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# DNA damage-inducible origins of DNA replication in *Escherichia coli*

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**Upon induction of the SOS response in *Escherichia coli*, the mode of initiation of DNA replication is altered such that it can occur in the absence of normally required protein synthesis. This type of DNA replication has been termed induced stable DNA replication (iSDR). We examined the origin usage during iSDR and found that the initiation of iSDR occurs primarily in the *oriC* and *terC* regions of the chromosome in a manner completely independent of transcription, translation and DnaA protein. Minichromosomes (*oriC* plasmids) pOC23 and pOC81 were induced to replicate in the absence of DnaA protein and transcription after SOS induction. The results localized one of the iSDR origin activities in a 596 bp region which includes the minimal *oriC*.**

*Key words:* DNA replication/*oriC*/recombination/SOS

## Introduction

The cellular response to different types of stress is the hallmark of the cell's strategy for survival in adverse environments. *Escherichia coli* is known to possess several distinct responses; e.g. the SOS response to genetic stress (Walker, 1984 for review), the heat shock response (Neidhardt and van Bogelen, 1987 for review), and the OxyR and SoxR response to oxidative stress (Farr and Kogoma, 1991 for review). The SOS response is elicited when DNA is damaged by UV radiation or when chromosome replication is arrested, for example, by incubation with nalidixic acid, a DNA gyrase inhibitor, or by thymine starvation of thymine-requiring cells. A set of at least 17 genes is known to be derepressed upon induction of the SOS response as a result of inactivation of the LexA repressor mediated by an activated form of RecA protein (Walker, 1984). The products of these DNA damage inducible genes function in concert to enhance the cell's survival. The SOS response includes an inducible DNA repair pathway and an accompanying mutagenic activity (SOS mutagenesis). An involvement of DNA polymerase II in this process has been proposed (Bonner *et al.*, 1988).

Chromosome replication in *E.coli* is initiated at a unique site on the chromosome, termed *oriC* (Hiraga, 1976). The process begins with binding of DnaA protein (encoded by the *dnaA* gene) to the 9 bp sequence (DnaA box) which is repeated four times in *oriC* (Oka *et al.*, 1980; see McMacken *et al.*, 1987 for review). This interaction results in melting of the duplex in the region allowing the entry of DnaB helicase and subsequent formation of a priming complex which lays down a small RNA primer for DNA synthesis by DNA polymerase III homoenzyme. *In vivo*, transcription in this region is essential, perhaps to facilitate the initial melting process (Lark, 1972; Messer, 1972). Also required for initiation *in vivo* is protein synthesis (Maaloe and Hanawalt, 1961; Kogoma and Lark, 1970). Thus, rifampin (RIF), an RNA polymerase inhibitor, and chloramphenicol (CAM), a protein synthesis inhibitor, block initiation while allowing completion of an ongoing round of DNA replication.

In *E.coli rnhA* mutants lacking ribonuclease H (RNase HI) activity, an altered mode of DNA replication, termed constitutive stable DNA replication (cSDR), is activated which can be initiated in the absence of protein synthesis (Kogoma, 1986 for a review). In this mode, DNA replication originates from several sites (collectively termed *oriK*) other than *oriC* in a DnaA independent manner. Thus the *oriC* site and *dnaA* gene are dispensable in *rnhA* mutants (Kogoma and von Meyenburg, 1983).

Changes of the DNA replication mode upon response to stress has not been systematically studied. Billen (1969) reported that after UV irradiation a new round of DNA replication is initiated from the origin of replication. Similarly, after thymine starvation, DNA replication resumes from the origin of replication as well as at the replication fork that has been arrested during thymine starvation (Pritchard and Lark, 1964). The DNA replication that occurs after UV irradiation, thymine starvation or nalidixic acid treatment was shown to be altered such that initiation becomes insensitive to the presence of CAM and was termed inducible stable DNA replication (iSDR) (Kogoma and Lark, 1970, 1975). Initiation of iSDR results in semi-conservative replication of the entire *E.coli* genome for many hours (Kogoma and Lark, 1970). iSDR is an SOS function and is considerably more resistant to UV irradiation than normal DNA replication (Kogoma *et al.*, 1979). It appears to be error prone (Lark and Lark, 1978). It has recently been determined that derepression of the *recA* gene and activation of the elevated level of RecA protein are the necessary and sufficient conditions for the induction of iSDR and that the initiation depends on some function of RecBC enzyme (Magee and Kogoma, 1990). In this study we have examined the origin usage during iSDR and found two primary regions on the chromosome in which initiation of iSDR occurs in the absence of transcription, translation and DnaA protein after induction of the SOS response: one in the *oriC* region and the other in *terC*, a region of termination of normal chromosome replication.

## Results

### *iSDR can occur in the complete absence of DnaA activity*

DNA synthesis in the presence of CAM after UV irradiation of a *dnaA46*(Ts) strain has been shown to be temperature resistant (Ciesla and Jonczyk, 1980). In order to determine whether this result could be extended to include other *dnaA* alleles and other SOS inducing treatments, iSDR activity was tested at 42°C in *dnaA5, dnaA203, dnaA204, dnaA205, dnaA211* and *dnaA508* mutants induced by a variety of SOS inducing conditions. It was found that none of the *dnaA*(Ts) mutations blocked iSDR at 42°C when it was induced by UV irradiation, nalidixic acid treatment or thymine starvation (data not shown). The results strongly suggested that iSDR did not require DnaA protein for initiation. It has been reported, however, that most *dnaA*(Ts) mutations are leaky and the residual DnaA activity in these mutants at the restrictive temperature is sufficient for some plasmids (e.g. F plasmid) that require DnaA protein activity for replication (Hansen and Yarmolinsky, 1986; Kline *et al.*, 1986; Kogoma and Kline, 1987; Murakami *et al.*, 1987). To exclude the possibility that iSDR occurred owing to the DnaA activity remaining in these Ts mutants at the restrictive temperature, the effect of insertional inactivation of the *dnaA* gene on iSDR was examined. The strain used as AQ5496 (*rnhA224 dnaA*::Tn*10*) in which the lethal effect of the insertional inactivation of *dnaA* was suppressed by the *rnhA* mutation which activates the cSDR pathway. Since cSDR depends on transcription (Kogoma, 1978; von Meyenburg *et al.*, 1987), cSDR in this strain can be inhibited by addition of RIF which, on the other hand, has no effect on iSDR (Lark, 1972). Thus, iSDR activity can be specifically measured by following incorporation of radioactive thymine into acid-insoluble material in the presence of both CAM and RIF. The result shown in Figure 1 indicated that DNA synthesis in this strain could occur in the presence of CAM without inducing treatments, i.e. replication by the cSDR pathway. However, when RIF was present, DNA synthesis occurred only slowly. This slow rate of DNA synthesis is due most likely to the iSDR activity that was partially induced in the strain in the absence of thymine starvation. The reduced rate of DNA synthesis relative to protein synthesis seen in *rnhA* mutants (von Meyenburg *et al.*, 1987) is a condition that is known to partially induce iSDR (Kogoma and Lark, 1975). A slow rate of RIF-resistant replication in *rnhA* mutants without thymine starvation is also seen in the experiment shown in Figure 2D (see below). After thymine starvation, DNA synthesis continued for several hours despite the presence of both CMA and RIF. The rate of DNA synthesis was proportional to the duration of thymine starvation. We concluded that iSDR can occur in the complete absence of DnaA protein.

### *Minichromosome replication after SOS induction*

It was previously proposed that initiation of iSDR occurs in the region of *oriC* (Kogoma and Lark, 1970). To test if the *oriC* region in fact contained an origin of replication for iSDR, minichromosomes which carry various segments of the *oriC* region of the chromosome were examined for replication in the presence of CAM and RIF after SOS induction by thymine starvation. pOC23 is a minichromosome capable of autonomous replication owing to the *oriC*

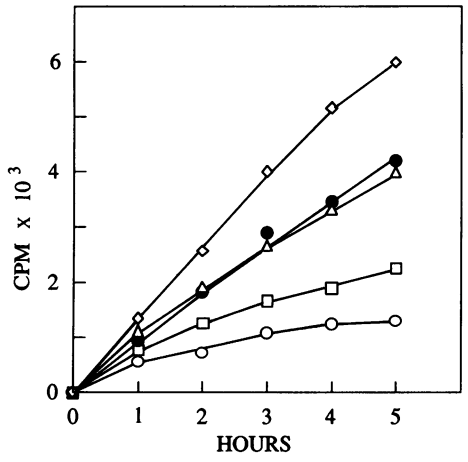


**Fig. 1.** Effect of *dnaA850*::Tn*10* on iSDR. A culture of AQ5496 (*rnhA224 dnaA850*::Tn*10*) was grown to 5 × $10^7$ cells/ml, and starved for thymine for 30 (□), 60 (△), and 90 (◇) min. Subsequently, [$^3$H]thymine (10 μCi/8 μg/ml), CAM (150 μg/ml) and RIF (200 μg/ml) were added. Control cultures were not starved for thymine but were immediately incubated with [$^3$H]thymine + CAM (●) or [$^3$H]thymine + CAM + RIF (○). Samples (0.1 ml) were removed at intervals and trichloroacetic acid (TCA)-precipitable counts were determined by scintillation counting as previously described (Kogoma and Lark, 1975).

site that it carries (Figure 2A). To prevent integration of the plasmid into the chromosome by homologous recombination, a strain (AQ4401) that had a 6 kb deletion including *oriC* was used (Figure 2A). The lethal effect of the *oriC* deletion in the strain was suppressed by the integration of a mini-F (*zif90*::pML*31kan*) near *oriC*. The strain transformed with pOC23 was labelled with [$^3$H]thymine for three generations. Radioactive thymine was removed by filtration, and the cells were resuspended in medium lacking required thymine. The cell suspension was split into two halves. Immediately, a mixture of thymine, CAM and RIF was added to one half of the culture (uninduced control). The other half of the culture was starved for thymine for 120 min and then the mixture was added. Samples were taken every 60 min for 5 h, and total DNA was isolated from the cells and digested with *Hin*dIII. Amounts of DNA samples normalized to give the same $^3$H counts were loaded for agarose gel electrophoresis. After electrophoresis the gel was blotted on a membrane and the resulting Southern blot was probed with $^{32}$P-labelled *Pst*I−*Bam*HI fragment (Figure 2A). The autoradiogram of the probed blot is shown in Figure 2B. The areas of the blot corresponding to the bands were cut out, and radioactivity was determined by liquid scintillation counting (Figure 2C). The result indicates that the minichromosome replicated continuously for 5 h despite the presence of CAM and RIF when cells were induced for iSDR whereas minichromosome replication in uninduced cells ceased immediately after the addition of drugs. Thus, pOC23 contains the DNA sequence that can be activated to permit continuous replication in the absence of both transcription and translation only in SOS induced cells.

Similarly, another minichromosome, pOC81, which consists of a 1674 bp *Hin*cII fragment containing *oriC* and a *kan* fragment (Figure 2A), was examined for the origin activity. Since pOC81 must be selected in medium containing kanamycin, the Δ*oriC zif90*::pML31*kan* strain could not be used as host cells. In this case, a strain (AQ5500) in which

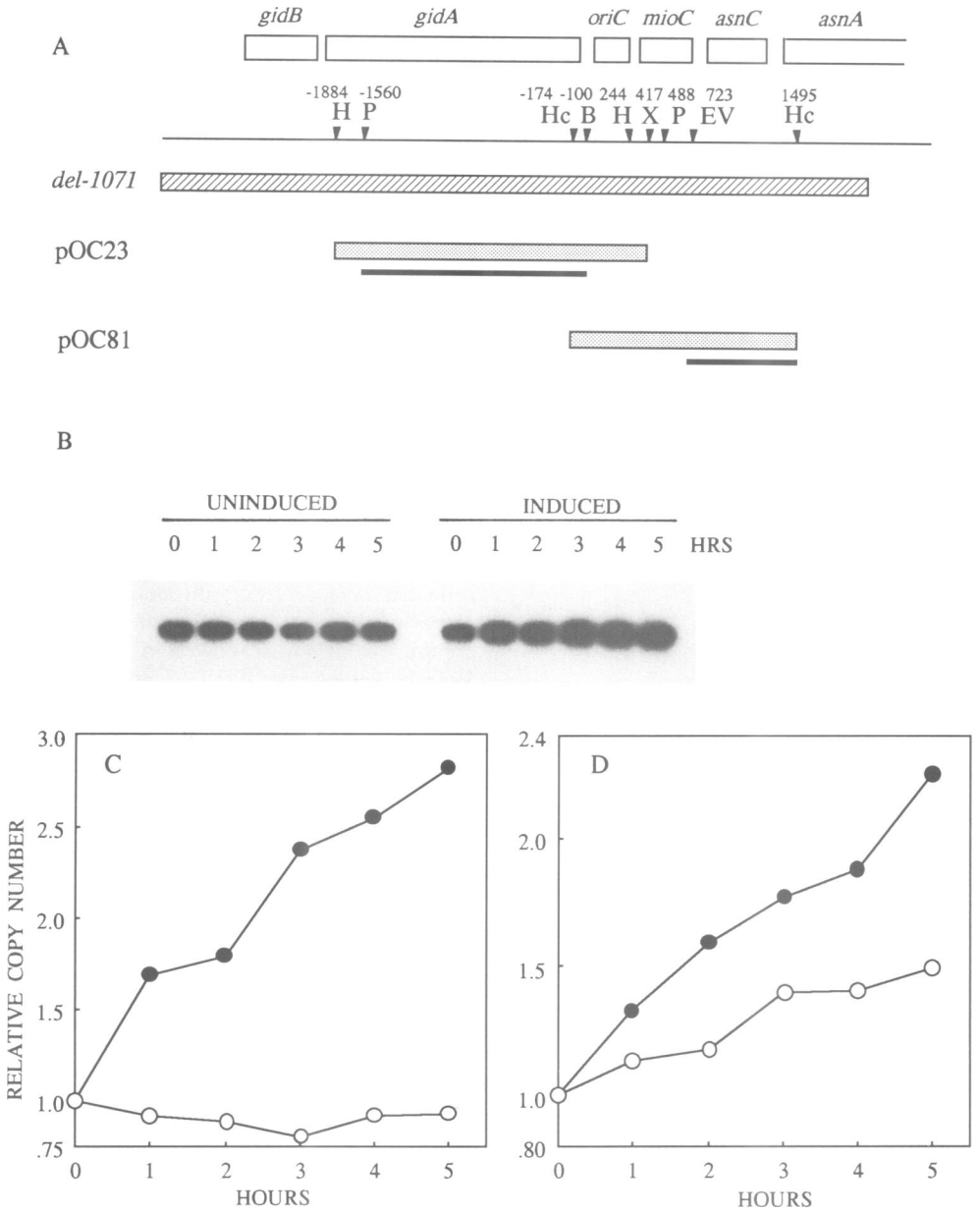


**Fig. 2.** Minichromosome replication after SOS induction. (**A**) The locations of restriction enzyme sites and known genes in the *oriC* region are shown alongside the segments covered by *oriCdel-1071* and carried by pOC23 and pOC81. The solid bars beneath the minichromosome segments indicate DNA fragments used to prepare labelled probes. B, *Bam*HI; H, *Hin*dIII; P, *Pst*I; X, *Xho*I; Hc, *Hin*cII; EV, *Eco*RV. The numbers above the restriction sites are the *oriC* region coordinates (Meijer *et al.*, 1979; Sugimoto *et al.*, 1979). (**B**) AQ4401 (*oriCdel-1071 zif90*::pML*31kan*) carrying pOC23 was grown in the presence of [$^3$H]thymine (5 μCi/8 μg/ml) for about three generations to 2 × $10^8$ cells/ml. The doubling time of the culture was 90 min under the above growth condition. Cells were then collected by filtration, washed extensively and resuspended in prewarmed medium lacking thymine. The cell suspension was split into two halves. One half (uninduced) immediately received a mixture of thymine, CAM and RIF. The other half (induced) was starved for thymine for 120 min, and then the mixture was added. Samples (1 ml) were taken immediately after addition of the mixture and at intervals thereafter as indicated. Total DNA was extracted from the cells as described in Materials and methods. Amounts of DNA samples normalized to give the same $^3$H counts were digested with *Hin*dIII and loaded on a 1% agarose gel and electrophoresed for 60 min. After electrophoresis, the gel was capillary-blotted on a Hybond-$N^+$ membrane, and the resulting Southern blot was probed with $^{32}$P-labelled *Pst*I–*Bam*HI DNA shown in (A) above. The autoradiogram was made by exposing the Southern blot to X-ray film. (**C**) The areas of the blot corresponding to the radioactive bands were cut out, and the radioactivity was determined by liquid scintillation counting. Induced (●); uninduced (○). (**D**) AQ5500 (*rnhA224 oriCdel-1071*) carrying pOC81 was similarly treated as described in (C) for AQ4401(pOC23). Radioactive bands on the blot were cut out and radioactivity was determined by liquid scintillation counting. Induced (●); uninduced (○).

the Δ*oriC* was suppressed by the *rnhA224* mutation was used. The result shown in Figure 2D indicates that pOC81 also replicated in CAM + RIF in thymine-starved cells. In contrast to the immediate cessation of pOC23 replication in uninduced Δ*oriC zif90*::pML*31* cells (Figure 2B and C), the replication of pOC81 in uninduced Δ*oriC rnhA* cells continued albeit at a slow rate. This is due most likely to the iSDR activity that was partially induced in the strain (see Figure 1 above). The results with the two minichromosomes described above together strongly suggest that an origin activity for iSDR resides in the region that is shared by the two plasmids, i.e. the 596 bp domain between the *Hin*cII (−174) and *Xho*I (+417) sites which includes the minimal *oriC* (Figure 2A).

### *Initiation of iSDR in the absence of oriC*

The above result (Figure 2) indicated that iSDR could be initiated from an origin in the *oriC* region. When a Δ*oriC* (*rnhA*) mutant was tested for iSDR after SOS induction by UV irradiation, the strain unexpectedly exhibited strong iSDR despite the deletion including the *oriC* region (Figure 3). A similar result was obtained when the strain was induced by nalidixic acid treatment (data not shown). The results suggested that iSDR could originate from other sites in the absence of the origin activity in the *oriC* region. In order to determine where initiation of DNA replication occurs after induction of iSDR, we utilized the procedure of marker frequency analysis (de Massy *et al.*, 1984; Hill *et al.*, 1987). An *oriC*-deleted strain AQ5500 (*rnhA224 oriC-del*) was induced for iSDR by thymine starvation, and DNA was labelled with [$^3$H]thymine during the subsequent iSDR in the presence of both CAM and RIF. Labelled DNA was analyzed for the marker frequency at multiple sites on the chromosome as described in Materials and methods. The result is depicted in Figure 4A. The highest marker frequency was seen in the region of *terC* whereas the frequency was lowest in the *oriC* region as expected. In addition to the *terC* region, two other regions around 0 and 70 min exhibited minor peaks. The inducible origin located in the terminus region was also active following UV irradiation or nalidixic acid treatment (data not shown).

The origin(s) in the *terC* region is active regardless of the presence or absence of *oriC* because a similar peak in the *terC* region was also seen in an *oriC*$^+$ strain induced by nalidixic acid (Figure 4B). The result of this experiment also rules out the possibility that the peak in the *terC* region reflected the completion of rounds of replication originated elsewhere because the peak in the *terC* region was seen in a culture which was allowed to replicate for only 15 min after the induction of iSDR (Figure 4B). We concluded that in addition to the origin activity in the *oriC* region, normally cryptic origins in the *terC* region and possibly in two additional regions elsewhere on the chromosome can be activated to function after SOS induction.

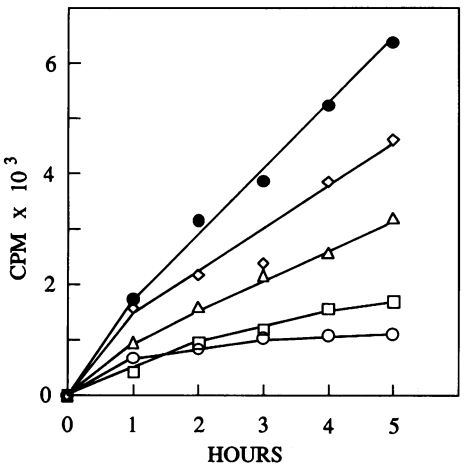


**Fig. 3.** Effect of *oriCdel-1071* on iSDR. A culture of AQ2079 (*rnhA224 oriCdel-1071*) was grown to 1 × 10$^8$ cells/ml and irradiated with UV (30 J/m$^2$). The cells were incubated for 30 (□), 60 (△) and 90 (◇) min and at the end of the incubation time, [$^3$H]thymine, CAM and RIF were added. Control cultures were not irradiated but instead were immediately incubated with [$^3$H]thymine + CAM (●) or [$^3$H]thymine + CAM + RIF (○). Samples (0.1 ml) were removed at intervals and TCA-precipitable counts determined.

### *Is the origin in the terC region a cryptic phage origin?*

Two cryptic lambdoid phages, *rac* and *kim*, are located in the terminus region of some strains of *E.coli* K-12 (Diaz *et al.*, 1979; Espion *et al.*, 1983). A functional origin of DNA replication (*oriJ*) has been cloned from the *rac* prophage (Diaz and Pritchard, 1978). Conceivably, one or both of these phages could have an origin of replication that

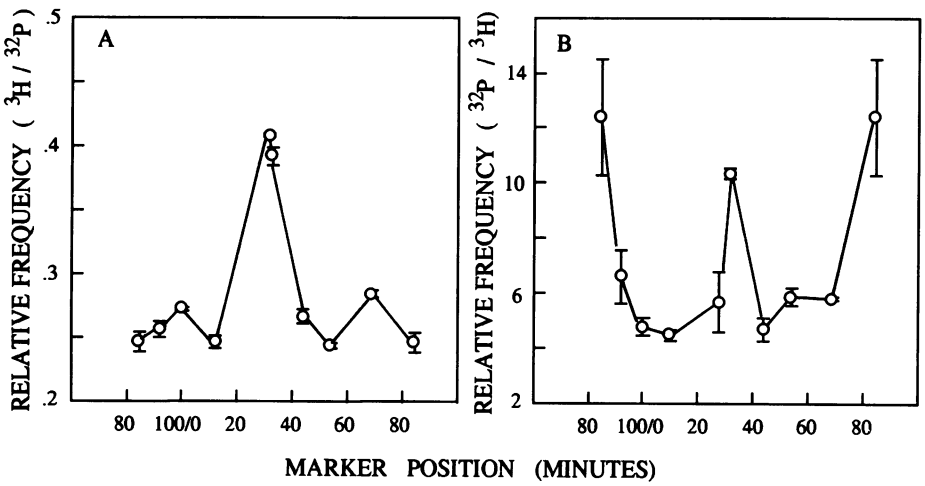


**Fig. 4.** Relative marker frequencies in AQ5500 and AQ634. (**A**) A culture of AQ5500 (*rnhA224 oriCdel-1071*) was grown to 2 × 10$^8$ cells/ml and starved for thymine for 90 min, after which [$^3$H]thymine (140 μCi/2 μg/ml; final specific activity, 8.1 Ci/mmol), CAM (150 μg/ml) and RIF (200 μg/ml) were added. Incubation was continued for 3 h whereupon the cells were quickly chilled, washed and processed for hybridization as described by Hill *et al.* (1987). The marker frequency determination was carried out as described in Materials and methods. Ratios of $^3$H (DNA from iSDR induced cells) and $^{32}$P (DNA from stationary phase cells) are plotted against the marker position on the *E.coli* linkage map (Bachmann, 1990), beginning at 80 min in the clockwise direction. Vertical bars indicate the standard error of duplicate samples. The probes used were (from left to right): pLC26-3, pDR2000, pLC17-22, pLC8-25, pTH51, pBS12, pLC14-29, pLC32-25 and pHA11. (**B**) A culture of AQ634 was grown to 2 × 10$^8$ cells/ml. Nalidixic acid was added and incubation continued for 70 min after which the culture was filtered, suspended in medium containing CAM (150 μg/ml), and incubated for 15 min. The cells were then quickly chilled, washed and processed for hybridization as described by de Massy *et al.* (1984). Ratios of $^{32}$P (DNA from iSDR induced cells) and $^3$H (DNA from stationary phase cells) are graphed as above. The probes used from left to right were pLC26-3, pDR2000, pLC17-22, pLC8-25, pLC4-6, pTH51, pLC14-29, pLC32-25 and pHA11.

becomes active after SOS induction. This possibility was tested by measuring replication of the terminus region in strains deleted for the *rac* or *kim* prophage. A $^{32}$P-labelled *terC* probe was used to measure the relative increase in terminus DNA concentration after induction of iSDR by thymine starvation. The results shown in Figure 5 indicate that terminus DNA concentration increased steadily for 5 h in the presence of RIF after induction for both the parental and Δ*rac* strains. Deletion of *kim* had no inhibitory effect on iSDR from the *terC* region (Figure 5). These results also indicate that replication from the terminus region is independent of transcription since replication occurred in the presence of RIF. The replication was also found not to require DnaA protein (data not shown).

## Discussion

Prichard and Lark (1964) demonstrated that after thymine starvation, DNA replication was resumed at the site at which chromosome replication during the regular replication cycle was initiated, i.e. the origin of replication (presumably *oriC*). Subsequent density shift experiments by Billen (1969) and Kogoma and Lark (1970) extended this finding to include both UV irradiation and nalidixic acid treatment, respectively, as treatments that induce initiation from the origin of replication. These results suggested that DNA replication originates from the *oriC* region following SOS induction. In this study we have demonstrated that after a period of thymine starvation, DNA replication is indeed initiated in a 596 bp region on the chromosome which includes the minimal *oriC*. The initiation can occur in the absence of both transcription and translation. It does not require DnaA protein (Figure 1). This was further substantiated by the observation that minichromosome replication occurred in the presence of CAM and RIF in *dnaA203*(Ts) mutants which were incubated at 42°C during and after thymine starvation (data not shown). In fact, the initiation from this region after thymine starvation probably does not require an active *oriC* site since an *oriC* site that was inactivated by mutations in three of the four DnaA boxes could function as an origin after thymine starvation (T.Asai and T.Kogoma, manuscript in preparation). We conclude that iSDR induced after DNA damage originates from the *oriC* region without the participation of DnaA protein.

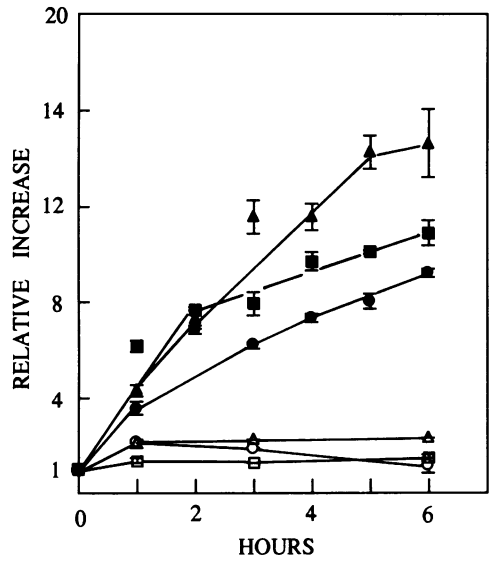


**Fig. 5.** Effect of deleting the *rac* and *kim* cryptic prophages on iSDR. Isogenic strains LSO489 (*rac*$^+$ *kim*$^+$), PK1567 (*rac-del*) and PK2220 (*kim-del*) were grown exponentially, starved of thymine for 70 min after which thymine (8 μg/ml) and RIF (200 μg/ml) were added. Controls were unstarved cultures. Duplicate samples (0.1 ml) were removed at intervals and processed for slot blot hybridization as described in Materials and methods. pJH113 was used to measure the relative increase in DNA in the *terC* region. LSO489, thymine-starved (●) and unstarved (○); PK1567, thymine-starved (■) and unstarved (□); PK2220, thymine-starved (▲) and unstarved (△).

We have also detected another DNA damage-inducible origin of replication localized at ~31 min in the *terC* region on the *E.coli* chromosome. The replication from the terminus after thymine starvation was previously suggested by the premature replication of the terminus region which was detected in an earlier density-shift experiment (see Figure 6 of Kogoma and Lark, 1970). Two cryptic lambdoid phages, *rac* and *kim* reside in some strains of *E.coli* K-12 (Diaz *et al.*, 1979; Espion *et al.*, 1983). We detected origin activity in the *terC* region of the strains that are deleted for *rac* or *kim*. This suggests that origin activity is not associated with either cryptic phage although the possibility that either one functions in the absence of the other cannot at present be ruled out. Previously, two origins of DNA replication (*oriK*s) that are activated by *rnhA* mutations were also mapped in the *terC* region (de Massy *et al.*, 1984). The relationship between these origins and the origin activity after SOS induction detected in the *terC* region has not been examined.

Although the origin activities in the *oriC* and *terC* regions are most conspicuous, other sites can also be activated for iSDR. In fact, the marker frequency analysis suggested activation of at least two other origins elsewhere in the *oriC*-deleted strain after thymine starvation (Figure 4A). It is not unlikely that additional sites undetectable by the marker frequency assay are activated in SOS induced cells, which contribute to the overall iSDR activity.

It has recently been reported that heat shock stress induces a transient non-scheduled initiation of chromosome replication in *E.coli* and, when combined with restricted RNA synthesis activity, leads to an elevated chromosome copy number (Guzman *et al.*, 1988). Whether this extra initiation occurs at *oriC* or other sites is unknown.

Clearly, during the SOS response to genetic stress, a new set of origins is activated as shown in this study. The recovery of the stalled replisomes [induced replisome reactivation (IRR)] after UV irradiation also occurs in SOS induced cells (Khidhir *et al.*, 1985). It appears therefore that there are at least two modes of DNA replication that operate after induction of SOS: the initiation of new rounds of DNA replication at fixed sites on the chromosome and the reactivation of stalled replisomes. The relationship between the two modes of replication has been discussed (Magee and Kogoma, 1990).

The initiation from these DNA damage inducible origins must occur in a manner radically different from the normal mechanism at *oriC* since the iSDR initiation involves neither DnaA protein nor transcription. Available evidence is insufficient at present to elucidate the mechanism of initiation iSDR at these origins. The following considerations, however, point to involvement of recombinational activities in the process. The iSDR process requires RecBC protein and an elevated level of activated RecA (Magee and

Kogoma, 1990). *recD* mutations which elevate the recombination frequencies also enhance iSDR activity after thymine starvation and nalidixic acid treatment (T.Asai and T.Kogoma, unpublished). It is possible that during the SOS response an activity is induced which introduces a nick or a double-strand break at or near *oriC*, providing a substrate for RecBCD enzyme to produce a single-stranded DNA with a 3′-OH end. Catalyzed by RecA protein, the 3′-OH end invades another molecule, forming a D-loop. Such a D-loop may be utilized not only for homologous recombination but also for an initiation site for replication. The notion that such a structure is competed by a recombination pathway and the iSDR initiation pathway is supported by our recent observation that *ruvC* mutations stimulate iSDR (T.Asai and T.Kogoma, manuscript in preparation). The *ruvC* mutations inactivate a nuclease that resolves Holliday junction intermediates in homologous recombination (Connolly *et al.*, 1991). An involvement of recombination activities has also been seen in the later stage of phage DNA replication in T4 phage-infected cells (Luder and Mosig, 1982; Kreuzer *et al.*, 1988). The availability of the sequence that functions as an origin of iSDR may facilitate identification of the specific activity that triggers the initiation process after DNA damage.

## Materials and methods

### Media and growth conditions

Unless otherwise stated, cells were grown at 37°C with aeration by shaking in M9 salts−glucose medium (Lark *et al.*, 1963) supplemented with casamino acids (0.2%; Difco Laboratories, Detroit, MI), required amino acids (50 μg/ml), thymine (8 μg/ml) and thiamine hydrochloride (2 μg/ml).

### E.coli strains and plasmids used

The bacterial strains are described in Table I. Strain AQ4241 was constructed by R.Rowland in this laboratory by EMS mutagenesis followed by trimethoprim selection of strain CM735 (Magee and Kogoma, 1991). The presence of the *oriC-1071* deletion in AQ5500 and the *dnaA850*::Tn*10* mutation in AQ5496 were verified by Southern blot hybridization as previously described (Kogoma and von Meyenburg, 1983). AQ5441 was a $Tc^s$ derivative of AQ5423 isolated by the procedure of Bochner *et al.* (1980). pOC23 and pOC81 (Messer *et al.*, 1978) were a gift of W.Messer. pJH113 has been described (Henson and Kuempel, 1985). Other plasmids used for the marker frequency determination experiments have also been described (de Massy *et al.*, 1984; Hill *et al.*, 1987).

### Enzymes

Restriction endonucleases and the nick translation kit were from Bethesda Research Laboratories (Gaithersburg, MD). Restriction endonuclease digestion and nick translation were performed according to the manufacturer's protocol.

### Induction of iSDR

Overnight cultures were diluted into fresh medium and allowed to grow for three mass doublings to ~2 × $10^8$ cells/ml. For thymine starvation, cells were collected by filtration, resuspended in thymine-minus medium and incubated for a duration of time as indicated. After thymine starvation, thymine (8 μg/ml) and either CAM (150 μg/ml) or CAM + RIF (200 μg/ml) were added to the culture. The controls were unstarved cultures which received thymine and CAM or CAM + RIF immediately after filtration without a period of thymine starvation. In the case of nalidixic acid treatment, nalidixic acid (50 μg/ml) was added to the culture. After a period of time, the culture was filtered and resuspended in medium containing thymine and either CAM or CAM + RIF. A control culture was filtered immediately after addition of nalidixic acid. In the case of UV irradiation, the culture was collected by centrifugation and resuspended in M9 medium, irradiated with UV (30 J/$m^2$) and resuspended in complete minimum medium, and incubated (Kogoma *et al.*, 1979). The control was unirradiated.

### Slot blot hybridization

Samples taken after induction of iSDR were processed essentially as described by Shields *et al.* (1986). Following hybridization and washing, hybridization filters were cut out and radioactivity determined by scintillation counting.

### Extraction of total DNA and Southern hybridization

Total cellular DNA was extracted from cells 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°C before the addition of SDS and proteinase K. Southern hybridization was carried out as described by Sambrook *et al.* (1989). The Hybond-$N^+$ membrane (Amersham, Arlington Heights, IL) was used for blotting.

### Determination of marker frequency

The marker frequency was determined essentially as described (Hill *et al.*, 1987). In brief, after thymine starvation, cells were labelled with [$^3$H]thymine (140 μCi/2 μg/ml; final specific activity = 8.1 Ci/mmol) for 3 h in the presence of CAM and RIF, and DNA was extracted and purified. DNA from stationary phase cells was similarly isolated and labelled with [α-$^{32}$P]dCTP by nick translation (Sambrook *et al.*, 1989). The radiolabelled DNAs were hybridized to marker frequency probes fixed on nitrocellulose filters. The filters were then washed, and $^3$H and $^{32}$P counts were determined by liquid scintillation counting. Some experiments in this study were done by a slightly different method that gave comparable results (de Massy *et al.*, 1984). In these cases, the DNAs from SOS induced cells and stationary phase cells were labelled by nick translation with [α-$^{32}$P]dCTP and [α-$^3$H]dCTP, respectively.

**Table I.** *E.coli* K-12 strains

| Strain | Relevant genotype[a] | Reference/construction |
|---|---|---|
| AQ634 | *rnh*$^+$ *thyA deoB* (or *C*) | Kogoma and von Meyenburg, 1983 |
| AQ978 | *rnhA224 zag*::Tn*10* | Subia and Kogoma, 1986 |
| AQ2079 | *rnhA224 oriCdel-1071* | Kogoma *et al.*, 1985 |
| AQ3519 | *dnaA850*::Tn*10 rnhA*::*cat* | Kline *et al.*, 1986 |
| AQ3746 | *dnaA5 zif90*::pML*31* | Kogoma and Kline, 1987 |
| AQ3788 | *oriCdel-1071 zif90*::pML*31* | Kogoma and Kline, 1987 |
| AQ4241 | *rnh*$^+$ *thyA deoB(C)* | Magee and Kogoma, 1991 |
| AQ4382 | *thyA deoB(C) zif90*::pML*31* | P1(AQ3788) × AQ4241, select $Km^r$ |
| AQ4401 | *thyA deoB(C) zif90*::pML*31 oriCdel-1071* | P1(AQ3788) × AQ4382, select $Tc^r$ |
| AQ5423 | *rnhA224 zag*::Tn*10 thyA deoB(C)* | P1(AQ978) × AQ4241, select $Tc^r$; screen for $Sdr^c$ |
| AQ5441 | *rnhA224 thyA deoB(C)* | $Tc^s$ of AQ5423 (this work) |
| AQ5496 | *dnaA850*::Tn*10 thyA deoB(C) rnhA224* | P1(AQ3519) × AQ5441, select $Tc^r$ |
| AQ5500 | *oriCdel-1071 thyA deoB(C) rnhA224* | P1(AQ3788) × AQ5441, select $Tc^r$ |
| LSO489 | *rac*$^+$ *kim*$^+$ | Binding *et al.*, 1981 |
| PK1567 | *del-1438* (*rac-del*) | P.Kuempel |
| PK2220 | *del-2038* (*kim-del*) | Hill *et al.*, 1987 |

[a]The remaining genotype: F$^-$ *trp his metE thi ara lacZ galK mtl rbs*$^+$ *rpsL tonA T6*$^r$ *sup-38* except for AQ634, AQ978, AQ3519, F$^-$ *ilv metB his-29 trpA9605 pro-2*; AQ2079, F$^-$ *proA3 his-29 trpA9605 lac-3 ilv-192 ansB50*::Tn*5*; AQ3788, F$^-$ *ilv-192 ansB32 relA spoT thi-1 fuc lysA.*

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