synthesis requires *umuDC* gene products in *recA718* mutant strains but not in *recA*$^+$ strains of *Escherichia coli*. Proc. Natl. Acad. Sci. USA **84**:6805–6809.