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Positioning of Replicated Chromosomes in *Escherichia coli*

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The positioning of replicated chromosomes at one-fourth and three-fourths of the cell length was inhibited when protein synthesis was inhibited by chloramphenicol or rifampin or by starvation for amino acids. Under these conditions, the progress of chromosome replication continued and replicated chromosomes were located close to each other as one nucleoid mass at midcell. Cells which already had two separate daughter chromosomes located at the cell quarters divided into two daughter cells under these conditions. When protein synthesis resumed, daughter chromosomes moved from midcell to the cell quarters, respectively, before any detectable increase in cell length was observed. The chromosome positioning occurred even under inhibition of the initiation of chromosome replication and under inactivation of DNA gyrase. The chromosome positioning presumably requires new synthesis of a particular protein(s) or translation itself.

During the bacterial cell cycle, replicated chromosomal DNA molecules are spatially separated from one another prior to cell division; therefore, daughter cells have at least one copy of the chromosome. In contrast to eucaryotes, microtubules or analogous structures have not been shown to exist in bacteria. Several hypotheses have been proposed to explain the chromosome partitioning in bacteria. In the first hypothesis, a specific site of the chromosome, for example, the replication origins (*oriC*) of chromosomes, binds with the cell envelope; and the attachment sites in the cell envelope move from midcell to opposite directions by elongation of the envelope, which is supposed to be synthesized in midcell (13, 18, 22–24). In most of these models, chromosome segregation is coupled with the process of chromosome replication, and the driving force for chromosome segregation is provided by insertion of new cell material between the attachment sites. In the second hypothesis, replication origins, replication forks, and replication termini of chromosomes bind to membrane sites; release; and bind again to other sites of the membrane during the cell cycle (15). In the third hypothesis (transcription-translation-mediated segregation model), replicating chromosomes move in opposite directions according to the progress of chromosome replication and they may be temporarily connected to the membrane or the cytoplasm by RNAs and polysomes, but there is no specific binding site on the chromosome (31). However, there is no decisive experimental evidence for any of these hypotheses.

We have described previously (20) that *oriC* plasmids or minichromosomes, which replicate by using the replication origin (*oriC*) of the *Escherichia coli* chromosome, do not have a partition mechanism, so that *oriC* plasmid DNA molecules are partitioned essentially at random into daughter cells. An *oriC* plasmid carrying the *sop* genes of F plasmid (10, 19, 20), which are essential for partitioning, is stably maintained even in nonselective media (20). To analyze the chromosome partitioning mechanism, we have described previously (9) the isolation of mutants which were defective in the precise partitioning of replicated chromosomes into daughter cells.

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A previously unknown organelle, the periseptal annular apparatus, which is present at the future division site before the onset of septum formation, has been described in recent years (4, 17). The periseptal annuli are two concentric rings that surround the cell at the site of cell division. In each annulus, the inner membrane, murein, and outer membrane lie in close apposition to each other. New periseptal annuli are generated from annuli that are already located in position at the midpoint of the newborn cell, and the nascent annuli are then displayed at positions at one-fourth and three-fourths of the cell length during cell elongation (4).

In this study we show that replicated chromosomes of *E. coli* require a newly synthesized protein or proteins to move from midcell to one-fourth and three-fourths of the cell length prior to septum formation. Daughter chromosomes that replicated under inhibition of protein synthesis were located close to each other at midcell. Resumption of protein synthesis caused the correct positioning of daughter chromosomes at the cell quarters without cell elongation. Most of the hypotheses described so far are inconsistent with our experimental results.

MATERIALS AND METHODS

Bacterial strains. All bacterial strains used in this study were derivatives of *E. coli* K-12. Strain W3110 was an F^- prototroph (16). Strain SH51 was F^- *dnaC2 leu thyA str tonB*. This strain was derived from strain PC2 (3), which was obtained from Y. Hirota. CBK110 [F^- *dapA* or *dapE*::Tn*5* *thyA* Δ(*lacA-argF*)] was obtained from A. Nishimura. Strain SH122 was F^- *dnaC2 ilvD leu metE thyA* (low) *mtl malA str tonB bfe* (our laboratory).

Media and chemicals. Synthetic minimal medium E (29) supplemented with 0.5% glucose and appropriate requirements was used to cultivate cells. L medium (1% tryptone [Difco Laboratories, Detroit, Mich.], 0.5% yeast extract, 0.5% NaCl [pH 7.4]) supplemented with thymine (50 μg/ml) was used for the cultivation of strain SH51. L-agar plates supplemented with thymine were used to measure the number of colony formers. The following antibiotics were used at the indicated concentrations: chloramphenicol, 300 μg/ml; streptomycin, 200 μg/ml; rifampin, 100 μg/ml; nalidixic acid,

50 μg/ml; novobiocin, 100 μg/ml; ampicillin, 50 μg/ml; furazlocillin, 50 μg/ml; mecillinam, 60 μg/ml.

Microscopic observation of cells and nucleoids. Cells were fixed with methanol and stained with 4′,6-diamidino-2-phenylindole solution by the fluo-phase combined method of Hiraga et al. (9) prior to observation and photography. Color photographic prints were used for all measurements of cell length and nucleoid position and number. However, only black and white prints are provided here. The brightness of chromosomal DNA stained with 4′,6-diamidino-2-phenylindole in each cell was measured with a graphic analyzer (9) in some critical cases but was generally evaluated by eye.

Microscopic observation of plasmolysis bays. Plasmolysis bays were observed by the method of Cook et al. (4). A 1-ml sample of a culture was removed; and cells were collected by centrifugation, suspended in 0.3 ml of 40% (wt/vol) sucrose solution, and kept at room temperature for 5 min to be plasmolyzed. To fix the cells, 0.3 ml of 6% glutaraldehyde in 40% (wt/vol) sucrose was added to the samples, and the mixture was kept at room temperature for at least 30 min. The cells were kept at 5°C before microscopic observation. They were observed and photographed through a phase-contrast microscope. The cell lengths and locations of plasmolysis bays were measured by using photographic prints. All cells that were in sharp focus were analyzed.

Measurement of cell number. Samples were removed at intervals from an exponentially growing culture and fixed with paraformaldehyde (final concentration, 1%). The cell number was estimated by using a Petroff-Hausser and Helber counting chamber (C. A. Hausser and Son, Philadelphia, Pa.) under a phase-contrast microscope.

Observation of nucleoids in living cells. Living cells were prepared for the phase-contrast microscopy as described by Valkenburg et al. (28), with minor modifications. In short, cells were allowed to adhere to object slides coated with poly-L-lysine and immersed in 3% (wt/wt) bovine serum albumin solution (fraction V; Sigma Chemical Co., St. Louis, Mo.) in medium E. For phase-contrast microscopy, a Nikon Optiphot microscope was used.

Measurement of the rate of DNA synthesis. For measurement of the rate of DNA synthesis, portions of a culture were removed at intervals and incubated with [^{3}H]thymidine for 5 min at 42°C. Incorporation into the acid-insoluble fraction was analyzed.

RESULTS

Effect of the addition of chloramphenicol and rifampin on the number of nucleoid masses in growing cells. The progress of chromosomal DNA replication is known to continue in the presence of chloramphenicol (300 μg/ml), rifampin (100 μg/ml), or both. The two antibiotics are inhibitors of protein and RNA synthesis, respectively. To determine the effect of these antibiotics on the positioning of replicated chromosomes, each antibiotic was added to an exponentially growing culture (doubling time, 90 min) of strain W3110. Under these growing conditions, multiple replication forks do not occur (5, 8). Cells showing two nucleoids at the separate positions, one-fourth and three-fourths of the cell length, represented 12 to 17% of the total cell population in exponentially growing cultures (Fig. 1). The proportion of this cell type decreased gradually after the addition of chloramphenicol or rifampin, reaching 0.5% after 1 h. This reduction as a result of chloramphenicol treatment was not observed in the presence of KCN, which blocked the synthesis of ATP (Fig. 1). This showed that the reduction in the proportion of cells with two separated nucleoids is dependent on cellular functions. A similar reduction in the number of cells with two separated nucleoids was observed after starvation for threonine, leucine, and methionine with a strain which was an auxotroph for these amino acids (data not shown). When chloramphenicol and furazlocillin, which is an inhibitor of septum formation, were added to a culture, the proportion of cells with two separate nucleoids was not reduced, but these cells had blunt constriction in midcell (data not shown).

After the addition of chloramphenicol or rifampin, each nucleoid condensed immediately (within 3 to 5 min) into a more compact structure. This phenomenon may be caused by disruption of polysome structures (1, 14, 33).

Effect of chloramphenicol in cultures synchronized for chromosome replication. Why does the proportion of cells with two spatially separated nucleoids decrease during incubation under inhibition of protein synthesis? One possibility is that the reduction of this type of cell is caused by cell division under these conditions. Another possibility is that separate nucleoids move to midcell under these conditions. To determine the reason for this, we tested the effect of chloramphenicol in cultures which were synchronized for chromosome replication. The *dnaC2* mutant is temperature sensitive for the initiation of chromosome replication (3, 11). Cells of *dnaC2* mutant SH51 were grown at 30°C (doubling time, 120 min at 30°C), transferred to the nonpermissive temperature of 42°C, incubated for 2 h at 42°C, and shifted back to 30°C (time zero). After the transfer to 30°C, chromosomal DNA replication initiates synchronously (11). Cells with two separated nucleoids at the one-fourth and three-fourths cell length positions represented 0.3% of the total cell population at the zero time of the shift back to 30°C (Fig. 2). The cell number remained constant for the first 1 h and then increased about twofold. The proportion of cells showing two spatially separated nucleoids was constant for the first 30 min and then increased to 38% at 1 h, before resumption of cell division. When samples of the culture were removed at intervals and incubated with chloramphenicol for 1 h, the proportion of cells with two separated nucleoids became very low (less than 1% of the total cell population in all samples) (Fig. 2).

In parallel experiments, colony formers were assayed. A pair of samples was removed at intervals; one was assayed immediately for colony formers, and another was incubated with chloramphenicol for 1 h and then assayed for colony formers. For the first 45 min, there was no difference in the number of colony formers between the control samples (before the addition of chloramphenicol) and the chloramphenicol-treated samples (Fig. 3B). However, 60 min after transfer to 30°C, the number of colony formers in each chloramphenicol-treated sample was always larger than that in each corresponding control sample. The ratio of colony formers of a chloramphenicol-treated sample to colony formers of a control sample is shown in Fig. 3A. The ratio was constant for the first 45 min and then increased to a peak of 1.8 at 75 min. This time corresponded to the time of the beginning of cell division in the culture without the antibiotic (Fig. 3A). The ratio decreased according to further incubation, and then it increased again.

When chloramphenicol was added to a *dnaC2* mutant culture (strain SH51) upon the shift back to 30°C (time zero), no increase in colony formers was observed (Fig. 3C). In contrast, when chloramphenicol was added to the same culture at 75 min after the shift back, the number of colony formers increased about twofold (Fig. 3C).

The results described above indicate that the reduction in

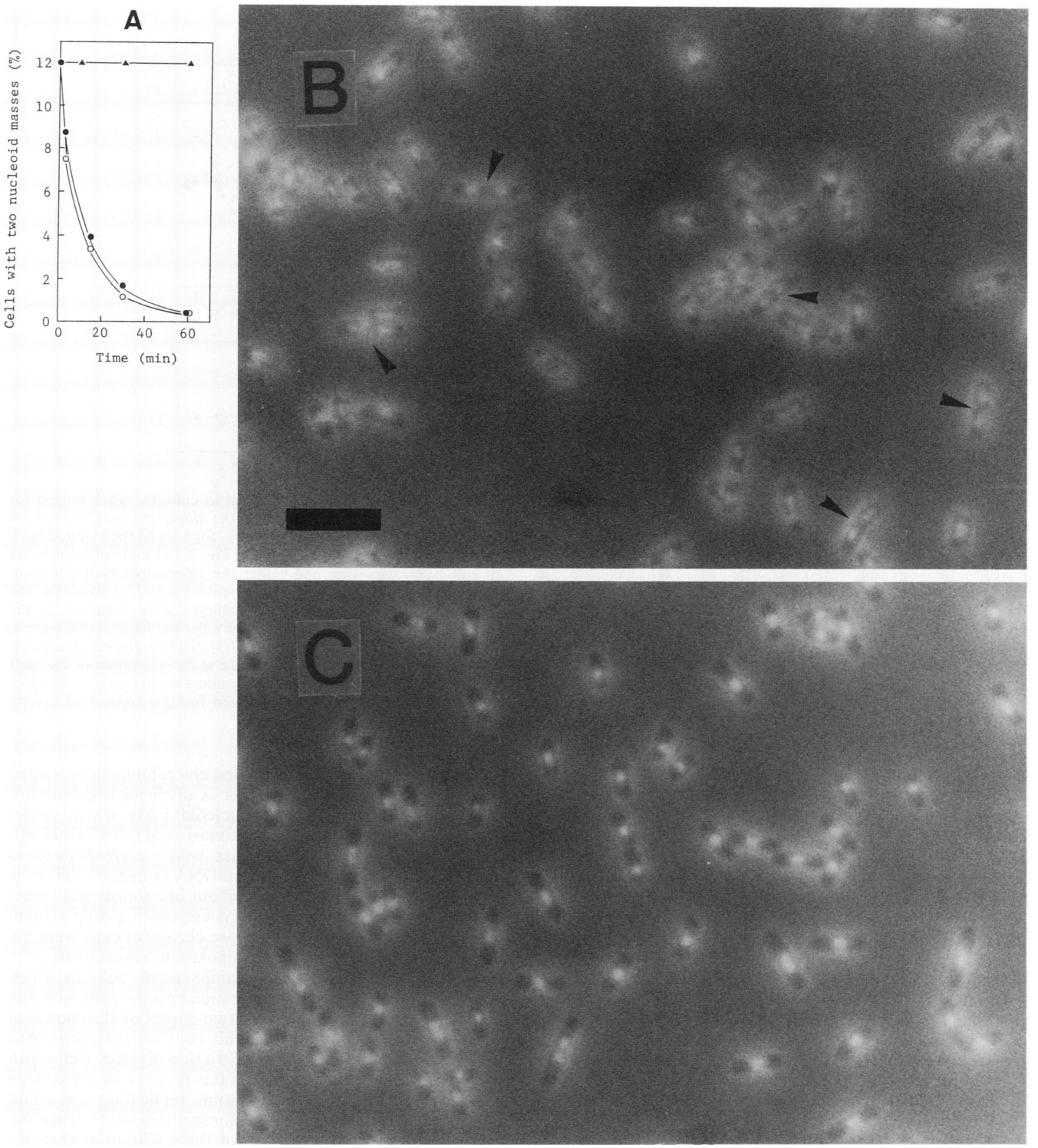

FIG. 1. Effect of chloramphenicol and rifampin on the number of nucleoid masses in growing cells. Cells of strain W3110 were grown at 37°C in a minimal medium containing 0.5% of glucose (doubling time, 90 min). Chloramphenicol or rifampin was added to the culture at time zero. Samples were removed and analyzed for the location of nucleoids by the fluo-phase combined method of Hiraga et al. (9). (A) The vertical axis shows the proportion of cells with two separated nucleoid masses as a percentage of the total cell population. Symbols: ●, chloramphenicol (300 μg/ml); ○, rifampin (100 μg/ml); ▲, chloramphenicol (300 μg/ml) plus KCN (50 mM). (B) Exponentially growing cells. Cells with arrowheads had two spatially separated nucleoids. Bar, 5 μm. (C) Cells treated with chloramphenicol for 60 min. Nucleoids were bright with fluorescence.

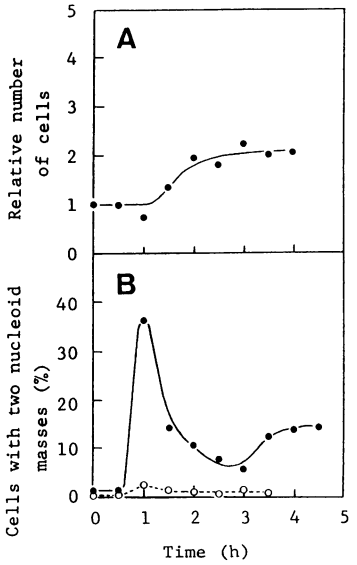

FIG. 2. Effect of chloramphenicol in cultures synchronized for chromosome replication. Cells of the temperature-sensitive *dnaC* mutant SH51 were grown at 30°C in L medium supplemented with 50 μg of thymine per ml (doubling time, 120 min). Exponentially growing cells were transferred to the nonpermissive temperature of 42°C and incubated for 2 h to terminate chromosome replication. These cells were shifted back to 30°C to initiate chromosome replication at time zero. Samples were removed at intervals and tested for total cell number (A) and nucleoids (B; ●), as described in the text. Samples were removed at the indicated times, incubated for 1 h with chloramphenicol (300 μg/ml), and then tested for nucleoids (B; ○).

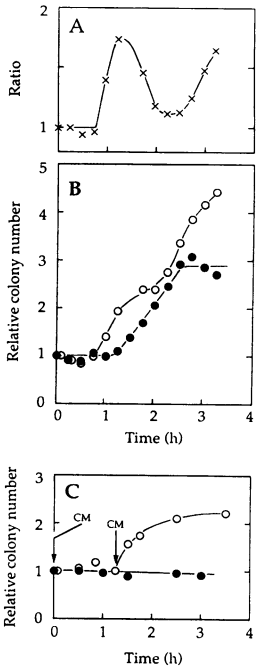

FIG. 3. Effect of chloramphenicol in cultures synchronized for chromosome replication. Cells of the *dnaC* mutant SH51 were grown at 30°C, and transferred to 42°C for 1 h and then shifted back to 30°C, as described in the legend to Fig. 2. (A) Ratio of the number of colony formers after and before the chloramphenicol treatment (see panel B). (B) Samples were removed and assayed for the number of colony formers (●). Samples were removed at the indicated times, incubated for 1 h with chloramphenicol (300 μg/ml), and then assayed for colony formers (○). (C) Chloramphenicol (CM; 300 μg/ml) was added to a subculture at the zero time of the shift back to 30°C (●) and to another subculture at 70 min (○). Samples were removed at intervals and tested for the number of colony formers.

the proportion of cells with two separate nucleoids is mainly due to cell division of this cell type. Chloramphenicol did not inhibit the division of cells in which the replicated chromosomes were already located at the separate positions of one-fourth and three-fourths of the cell length. The antibiotic inhibited cell division in other stages of the cell cycle. The antibiotic inhibited the positioning of the replicated daughter chromosomes at one-fourth and three-fourths of the cell length. Chloramphenicol did not inhibit the progression or termination of chromosome replication, although it does inhibit the reinitiation of chromosome replication, as described previously (11). Daughter chromosomes that replicated in the presence of chloramphenicol were presumably located close to each other, as they were observed as one nucleoid mass at midcell. The proportion of cells with two separated nucleoids was gradually decreased after the addition of chloramphenicol (Fig. 1).

Proportion of various classes of cells in chloramphenicol-treated cultures. The locations of nucleoids were analyzed in exponentially growing and chloramphenicol-treated cells. We classified exponentially growing cells (547 total) into four classes, as distinguished morphologically: (i) cells with one small nucleoid which was not, presumably, in the process of replication; (ii) cells with one irregularly formed nucleoid which was probably in the process of replication (a small portion may have been unseparated double nucleoids); (iii) cells with two separate nucleoids at about one-fourth and three-fourths of the cell length; and (iv) cells with a deep septum constriction or pairs of cells. These classes of cells were 41, 38, 17, and 4% of the total cell population, respectively.

On the other hand, we classified chloramphenicol-treated cells (504 total) into four classes as distinguished morphologically; (i) cells with one nucleoid mass which corresponded to about one copy of chromosomal DNA at midcell, (ii) cells with one nucleoid mass which corresponded to about two copies of chromosomal DNA at midcell, (iii) cells with two separate nucleoids at about one-fourth and three-fourths of the cell lengths, and (iv) cells with a deep septum constriction or pairs of cells. These classes of cells were 59, 32, 1, and 8% of the total cell population, respectively.

During incubation with chloramphenicol, cells in which DNA was in the process of replication became cells with one nucleoid mass corresponding to two copies of chromosomal DNA at midcell. On the other hand, cells with two separated nucleoids or a deep septum constriction divided. Cells with one nucleoid which was not in the process of replication were unable to initiate chromosome replication and could not divide in the presence of chloramphenicol, because the synthesis of proteins essential for the initiation of chromosome replication was blocked by the antibiotic. Therefore, it was expected that 31% of cells with one nucleoid mass corresponding to two copies of chromosomal DNA at midcell existed in the culture treated with chloramphenicol for 1 h. This type of cell, indeed, represented 32% of the cells in the chloramphenicol-treated culture.

Location of nucleoids and plasmolysis bays in chloramphenicol-treated cells. The effect of chloramphenicol treat-

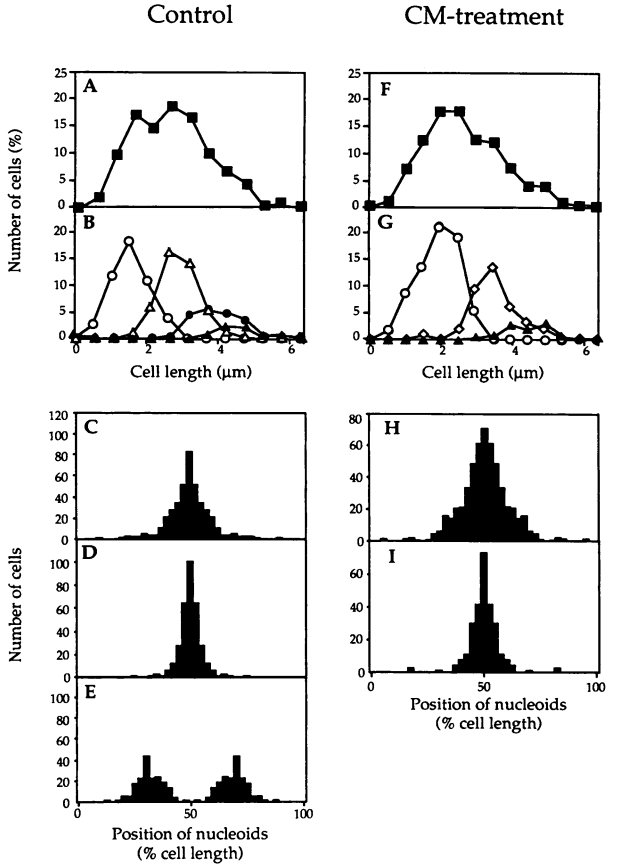

FIG. 4. Cell length distribution and location of nucleoid masses. Strain W3110 cells grown exponentially at 37°C in a minimal glucose medium were incubated with chloramphenicol (CM; 300 μg/ml) for 1 h at 37°C. Cells were analyzed for the cell length and location of nucleoid masses. (A to E) Before the addition of chloramphenicol. (F to I) After chloramphenicol treatment for 1 h. (A and F) Cell length distribution of total cells. The numbers of cells analyzed were 612 (A) and 462 (F). (B and G) Proportion of various classes of cells. Cells with one small round nucleoid mass corresponding to one copy of chromosomal DNA (○), one irregularly formed nucleoid mass (△), two separate nucleoid masses (●), one large nucleoid mass corresponding to two copies of chromosomal DNA (◇), and a deep septum constriction (▲) are indicated. (C) Location of nucleoid masses in cells with one small round nucleoid mass which was presumably not in replication. (D) Location of nucleoid masses in cells with one irregularly formed nucleoid mass which was presumably in the process of replication. (E) Location of nucleoid masses in cell showing two separate nucleoid masses. (H) Location of nucleoid masses in cells with one nucleoid mass corresponding to about two copies of chromosomal DNA in the chloramphenicol-treated culture. (I) Location of nucleoids in cells with one nucleoid mass corresponding to two copies of chromosomal DNA in the chloramphenicol-treated culture. Distances between the center of a nucleoid and each the cell pole are given as a percentage of cell length.

ment on the locations of nucleoids and plasmolysis bays was analyzed. In an exponentially growing W3110 culture, cells were classified into the four classes described above (Fig. 4A to E). The first class of cells with one small round nucleoid mass were small in size (Fig. 4B). The second class of cells showing an irregularly formed nucleoid were middle-sized. The third class of cells showing two separate nucleoids and the fourth class of cells with a deep septum constriction were large. The first and second classes of cells had one nucleoid

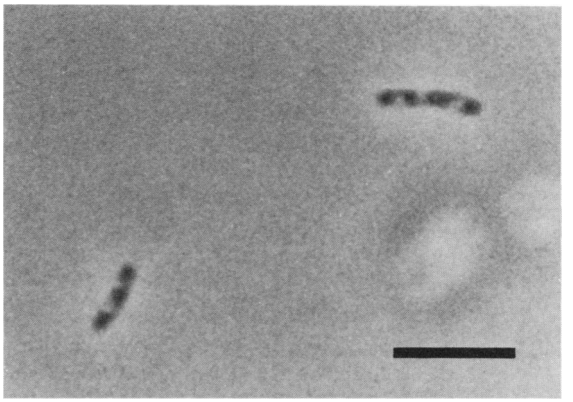

FIG. 5. Photograph of cells with plasmolysis bays. Growing cells of strain W3110 were harvested and treated with 40% sucrose solution as described in the text. Plasmolysis bays were observed as transparent bands. Bar, 5 μm.

mass in midcell (Fig. 4C and D), and the third class of cells had two nucleoids at separate positions, at one-fourth and three-fourths of the cell length (Fig. 4E). The third class of cells was 17% of the total cell population.

In contrast, in a chloramphenicol-treated culture, cells showing two separate nucleoids were only 1% of the total cell population, but about 90% of the cell population showed one nucleoid mass at midcell (Fig. 4F through I). Small cells had small amounts of DNA corresponding to about one copy of the chromosome, and large cells generally had a large amount of DNA which corresponded to about two copies of the chromosome (Fig. 4G). This indicates that replicated chromosomes are always located close to each other, and they were observed as one nucleoid mass at midcell in cells from the chloramphenicol-treated cultures.

To determine the locations of periseptal annuli, we analyzed plasmolysis bays (Fig. 5). In an exponentially growing culture, small cells had plasmolysis bays located at midcell and the cell poles (Fig. 6A and B). Large cells had plasmolysis bays at the one-fourth and three-fourths cell length

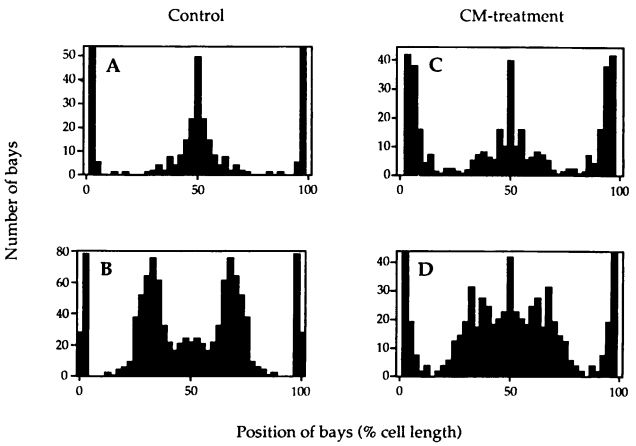

FIG. 6. Location of plasmolysis bays. Strain W3110 cells were treated with chloramphenicol as described in the legend to Fig. 4. (A and B) Before the addition of chloramphenicol. (C and D) After the 1-h chloramphenicol (CM) treatment. (A and C) Small cells with lengths of 1.0 to 2.4 μm. (B and D) Large cells with lengths of 2.5 to 4.0 μm. Distances between the center of a plasmolysis bay and each of the cell poles are shown as a percentage of the total cell length.

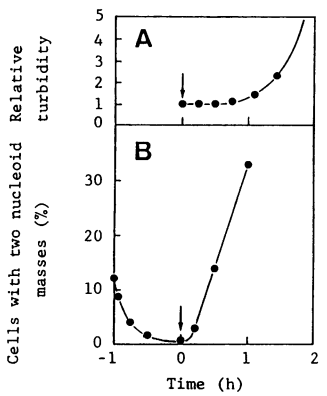

FIG. 7. Positioning of replicated chromosomes to one-fourth and three-fourths of the cell length after the removal of chloramphenicol. Strain W3110 cells were incubated with chloramphenicol for 1 h, as described in the legend to Fig. 1. The chloramphenicol-treated cells were harvested by centrifugation at 5°C and washed with the same volume of ice-cold medium E to remove chloramphenicol. The washed cells were suspended in a prewarmed minimal glucose medium at time zero and incubated at 37°C. Samples were removed and analyzed for nucleoid location.

positions, in addition to midcell and cell poles. These results were consistent with the results described by Cook et al. (4). There were many large cells which had plasmolysis bays located at the one-fourth and three-fourths cell length positions in the chloramphenicol-treated culture (Fig. 6D), indicating that periseptal annuli are located in these separate positions; on the other hand, replicated chromosomes were located at midcell in large cells of the chloramphenicol-treated cultures (Fig. 4I). The proportion of cells with plasmolysis bays at the cell quarters was relatively lower and broader compared with that of cells in control cultures without chloramphenicol (cf. Figs. 6B and D). This can be explained by the division of cells which already had two separated chromosomes at the cell quarters when the antibiotic was added.

Positioning of replicated chromosomes after removal of chloramphenicol from the medium. As described above, when cultures of strain W3110 were treated with chloramphenicol for 1 h, most cells had one compact nucleoid mass in midcell (Fig. 4H and I), and cells with two spatially separated nucleoid masses represented only 0.5% of the total cell population. When chloramphenicol was removed from the medium after a 1-h incubation with the antibiotic, the number of cells with two separated nucleoids increased and represented 14% of the total cell population at 30 min and 32% at 60 min after the removal of chloramphenicol (Fig. 7B). After 30 min, the remaining cells had one compact nucleoid mass in midcell or the irregular form of one nucleoid. After 60 min, the remaining cells had the irregular form of one nucleoid mass which was probably in the process of replication. No detectable increase (less than 5%) of turbidity was observed for the first 30 min (Fig. 7A); however, 14% of the cells already had two separated nucleoids at 30 min. Turbidity increased only 1.2-fold at 60 min (Fig. 7A), when 32% of cells had two separated nucleoids. The number of colony formers was constant for at least 30 min after the removal of chloramphenicol.

Before the removal of chloramphenicol, about 30% of cells had one nucleoid mass which contained two daughter chromosomes (Fig. 4G). In this cell type, the daughter chromosomes presumably moved from midcell to the one-fourth and three-fourths cell length positions during incubation in the absence of antibiotic. Cells which had one copy of chromosome before the removal of chloramphenicol initiated chromosome replication after the removal of chloramphenicol. To confirm the results presented above, we observed living cells in bovine serum albumin solutions as described earlier in this report. The same results were obtained after the removal of chloramphenicol (data not shown).

We measured cell lengths in cultures before and after the removal of chloramphenicol from the medium. The average cell length was 2.6 μm in the cultures that were incubated with chloramphenicol for 1 h. After the removal of chloramphenicol, the average cell length did not increase during the 30-min incubation in the medium lacking chloramphenicol. Before the removal of chloramphenicol, the average cell length of cells with one nucleoid mass corresponding to two copies of chromosomal DNA was 3.3 μm. The average cell length of cells with two separate nucleoids was 3.2 μm in the sample incubated in the absence of the antibiotic. This indicates that daughter chromosomes move from midcell to the positions at one-fourth and three-fourths of the cell length without cell elongation.

We tested a *dap* mutant (CBK110) for chromosome positioning. This mutant requires diaminopimelic acid, which is a substrate for peptidoglycan synthesis. When chloramphenicol was removed from the medium, the chromosome positioning occurred similarly in the presence and absence of diaminopimelic acid (data not shown).

Effects of various antibiotics on the positioning of replicated chromosomes after the removal of chloramphenicol. As described above, replicated chromosomes moved to the one-fourth and three-fourths cell length positions after the removal of chloramphenicol. The effects of the addition of various antibiotics upon removal of chloramphenicol from the medium were observed. Rifampin (100 μg/ml) and streptomycin (200 μg/ml) inhibited chromosome positioning; cells with two separated nucleoids were therefore still 0.5% of the total cell population after 1 h. This suggested that transcription and translation are required for the positioning.

Nalidixic acid (50 μg/ml) and novobiocin (100 μg/ml), which are inhibitors of DNA gyrase, did not inhibit positioning. Ampicillin (50 μg/ml) and furazlocillin (50 μg/ml), which inhibits septum formation, did not inhibit chromosome positioning. Mecillinam (60 μg/ml), which causes the cell to change from a rod to a round shape, also did not inhibit chromosome positioning. In the cases of rifampin and streptomycin, there was no increase in cell mass after 1 h. In the cases of nalidixic acid, novobiocin, ampicillin, furazlocillin, and mecillinam, the cell mass increased by less than 5% after 30 min and 20% after 1 h, similar to the control culture without antibiotics. Cells remained rods after at least 1 h in all cases.

Positioning of daughter chromosomes under inhibition of the initiation of chromosome replication. To determine whether the initiation of chromosome replication is required for the positioning of daughter chromosomes, we used a thermosensitive *dnaC2* mutant strain (SH122) which was auxotrophic for methionine, leucine, isoleucine, valine, and thymine. Cells of this strain were grown at 30°C in minimal glucose medium containing these amino acids and thymine (doubling time, 300 min at 30°C), starved for these four amino acids for one generation at 30°C, and then incubated at 42°C for 30 min to inactivate the DnaC protein. After incubation at 42°C, these amino acids were added to the culture (time zero) and the culture was incubated continu-

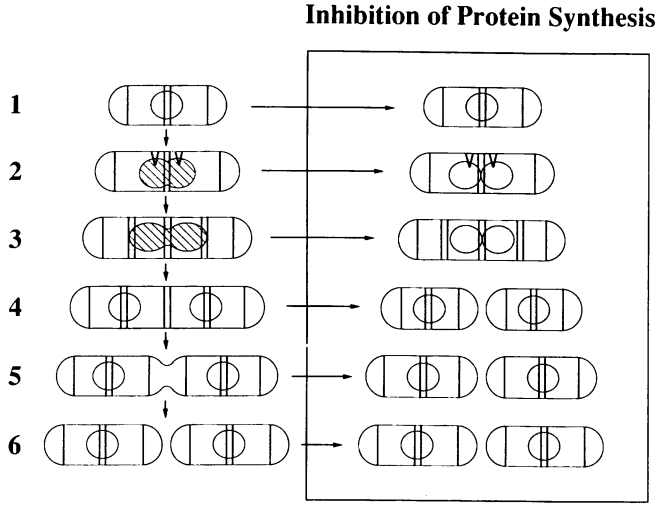

FIG. 8. Schematic representation of nucleoids and plasmolysis bays under the inhibition of protein synthesis. Hatched areas represent replicating nucleoids. Meshed areas represent nucleoids which did not undergo DNA replication. Vertical lines in the cells represent periseptal annuli. The two triangles in stage 2 represent newly generated periseptal annuli. After incubation under the inhibition of protein synthesis, two replicated daughter chromosomes were located close to each other and observed as one nucleoid mass (see text).

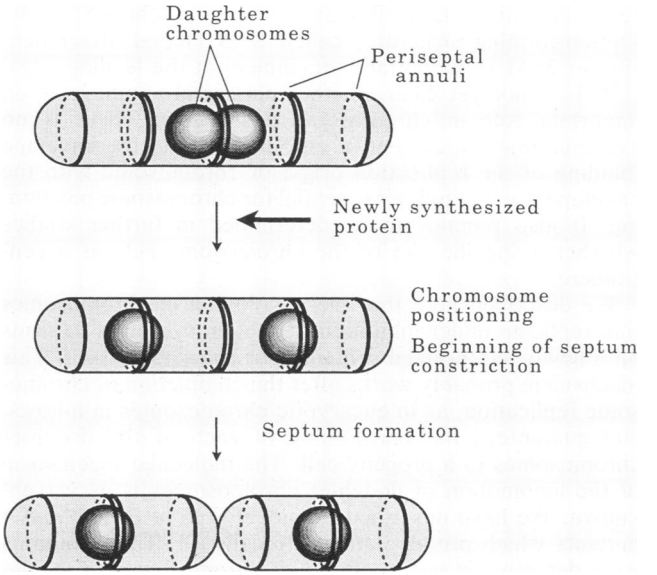

FIG. 9. Model of positioning of daughter chromosomes from midcell to one-fourth and three-fourths of the cell length.

ously at 42°C. The rate of DNA synthesis was analyzed by pulse incorporation of [^{3}H]thymidime into the acid-insoluble fraction, as described above. Under these conditions, the initiation of chromosome replication was completely inhibited. The proportion of cells with two separate nucleoids was increased: 0.3% of the total cell population at time zero, 19% at 15 min, and 18% at 30 min. For the first 15 min, the increase in turbidity was less than 5%. After 30 min, the turbidity increased only 5% over that at time zero. This indicates that the positioning of daughter chromosomes at the cell quarters is independent of the initiation of chromosome replication and cell elongation.

DISCUSSION

The results of this study indicate that replicated chromosomes require a newly synthesized protein(s) or translation itself for the positioning from midcell to one-fourth and three-fourths of the cell length. Under conditions of inhibition of protein synthesis, chromosome replication progresses (11) and terminates (7); however, replicated chromosomes remain in midcell (Fig. 8). The positioning of replicated chromosomes to the one-fourth and three-fourths positions was not coupled with the process of chromosome replication or was independent of the initiation of chromosome replication.

In large cells of chloramphenicol-treated cultures, plasmolysis bays were located at the one-fourth and three-fourths positions and were also located at midcell and the cell poles (Fig. 6D). This shows that the positioning of daughter chromosomes at the cell quarters is independent of the displaying of periseptal annuli at these positions. In the presence of chloramphenicol, periseptal annuli at midcell were allowed to form a division septum only in cells which already had two separated chromosomes located at the cell quarters, respectively (Fig. 8). However, periseptal annuli in other stages of the cell cycle were presumably fixed as they were at the time of antibiotic addition. The generation of new periseptal annuli was probably inhibited by the antibiotic.

When protein synthesis resumed after the removal of chloramphenicol, each daughter chromosome at midcell moved to the one-fourth and three-fourths positions before a detectable increase of cell length was observed. These results suggest that the positioning of daughter chromosomes is independent of cell elongation. Ampicillin and furazlocillin did not inhibit the positioning. Treatments of *E. coli* with ampicillin and furazlocillin result in blunt constrictions (21). Furazlocillin is supposed to be specific for penicillin-binding protein (PBP) 3, which is involved in cell division (2) and which is associated with transglycosylase and transpeptidase activities (12). PBP 3 might not be needed for the initiation of cell division but, rather, for later steps (21). Ampicillin inhibits all PBPs. The antibiotic mecillinam, which interacts specifically with PBP 2 and transforms rods into spheres (26), does not inhibit the positioning.

DNA gyrase is known to function in the decatenation of replicated chromosomes (27) and the folding of chromosomes (32). Nalidixic acid and novobiocin, which inhibit the activity of DNA gyrase (6, 25, 30, 32), do not inhibit chromosome positioning after the removal of chloramphenicol. This result indicates that the activity of DNA gyrase is not necessary for chromosome positioning after the removal of chloramphenicol.

We proposed here a model in which replicated chromosomes moved rapidly from midcell to the one-fourth and three-fourths cell length positions and in which chromosome positioning was not coupled with the process of chromosome replication and was not dependent on the elongation of cell length. The chromosome positioning required a specific, newly synthesized protein(s) as a trigger(s). The new synthesis of protein was also required for the formation of the septum constriction in midcell and the maturation of periseptal annuli to form this septum constriction (Fig. 9).

When protein synthesis resumed after the removal of chloramphenicol, septum formation also resumed; the peri-

septal annuli at midcell may be able to change to the septum-forming structure, called SAS (septal attachment site) or MALE (membrane attachment at the leading edge) (17). It is not yet clear whether periseptal annuli have an important role in chromosome positioning. There is no evidence that attachment of chromosomes to the envelope (binding of the replication origin of chromosome with the envelope, for example) is essential for chromosome positioning. It also remains to be determined in further studies whether a specific site of the chromosome acts as a centromere.

We described here that the positioning of chromosomes that replicate under inhibition of protein synthesis depends on a newly synthesized protein(s) or translation itself. This mechanism probably works after the completion of chromosome replication, as in eucaryotic chromosomes in mitosis, and guarantees the partitioning of each of the daughter chromosomes to a progeny cell. The molecular mechanism of the locomotion of daughter chromosomes is as yet unknown. We have described the novel type of *E. coli mukA* mutants which produce anucleate cells (9). These mutants were defective in positioning their chromosomes to regular intracellular positions and frequently failed to partition the replicated daughter chromosomes into both daughter cells, resulting in the production of one anucleate daughter cell and one cell with two chromosomes. In these mutants, replicated daughter chromosomes were generally located close to each other and the proportion of cells with two spatially separate nucleoids was lower than that of the wild-type strain. These mutants were defective in an outer membrane protein, and the mutations directly or indirectly affected the chromosome positioning. Thus, chromosome positioning may be controlled by particular gene products.

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