JOURNAL OF BACTERIOLOGY, Jan. 1997, p. 163–169 Vol. 179, No. 1
0021-9193/97/$04.00+0

Gene Transcription and Chromosome Replication in *Escherichia coli*

PING ZHOU,[1] JOSEPH A. BOGAN,[1] KEVEN WELCH,[1]† SAMUEL R. PICKETT,[1] HSIU-JU WANG,[1] ARIEH ZARITSKY,[2] AND CHARLES E. HELMSTETTER[1]*

Department of Biological Sciences, Florida Institute of Technology, Melbourne, Florida 32901,[1] and Department of Life Sciences, Ben-Gurion University of the Negev, Be'er-Sheva 84105, Israel[2]

Transcript levels of several *Escherichia coli* genes involved in chromosome replication and cell division were measured in *dnaC2*(Ts) mutants synchronized for chromosome replication by temperature shifts. Levels of transcripts from four of the genes, *dam*, *nrdA*, *mukB*, and *seqA*, were reduced at a certain stage during chromosome replication. The magnitudes of the decreases were similar to those reported previously for *ftsQ* and *ftsZ* (P. Zhou and C. E. Helmstetter, J. Bacteriol. 176:6100–6106, 1994) but considerably less than those seen with *dnaA*, *gidA*, and *mioC* (P. W. Theisen, J. E. Grimwade, A. C. Leonard, J. A. Bogan, and C. E. Helmstetter, Mol. Microbiol. 10:575–584, 1993). The decreases in transcripts appeared to correlate with the estimated time at which the genes replicated. This same conclusion was reached in studies with synchronous cultures obtained with the baby machine in those instances in which periodicities in transcript levels were clearly evident. The transcriptional levels for two genes, *minE* and *tus*, did not fluctuate significantly, whereas the transcripts for one gene, *iciA*, appeared to increase transiently. The results support the idea that cell cycle timing in *E. coli* is not governed by timed bursts of gene expression, since the overall findings summarized in this report are generally consistent with cell cycle-dependent transient inhibitions of transcription rather than stimulations.

The duplication of an *Escherichia coli* cell features two events initiating critical processes: chromosome replication and cell constriction. There has been considerable interest in the molecular mechanisms responsible for the timing of these events and the processes associated with them. At the very least, a full complement of the components needed to start the individual processes must be formed. For instance, chromosome replication and cell constriction are not initiated until the required threshold levels of active DnaA and FtsZ proteins, respectively, have assembled (26, 32). The actual kinetics of the formation of the required components, however, could be very complex. At one extreme, the relevant gene could be transcribed, and the mRNA could be translated, throughout the cell cycle, yielding a continuous increase in the encoded protein. At the opposite extreme, the gene product could be produced with strong periodicity in the cycle so that the required amount is formed just prior to its utilization. Both situations have been well documented in eukaryotes (1), where, for instance, the synthesis of cyclins (protein components of kinases required for cell cycle progression) can be either continuous or periodic. The situation in bacteria is less well understood, although there is evidence for programmed gene expression in the dimorphic bacterium *Caulobacter crescentus* (4) and for some cycle-dependent aspects of gene expression in *E. coli.*

There have been several reports suggesting that certain genes in *E. coli* are expressed periodically with respect to either chromosome replication or the cell division cycle. Especially notable among these are genes that encode products involved in an aspect of chromosome replication. One of these is *dnaA*, the product of which binds to sites (DnaA boxes) in the origin of replication, *oriC*, to facilitate the opening of the DNA duplex to start replication (3). The timing of initiation of replication during steady-state growth may be set by the binding of a fixed quantity of active DnaA to *oriC* (16, 24). Thus, cell cycle-dependent controls on the kinetics of formation of the protein could conceivably contribute to initiation timing. In fact, transcription of *dnaA* fluctuates during the cell cycle due to a transient inhibition immediately after the gene replicates (2, 5, 29, 35). Transcription of *gidA*, located immediately to the left of *oriC*, oscillates in a manner identical to that of *dnaA* (2, 29, 35). Transcription of *mioC*, adjacent to *oriC* but on the right side, also varies periodically but with considerably different kinetics. *mioC* transcription is shut off prior to initiation of chromosome replication, due to the binding of DnaA upstream of its promoter, and remains off for several minutes after initiation of replication (2, 29, 35). Transcription of the genes encoding ribonucleotide reductase has also been reported to fluctuate in the cycle, with a maximum at the time of initiation of chromosome replication (33, 34). In addition to these replication-associated genes, transcript levels of some genes associated with cell fission, residing within the 2-min region on the genetic map and including *ftsZ*, have been found to fluctuate during the cycle (13, 37).

Transcriptional periodicities could play roles in regulating initiation of processes in the cycle if genes required for a process were maximally expressed just prior to its initiation and then shut down afterwards as part of the mechanism to prevent premature reinitiation of the process. Conversely, genes whose transcripts prevented initiation of a process might need to be shut down for the process to begin. A decrease in the rates of formation of the protein products would be anticipated while transcription was shut down and the existing mRNAs were degraded. The magnitude of the decrease would depend, of course, on the duration of transcriptional repression. In fact, no dramatic cell cycle-dependent variations in synthesis of individual proteins have been detected (9, 27), although there may be variations in enzyme activities, such as have recently been reported for an autolysin activity in *Pseudomonas aeruginosa* (22).

In an effort to identify possible relationships between gene transcription and cell duplication, we have undertaken an anal-

* Corresponding author. Mailing address: Department of Biological Sciences, Florida Institute of Technology, 150 W. University Blvd., Melbourne, FL 32901. Phone: (407) 768-8000, ext. 8788. Fax: (407) 968-1818. E-mail: chelmste@fit.edu.

† Present address: Human Genome Sciences, 9620 Medical Center Drive, Rockville, MD 20852.

ysis of the transcription of a variety of genes important for the cell cycle. This paper contains a summary of the transcriptional patterns observed.

MATERIALS AND METHODS

Bacteria, growth conditions, and radioactive labeling. The strains employed were *E. coli* K-12 PC2 (*dnaC2 thyA47 leu-6 deoC3* Str^r), K-12 DG76 (the parental strain) (6), and B/rA (ATCC 12407). Cultures were grown in minimal salts medium (19) supplemented with 0.1% glycerol, with 0.1% glucose, or with 0.1% glucose plus 0.2% Casamino Acids (Difco Laboratories). When necessary, thymine was added at 10 μg/ml. For temperature shift experiments, cultures were grown for a minimum of five doublings at 30°C, shifted to 40°C for 60 or 75 min, and then returned to 30°C (19). For measurement of DNA replication, 1.0-ml samples were removed from the cultures and placed in a test tube in a shaking water bath at the growth temperature along with 1 μCi of [^{3}H]thymidine (approximately 80 Ci/mM; NEN) for 5 min, and uptake was determined as described previously (35). Bacterial concentrations were determined with a model ZB Coulter electronic particle counter. Absorbances of the cultures at 600 nm were measured with a Milton Roy Spectronic 601 spectrophotometer.

Synchronous growth experiments. Cultures of *E. coli* B/r growing exponentially at 37°C (100 ml) containing 0.5×10^8 to 1.0×10^8 cells/ml were filtered onto the surface of a type GS 142-mm-diameter Millipore membrane filter (Millipore Corp.). The filter was inverted in a Full-View incubator (Precision Scientific) at 37°C, and fresh medium (ca. 200 ml) was poured into the upper part of the filter holder. The upper chamber of the holder was connected to a peristaltic pump. The pump was operated at a rate of 15 ml per min for 2 min to remove unattached cells, and then the rate was reduced to 2 ml per min. After a delay of 12 to 15 min, to allow for the release of weakly attached cells, consecutive 3-min (glucose-Casamino Acids cells), 4-min (glucose cells), or 5-min (glycerol cells) samples were collected (usually 28) from the effluent. An aliquot was taken from each sample for determination of cell concentration, and the rest of the sample was transferred to a culture flask and placed in a 37°C shaking water bath. Cells at different stages of the cell cycle were obtained by incubating each sample for a different length of time. At the end of the period of synchronous growth, a second aliquot of cells was taken for determination of cell concentration and the balance of the synchronous culture was placed on ice for isolation of total cellular RNA.

Quantitative S1 nuclease transcription assays. Isolation of total cellular RNA and the S1 nuclease protection assays were performed as described in detail previously (35). The probes used in the S1 nuclease assay were synthetic oligonucleotides, purchased from Keystone Scientific (Menlo Park, Calif.) or Oligos, Etc. (Wilsonville, Oreg.), which complemented the transcripts of interest. The probes were designed so that, following hybridization to total cellular RNA, S1-protected fragments would be a minimum of 10 nucleotides shorter than the undigested probe to prevent interference in the subsequent analysis. This was achieved by overlapping the transcriptional start site and/or by tailing the 3′ end of the probe with a mismatch to the transcript. The probe sequences for the various genes were as follows: *dam*, 5′-GGGCAAATGCCGTTTAATATCATC AAGCAGGGGATACTTGCCCCCGTAAACAGGA; *gidA*, 5′-GGTGATTGC CTCGCATAACGCGGTATGAAAATGGATTGAAGCCCGGGCCGTGGA TTCCGTCTGGTCC; *iciA*, 5′-GCGCCTGATGTTGAATACTCACCGGCCG ACCACTTCGCCGCGGCGCAGACGTTCCTGAGTGCGGGGGGACGA GGA; *minE*, 5′-TTCTGCATCGCTGCGACGGCGTTCAGCAACAATAATC TGCAGCCGGGAGGGGTAC; *mukB*, 5′-CTTACCGTGCAGACCTTTATCG CGCGAACCGCTGGTGGCCCCGGCGGAATGCTGT; *nrdA*, 5′-TACTGAT AATCCGGCGCATCACGGGAGATCAGGTCTGCGGCAGCCGGTCGCC GTT; *rpoA*, 5′-GGCGCGGTTTTAGAAACTCTGTCACAGAACCCTGCCG GTGTGAAGAGAGGGTTGC; *seqA*, 5′-TTCGACGATAGCAGGTGACGCA ACGCGAACCTCTTTCGTCACCGGCTACTACTTA; *tus*, 5′-GAACACGCG GGCAACCAATAGCTTGTGTTGCTCAAGATGAGCGGCCCCGCGC TAA. The oligonucleotide probes were 5′ end labeled with polynucleotide kinase (U.S. Biochemicals) and [γ-^{32}P]ATP (greater than 3,000 Ci/mM; NEN). To quantitate transcripts, 10 μg of total cellular RNA was hybridized with 0.3 to 0.6 ng of the relevant 5′-^{32}P-end-labeled oligonucleotide (50,000 to 500,000 cpm). After S1 digestion and electrophoresis (35), the resultant autoradiograms were scanned with a UMAX UC630 flat-bed scanner and Adobe photoshop. The images were quantitated with NIH Image 1.52. Because of the limited linear response range of the film and the variation in intensity of the signals produced by the different probes used simultaneously in the S1 transcription assays, multiple exposures of most autoradiograms were scanned to allow accurate quantitation.

RESULTS

Alignment of chromosome replication by temperature shifts of *E. coli* PC2 *dnaC2*(Ts). The relationship between chromosome replication and gene transcription was analyzed in cultures of *E. coli* K-12 PC2 *dnaC2*(Ts) which had been aligned for initiation of chromosome replication by temperature shifts.

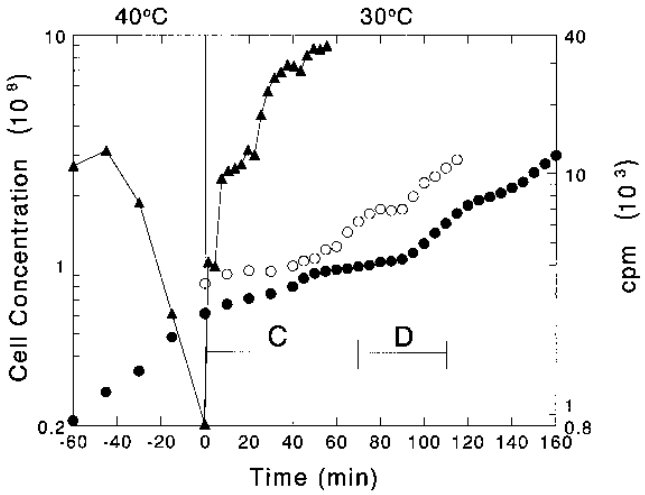

FIG. 1. Chromosome replication and cell division during temperature shifts of *E. coli* K-12 PC2 *dnaC2*(Ts). Cultures growing exponentially at 30°C in glucose-Casamino Acids minimal medium were shifted to 40°C for 60 min and then returned to 30°C. Samples were taken at intervals to measure cell concentration (●) and counts per minute (▲) after pulse-labeling with [^{3}H]thymidine for 5 min. In a second, parallel culture, cell concentration was measured after exposure of each sample to 200 μg of chloramphenicol/ml for 90 min (○). Since cells in the C period cannot divide in the presence of the drug but those in the D period can (18), the abrupt increase in cell concentration, with a midpoint at 70 min, corresponds to the end of the C period. The average durations of the C and D periods are indicated.

Cultures growing in minimal medium containing glucose and Casamino Acids at 30°C (permissive temperature) were shifted to 40°C (nonpermissive temperature) for approximately two mass doublings and then were returned to 30°C. At 40°C, ongoing rounds of replication are completed, new rounds are not initiated, and potential for initiation of two subsequent rounds of replication has accumulated (2, 6, 10, 11, 15, 19, 31). Upon return of the cultures to 30°C, the first round of replication is initiated immediately and the second is initiated about 25 to 30 min later (2). Figure 1 shows a representative temperature shift experiment, with the two initiations at 30°C corresponding to the two abrupt increases in [^{3}H]thymidine uptake. Cell concentration changed little at 30°C until the parasynchronized division, which took place C + D minutes (about 110 min, on average) after the synchronized initiation of replication at time zero. The figure also shows cell concentration after exposure of the samples to chloramphenicol for 90 min. Since cells in the D (cell division) period divide in the presence of the drug (reviewed in reference 18), the 40-min leftward shift of the first abrupt increase in cell concentration establishes the relative durations of the C (chromosome replication) and D periods, as indicated. The slight increase in cell concentration at 40 min in the untreated culture suggests that some cells may have been blocked at entry into D at 40°C and then progressed as a group through D upon the shift to 30°C. The absence of a measurable increase in cell concentration between 40 min and the first synchronized division (e.g., between D and C + D min) demonstrates that essentially all rounds of replication were completed at 40°C, for if they had not been, cells would have divided during this interval.

For measurement of transcript levels, RNA was extracted from samples taken at intervals during the temperature shifts. Transcripts in the RNA samples were quantitated based on the level of protection of 5′-end-labeled complementary oligonucleotides from digestion with nuclease S1 after hybridization. The protected fragments were electrophoresed on denaturing

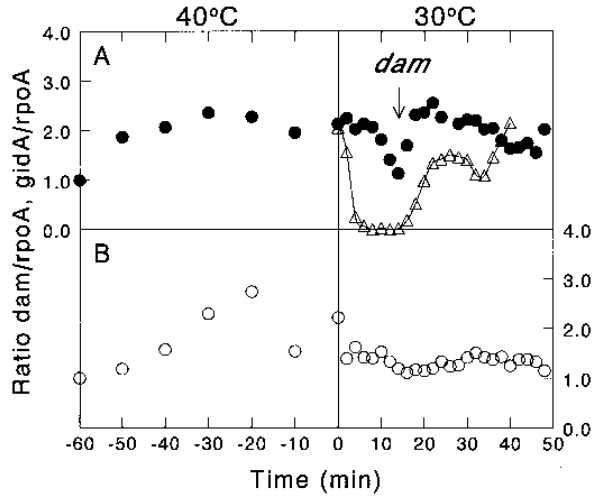

FIG. 2. Transcript levels of *dam* during temperature shifts of PC2 *dnaC2*(Ts) (A) and DG76 *dnaC*$^+$ (B). Cultures growing exponentially at 30°C in glucose-Casamino Acids minimal medium were shifted to 40°C for 60 min and then returned to 30°C. At intervals, 10 μg of total cellular RNA was isolated, hybridized to ^{32}P-labeled oligonucleotide probes, and assayed by S1 protection. The resultant autoradiograms were quantitated by densitometry, and the intensities of the signals are expressed as ratios to the *rpoA* protected signal, normalized to 1.0 at the shift to 40°C (−60 min). The relative transcript levels of *dam* during the shift of PC2 (●) and during the shift of DG76 (○) and the relative transcript levels of *gidA* in PC2 (△) (included for comparison) are shown. The approximate time of *dam* gene replication (map position, 74 min) is indicated by the arrow and was calculated to be (84 − 74)/50 × C, with a C period of 70 min at 30°C.

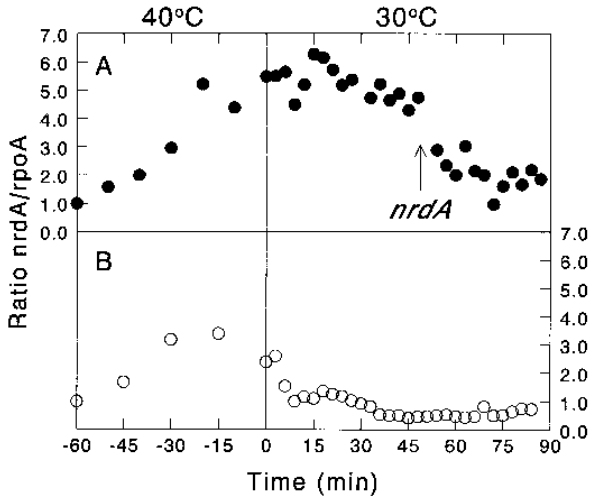

FIG. 3. Transcript levels of *nrdA* during temperature shifts of PC2 *dnaC2* (Ts) (A) and DG76 *dnaC*$^+$ (B). The approximate time of *nrdA* gene replication is indicated by the arrow.

polyacrylamide gels, and the radioactive bands in the resultant autoradiograms were scanned and quantitated. Each experiment was performed at least twice in its entirety to ensure reproducibility of the results. The variations in transcript levels for eight different genes are shown in Fig. 2 through 8. The top panels in the figures show transcript levels during the temperature shift with PC2 *dnaC2*(Ts), and the bottom panels show levels in the *dnaC*$^+$ parental strain, DG76, which serve as controls for the effects of the temperature change itself. The data are plotted as ratios to the level of *rpoA* transcript, which does not fluctuate in these experiments (35), to correct for any variabilities in the processing of each sample. The estimated time at which each of the genes replicated is shown in each figure and was calculated as (m/50) × C, where m is the map distance in minutes between *oriC* (located at 84 min) and the gene of interest and 50 is the map distance replicated during the C period (70 min at 30°C).

***dam* and *gidA*.** Transcript levels of *dam* (Fig. 2A), encoding *dam* methyltransferase, which is responsible for methylation at N-6 of adenine in GATC sequences (17, 21), increased somewhat at 40°C. The transcript level decreased shortly after the shift to 30°C, with a minimum at 14 min, and then again several minutes later. As indicated, the first minimum coincided with the approximate time of gene replication. Comparable fluctuations at 30°C were not discernible in the control experiment with DG76 (Fig. 2B). The levels of *gidA* transcripts during synchronized chromosome replication at 30°C are also shown in the figure. As reported previously (35), *gidA* mRNA levels decreased dramatically after replication of the gene (immediately after the shift and then again at 30 min). The decrease in *dam* transcript levels, while clearly detectable, was not nearly as pronounced as that for *gidA*.

***nrdA*.** Transcript levels of *nrdA* (Fig. 3A), encoding one of the two subunits of nucleotide diphosphate reductase (7), increased about fivefold at 40°C and then remained essentially unchanged for 45 min after the shift to 30°C. At 45 min postshift, there was an abrupt decrease in transcript levels, which again corresponded to the approximate time the gene replicated. In the control nonaligned culture (Fig. 3B), transcript levels increased to a lesser extent at 40°C, and there was no decrease at 45 min after the return to 30°C.

***seqA*.** Transcript levels of *seqA* (Fig. 4A), a negative effector of initiation of chromosome replication (25, 36), did not vary at 40°C or during the first 45 min at 30°C but decreased transiently about the time the gene replicated and subsequently increased about twofold. These fluctuations were not seen in the control experiment (Fig. 4B).

***mukB*.** Transcript levels of *mukB* (Fig. 5A), involved in chromosome partitioning (28), increased considerably at 40°C, and there was again an abrupt transient decrease at 30°C with a minimum at about 45 min. In this case, the minimum transcript level was observed just prior to the estimated time of *mukB* replication.

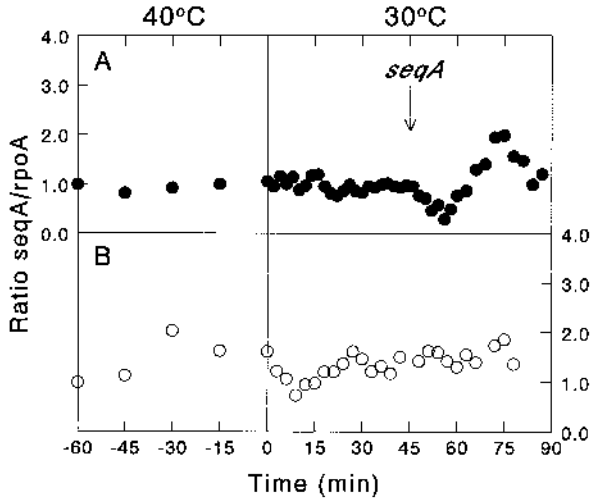

FIG. 4. Transcript levels of *seqA* during temperature shifts of PC2 *dnaC2*(Ts) (A) and DG76 *dnaC*$^+$ (B). The approximate time of *seqA* gene replication is indicated by the arrow.

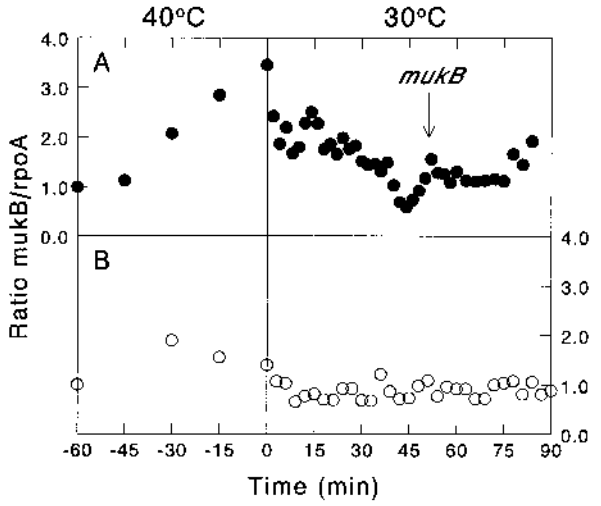

FIG. 5. Transcript levels of *mukB* during temperature shifts of PC2 *dnaC2* (Ts) (A) and DG76 *dnaC*$^+$ (B). The approximate time of *mukB* gene replication is indicated by the arrow.

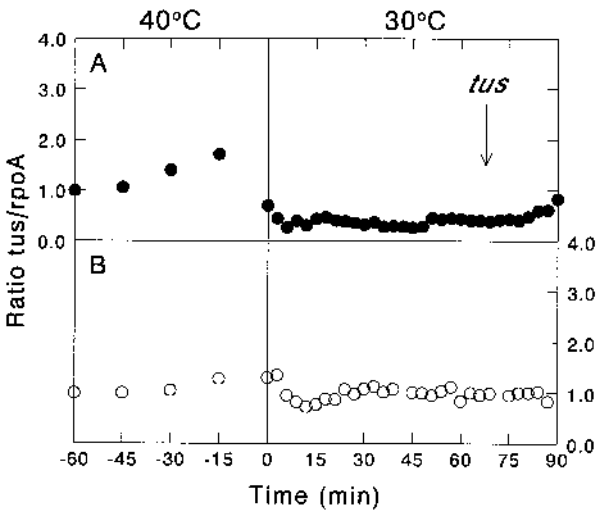

FIG. 7. Transcript levels of *tus* during temperature shifts of PC2 *dnaC2*(Ts) (A) and DG76 *dnaC*$^+$ (B). The approximate time of *tus* gene replication is indicated by the arrow.

***minE* and *tus*.** Transcript levels of *minE* (Fig. 6), involved in division positioning (8), and of *tus* (Fig. 7), involved in termination of chromosome replication (30), did not fluctuate detectably during synchronized chromosome replication at 30°C. One striking aspect of *minE* transcript levels is that they rose to a peak at about 50 min at 40°C and then declined. Figure 6 shows incubation at 40°C for 75 min, rather than for 60 min as in the other figures, to demonstrate that the peak and subsequent decline in levels occurred at 40°C and was unrelated to the shift back to 30°C. The results at 30°C were identical when the incubation at 40°C was restricted to 60 min (data not shown).

***iciA*.** Transcript levels of *iciA* (Fig. 8), whose product inhibits replication from *oriC* in vitro (20), displayed a pattern that differed from all of the preceding. The levels were consistently found to increase transiently, with a peak about 30 min after the shift to 30°C. This increase occurred about the same time as both replication of the gene and the second initiation of replication after the shift to 30°C.

Synchronously dividing cultures. To investigate cell cycle-dependent transcription in cells that had not been subjected to temperature shifts to align initiation of chromosome replication, the baby machine was used to obtain synchronous cultures. In general, small changes in levels are difficult to detect with synchronous cultures, due to the broad age distribution of initiation of chromosome replication (14). However, genes whose transcripts fluctuate dramatically in temperature shift experiments, i.e., *dnaA* and *mioC*, also show clear fluctuations in synchronous cultures (35). This is also the case for transcript levels of *gidA* (Fig. 9). Transcripts from *gidA* reached a maximum near initiation of chromosome replication and a minimum after initiation. The results are consistent with the conclusion from the data in Fig. 2 that *gidA* transcription is blocked after replication of the gene. The only other gene

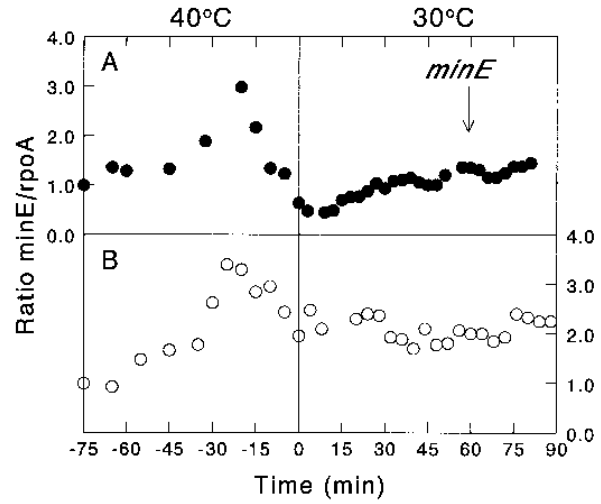

FIG. 6. Transcript levels of *minE* during temperature shifts of PC2 *dnaC2* (Ts) (A) and DG76 *dnaC*$^+$ (B). The approximate time of *minE* gene replication is indicated by the arrow.

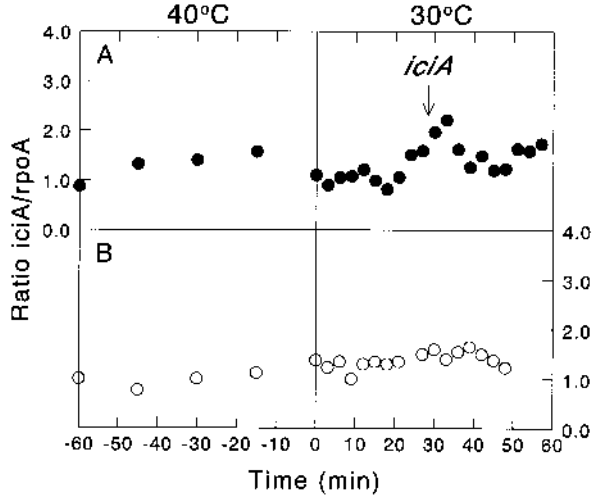

FIG. 8. Transcript levels of *iciA* during temperature shifts of PC2 *dnaC2*(Ts) (A) and DG76 *dnaC*$^+$ (B). The approximate time of *iciA* gene replication is indicated by the arrow.

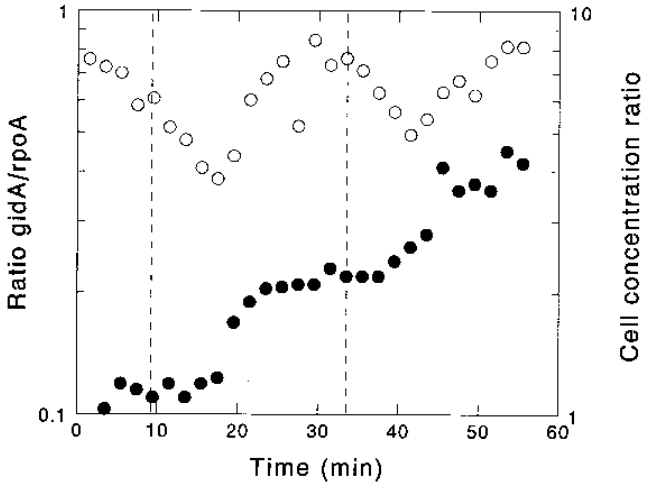

FIG. 9. Transcript levels of *gidA* in the cell cycle. A synchronous culture of B/rA growing in glucose-Casamino Acids medium at 37°C (doubling time [τ] = 24 min) was generated with the baby machine. ○, *gidA*/*rpoA* ratios; ●, synchronous growth curve obtained by dividing the cell concentration at the time of cell harvest by the cell concentration at the end of synchronous growth. The vertical dashed lines show the average times of initiation of chromosome replication in the cell cycle calculated from the equation a_i (cell age at initiation) = $(n + 1)\tau - (C + D)$, where n is the smallest integer so that $(n + 1)\tau \geq (C + D)$ and C + D is 64 min for *E. coli* B/rA growing with this doubling time at 37°C (18).

tested that showed indications of cell cycle-dependent transcriptional periodicities in synchronous cultures was *nrdA*. Figure 10 shows *nrdA* transcript levels during synchronous growth in media supporting two different growth rates. Although more difficult to interpret than the data from experiments with the *gidA* gene, these data are consistent with maxima in transcript levels at roughly the times of initiation of replication and minima between initiations. As indicated in the figure, the minima were also generally located at the estimated time of *nrdA* gene replication.

DISCUSSION

The results of studies on gene transcription during synchronized chromosome replication in *E. coli* K-12 PC2 *dnaC2*(Ts) presented here and published previously from this laboratory (2, 35, 37) are summarized in Table 1. The genes are listed in the sequence in which they replicate in the C period at 30°C, ranging from 0 to 68 min (column 3). The most striking observation is that there was a decrease in mRNA levels of 8 of the 12 genes, and in 7 of these the decrease was within 10% of the estimated time of gene replication. The only exception to this relationship was *mukB*, for which the decrease occurred about 20% before the estimated gene replication time. No other relationship between transcript levels and events of the chromosome replication cycle, such as initiation of replication, can be distinguished. The absence of detectable fluctuations in transcript levels for *rpoA*, *minE*, and *tus* could imply a differing relationship between replication and transcription for these genes or a change in transcript levels that was too small to be detected. It should be noted, however, that the absence of a transient decrease in transcripts for *minE* and *tus* could be due to a decay in synchrony of chromosome replication over time, since these genes replicate late in the C period, but this explanation would not obtain for *rpoA*.

The one gene that seemed to behave differently from those described above was *iciA*. A transient increase in transcript levels, with a maximum at 30 min after the shift to 30°C, was consistently found with this gene. The timing of the increase could correspond either to gene replication or to the second initiation of chromosome replication after the shift. If it is due to gene replication, the increase could reflect a relationship between transcription and gene dosage, although gene dosage effects were not obvious for any other gene examined, probably due to both the insensitivity of the assay and the imperfect synchronization of replication. There could also be a coupling between initiation of replication and *iciA* transcription if the act of initiation stimulated transcription of *iciA* through the titration of existing IciA by binding to the 13-mers in the newly formed origins. In this scenario, the stimulation of transcription must occur prior to the functioning of DnaC at initiation because the increase was not seen during the first initiation at 0 min postshift.

Transcript levels have also been quantitated in synchronous cultures obtained with the baby machine. Except for *dnaA*, *mioC*, and *ftsZ* transcripts, which were reported on previously (35, 37), and *gidA* and *nrdA*, reported on here, obvious fluctuations in levels were not detectable. Since it is not surprising that small changes in levels would be difficult to detect with synchronous cultures, the current study was undertaken with cells aligned for initiation with temperature shifts. Such shifts

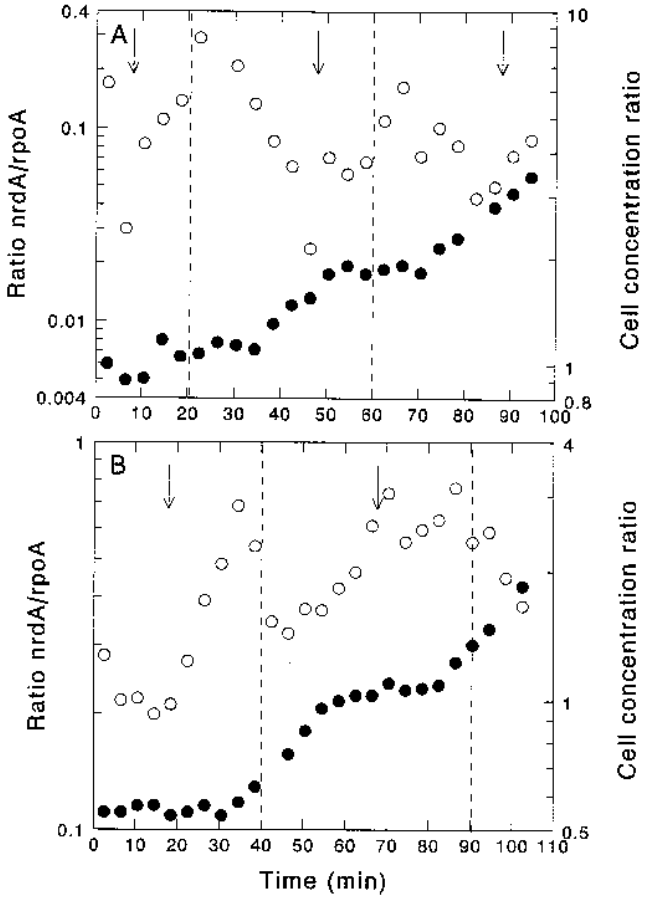

FIG. 10. Transcript levels of *nrdA* in the cell cycle. Synchronous cultures of B/rA growing in glucose minimal medium (A) and glycerol minimal medium (B) at 37°C were generated with the baby machine. ○, *nrdA*/*rpoA* transcript ratios; ●, synchronous growth curves. The vertical dashed lines show the average times of initiation of chromosome replication. The arrows indicate the average times of replication of *nrdA*, at 48 min on the genetic map; these were calculated as $(84 - 48)/50 \times C$, with a C period of 42 min at 37°C.

TABLE 1. Gene replication and expression in *E. coli* at 30°C

Gene	Map position (min)	Gene replication (min)[a]	mRNA decrease (min)[b]	Orientation[c]
gidA	84	0	2	Same
mioC	84	0	Before 0	Opposite
dnaA	83	1.4	3	Same
rpoA	73	14	None	Same
dam	74	15	11	Same
ftsZ	2	25	26	Same
iciA	63	28	25 (increase)	Opposite
seqA	16	45	50	Same
nrdA	48	49	50	Opposite
mukB	21	52	40	Same
minE	26	59	None	Same
tus	35	68	None	Opposite

[a] The times of replication of the genes in the first C period after the shift from 40°C to 30°C were estimated based on their genetic map positions, a C period of 70 min initiated at time zero, and a constant rate of DNA polymerization.
[b] The times refer to the midpoints of the decreases in mRNA levels.
[c] Indicates whether the replication fork and transcription of the gene progress in the same or opposite directions.

could themselves cause disturbances in gene expression, due to either the temperature change or the inhibition of initiation of DNA replication. Comparing the results obtained with the *dnaC2*(Ts) mutant to those obtained with the parental *dnaC*$^+$ strain in every experiment obviated some of that concern. Furthermore, the periodicities detectable in synchronous cultures were all consistent with the results obtained with aligned cells, namely, minimum levels at the approximate time of gene replication. Finally, the magnitudes of any periodicities in transcript levels due to transient inhibitions of transcription are also dependent on the stability of the mRNAs. Half-lives have been measured for each of the genes examined, based on levels in cells exposed to 200 μg of rifampin/ml, and have been found to be in the range of 1 to 3 min at 30°C, with the exception of *ftsZ*, whose half-life was approximately 10 min (data not shown).

The specific explanations for the possible inhibition of transcription by replication suggested in the in vivo studies reported here could differ for different genes. The extended inhibition of transcription of *dnaA*, and possibly of *gidA*, is most likely caused by the lengthy period of hemimethylation of the N-6 of adenine in GATC sequences in *dnaA* and *oriC* (5, 29, 35). The less pronounced decreases in transcript levels for other genes, presumably due to a shorter period of transcription inhibition, could also be explained on this basis because the period of hemimethylation can vary at different GATC sequences (5). There is also the possibility that progression of the replication fork through a gene aborts existing transcriptional complexes by dislodging them from it. This is a controversial concept (12, 23), but even if it is correct, it is likely to produce a transcriptional delay that would be too short to produce a detectable decrease in transcript levels in a batch culture in vivo. In any case, the transcriptional patterns reported here were unrelated to orientation, i.e., whether the replication fork and transcription complex moved in the same or opposite directions (Table 1). Another possibility is that the replication fork alters gene expression by dislodging negative or positive effectors of transcription. Evidence for the former in the case of *mioC* has recently been described (2), and evidence for the latter, in the case of *nrdA*, will be presented elsewhere.

The primary aim of this study was to determine if the timing of periodic cell cycle events could be set by periodic expression of genes essential for the events. On the contrary, the findings are generally consistent with cell cycle-dependent inhibitions of transcription rather than stimulations. Previous reports of cycle-dependent periodicities in transcription of *ftsZ* (13) and *nrdA* (33), interpreted to be enhancements of transcription around the time of initiation of replication, may be explained equally well on this basis. Considering the information currently available, we concluded that there is no compelling evidence for cell cycle timing in *E. coli* being governed at the transcriptional level by any of the genes examined in this study.

ACKNOWLEDGMENTS

This work was supported, in part, by grant GM26429 from the National Institute of General Medical Sciences and grant 91-00190 from the United States-Israel Binational Science Foundation.

REFERENCES

1. **Andrews, B. J., and I. Herskowitz.** 1990. Regulation of cell cycle-dependent gene expression in yeast. J. Biol. Chem. **265:**14057–14060.
2. **Bogan, J. A., and C. E. Helmstetter.** 1996. *mioC* transcription, initiation of replication, and the eclipse in *Escherichia coli*. J. Bacteriol. **178:**3201–3206.
3. **Bramhill, D., and A. Kornberg.** 1988. Duplex opening by DnaA protein at novel sequences in initiation of replication at the origin of the *E. coli* chromosome. Cell **52:**743–755.
4. **Brun, Y., G. Marcynzski, and L. Shapiro.** 1994. The expression of asymmetry during *Caulobacter* cell differentiation. Annu. Rev. Biochem. **63:**419–450.
5. **Campbell, J. L., and N. Kleckner.** 1990. *E. coli oriC* and the *dnaA* gene promoter are sequestered from *dam* methyltransferase following passage of the chromosomal replication fork. Cell **62:**967–979.
6. **Carl, P. L.** 1970. *Escherichia coli* mutants with temperature-sensitive synthesis of DNA. Mol. Gen. Genet. **109:**107–122.
7. **Carlson, J., J. A. Fuchs, and J. Messing.** 1984. Primary structure of the *Escherichia coli* ribonucleoside diphosphate reductase operon. Proc. Natl. Acad. Sci. USA **81:**4294–4297.
8. **de Boer, P. A. J., R. E. Crossley, and L. I. Rothfield.** 1988. Isolation and properties of *minB*, a complex genetic locus involved in correct placement of the division site in *Escherichia coli*. J. Bacteriol. **170:**2106–2112.
9. **Donachie, W. D.** 1979. The cell cycle of *Escherichia coli*, p. 11–35. *In* J. H. Parish (ed.), Developmental biology of prokaryotes. University of California Press, Berkeley.
10. **Eberle, H., N. Forrest, J. Hrynyszyn, and J. Van Knapp.** 1982. Regulation of DNA synthesis and capacity for initiation of DNA temperature sensitive mutants of *Escherichia coli*. I. Reinitiation and chain elongation. Mol. Gen. Genet. **186:**57–65.
11. **Evans, I. M., and H. Eberle.** 1975. Accumulation of the capacity for initiation of deoxyribonucleic acid replication in *Escherichia coli*. J. Bacteriol. **121:**883–891.
12. **French, S.** 1992. Consequences of replication fork movement through transcription units in vivo. Science **258:**1362–1365.
13. **Garrido, T., M. Sanchez, P. Palacios, M. Aldea, and M. Vicente.** 1993. Transcription of *ftsZ* oscillates during the cell cycle of *Escherichia coli*. EMBO J. **12:**3957–3965.
14. **Grover, N. B., and C. E. Helmstetter.** 1995. Characterization of cell-cycle-specific events in synchronous cultures of *Escherichia coli*: a theoretical evaluation. Microbiology **141:**59–62.
15. **Hanna, M. L., and P. L. Carl.** 1975. Reinitiation of deoxyribonucleic acid synthesis by deoxyribonucleic acid initiation mutants of *Escherichia coli*: role of ribonucleic acid synthesis, protein synthesis, and cell division. J. Bacteriol. **121:**219–226.
16. **Hansen, F. G., B. B. Christensen, and T. Atlung.** 1991. The initiator titration model: computer simulation of chromosome and minichromosome control. Res. Microbiol. **142:**161–167.
17. **Hattman, S. M., J. E. Brooks, and M. Masurekar.** 1978. Sequence specificity of the P1 modification methylase (M. Eco P1) and the DNA methylase (M. Eco dam) controlled by the *Escherichia coli dam* gene. J. Mol. Biol. **126:**367–380.
18. **Helmstetter, C. E.** 1996. Timing of synthetic activities in the cell cycle, p. 1627–1639. *In* F. C. Neidhardt, R. Curtiss III, J. L. Ingraham, E. C. C. Lin, K. B. Low, B. Magasanik, W. S. Reznikoff, M. Riley, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella*: cellular and molecular biology, 2nd ed. ASM Press, Washington, D.C.
19. **Helmstetter, C. E., and C. A. Krajewski.** 1982. Initiation of chromosome replication in *dnaA* and *dnaC* mutants of *Escherichia coli* B/r F. J. Bacteriol. **149:**685–693.
20. **Hwang, D. S., B. Thony, and A. Kornberg.** 1992. IciA protein, a specific inhibitor of initiation in *Escherichia coli* chromosomal replication. J. Biol. Chem. **267:**2209–2213.

21. **Lacks, S., and B. Greenberg.** 1977. Complementary specificity of restriction endonucleases of *Diplococcus pneumoniae* with respect to DNA methylation. J. Mol. Biol. **114:**153–168.
22. **Li, Z., A. J. Clarke, and T. J. Beveridge.** 1996. A major autolysin of *Pseudomonas aeruginosa*: subcellular distribution, potential role in cell growth and division, and secretion in surface membrane vesicles. J. Bacteriol. **178:**2479–2488.
23. **Liu, B., M. L. Wong, R. L. Tinker, E. P. Geiduschek, and B. M. Alberts.** 1993. The DNA replication fork can pass RNA polymerase without displacing the nascent transcript. Nature **366:**33–39.
24. **Løbner-Olesen, A., K. Skarstad, F. G. Hansen, K. von Meyenburg, and E. Boye.** 1989. The DnaA protein determines the initiation mass of *Escherichia coli* K-12. Cell **57:**881–889.
25. **Lu, M., J. L. Campbell, E. Boye, and N. Kleckner.** 1994. SeqA: a negative modulator of replication initiation in *E. coli*. Cell **77:**413–426.
26. **Lutkenhaus, J.** 1993. FtsZ ring in bacterial cytokinesis. Mol. Microbiol. **9:**403–409.
27. **Lutkenhaus, J. F., B. A. Moore, M. Masters, and W. D. Donachie.** 1979. Individual proteins are synthesized continuously throughout the *Escherichia coli* cell cycle. J. Bacteriol. **138:**352–360.
28. **Niki, H., A. Jaffe, R. Imamura, T. Ogura, and S. Hiraga.** 1991. The new gene *mukB* codes for a 177 kd protein with coiled-coil domains involved in chromosome partitioning of *E. coli*. EMBO J. **10:**183–193.
29. **Ogawa, T., and T. Okazaki.** 1994. Cell cycle-dependent transcription from the *gid* and *mioC* promoters of *Escherichia coli*. J. Bacteriol. **176:**1609–1615.
30. **Roecklein, B. A., and P. L. Kuempel.** 1992. *In vivo* characterization of *tus* gene expression in *Escherichia coli*. Mol. Microbiol. **6:**1655–1661.
31. **Schuback, W., J. D. Whitmer, and C. I. Davern.** 1973. Genetic control of DNA initiation in *Escherichia coli*. J. Mol. Biol. **74:**205–221.
32. **Skarstad, K., and E. Boye.** 1994. The initiator protein DnaA: evolution, properties and function. Biochim. Biophys. Acta **1217:**111–130.
33. **Sun, L., and J. A. Fuchs.** 1992. *Escherichia coli* ribonucleotide reductase expression is cell cycle regulated. Mol. Biol. Cell **3:**1095–1105.
34. **Sun, L., B. A. Jacobson, B. S. Dien, F. Srienc, and J. A. Fuchs.** 1994. Cell cycle regulation of the *Escherichia coli nrd* operon: requirement for a *cis*-acting upstream AT-rich sequence. J. Bacteriol. **176:**2415–2426.
35. **Theisen, P. W., J. E. Grimwade, A. C. Leonard, J. A. Bogan, and C. E. Helmstetter.** 1993. Correlation of gene transcription with the time of initiation of chromosome replication in *Escherichia coli*. Mol. Microbiol. **10:**575–584.
36. **von Freiesleben, U., K. V. Rasmussen, and M. Schaechter.** 1994. SeqA limits DnaA activity in replication from *oriC* in *Escherichia coli*. Mol. Microbiol. **14:**763–772.
37. **Zhou, P., and C. E. Helmstetter.** 1994. Relationship between *ftsZ* gene expression and chromosome replication in *Escherichia coli*. J. Bacteriol. **176:**6100–6106.