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DnaA Protein Overproduction Abolishes Cell Cycle Specificity of DNA Replication from *oriC* in *Escherichia coli*

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Initiation of DNA replication from *oriC* in *Escherichia coli* takes place at a specific time in the cell division cycle, whether the origin is located on a chromosome or a minichromosome, and requires participation of the product of the *dnaA* gene. The effects of overproduction of DnaA protein on the cell cycle specificity of the initiation event were determined by using minichromosome replication as the assay system. DnaA protein was overproduced by inducing the expression of plasmid-encoded *dnaA* genes under control of either the p*tac* or lambda p_L promoter. Induction of DnaA protein synthesis caused a burst of minichromosome replication in cells at all ages in the division cycle. The magnitude of the burst was consistent with the initiation of one round of replication per minichromosome in all cells. The replication burst was followed by a period of reduced minichromosome replication, with the reduction being greater at 30 than at 41°C. The results support the idea that the DnaA protein participates in *oriC* replication at a stage that is limiting for initiation. Excess DnaA protein enabled all cells to achieve the state required for initiation of DNA polymerization by either effecting or overriding the normal limiting process.

In *Escherichia coli*, DNA synthesis initiates at the chromosomal origin of replication, *oriC*, at a specific time during the division cycle (14–17, 20). The biochemical process responsible for this timing mechanism has not been identified, but it has been suggested that the product of the *dnaA* gene, which is required for initiation of replication from *oriC*, could be involved (3, 7, 24, 30, 39). The DnaA protein binds cooperatively to several sites (DnaA boxes) located within *oriC* (10, 13, 22). In so doing, it participates in the assembly of the complex of initiation proteins that eventually leads to the unwinding, priming, and polymerization of the *oriC* region (4, 11, 36). The DnaA protein also binds to DnaA boxes within the promoter of the *dnaA* gene itself (1, 8, 19, 40), the *mioC* gene adjacent to *oriC* (21, 31, 33), and several other genes, some of which are involved in DNA metabolism (10). Thus, in addition to its direct role in initiation of replication at *oriC*, the DnaA protein may also act to regulate transcription of a number of DNA synthesis-related genes (23).

When the intracellular quantity of DnaA protein is increased, through induction of synthesis from plasmid-encoded *dnaA* genes, replication from *oriC* is stimulated (2). This is the case whether *oriC* is located on the chromosome or on an extrachromosomal plasmid (a minichromosome). Replication of the minichromosomes progresses to completion. The newly initiated chromosomal replication forks are stalled shortly after initiation (2, 41), although addition of rifampin appears to enable the stalled forks to proceed along the chromosome (28). These findings suggest that overproduction of DnaA protein could alter the normal timing of initiation of DNA replication from *oriC* during the division cycle and, as a result, cause initiation events at earlier-than-normal times in the cycle. To examine this issue, we determined the kinetics of minichromosome replication during overproduction of DnaA protein at various times during the division cycle of synchronously growing *E. coli* B/r F (15). It was found that minichromosome replication could be induced in cells of all ages.

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MATERIALS AND METHODS

Bacteria and plasmids. Experiments were performed with *E. coli* B/r F *thyA his* (15) harboring the minichromosome pAL49 or pAL4 (20). The *oriC* region of pAL49, but not that of pAL4, contains the *mioC* gene (20). Plasmids pLSK5 and pTTQ9 both harbor the *lacI*^{q} repressor gene and the *trp-lac* promoter (p*tac*). The p*tac* promoter is upstream of the *dnaA* gene in pLSK5 and upstream of the beta-galactosidase alpha-fragment gene in pTTQ9 (38). Plasmid pLSK5 was obtained from W. Messer, via J. Zyskind. Plasmid pTAC1445 carries the *dnaA* structural gene under the control of the lambda p_L promoter (2). In plasmid pTAC1584, a derivative of plasmid pTAC1445, the *dnaA* gene contains a 121-base-pair deletion and produces a nonfunctional DnaA protein (2). Plasmid pALO8 contains the cI857 gene, encoding the thermolabile lambda repressor protein (2). Induction of plasmid-coded *dnaA* gene expression was obtained in *E. coli* B/r F(pLSK5) by exposure of the cells to the inducer isopropyl-β-D-thiogalactopyranoside (IPTG; final concentration, 10^{-3} M) and in B/r F(pALO8, pTAC1445) by a temperature shift from the growth temperature, 30°C to 41°C, the temperature of inactivation of the lambda repressor protein.

Growth media and chemicals. Cells were grown at 30°C in minimal salts medium (15) supplemented with thymine (10 μg/ml), histidine (20 μg/ml), and methionine (20 μg/ml). Glucose (0.2%) was used as the carbon source. Ampicillin (100 μg/ml), chloramphenicol (10 μg/ml), kanamycin (100 μg/ml), and tetracycline (10 μg/ml) were present for plasmid-containing cells, as necessary. IPTG, ampicillin (sodium salt), and tetracycline hydrochloride were from Sigma Chemical Co., St. Louis, Mo. Chloramphenicol and kanamycin sulfate were from United States Biochemical Corp., Cleveland, Ohio. Rifampin was from Boehringer GmbH, Mannheim, Federal Republic of Germany. Plasmid pTTQ9 was from Amersham Corp., Arlington Heights, Ill.

Minichromosome replication. The rates of DNA replication were measured in samples (1 or 2 ml) of cultures exposed to

[*methyl*-^{3}H]thymidine (10 μCi/ml; specific activity, 80 Ci/mmol) for 4 or 5 min. For both minichromosomes and chromosomes, the relative changes in DNA content were determined in samples (2 ml) of cultures continuously exposed to [*methyl*-^{3}H]thymidine (8 μCi/ml; specific activity, 60 mCi/mmol). Radioactivity incorporation was stopped by the addition of excess nonradioactive thymidine (final concentration, 200 μg/ml) for 10 min. A portion (0.1 or 0.2 ml) of each sample was placed in ice-cold, 5% trichloroacetic acid (TCA) for estimation of the total cellular radioactivity incorporation. The remaining portion was used for the analysis of minichromosome radioactivity incorporation by the procedure described by Projan et al. (29). Twenty microliters of whole-cell lysate (total volume, 50 or 80 μl) was loaded into each well of a 0.7% horizontal agarose gel. Electrophoresis in Tris borate-EDTA buffer was performed for 18 h at 40 V. Gels were prepared for fluorography as described previously (28), and the dried gels were exposed to X-Omat AR X-ray film (Eastman Kodak Co., Rochester, N.Y.) at −70°C. The autoradiographs of the minichromosome and plasmid DNA bands were scanned with a video densitometer (Bio-Rad Laboratories, Richmond, Calif.).

Cell cycle analysis during short periods of DnaA protein overproduction. The effects of short periods of enhanced *dnaA* gene expression on the initiation of DNA replication were analyzed in multiple samples of synchronously growing cells obtained as previously described (15, 20). Briefly, overnight cultures grown at 30°C in media supplemented with the appropriate antibiotics were diluted (1:200) in 100 ml of medium lacking the antibiotics and grown at the same temperature for at least four mass doublings. At a cell concentration of 10^8 cells per ml, the cultures were filtered onto the surface of a type GS Millipore nitrocellulose filter and washed with 100 ml of minimal medium, and the filters were inverted. Newborn cells were eluted at 30°C; 18 consecutive samples taken at 2-min intervals were collected, and portions (5 ml) were grown synchronously at 30°C for different time periods, namely 90, 85, . . . 10, and 5 min. After these periods, one-half of each sample was exposed to IPTG for 7 or 10 min. During the last 5 min of the induction period, 2 ml of the induced and uninduced portions of each synchronous sample was exposed to [*methyl*-^{3}H]thymidine (10 μCi/ml). Cell concentration was determined both at the time of collection and 1 min prior to the radiolabeling.

Cell cycle analysis during continuous DnaA protein overproduction. The effect of long-term induction of *dnaA* gene expression on minichromosome replication was analyzed in cells of specific ages in the cycle. Twenty consecutive samples of newborn cells taken at 1-min intervals were collected, and a 4-ml portion of each sample was grown at 30°C for either 0 (age 0) or 35 (age 0.65) min. IPTG was added to one-half of each sample of the chosen cell age, and growth was continued thereafter for different time periods, namely 96, 91, . . . 6, and 1 min. At these times the samples of both the induced and uninduced cells were exposed to [*methyl*-^{3}H]thymidine for 5 min.

The rates of minichromosome replication during the division cycle were estimated as the ratio of the intensity of the minichromosome DNA bands obtained from synchronous cells to the cell number at the time of collection. The doubling times were calculated as the times required for the cell concentrations to increase 1.5-fold during the synchronous growth. The cell ages at the time of exposure to radioactive thymidine were computed as the ratios of the midtimes of the labeling periods during synchronous growth to the doubling times of the synchronous cultures.

Absorbance, cell concentration, and radioactivity. The A_{450} of cultures was determined 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 ICN Radiochemicals, Irvine, Calif. Incorporation of [^{3}H]thymidine into cellular DNA was measured by cold 5% TCA-precipitated radioactivity retained on filter disks. The radioactivity in samples was counted in Spectrafluor (Amersham) with a liquid scintillation counter (LS-7000; Beckman Instruments, Inc., Fullerton, Calif.).

RESULTS

Minichromosome replication upon induction of plasmid-encoded *dnaA* gene expression. Minichromosome replication was analyzed during DnaA protein overproduction in steady-state cultures of *E. coli* B/r F containing the *oriC* plasmid pAL49 or pAL4. DnaA protein overproduction was achieved in minichromosome-containing cells in two ways: (i) by the addition of IPTG to cells harboring pLSK5 or (ii) by thermoinduction of cells harboring pTAC1445. The kinetics of minichromosome replication were determined by pulse-labeling samples with [^{3}H]thymidine at various times after induction of *dnaA* gene expression and visualizing the incorporation by agarose gel electrophoresis of whole-cell lysates.

The radioactivity in pAL49 minichromosome bands at consecutive 2-min intervals following induction of *dnaA* gene expression from plasmid pLSK5 by IPTG addition is shown in Fig. 1, row a. Overproduction of the DnaA protein resulted in a burst of minichromosome replication. An increase in the rate of pAL49 replication was not seen when IPTG was added to cells containing plasmid pTTQ9, which encodes the beta-galactosidase alpha-fragment gene in place of the *dnaA* structural gene (row b). The effects of thermoinduction of DnaA protein synthesis in *E. coli* B/r F(pALO8, pTAC1445) containing pAL49 or pAL4 are shown in rows c and f, respectively. Replication was enhanced immediately for both minichromosomes. The enhancement was absent in B/r F(pALO8, pTAC1584), in which the DnaA protein was defective (rows d and g), and B/r F, which did not contain an inducible *dnaA* gene (rows e and h). However, a transient inhibition of minichromosome replication was associated with the temperature shift from 30 to 41°C in cells in which a functional DnaA protein was not induced.

The enhanced minichromosome replication resulting from overproduction of DnaA protein was followed by a period of reduced replication (Fig. 1, rows a, c, and f). The rate of replication during this period was higher when the DnaA overproduction was induced by a shift from 30 to 41°C (rows c and f) than when the temperature remained at 30°C (row a). This higher level of subsequent replication was a consequence of the temperature upshift; indeed, when DnaA protein synthesis was induced by IPTG in B/r F(pLSK5) concomitant with an upshift in temperature (row i), the rate of minichromosome replication was similar to that observed following thermoinduction of DnaA protein synthesis. Again, the temperature shift alone in the absence of induced DnaA protein synthesis was associated with a temporary inhibition of minichromosome replication (row j).

The kinetics of pAL49 minichromosome replication in selected experiments from Fig. 1 are shown in quantitative form in Fig. 2. The absorbances of the DNA bands in Fig. 1, row a [B/r F(pLSK5)] and row b [B/r F(pTTQ9)], upon induction of DnaA protein and the beta-galactosidase alpha

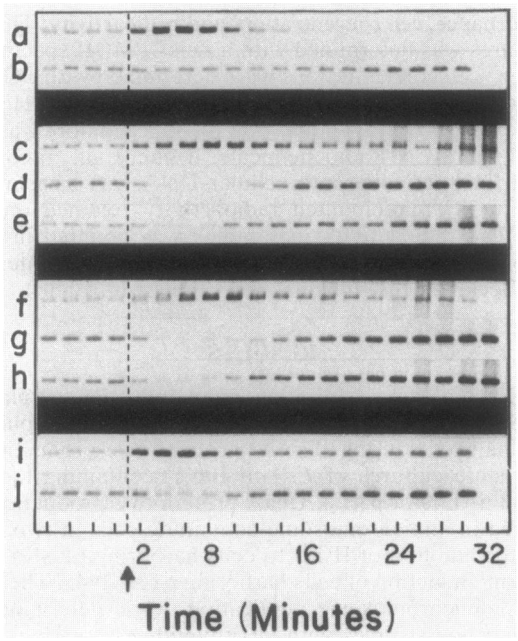

FIG. 1. Minichromosome replication during overproduction of the DnaA protein: fluorographs of minichromosome DNA bands. Steady-state cultures of *E. coli* B/r F harboring the minichromosome pAL49 or pAL4 were grown under selective pressure at 30°C. At a cell concentration of about 5×10^7 cells per ml, cells carrying the pLSK5-coded *dnaA* gene were induced by IPTG and cells harboring the pTAC1445-coded *dnaA* gene were thermoinduced by a temperature shift from 30 to 41°C. At various times before and after DnaA induction, samples (1 ml) were radioactively labeled with 10 μCi of [^{3}H]thymidine per ml for 4 min. After lysis, electrophoresis, and fluorography, the minichromosome DNA bands were photographed. Rows: a and b, pAL49 minichromosome DNA bands during exposure to IPTG at 30°C [a, B/r F(pLSK5); b, B/r F(pTTQ9)]; c, d, and e, pAL49 minichromosome DNA bands after a temperature shift from 30 to 41°C [c, B/r F(pALO8, pTAC1445); d, B/r F(pALO8, pTAC1584); e, B/r F]; f, g, and h, pAL4 minichromosome DNA bands after a temperature shift from 30 to 41°C [f, B/r F(pALO8, pTAC1445); g, B/r F(pALO8, pTAC1584); h, B/r F]; i and j, pAL49 minichromosome DNA bands from B/r F(pLSK5) after a temperature shift from 30 to 41°C [i, with IPTG; j, without IPTG]. The broken vertical line indicates the time of IPTG addition and/or the temperature shift. The midpoints of the periods of radioactive-thymidine labeling are indicated at the bottom.

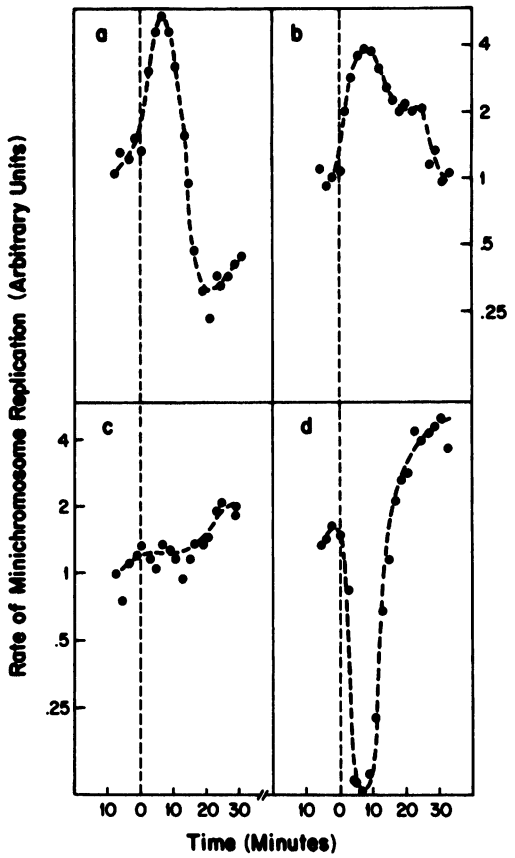

FIG. 2. pAL49 minichromosome replication during induction of *dnaA* gene expression. The intensities of the pAL49 minichromosome DNA bands from fluorographs such as those shown in Fig. 1 were determined by two-dimensional densitometry and plotted as a function of time after the induction of DnaA protein synthesis. Shown are the rates of pAL49 minichromosome replication in B/r F(pLSK5) (a) and B/r F(pTTQ9) (c) upon IPTG induction at 30°C and in B/r F(pALO8, pTAC1445) (b) and B/r F(pALO8, pTAC1584) (d) upon a shift in temperature from 30 to 41°C. The broken vertical lines indicate the time of IPTG addition or the temperature shift.

fragment at 30°C are shown in Fig. 2a and c, respectively. Replication was enhanced approximately fivefold at 6 min after induction of DnaA protein synthesis and was slightly depressed in the absence of induction. Figures 2b and d show the absorbances of the DNA bands in Fig. 1, rows c and d, as a function of time after thermoinduction of B/r F(pALO8, pTAC1445) and B/r F(pALO8, pTAC1584), respectively. In these experiments, the rate of replication was enhanced about 4-fold when functional DnaA protein was produced (Fig. 2b) but transiently depressed about 10-fold when it was not (Fig. 2d).

Chromosome replication in the presence of rifampin. We have previously shown (28) that new, functional replication forks are activated on the chromosome upon thermoinduction of DnaA protein synthesis in B/r F(pTAC1445, pALO8), based on the extent of runout replication in the presence of rifampin. The rapid enhancement of minichromosome replication from plasmid pLSK5 at 30°C upon induction of the *dnaA* gene product with IPTG (Fig. 1 and 2) suggests that new replication forks might be similarly activated on the chromosome, independent of a temperature upshift. To analyze this possibility, steady-state cultures of B/r F(pLSK5) were grown at 30°C for at least 20 generations in the presence of [^{3}H]thymidine to fully label chromosomal DNA and then divided into four portions. One portion of the culture received IPTG at 30°C, while another portion received IPTG and was simultaneously shifted to 41°C. The third and fourth portions were maintained at 30°C, or shifted to 41°C, without the addition of IPTG. After 12 min, rifampin was added to each portion. The radioactivity incorporated into cold TCA-precipitable material was plotted as a function of time after rifampin addition (Fig. 3). In cultures induced with IPTG, the incorporation reached identical plateaus, at 4 h for 30°C and 2 h for 41°C (Fig. 3a). This plateau was at a value about 2.4 times that at the time of rifampin addition, independent of the temperature shift. In uninduced cells (Fig. 3b), incorporation reached maximum values of 1.4 and 1.6 times the initial value at 30 and 41°C, respectively. Thus, induction of the *dnaA* gene product with IPTG was associated with an increased level of chromosomal runout replication, consistent with the activation of functional replication

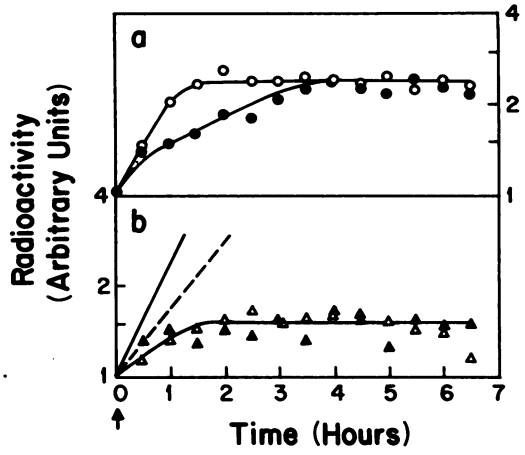

FIG. 3. Runout chromosome replication in the presence of rifampin. A culture of B/r F(pLSK5) was grown for approximately 20 generations in the presence of 8 μCi of [^{3}H]thymidine per ml (specific activity, 60 mCi/mmol) under selective pressure. (a) Portions of the culture received IPTG at 30°C (●) or concomitant with a temperature shift to 41°C (○); (b) portions of the culture were maintained at 30°C (▲) or shifted to 41°C (△) without addition of IPTG. Twelve minutes later, rifampin (final concentration, 100 μg/ml) was added to all the cultures. The radioactivity incorporated into cold 5% TCA-insoluble material at various times after rifampin addition is plotted as a function of time. The arrow indicates the time of rifampin addition. The broken and solid lines in panel b indicate incorporation at 30°C and during a shift from 30 to 41°C, respectively, in the absence of rifampin.

forks on the chromosome, independent of a temperature shift.

Minichromosome replication in synchronously growing *E. coli* B/r F during short periods of DnaA protein overproduction. Minichromosome replication is normally restricted to a discrete interval in the cell division cycle, coincident with initiation of chromosome replication (14). The burst of minichromosome replication observed during induction of DnaA protein synthesis in exponentially growing cultures could be a consequence of enhanced synthesis of minichromosomes during this replication interval or of the activation of replication potential in cells at other (earlier) ages in the division cycle. To discriminate between these possibilities, samples of *E. coli* B/r F(pLSK5) of various ages in the division cycle were divided into two equal portions. In one, synthesis of the *dnaA* gene product was induced for either 7 or 10 min, while the other portion served as an uninduced control. Induced and uninduced portions of each synchronous sample were labeled with [^{3}H]thymidine during the last 5 min of the induction period and processed and analyzed under the same conditions. Figures 4 and 5 show the DNA bands and densitometric scans of the bands, respectively, for the induced and uninduced samples in both experiments. Minichromosome replication in the uninduced cells was periodic, with a maximum value about midway through the division cycle. DnaA protein overproduction stimulated minichromosome replication in cells of all ages. In the induced cells, the rate of replication followed a stepwise pattern, with a doubling shortly after the interval of pAL49 replication in the uninduced culture. The amount of radioactivity in each sample of the induced culture was approximately equal to the total incorporation in all samples from the corresponding cell cycle of the uninduced culture. This is consistent with the initiation of one round of replication per minichromosome upon induction of DnaA protein synthesis, independent of the stage in the cell cycle. Thus, the stepwise doubling in [^{3}H]thymidine incorporation during the cell cycle reflected the average number of minichromosomes present per cell at the time DnaA protein synthesis was induced. Similar enhancement in minichromosome replication was seen in B/r F(pALO8, pTAC1445) during thermoinduction of DnaA protein synthesis (data not shown).

Minichromosome replication in synchronously growing *E. coli* B/r F during continuous DnaA protein overproduction. The preceding information indicates that cells of all ages were stimulated to replicate their minichromosome contents during the initial stages of DnaA overproduction, but the experiments did not indicate whether the kinetics of replication differed in cells of different ages or whether there was more than one burst of replication. To answer these questions, synchronous samples of cells with ages of 0 and 0.65 generations were divided in half and IPTG was added to one portion. Synchronous growth was continued, and portions were pulse-labeled with [^{3}H]thymidine at consecutive intervals. The absorbances of the radioactive minichromosome bands were determined and plotted as a function of cell age after IPTG addition (Fig. 6). In both experiments, overproduction of the DnaA protein was associated with an immediate enhancement in minichromosome replication. The initial burst of minichromosome replication was followed by a period of significantly reduced replication. At about one generation after the first burst, there may have been a slight second burst of replication, but the level of incorporation was too low to accurately assess the extent of replication at this time. Finally, the total radioactivity in the initial peak in the induced culture was approximately equal to the total incorporation in all samples of the uninduced culture in the same cell cycle, again consistent with one replication per minichromosome upon DnaA overproduction.

DISCUSSION

Overproduction of the DnaA protein was associated with an abrupt increase in the rate of minichromosome replication, independent of the presence or absence of the *mioC* gene on the minichromosome. The extent of replication was

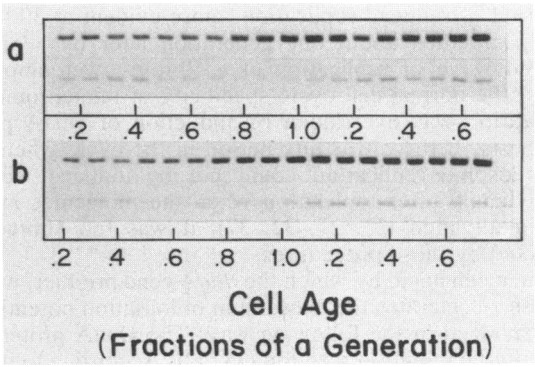

FIG. 4. Minichromosome replication in the division cycle during short periods of DnaA protein overproduction. The fluorographs of the pAL49 minichromosome DNA bands from synchronous B/r F(pLSK5) cells IPTG induced for 7 min (a) and 10 min (b) and radiolabeled for the last 5 min of induction are shown. In each set, the upper row represents the DNA bands of the induced samples and the lower row represents those of the corresponding uninduced samples. The ages of the synchronous cells at the start of induction are indicated at the bottom of each set.

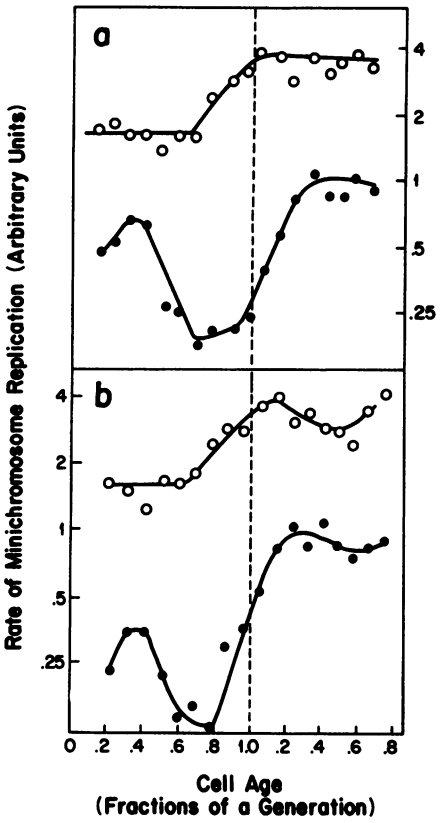

FIG. 5. Rate of minichromosome replication in the cell division cycle during short periods of DnaA protein overproduction. The intensities of the pAL49 minichromosome DNA bands shown in Fig. 4 were normalized to the cell number at the start of synchronous growth and plotted as a function of cell age at the time of radiolabeling. DnaA protein synthesis induction was for 7 min (a) and 10 min (b). Symbols: ○, induced cells; ●, uninduced cells. The vertical broken lines indicate the midpoints of synchronous division.

consistent with one round of replication per minichromosome, in agreement with previous findings by Atlung et al. (2). This stimulation of replication took place at all stages in the division cycle. The burst of replication was followed by a period of reduced replication, more evident at 30 than at 41°C, and then about one generation later by a possible second wave of replication at a 10-fold-lower amplitude. Thus, the normal cell cycle specificity of minichromosome replication was overridden by induction of DnaA protein synthesis. If there was any period in the cycle when minichromosome replication could not be induced, such as immediately after replication when the molecules are still hemimethylated (25, 27, 32, 37), it was too short to be detected by these procedures.

The mechanism by which the *dnaA* gene product, when in excess, accelerates the expression of initiation potential can be explained in the following way. The DnaA protein acts early in the initiation reaction (35, 42). According to in vitro studies of minichromosome replication, the active ATP-DnaA form (34) participates in the opening of the DNA duplex at *oriC* (7, 18) to facilitate entry of additional proteins involved in subsequent priming and polymerization steps (4, 6). Overexpression of DnaA protein could induce initiation if an excess of the protein was sufficient to cause formation of the open complex (7) and the other components involved in the later stages of replication were not limiting. This condition would obtain if DnaA was normally limiting for initiation or if the normal limiting step in open complex formation was circumvented by the excess DnaA protein. It has also been suggested that RNA polymerase acts early in initiation and facilitates open complex formation by transcriptional activa-

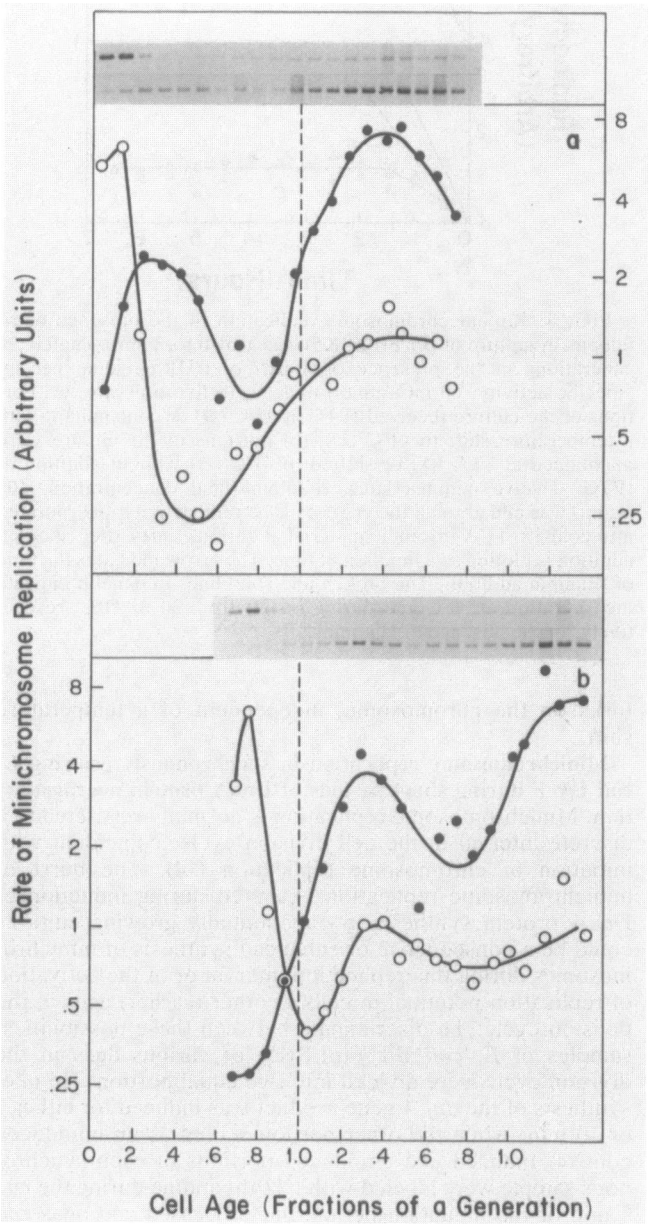

FIG. 6. Rate of minichromosome replication during continuous expression of the *dnaA* gene in the division cycle. Synchronous samples of B/r F(pLSK5) were grown for either 0 min (age 0) (a) or 35 min (age 0.65) (b), and then one-half of each sample received IPTG and the other did not. Growth was continued, and the samples were radioactively labeled at intervals with [^{3}H]thymidine for 5 min. Photographs of the radioactive minichromosome DNA bands are shown at the tops of panels a and b. In each set, the upper row represents the minichromosomes of the induced samples and the lower row represents those of the corresponding uninduced samples. The rate of minichromosome replication was determined as for Fig. 5 and plotted as a function of the age at the time of radiolabeling. The vertical broken lines indicate the times of synchronous division. The rates of minichromosome replication in induced (○) and uninduced (●) cells are shown.

tion at *oriC* (5). The two proteins might thus act to achieve the same topological alteration of the origin region. In fact, initiation of replication from *oriC* is also stimulated by an increase in the availability of RNA polymerase (40a). An elevated ATP-DnaA level could obviate the need for direct polymerase function at the origin. Thus, RNA polymerase action might no longer be needed in the presence of ATP-DnaA in excess, and conversely, initiation might take place in the presence of lower levels of DnaA when RNA polymerase availability is increased.

The period of depressed minichromosome replication after the initial stimulation by DnaA protein could be a reflection of several factors, including modification of DNA topology during overinitiation at *oriC* (6; D. W. Smith and J. W. Zyskind, *in* K. W. Adolph, ed., *Chromosomes: Eucaryotic, Procaryotic and Viral*, vol. 2, in press) or DnaA-mediated effects on the transcription of essential DNA replication-related genes (30). Inhibition of chromosome replication has previously been reported upon IPTG induction of the *dnaA* gene under p*tac* promoter control (31). The inhibition was associated with a decrease in the extent of counterclockwise transcription entering *oriC* during increased DnaA protein synthesis.

The effects of the treatments used for the induction of plasmid-coded *dnaA* gene expression in cells lacking an inducible plasmid-coded *dnaA* gene were also of interest. Temperature upshifts, in the absence of induced DnaA protein synthesis, were associated with a temporary inhibition of minichromosome replication. This early, transient inhibition of replication was specific for the minichromosome; i.e., the rates of replication of plasmids pLSK5, pALO8, pTAC1445, and pTAC1584 all increased immediately upon a shift in temperature from 30 to 41°C. When minichromosome replication resumed, it was at a rate higher than that in unshifted cells. The explanation for this thermal inhibition of minichromosome replication, which was reversed by DnaA protein overproduction, is unknown but could be related to changes in transcriptional patterns (9). Enhanced transcription from heat shock genes (26) could serve to reduce the availability of RNA polymerase for initiation. Excess DnaA protein might serve to overcome the reduced polymerase availability by opening the DNA duplex at *oriC* without the need for transcriptional activation. It should be noted that our findings in this regard are in opposition to those of a recent study by Guzman et al. (12), in which initiation of rounds of chromosome replication were stimulated during a temperature shift of cells in which RNA synthesis activity was reduced. The investigators suggested that the displacement of RNA polymerase toward the heat shock genes reduced the transcription of a replication inhibition gene. We found no evidence of this phenomenon.

In our experiments, the cell cycle specificity of minichromosome replication was similarly affected whether overproduction of the DnaA protein was obtained by IPTG induction of the p*tac* promoter in plasmid pLSK5 at 30°C or upon thermoinduction of the p_L lambda promoter in plasmid pTAC1445. Furthermore, runout replication of chromosomes was similarly enhanced. On the other hand, minichromosome replication lasted for a longer period of time when DnaA overproduction was combined with a temperature upshift. Thus, the expression of DnaA-dependent initiation potential appears to be positively affected by an upshift in temperature, as observed previously by Xu and Bremer (41) for runout chromosome replication and Kornberg et al. (18) for *oriC* replication in vitro.

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