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# Overexpression of the *dnaA* Gene in *Escherichia coli* B/r: Chromosome and Minichromosome Replication in the Presence of Rifampin

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**The replication of chromosomes and minichromosomes in *Escherichia coli* B/r was examined under conditions in which the *dnaA* gene product was overproduced. Increased levels of the DnaA protein were achieved by thermoinduction of the *dnaA* gene, under the control of the lambda $p_L$ promoter, or by cellular maintenance of multicopy plasmids carrying the *dnaA* gene under the control of its own promoters. Previous work has shown that overproduction of DnaA protein stimulates replication of the chromosomal origin, *oriC*, but that the newly initiated forks do not progress along the length of the chromosome (T. Atlung, K. V. Rasmussen, E. Clausen, and F. G. Hansen, p. 282–297, *in* M. Schaechter, F. C. Neidhardt, J. L. Ingraham, and N. O. Kjeldgaard, ed., *The Molecular Biology of Bacterial Growth*, 1985). In the present study, it was found that overproduction of DnaA protein caused both a two- to threefold increase in the amount of residual chromosome replication and an extended synthesis of minichromosome DNA in the presence of rifampin. The amount of residual chromosome replication was consistent with the appearance of functional replication forks on the majority of the chromosomes. Since the rate of DNA accumulation and the cellular DNA/mass ratios were not increased significantly by overexpression of the *dnaA* gene, we concluded that the addition of rifampin either enabled stalled replication forks to proceed beyond *oriC* or enabled new forks to initiate on both chromosomes and minichromosomes, or both.**

The molecular mechanism which times the initiation of chromosome replication in *Escherichia coli* has not been determined. Although many of the proteins involved in the process have been identified, the kinetics of their assembly into an active initiation complex has not been studied in vivo. One component of the initiation complex, the *dnaA* gene product, has many properties which suggest that it could be a rate-limiting determinant of complex activity. The DnaA protein is required for initiation from the chromosomal origin of replication, *oriC*, both in vivo and in vitro (2, 7, 8, 12, 13, 15, 37, 39), and mutations in the *dnaA* gene can lead to either a defect in initiation (7, 10, 18, 19, 34, 38) or overinitiation (11, 21). The DnaA protein binds to a 9-base-pair nucleotide sequence which is found four times within *oriC* (13, 20, 24, 28), between the two promoters in the *dnaA* gene (1, 4, 14, 37), upstream of the *mioC* gene promoter adjacent to *oriC* (23, 35), and within several other genes on the chromosome (for a review, see reference 12). The cellular concentration of the DnaA protein is autoregulated by the binding of the protein within the *dnaA* gene promoter region (1, 4).

Recently, chromosome replication in *E. coli* strains which overproduced functional DnaA polypeptide was studied (1a, 2, 5, 9). The amount of markers near *oriC* was found to be increased in the cells with elevated levels of *dnaA* gene product, as would be expected if DnaA protein were rate limiting for initiation (1a, 2). However, the rate of DNA accumulation and the extent of residual DNA replication during inhibition of protein synthesis (runout replication) were not increased appreciably by induced overproduction of DnaA protein (2, 9). These observations led to the hypothesis that the newly initiated replication forks become stalled near the origin and are not engaged in normal chromosome replication (2).

To begin evaluation of the effects of enhanced DnaA protein levels on the timing of initiation at *oriC* in the cell division cycle, we have examined chromosome replication under conditions of increased *dnaA* gene product by using *E. coli* B/r F, a strain in which cell cycle properties have been well characterized (17). Overproduction of the *dnaA* gene product was achieved through the use of plasmids harboring the *dnaA* gene under the control of the lambda $p_L$ promoter (2), the *dnaA* promoter, or both (13). It was found that enhanced *dnaA* gene expression in *E. coli* B/r F resulted in the production of additional initiation events on chromosomes and minichromosomes, which were functional in DNA chain elongation in the presence of rifampin.

## MATERIALS AND METHODS

**Bacterial strains and plasmids.** Experiments were performed with *Escherichia coli* B/r F *his thyA* (17). P1 *vir* lysates of *E. coli* K-12 6405 *c*I857 *nad*::Tn*10* were used for P1 transduction of the *c*I857 temperature-sensitive lambda repressor gene into *E. coli* B/r F. Spontaneous tetracycline-sensitive mutants of B/r F *c*I857 *nad*::Tn*10* were selected on minimal medium plates not containing nicotinic acid. P1 transduction and P1 lysate preparation were as described by Miller (26). *E. coli* K-12 6405 *pro leu thr bio c*I857 was obtained from M. Gottesman.

The plasmids pTAC1445 and pTAC1584, which carry the *dnaA* gene under the control of the lambda $p_L$ promoter, were obtained from T. Atlung and have been described

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previously (1). These plasmids lack the *dnaA* promoters upstream of the *dnaA* gene. The *dnaA* gene on pTAC1584 contains a 121-base-pair deletion and produces nonfunctional DnaA protein. In some experiments, pTAC1445 or pTAC1584 was harbored coincidentally with pALO8 (obtained from A. Lobner-Olesen) which carries the lambda *c*I857 gene encoding thermolabile *c*I repressor protein. An additional plasmid, pBF110, carrying the *dnaA* gene under lambda $p_L$ promoter control was also used in some studies. This plasmid, obtained from R. Fuller, also carries the promoter region from the *dnaA* gene (13). For our studies, a deletion derivative of pBF110 lacking the *Eco*RI fragment which harbors the lambda $p_L$ promoter was constructed. This derivative, pMW110, expresses the *dnaA* gene product solely under the control of the *dnaA* gene promoters. The *Eco*RI fragment from pBF110 which carries the promoter region of the *dnaA* gene, but not the gene itself, was introduced into the unique *Eco*RI site on plasmid pKO1 (25). The resultant 4.9-kilobase plasmid was designated pAL1600. The minichromosome pAL55 contains the *E. coli* chromosomal origin of replication (*oriC*) from nucleotides −41 to 286 (28; C. E. Helmstetter and A. C. Leonard, submitted for publication). Plasmids pTAC1445, pBF110, and pMW110 phenotypically suppressed the *dnaA5* mutation in strain 6405 *dnaA5 zib-501*::Tn*10*, whereas plasmids pTAC1584 and pAL1600 did not (data not shown).

**Growth media and inhibitors.** Cells were grown in glucose minimal medium (17) supplemented with thymine (10 μg/ml), histidine (20 μg/ml), and, for some studies, Casamino Acids (0.2%; reagent grade; Difco Laboratories, Detroit, Mich.). Nicotinic acid (0.1 mM) and biotin (0.1 mM) were provided as required. Chloramphenicol (10 μg/ml), ampicillin (24 μg/ml), tetracycline (10 μg/ml), and kanamycin (100 μg/ml) were supplied as necessary for plasmid-containing cells or cells harboring a transposon. Rifampin was added to cultures during runout replication experiments. The rate of uridine incorporation into cold 5% trichloroacetic acid (TCA)-insoluble material was reduced by rifampin (100 μg/ml) in cultures growing at 25°C (or after a shift to 41°C) to less than 5% during the first 1/10 of a generation and to 2.5% thereafter (data not shown).

**Absorbance, cell concentration, and radioactivity.** $A_{450}$ determinations for cultures were done with a Zeiss PMQII spectrophotometer. Cell concentrations were determined with a Coulter Counter (model ZB; Coulter Electronics, Inc., Hialeah, Fla.). [*methyl*-$^3$H]thymidine (78 to 80 Ci/mmol) was purchased from New England Nuclear Corp., Boston, Mass. For short-term incorporation of radioactive thymidine, [*methyl*-$^3$H]thymidine was added at 20 μCi/ml (final specific activity of 18.5 Ci/mmol). For continuous incorporation, [*methyl*-$^3$H]thymidine was present at 2 μCi/ml (final specific activity of 60 mCi/mmol). Incorporation of [$^3$H]thymidine into DNA was measured by cold TCA-precipitated radioactivity retained on filter disks. Samples were counted in a Spectrafluor (Amersham Corp., Arlington Heights, Ill.) with a liquid scintillation counter (LS-7000; Beckman Instruments, Inc., Fullerton, Calif.).

**Minichromosome replication.** Minichromosome replication was measured by exposing a 2.5-ml sample of culture to [$^3$H]thymidine (20 μCi/ml) for 4 min, followed by the addition of nonradioactive thymidine (100 μg/ml) for 10 min. After being labeled, samples were centrifuged at 12,000 × *g* for 10 min, and whole-cell lysates were prepared as described by Projan et al. (31) and analyzed as indicated elsewhere (22). Thirty-five microliters of whole-cell lysate (total volume, 70 μl) was loaded into each well of either 1.5 or 0.7% horizontal agarose gels, and electrophoresis in Tris-borate-EDTA buffer was performed for 19 h at 65 or 20 V, respectively. After fluorography, the autoradiographs were scanned with a laser densitometer (LKB Instruments, Inc., Rockville, Md.).

**DNA content.** DNA content was determined with the diphenylamine color reaction (6). Cells were harvested by centrifugation from 50 ml of rifampin-treated cultures, and they were washed free of color with a solution of 75% methanol and 5% TCA and then with 0.75 ml of 5% TCA. Pellets were suspended in 0.75 ml of 5% TCA and hydrolyzed at 90°C for 30 min. To 0.5 ml of supernatant, 1.0 ml of diphenylamine reagent was added, and the color was developed overnight at room temperature in the dark. The average $A_{600}$ from duplicate samples was compared with that of standard deoxyadenosine solutions treated in a similar manner. The amount of DNA was estimated as being twice the amount of deoxyadenosine that gave the same $A_{600}$.

## RESULTS

**Chromosome replication during enhanced *dnaA* gene expression.** We examined chromosome replication and cell mass synthesis in *E. coli* B/r F during overproduction of the *dnaA* gene product. Enhanced levels of DnaA protein were achieved by thermally induced gene expression on plasmid pTAC1445, which contained the lambda $p_L$ promoter upstream of the *dnaA* structural gene. The cells also harbored the thermosensitive *c*I857 lambda repressor gene, either on a plasmid (pALO8) or integrated into the chromosome (B/r F *c*I857). The *c*I857 lambda repressor prevents transcription of the plasmid-encoded *dnaA* gene at the permissive temperature of 25°C, but at 41°C, the *c*I857 lambda repressor is inactivated and the lambda $p_L$ promoter-controlled genes are expressed. Figure 1 shows [$^3$H]thymidine incorporation and $A_{450}$ in cultures of B/r F(pTAC1445) during a shift from 25 to 41°C. In B/r F(pTAC1445, pALO8), [$^3$H]thymidine incorporation increased slightly faster than $A_{450}$ for the first 90 min after the shift and increased in parallel thereafter (Fig. 1a). The same experiment was also performed with cells which harbored plasmid pTAC1584, which is identical to pTAC1445 except that it has a 121-base-pair deletion in the *dnaA* gene (1). In these cells, [$^3$H]thymidine incorporation and $A_{450}$ increased at identical rates after the temperature shift (Fig. 1b). A similar relative enhancement in [$^3$H]thymidine incorporation into cells containing pTAC1445, as opposed to pTAC1584, was observed when the *c*I857 gene was integrated into the chromosome (Fig. 1c and d). In this case, [$^3$H]thymidine incorporation and $A_{450}$ increased at similar rates in B/r F *c*I857(pTAC1445) after the temperature shift, but [$^3$H]thymidine incorporation increased more slowly than $A_{450}$ in B/r F *c*I857(pTAC1584). Thus, the postshift DNA/mass ratio was about 30% higher in cells containing pTAC1445 than in those containing pTAC1584.

**Chromosome replication in the presence of rifampin during DnaA protein overproduction.** The slight acceleration in DNA replication relative to cell mass synthesis and the resultant increase in DNA/mass ratio in cells harboring pTAC1445 are consistent with an increase in the number of active chromosome replication forks initiated on overproduction of DnaA protein. To further examine this possibility, chromosome replication was measured during rifampin-induced inhibition of RNA and protein syntheses (15). In cultures undergoing steady-state growth, ongoing rounds of chromosome replication are completed during runout repli-

cation, and new rounds are not initiated. In populations of *E. coli* growing in glucose minimal medium, runout replication leads to a 1.4-fold increase in DNA content in the culture (30). If, on the other hand, extra replication forks were induced on all chromosomal origins just before the addition of rifampin, runout replication would yield a 2.8-fold increase in DNA content, assuming that all the rounds were completed. To measure runout replication during overproduction of the *dnaA* gene product, cultures of B/r F containing the *c*I857 gene and either pTAC1445 or pTAC1584 were grown in the presence of [3H]thymidine at 25°C and then exposed to rifampin either at 25°C or after a shift to 41°C (Fig. 2). When rifampin was added at 25°C to cells harboring either plasmid, [3H]thymidine radioactivity reached a plateau after a 1.4-fold increase in incorporation (Fig. 2a and c). However, when rifampin was added 10 min after a shift to 41°C, incorporation increased 2.1-fold in the presence of pTAC1445, but it increased only 1.4-fold in the presence of pTAC1584 (Fig. 2b and d).

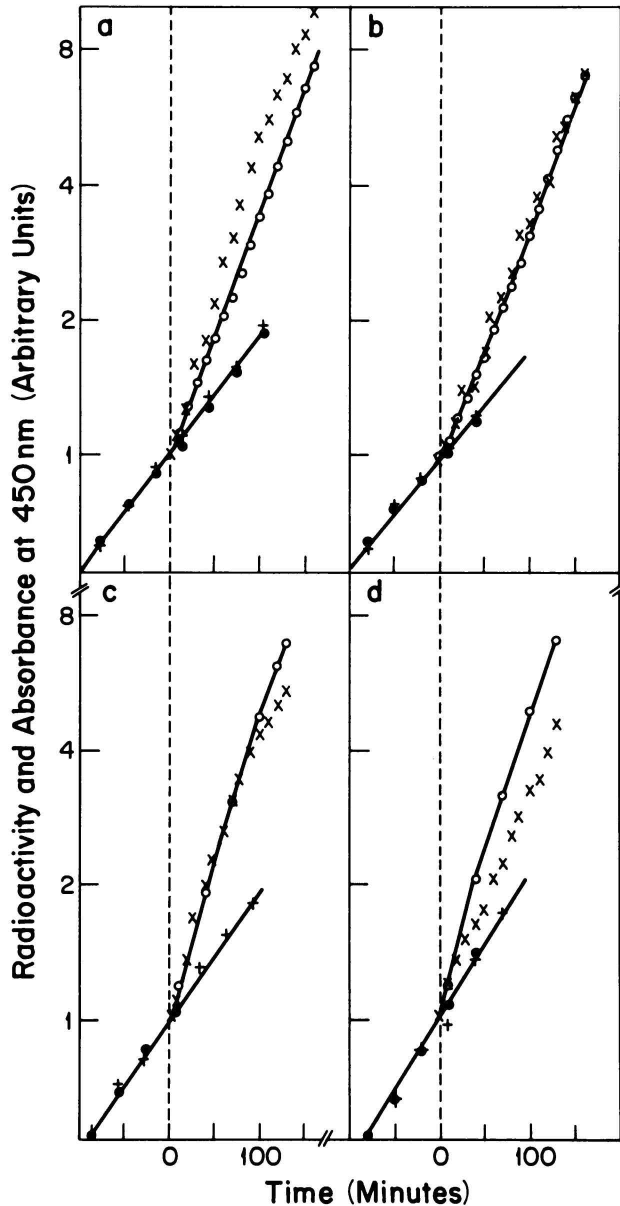


FIG. 1. DNA replication and cell mass synthesis in *E. coli* B/r F during thermal induction of *dnaA* gene expression. Cultures (300 ml) were grown for at least 12 generations at 25°C in glucose minimal medium supplemented with [3H]thymidine (2 μCi/ml). Part of each culture was shifted to 41°C at an $A_{450}$ of 0.11 to 0.13. The time of a temperature shift is indicated (- - -). $A_{450}$ and [3H]thymidine incorporation, measured at the indicated times, are expressed as ratios of the values at the time of the temperature shift. Strains of B/r F which harbored the thermolabile lambda repressor gene integrated into their chromosomes are designated *c*I857. (a) B/r F(pALO8, pTAC1445); (b) B/r F(pALO8, pTAC1584); (c) B/r F *c*I857(pTAC1445); and (d) B/r F *c*I857(pTAC1584). Symbols: ● and ○, $A_{450}$ at 25 and 41°C, respectively; + and ×, radioactivity at 25 and 41°C, respectively.

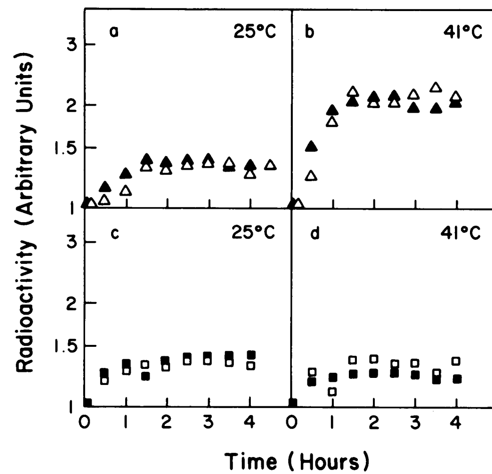


FIG. 2. Runout DNA replication in *E. coli* B/r F in the presence of rifampin after thermal induction of *dnaA* gene expression. Portions of cultures containing pTAC1445 or pTAC1584, incubated as described in the legend to Fig. 1, were exposed to rifampin (100 μg/ml) at 25°C or 10 min after a shift from 25 to 41°C. Each datum point represents the amount of [3H]thymidine incorporation relative to the amount at the time of rifampin addition (time zero). (a and c) Rifampin was added at and cells were maintained at 25°C. (b and d) Rifampin was added 10 min after a shift to 41°C. Symbols: △, B/r F(pALO8, pTAC1445); ▲, B/r F *c*I857(pTAC1445); □, B/r F(pALO8, pTAC1584); ■, B/r F *c*I857(pTAC1584).

To determine whether the duration of DnaA protein overproduction affected the extent of runout replication, the [3H]thymidine incorporation maxima reached during runout replication were measured as a function of the time of drug addition after a shift from 25 to 41°C (Fig. 3). When rifampin was added to cells containing pALO8 and pTAC1445 within 5 min of the temperature shift, incorporation of radioactivity reached a plateau after approximately a twofold increase (Fig. 3a). At later times of rifampin addition, these values were slightly higher, corresponding to a 2.4-fold increase. In cells containing pTAC1584, the increase was 1.4-fold, independent of the time of addition of rifampin. Similar results were obtained when the *c*I857 gene was integrated into the chromosome (Fig. 3b).

**Minichromosome replication in the presence of rifampin during DnaA protein overproduction.** To further establish that overproduction of DnaA protein yielded extra initiations of active replication forks from *oriC*, the extent of minichromosome replication in the presence of rifampin was determined. The minichromosome used was pAL55, which harbors 327 base pairs of *oriC*-containing chromosomal DNA as the sole origin of replication. When rifampin was added 13 min after the shift of a culture of strain B/r F

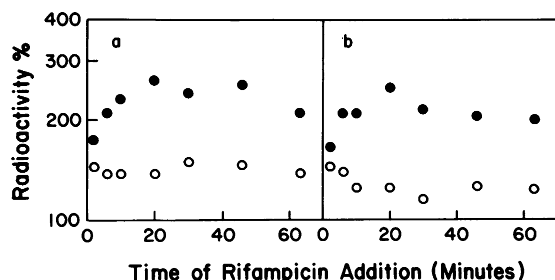


FIG. 3. Plateau values achieved in runout replication on addition of rifampin at various times after thermal induction of *dnaA* gene expression. Portions of cultures containing pTAC1445 or pTAC1584 were exposed to rifampin (100 μg/ml) at various times after a shift from 25 to 41°C. Each datum point shows the plateau value of [$^3$H]thymidine incorporation reached during runout replication at 41°C. Symbols (a): ●, B/r F(pALO8, pTAC1445); ○, B/r F(pALO8, pTAC1584). Symbols (b): ●, B/r F *c*I857(pTAC1445); ○, B/r F *c*I857(pTAC1584).

*c*I857(pTAC1445, pAL55) from 25 to 41°C, minichromosome replication decreased initially and then resumed for about 30 min before decreasing again (Fig. 4). The decrease in uptake of [$^3$H]thymidine into minichromosomes immediately after the temperature shift was also seen in the absence of

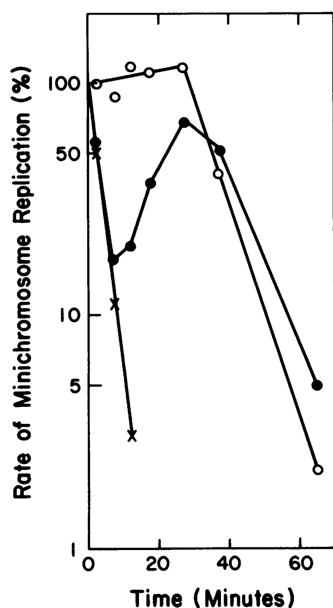


FIG. 4. Rate of minichromosome replication in *E. coli* B/r F *c*I857 in the presence of rifampin after thermal induction of *dnaA* gene expression. Cultures containing the minichromosome pAL55 and either pTAC1445 or pTAC1584 were shifted from 25 to 41°C to induce overexpression of the *dnaA* gene. At 13 or 55 min after the temperature shift, rifampin (200 μg/ml) was added. The rate of minichromosome replication was measured by pulse-labeling portions of the culture (for 4 min) with [$^3$H]thymidine at various times after the addition of rifampin (time zero) and determining the radioactivity incorporated into minichromosome plasmid DNA. Each datum point shows the rate of minichromosome replication relative to the rate at time zero. Symbols: ● and ○, minichromosome replication in B/r F *c*I857(pTAC1445, pAL55) exposed to rifampin at 13 and 55 min after the temperature shift, respectively; ×, minichromosome replication in B/r F *c*I857(pTAC1584, pAL55) exposed to rifampin at 55 min after the temperature shift.

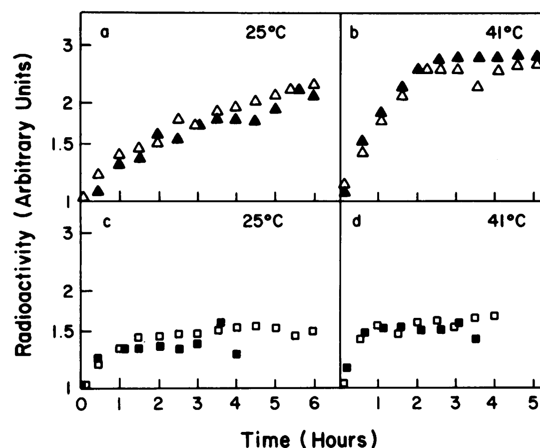


FIG. 5. Runout DNA replication in *E. coli* B/r F during constitutive plasmid-encoded *dnaA* gene expression. Cultures harboring pBF110, pMW110, or pAL1600 or having no plasmid were grown in glucose minimal medium in the presence of [$^3$H]thymidine at 25°C for several generations. (a and c) Rifampin (100 μg/ml) was added and the cells were maintained at 25°C. (b and d) Rifampin was added at 25°C, and after 5 min, the cells were shifted to 41°C. The amount of [$^3$H]thymidine incorporation relative to the amount at the time of rifampin addition (time zero) is shown. Symbols: ▲ and △, radioactivity in B/r F *c*I857(pBF110) and B/r F(pMW110), respectively; ■ and □, radioactivity in B/r F *c*I857 and B/r F(pAL1600), respectively.

rifampin (data not shown). When rifampin was added 55 min after the shift to 41°C, minichromosome replication in pTAC1445-containing cells continued for about 30 min and then gradually decreased. Addition of rifampin to cells which contained pTAC1584 instead of pTAC1445 resulted in a rapid inhibition of minichromosome replication. Thus, on inhibition of RNA and protein syntheses, replication of minichromosomes continued in cells containing pTAC1445, but not in cells containing pTAC1584, indicating that initiation of replication continued in the presence of rifampin after overproduction of DnaA protein.

**Chromosome replication during constitutive plasmid-encoded *dnaA* gene expression.** To analyze the effects of elevated constitutive *dnaA* gene expression on chromosome replication, plasmids pBF110, pMW110, and pAL1600 were introduced into strain B/r F or B/r F *c*I857. Plasmid pBF110 contains both the lambda $p_L$ promoter and the *dnaA* gene promoters upstream of the *dnaA* structural gene. Plasmid pMW110 is a deletion derivative of pBF110, which contains only the *dnaA* promoters upstream of the *dnaA* structural gene. It was previously reported that a plasmid comparable to pMW110 resulted in a constitutive fivefold increase in cellular DnaA protein concentration (37). Plasmid pAL1600 harbors the *dnaA* gene promoters but no *dnaA* structural gene. When B/r F(pMW110) and B/r F *c*I857(pBF110) growing at 25°C were exposed to rifampin, [$^3$H]thymidine incorporation continued for at least 6 h without reaching a plateau (Fig. 5a). At 6 h, the level of incorporation was about twice that seen at the time of rifampin addition. When cells growing at 25°C were exposed to rifampin and then shifted to 41°C, [$^3$H]thymidine incorporation increased at a faster rate and a plateau in radioactivity, at 2.8 times the initial value, was attained (Fig. 5b). In contrast, addition of rifampin to either B/r F *c*I857 or B/r F(pAL1600) yielded increases in radioactivity of 1.5-fold at 25°C and 1.6-fold after a shift to

41°C. Analogous studies of minichromosome replication could not be performed because plasmid pMW110 was unstable in the presence of pAL55 (data not shown).

**Colorimetric determination of DNA content during runout replication.** Colorimetric determination of DNA content was used to demonstrate that the runout [$^3$H]thymidine incorporation represented actual increases in the total amount of DNA. Steady-state cultures of B/r F, B/r F(pMW110), and B/r F(pAL1600) growing in glucose minimal medium at 25°C were exposed to rifampin, and then half of each culture was immediately precipitated with cold TCA. The other half was shifted to 41°C for 4 h and was then precipitated. At the time of rifampin addition, the DNA/mass ratios were 3.5, 3.8, and 3.2 μg of DNA per 1.0 U of $A_{450}$ for B/r F, B/r F(pMW110), and B/r F(pAL1600), respectively. At 4 h after rifampin addition, the DNA/mass ratios had increased 1.44-fold for B/r F, 2.34-fold for B/r F(pMW110), and 1.66-fold for B/r F(pAL1600). These results compare favorably with the increases of 1.5-, 2.8-, and 1.6-fold, respectively, determined by [$^3$H]thymidine incorporation.

## DISCUSSION

In *E. coli* B/r F, overproduction of the *dnaA* gene product resulted in the appearance of extra replication forks on chromosomes and minichromosomes in the presence of rifampin. This was the case whether the enhanced *dnaA* gene expression was due to the induced transcription of the gene from a thermoinducible lambda $p_L$ promoter or was due to the constitutive synthesis from the *dnaA* gene on a multicopy plasmid. The findings were consistent with either the initiation of new replication forks or the continuation of stalled forks from the majority of origins in the presence of the inhibitor.

It is important to note that the pronounced effect of increased *dnaA* gene product on chromosome replication reported here was observed during a period of growth perturbation, namely, on the inhibition of RNA and protein syntheses. When cells containing the *dnaA* structural gene under the control of the thermoinducible lambda $p_L$ promoter were merely shifted to 41°C without addition of rifampin, the rate of chromosome replication increased only slightly more than the mass synthesis, resulting in about a 30% increase in the DNA/mass ratio, after one mass doubling. If functional replication forks had been initiated at all chromosomal origins by enhanced *dnaA* gene expression, this ratio might have been expected to increase by 100 to 200%, as was seen, for instance, during overinitiation in a conditional RNA polymerase mutant (32). Furthermore, the DNA/mass ratio of cells containing the *dnaA* gene on a multicopy plasmid (pMW110) was not significantly different from that of cells lacking the plasmid, and yet the DNA content increased 2.8-fold during runout replication at 41°C. Thus, it would appear that overproduction of DnaA protein rendered the cells capable of initiation of new rounds of replication, but this initiation potential was not fully expressed in DNA chain elongation unless rifampin was added. The addition of rifampin might have allowed either replication from forks which were initiated, but which became stalled, during DnaA protein overproduction or de novo initiation of new forks from *oriC*. The extended minichromosome replication during rifampin exposure was indeed consistent with the initiation of new forks in the presence of the inhibitor.

Churchward et al. (9) reported that overproduction of the *dnaA* gene product had no effect on chromosome replication as determined by DNA accumulation, DNA/mass ratios, and runout replication. In their study, the *dnaA* gene was under the control of the *E. coli lacUV5* promoter, and induction of *dnaA* gene expression was achieved by addition of isopropyl-β-D-thiogalactopyranoside. Atlung and co-workers (1a, 2) also found that the DNA/mass ratio did not increase significantly, i.e., not more than 20%, and that the extent of runout replication in the presence of chloramphenicol was unaltered during DnaA protein overproduction. In their studies, as in ours, a temperature shift-up was used to induce DnaA protein synthesis from plasmid-encoded copies of the *dnaA* structural gene under the control of the lambda $p_L$ promoter. However, they found that the frequency of genes near *oriC* was increased. Within 60 min after induction, the number of genes adjacent to *oriC* increased about threefold. These results led to the concept of abortive initiation, in which the new replication forks initiated by enhanced DnaA protein synthesis were aborted (or stalled) after replication of several kilobases of DNA and were not functional in the complete synthesis of the chromosome.

Our results support the findings of Atlung and co-workers that increased *dnaA* gene expression stimulates initiation. There is, however, a major difference between their results and ours regarding the expression of initiation potential during runout replication. We found that a significant portion, if not all, of the newly initiated replication forks were active in DNA replication. The difference in results was not a consequence of the use of chloramphenicol rather than rifampin because we had essentially the same results with chloramphenicol (data not shown). The difference is probably related to the strains used in the studies. With the B/r F strain that we used, a shift from 25 to 41°C caused a rapid twofold increase in the rates of DNA and mass syntheses and of cell division, as would be expected given the temperature coefficients of these processes (29, 33). On the other hand, at least in the case of the K-12 strains used by Atlung et al. (2), a shift in temperature from 30 to 41°C had little effect on the rates of DNA and mass synthesis. The reason for the differences between the strains with respect to expression of initiation potential during inhibition of RNA or protein synthesis is being investigated. It can be concluded, however, not only that overproduction of DnaA protein stimulates initiation from *oriC*, but also that conditions exist, i.e., addition of rifampin, in which these newly initiated rounds progress in chromosomal DNA replication.

The mode of involvement of rifampin in the expression of the initiation potential is unclear at present. One possible explanation may relate to the effect of the inhibitor on the availability of RNA polymerase for initiation of replication. During exposure to rifampin, the rate of synthesis of RNA polymerase subunits is transiently increased (16, 27), and RNA polymerase molecules might be redistributed on the DNA templates (3). There is considerable evidence for a direct interaction between RNA polymerase and DnaA protein, and particularly relevant are mutations in RNA polymerase subunits which lead to initiation of chromosome replication at a lower-than-normal cell mass (32, 36). An altered interaction between RNA polymerase and *oriC* might enable both de novo initiation and the expression of latent replication forks produced by excess DnaA protein. Alternatively, the rifampin might have acted by disengaging the mechanism which normally inhibits initiation and fork movement after DnaA protein overproduction. Whatever the explanation may be, the findings indicate that the *dnaA* gene product per se is not sufficient for the initiation of new, functional chromosomal replication forks and that their

appearance requires another element which might become active during inhibition of RNA or protein synthesis.

## ACKNOWLEDGMENTS

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## LITERATURE CITED

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