JOURNAL OF BACTERIOLOGY, Oct. 1989, p. 5405–5409
0021-9193/89/105405-05$02.00/0

Vol. 171, No. 10

Chromosome Partition in *Escherichia coli* Requires Postreplication Protein Synthesis

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Received 25 May 1989/Accepted 26 June 1989

After inhibition of protein synthesis, the number of nuclear bodies (nucleoids) visible in cells of *Escherichia coli* B/rA corresponded closely to the number of completely replicated chromosomes. We calculated that nucleoid partition follows almost immediately after replication forks reach the chromosome terminus. We show that such a partition is dependent on protein synthesis and that this may reflect the requirement that cells must achieve a certain minimum length before partition (and subsequent cell division) can take place.

A number of genes and proteins are known to be required specifically for cell division in *Escherichia coli* (for reviews, see references 5 and 7), but it is not known what change of state of the growing cell brings these periodically into action. Physiological studies have shown that there are two prerequisites for cell division under normal circumstances. These are the presence of at least two complete genomes and growth of the cell to a particular minimum length (6, 8, 13). It is known that, normally, completion of chromosome replication and the achievement of the required cell length take place at about the same time and are soon followed by the onset of septation (3, 6, 8). Since each sister cell receives a copy of the genome after division, it follows that partition of the sister genomes into appropriate parts of the cell must take place some time between the termination of a round of DNA replication and the completion of septation, but this is a process which is very little understood. We show here that separation of sister genomes into two visible nuclear bodies (nucleoids) takes place almost immediately after termination of each round of replication but that this process has an absolute requirement for a period of protein synthesis. The kinetics of nucleoid partition during postreplication growth are consistent with the idea that a period of protein synthesis is required to bring the cell to a critical length that is required before genome partition can take place.

MATERIALS AND METHODS

Bacterial strains and growth conditions. *E. coli* B/rA and *E. coli* K-12 TKF12 (*ftsA12 thyA leu*) were cultured in L broth (Difco Laboratories, Detroit, Mich.) or Vogel-Bonner salts with a carbon source and other supplements, as required to obtain various growth rates. Strain B/rA was grown at 37°C, and TKF12 was grown at 30°C. Cultures were maintained at low densities (optical density at 540 nm of less than 0.2 and usually less than 0.15) in vigorously aerated cultures. Growth in total cell mass was followed as the increase in the optical density at 540 nm in a 1-cm light path. Cell numbers and median cell volumes were measured on samples that were fixed in 10% Formalin. Cell length measurements were made on photomicrographs. Nucleoids were made visible by staining them with 4,6-diamidino-2-phenylindole (DAPI) and were observed by using a mixture of phase-contrast and fluorescence microscopy by the procedure of Hiraga, as described elsewhere (4). Color photographs were used for all measurements of nucleoid position and number (although only black and white photographs are presented with this report).

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RESULTS

The number of nucleoids per cell is proportional to the number of complete genomes. In *E. coli* initiation of rounds of chromosome replication takes place whenever the ratio of cell mass (or volume) to the number of copies of the chromosome origin of replication (*oriC*) reaches a certain critical value (initiation mass [2]). Replication then takes place at a more or less constant rate at a diverging pair of replication forks which meet at the terminus (*terC*) of the circular chromosome some 41 min later (in strain B/rA at 37°C [1]). In cells that are growing in medium in which a doubling in mass takes less than 41 min, new rounds of replication are initiated before previous ones have been completed, so as to produce chromosomes with multiple replication forks. In growth medium in which it requires more than 41 min to double the cell mass, there are gaps between successive periods of DNA replication. Cell division is normally completed some 22 min after the completion of each round of chromosome replication (in strain B/rA at 37°C [1]). As a consequence of this regulatory system, the average number of completely replicated chromosomes per cell (i.e., the average number of copies of *terC* per cell) is a function of the growth rate, such that (*terC*/cell) = $2^{(22/T)}$, where T is the mass doubling time of the culture. For example, the average number of complete genomes per cell will be 2 for cells with a generation time of 22 min and 1.26 for cells with a doubling time of 66 min.

Nucleoids in actively growing cells often take up complicated shapes which make it difficult to accurately estimate their number and location within the cell (Fig. 1a). However, if protein synthesis is inhibited, the DNA rapidly condenses into regularly shaped discrete nucleoids and nucleoid numbers may be easily determined, either in live cells by phase-contrast microscopy in a medium with a suitable refractive index (3) or in fixed cells stained with a specific DNA stain, such as DAPI (4) (Fig. 1b) or Hoechst 33258 (9). Figure 2 shows the average number of nucleoids per cell at different times after the inhibition of protein synthesis (by chloramphenicol at 200 μg/ml) in a culture of *E. coli* B/rA. This number fell continuously over approximately the first 20 min to reach a final value of about 1.18. This fall can be accounted for by the continuation of cell division during this

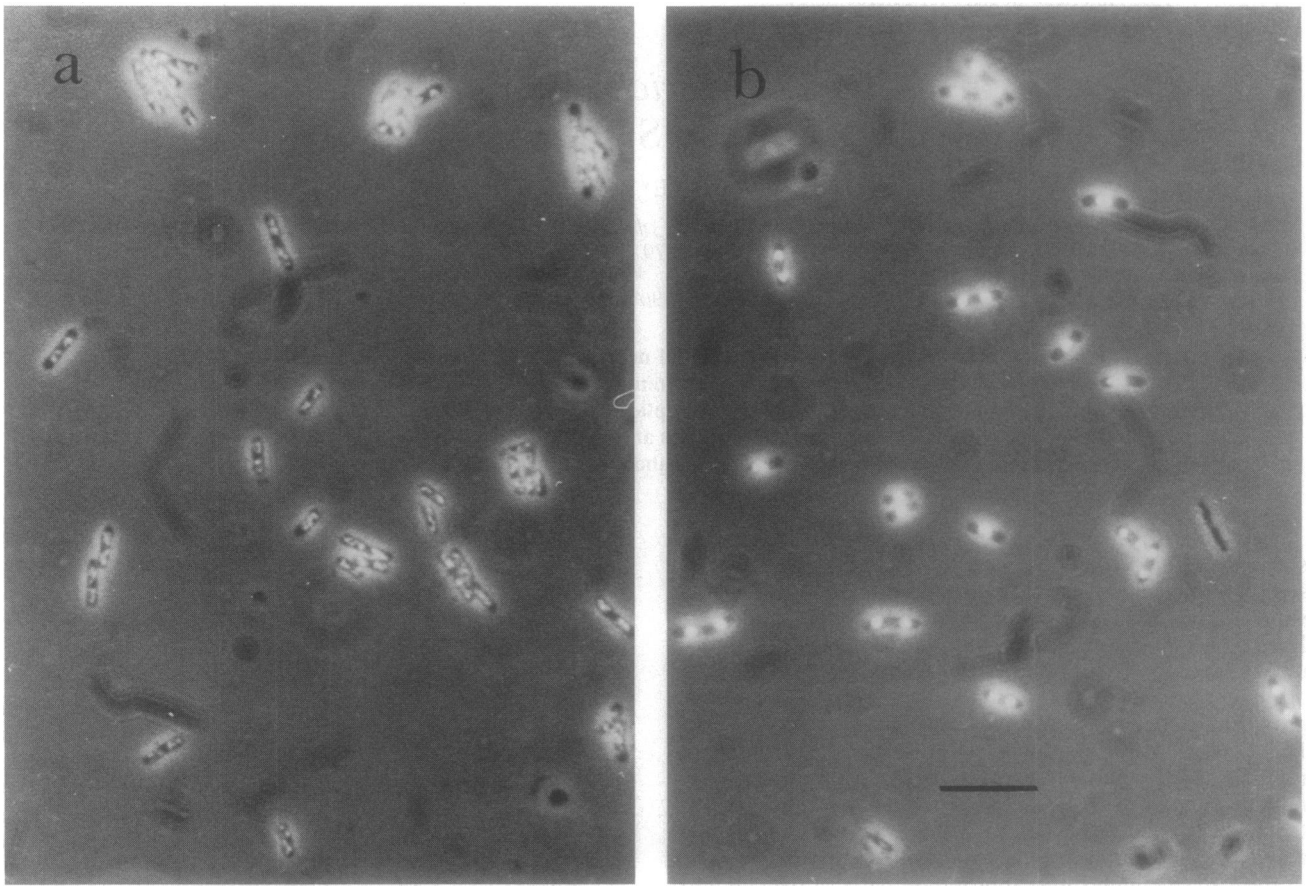

FIG. 1. DAPI-stained nucleoids in *E. coli* B/rA. (a) Actively growing cells. (b) Cells after 10 min in the presence of chloramphenicol (200 μg/ml). Bar, 10 μm.

period (10, 13). Furthermore, this observation shows that it is cells which already have two nucleoids which are able to divide without further protein synthesis. A small fraction of these binucleate cells failed to divide in the presence of chloramphenicol. This may be because a further short period of postpartition protein synthesis is required before cells become able to divide. In addition, estimation of the average number of nucleoids per cell and the total number of cells per milliliter in the culture at the beginning and end of the experiment showed that the total number of nucleoids per milliliter remains constant after inhibition of protein synthesis. This was surprising, because chromosome replication continues (although initiation of new rounds of replication does not take place) after inhibition of protein synthesis (11, 12, 15). The average number of completed chromosomes per nucleoid therefore increased during the initial 40 to 60 min after the addition of chloramphenicol.

The continuation of cell division during the period required for nucleoid condensation after the addition of chloramphenicol thus made it difficult to make an accurate estimate of the average number of nucleoids per cell in the growing culture. To avoid this problem cell division must be inhibited during the period of condensation. We were able to do this when we discovered that the simultaneous addition of chloramphenicol and a shift of temperature from 37 to 42°C immediately blocked cell division. Specific inhibitors of cell division, such as furazlocillin, were found to be less useful because they allowed some residual division. Figure 3 shows the distribution of nucleoid numbers in cultures of B/rA that were obtained after the simultaneous addition of chloramphenicol and a shift of temperature from cultures which were in balanced growth in different media. The number of nucleoids per cell also remained constant for at least 90 min after this treatment (data not shown). Figure 3 also shows the predicted proportions of cells with 1 and 2 copies of *terC* calculated for these growth rates (as described above). In each case the observed proportion of binucleate to uninucleate cells was almost identical to the calculated ratios of cells with 2 copies of *terC* to those with 1 copy. Thus, we showed that the number of visible nuclear bodies in cells growing at different rates is close to or the same as the calculated number of chromosome termini (i.e., the number of completed chromosomes).

Partition of sister chromosomes requires postreplication protein synthesis. The experiments described above suggest that chromosome replication is not, by itself, sufficient to allow the partition of sister genomes into spatially separated nucleoids and that inhibition of protein synthesis prevents the physical separation of sister chromosomes. In order to test this idea further, a population of cells was blocked in protein synthesis for a period of time sufficient to allow the completion of all ongoing rounds of chromosome replication, and then the cells were allowed to resume protein synthesis under conditions which prevented further DNA synthesis. In

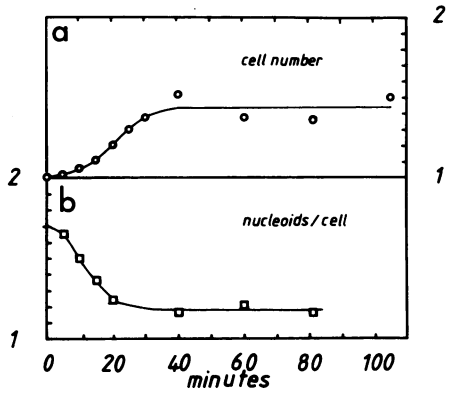

FIG. 2. Increase in cell number and decrease in average number of nucleoids per cell following inhibition of protein synthesis by the addition of chloramphenicol (200 μg/ml) at 0 min to a population of strain B/rA in balanced growth (doubling time, 25 min) in nutrient broth (Oxoid Ltd., London, England) at 37°C. (a) Increase in cell number per milliliter relative to the number of cells (4.05×10^7/ml) at the time of chloramphenicol addition. (b) Decrease in the mean number of nucleoids per cell in DAPI-stained samples. The first sample in which nucleoids were condensed and were able to be scored was 5 min after chloramphenicol addition. The extrapolated value for 0 min (1.7) was calculated from the relative increase in cell number.

this way it was hoped to see that postreplicative protein synthesis permitted partition of nucleoids which consisted of 2 (or more) chromosomes. In order to make the scoring of nucleoid numbers and locations easy, cell division was blocked during the whole experiment (Fig. 4). Protein synthesis in a *leu* mutant auxotroph was stopped by removing leucine from the synthetic medium and was restarted by the readdition of leucine 60 min later. At that time thymine was removed from the medium to prevent further DNA synthesis (because the strain was also a *thyA* mutant). The cells were also temperature sensitive for cell division (because they carried the *ftsA12* allele), and cell division was blocked throughout by keeping the culture at 42°C from the time of leucine removal. Figure 4 shows that cell growth and division stopped immediately after the removal of leucine and the shift to 42°C and that cell growth (but not cell division) resumed after the readdition of leucine and the removal of thymine. Figure 4 also shows that the average number of nucleoids per cell remained almost constant during the period when protein synthesis was inhibited but increased, in parallel with cell growth, when protein synthesis was resumed, even though DNA replication was then blocked. The average number of nucleoids per cell approximately doubled during the period in which the average cell size (length, volume, and mass) doubled. Growth continued thereafter without a further increase in nucleoid numbers. Measurement of total DNA in this strain under these condi-

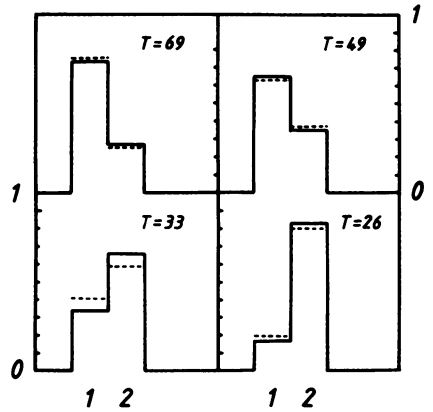

FIG. 3. Fraction of cells with 1 or 2 nucleoids per cell (solid line) in cultures of strain B/rA growing at different doubling times (T) at 37°C. Also shown is the calculated fraction of cells with 1 or 2 copies of the chromosome terminus, i.e., complete chromosomes (dashed line). The fraction of cells with 1 terminus (f_1) was taken as $f_1 = 2 - 2^{(22/T)}$.

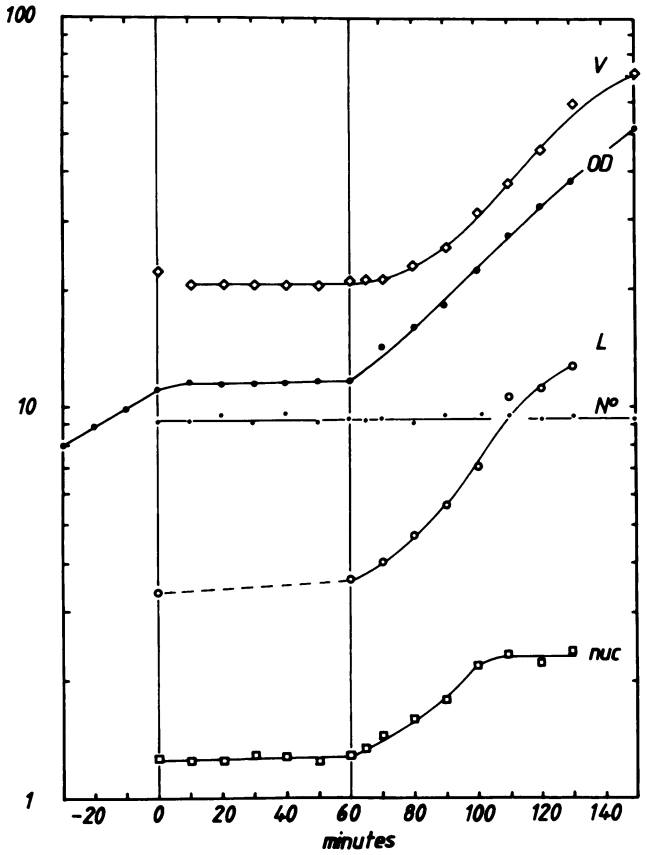

FIG. 4. Nucleoid partition and cell growth. Cells of strain TKF12 [*ftsA12*(Ts) *thyA leu*] were grown at 30°C in superminimal medium (6). At 0 min the culture was harvested, washed, suspended in prewarmed medium without leucine (to block protein synthesis), and shifted to 42°C (to block cell division). A total of 60 min was allowed for the completion of all rounds of chromosome replication (without protein synthesis), after which the cells were again washed and suspended in prewarmed medium with leucine (to allow resumption of protein synthesis) but without thymine (to prevent further DNA synthesis), and growth was continued at 42°C. The values of the measurements were corrected for dilution at the washing steps. Samples were taken at intervals to measure the optical density (OD) (●), median cell volume (V) (◇), cell number per milliliter (•), mean cell length (○), and average number of nucleoids per cell (□). One unit on the ordinate (log scale) corresponds to optical densities at 30°C of 0.0115, at 42°C without leucine of 0.0083, and at 42°C without thymine of 0.0042; to cell numbers per milliliter at 42°C without leucine of 5.85×10^6 and at 42°C without thymine of 3.04×10^6; to a mean cell length of 1 μm; to a mean number of nucleoids per cell of 1; and to a median cell volume in arbitrary units.

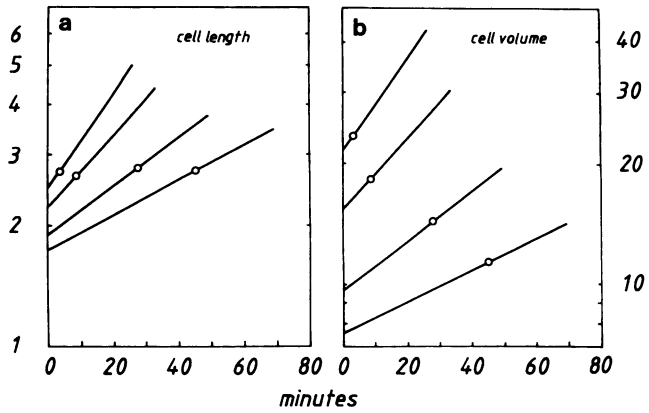

FIG. 5. Average time of nucleoid partition (○) shown as a function of cell length (a) and cell volume (b) for cells growing at different rates. Growth was assumed to be exponential over each cell cycle for both mass and volume. As a first approximation, cell length or volume at the beginning of each cycle was assumed to be the mean value multiplied by ln 2 (14). The doubling times, median cell volumes, and mean cell lengths were taken from the B/rA cultures described in the text (except for the cell length for the culture with the 33-min generation time, for which no measurement was available and for which the length was calculated from the growth rate ([3, 6, 7]). The average time of nucleoid partition was calculated from the observed frequencies of cells with 1 or 2 nucleoids in each case. Cell lengths are given in micrometers, and cell volumes are given in the arbitrary units of the Channelyser (Coulter Electronics, Inc., Hialeah, Fla.).

tions showed that DNA synthesis went on as expected. Total DNA increased by about 44% during the first 30 to 40 min after removal of the required amino acids (at 42°C), which is consistent with the fact that all ongoing rounds of replication were completed. No further DNA synthesis took place thereafter, even when amino acids were restored and thymine was removed.

The doubling in nucleoid number, in parallel with a doubling in cell size but in the absence of any further DNA replication, suggests that nucleoid separation took place as different cells successively reached some particular critical size. Because of the pretreatment, the population contained the complete range of cell sizes found in the original asynchronous, exponentially growing population, but each of these cells had at least 2 complete chromosomes per nucleoid. It is not possible, however, to determine from these data whether nucleoid partition took place as cells reached a particular size or whether this segregation took place at random times after the resumption of protein synthesis, independently of cell size. If the latter is the correct interpretation, then we must suppose that the probability of nucleoid partition is such that essentially every cell carried this out by the time the average cell size doubled in the whole population. What is indisputable is that nucleoid partition is absolutely dependent on postreplication protein synthesis.

DISCUSSION

The number of physically separate nucleoids in cells increased from 1 to 2 almost immediately after the time of completion of chromosome replication, but only if postreplication protein synthesis was allowed. What is the nature of this required period of protein synthesis? The observation that the average length of cells with 1 nucleoid was less than that for cells with 2 nucleoids is in accordance with the knowledge that initiation of chromosomal DNA replication takes place at particular cell sizes and shows that, unsurprisingly, termination of rounds of replication and the subsequent separation of sister nucleoids are also correlated with cell size. The actual cell length at which partition takes place, on average, can easily be calculated from the observed proportion of uninucleoid cells at different growth rates. By using the data in Fig. 3 and the average cell lengths in these cultures, the average length at which partition occurred at the four different growth rates was found to be very similar, although the cell mass or volume at these times was very different (Fig. 5). In a separate study (4), we have shown that mutant cells with an altered cell shape (coccal forms) behave as if cell division and nucleoid partition both depend on the attainment of a fixed cell length, which is also the case for normal rod-shaped cells. Together with the results reported here, this reinforces our belief that it is the achievement of a minimum cell length which is required for physical separation is sister chromosomes. However, the evidence for this remains circumstantial, and the only conclusion which is beyond dispute is that postreplication protein synthesis is required for nucleoid partition.

ACKNOWLEDGMENTS

Some of this work was carried out (at least in preliminary form) during working visits by W. D. Donachie to L'Institut Jacques Monod, Paris, France. We are grateful to the European Molecular Biology Organisation for the short-term fellowships which made this possible. We are especially grateful for the warm hospitality, encouragement, and critical discussion given by Dick D'Ari, Aline Jaffé, and other members of the laboratory in Paris.

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