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Experiments on Chromosome Separation and Positioning in *Escherichia coli*

Kenneth J. Begg and William D. Donachie

The way in which sister genomes are spatially separated after replication and positioned in sister cells after division remains unknown for prokaryotes. Experiments with *Escherichia coli* suggest that individual "chromosomes" (folded, covalently closed circular DNA molecules) are fixed in position within growing cells both before and during replication, but that they are rapidly moved apart by a fixed distance (unit length) immediately after replication has been completed. Such a mitosis-like mechanism accounts for the aberrant positions of DNA and septa in cells in which the normal coordination between DNA replication and cell elongation has been perturbed.

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Prokaryotic cells, such as *Escherichia coli,* have a single "chromosome" in the form of a covalently closed circular DNA molecule. The circumference of this molecule is about 1.4 mm, or about 1,000 times the length of the bacterial cell, and the chromosome is folded as an intracellular mass of DNA known as the "nucleoid." The nucleoid is not bounded by a separate membrane, and the way in which the DNA is maintained in this folded form during replication and transcription is not known. The nucleoid can take up a variety of shapes in growing cells but contracts into a more-or-less spherical form if protein synthesis is inhibited. A cell may contain more than one copy of the chromosome, but each chromosome normally forms a separate nucleoid (Donachie and Begg, 1989a,b). The mechanism by which sister chromosomes are separated from one another after replication, and located in the correct positions to be partitioned into sister cells after cell division, in the absence of any observable mitotic apparatus, is a long-standing mystery. That it has remained so for so long is partly due to the lack of mutants that are altered in partition, and partly to the small size of bacterial cells and the difficulty of deciding the exact position and number of nuclear bodies in individual cells.

Until recently, the only mutants known in which chromosome partition is affected are some with mutations in genes for enzymes of DNA replication (Norris et al., 1986; Kato et al., 1988), such as DNA gyrase (or topoisomerase II) (Orr et al., 1979; Hussain et al., 1987a,b; Kato et al., 1989), or topoisomerase IV (Kato et al., 1990). DNA gyrase is thought to be necessary for decatenation of replicated circular DNA molecules (Steck and Drlica, 1984), which would account for the inability of gyrase-deficient mutant cells to separate their sister chromosomes after replication. More recently, new classes of mutants have been reported (Hiraga, 1990; Hiraga et al., 1990) in which nucleoid separation can take place but in which their positioning within the cell appears to be disturbed. These mutants may provide a way to study the molecular basis of chromosome-membrane attachment and partition.

Another recent advance has been the development of a simple procedure to condense the cell's DNA into discrete bodies and then to measure the number and position of these by means of fluorescence and phase microscopy (Hiraga et al., 1989). By this means it has been shown that each visible condensed nuclear body consists of a single chromosome (Donachie and Begg, 1989a,b) (which is usually in the process of replication) and that partition into two bodies follows immediately after completion of replication and is dependent on a short period of de novo protein synthesis (Donachie and Begg, 1989b; Hiraga et al., 1990). In studies of mutant cells with altered morphologies, we have shown that partition into pairs of nuclear bodies depends also on the linear dimensions of the cell, as if a minimum separation distance is required for the movement apart of nuclear bodies (Donachie and Begg, 1989a).

Here we report on the way in which nuclear bodies are located in cells that are growing without DNA replication, and also on the way in which they reassort themselves within the cell following a resumption of DNA synthesis. Our measurements seem to limit the

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number of possible mechanisms for chromosome partition. The smallest number of assumptions needed to account for our data are (i) that incompletely replicated chromosomes maintain their relative positions within the growing cell, e.g., by attachment to the cell envelope, (ii) that they are free to move after completion of replication, perhaps by detachment from the envelope; and (iii) that the sister chromosomes are then rapidly moved apart, by an approximately constant distance, before becoming once more fixed in their new positions.

This model is in accord with earlier work that was interpreted to mean that chromosomes are attached to cell membranes during replication but that they detach after replication (Worcel and Burgi, 1974; Jones and Donachie, 1974). De novo protein synthesis also appears to be required both for detachment of the replicated chromosome from the membrane (Jones and Donachie, 1974), and for nucleoid partition after completion of chromosome replication (Donachie and Begg, 1989b; Hiraga et al., 1990).

RESULTS AND DISCUSSION

Maintenance of Nucleoid Position in Growing Uninucleate Cells

Normal cells of *E. coli* have either one or two nucleoids, depending on the number of completely replicated chromosomes (Donachie and Begg, 1989a,b). In uninucleate cells the nucleoid is usually more-or-less in the cell center, whereas in binucleate cells the two nucleoids are most often well separated (Hiraga et al., 1989; Donachie and Begg, 1989a,b; Hiraga et al., 1990). However, even after condensation (by inhibition of protein synthesis; see Donachie and Begg, 1989a,b) the nucleoids are large bodies whose diameter is a significant proportion of the cell length. Consequently, it is hard to be precise about their location in the cell, or to decide whether they are randomly located or attached to the cell at some point. To make this easier to measure, we allowed cells to double in length in the absence of DNA synthesis and then measured nucleoid position in the resulting long cells. Figure 1 shows cells of a *dnaE*486 *thyA* mutant that were grown for 110 min at 42°C without thymine. Almost all cells were then uninucleate (the binucleate cells in the initial population having divided during the initial period at 42°C without thymine) and averaged about 20 μm in length. The measurements of relative nuclear positions (Fig. 2) confirmed the impression that nucleoids remain close to the cell center during cell elongation without DNA synthesis. (Note that cell division was also blocked in these cells because of the induction of the SOS-dependent division inhibitor protein, SfiA, as a consequence of the inhibition of DNA synthesis [Huisman and D'Ari, 1981].)

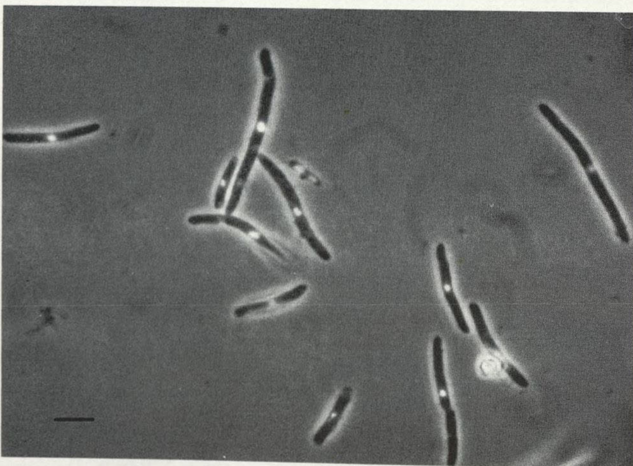

Figure 1. Nucleoids (bright bodies) in filamentous cells of the *dnaE*486 (ts) *thyA* mutant of *E. coli*.

The cells were grown for 110 min at 42°C in the absence of thymine. DNA was stained with DAPI and the cells were photographed as described (see Materials and Methods). Scale bar, 5 μm.

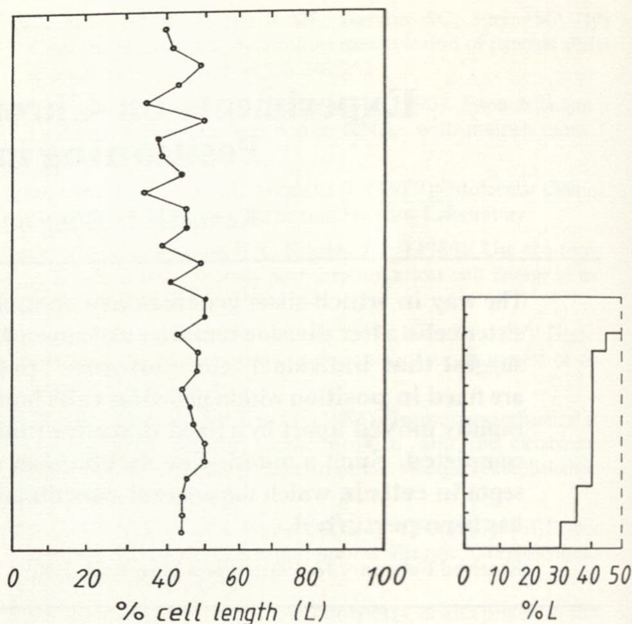

Figure 2. Nucleoid position in uninucleate *dnaE*486 (ts) *thyA* cells after 110 min of growth at 42°C without thymine.

Nucleoid centers were measured from the nearer cell pole and the results were plotted as a percentage of cell length. Data for individual cells are shown in the left panel and the cumulative histogram is shown in the right panel.

Maintenance of Nucleoid Position in Growing Cells With More Than One Nucleoid

Probably because of a reduced rate of DNA replication in the *dnaE* mutant, few of the cells in the

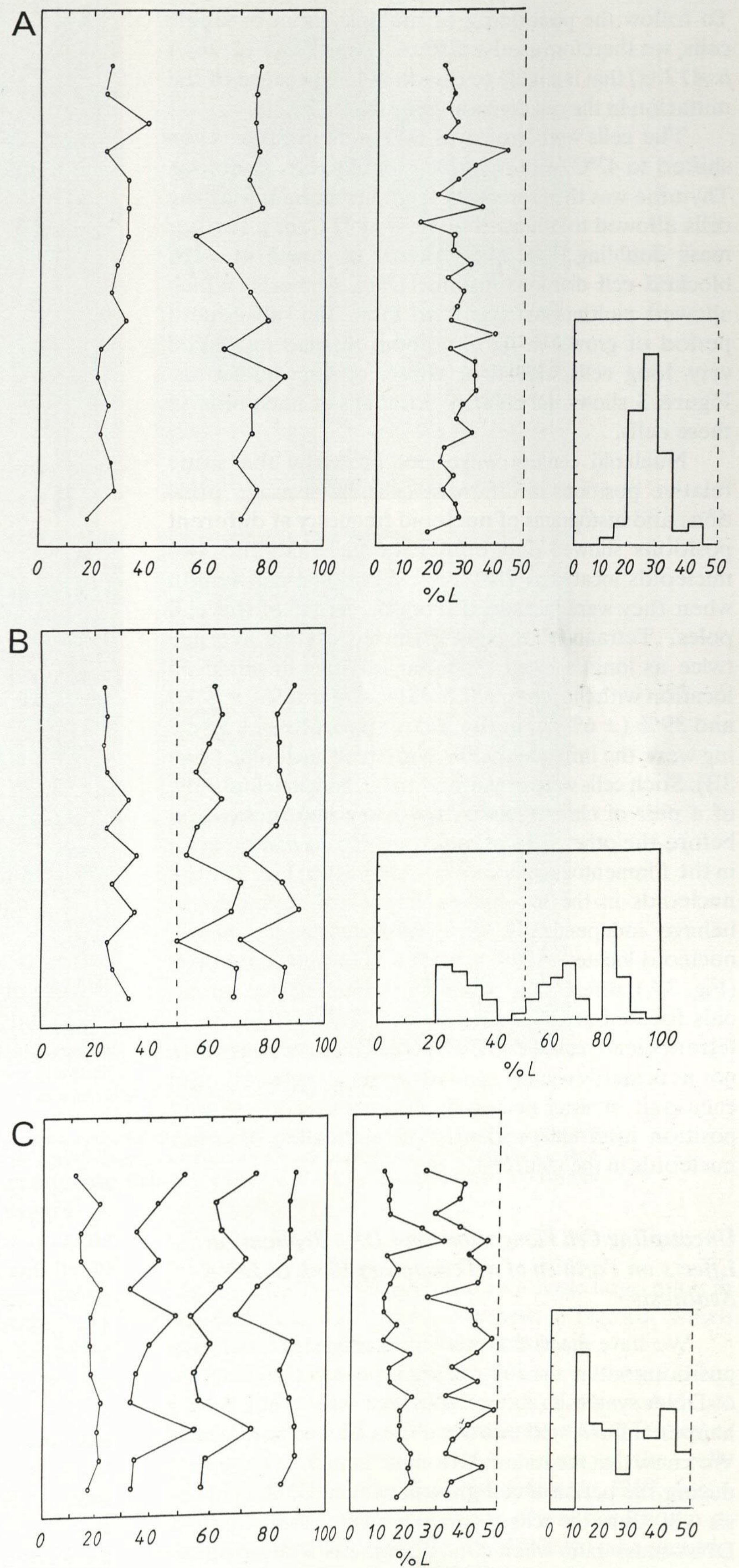

Figure 3. Nucleoid positions in cells with two, three, or four nucleoids after growth for one mass doubling in the absence of DNA synthesis.

Cells of TKF12 *ftsA*12 (ts) were grown for one mass doubling at 42°C with thymine (to increase the average number of nucleoids per cell) and then for one more mass doubling at 42°C in the absence of thymine (to allow cell elongation without increase in nucleoid number). Positions of nucleoid centers are plotted as in Fig. 2. (**A**) Cells with two nucleoids. (**B**) Cells with three nucleoids. (**C**) Cells with four nucleoids. In A and C, the central panel shows the sum of the two cell halves. Histograms are on the right.

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first strain were binucleate, even when grown at 30°C. To follow the positioning of multiple nuclei in single cells, we therefore used a different strain (TKF12: *thyA ftsA*12.ts) that is unable to divide at 42°C because of the mutation in the cell division gene *ftsA*.

The cells were grown at 30°C with thymine, then shifted to 42°C (with thymine) for one mass doubling. Thymine was then removed from the medium and the cells allowed to continue to grow at 42°C for a further mass doubling. The initial period of growth at 42°C blocked cell division but not DNA synthesis, which allowed multinucleate cells to form. The subsequent period of growth at 42°C without thymine produced very long cells with two, three, or four nucleoids. Figure 3 shows the relative locations of nucleoids in these cells.

Nucleoid centers were not in exactly the same relative positions in different cells, but average positions and histograms of nucleoid frequency at different positions showed that binucleate cells had the two nucleoids located at 28% (± 6%) of total cell length when they were measured from the nearer of the cell poles. Tetranucleate cells (which were on average twice as long) showed a similar variation in nucleoid location with the four nucleoids located at 17% (± 5%) and 39% (± 6%) from the nearer pole. Most interesting were the infrequent cells with three nucleoids (Fig. 3B). Such cells were presumed to be those in which one of a pair of chromosomes had completed replication before the other. This asynchrony in pairs can be seen in the filamentous cells shown in Fig. 4B. In Fig. 3B, the nucleoids in the two halves of the cell appeared to behave independently. One half contained a single nucleoid located at 29% (± 5%), as in binucleate cells (Fig. 3A), whereas the other half contained two nucleoids located at 18% (± 6%) and 38% (± 7%), as in tetranucleate cells (Fig. 3C). Nucleoids are therefore not necessarily equally spaced within a single cell, and each pair of sister nucleoids appears to separate and position itself independently of the location of other nucleoids in the same cell.

Uncoupling Cell Elongation and DNA Replication: Effects on Partition of a Temporary Block in DNA Synthesis

We have shown that nucleoids retain their relative positions within the cell during growth in the absence of DNA synthesis (above). We next asked what would happen if DNA synthesis were then allowed to resume. We know that the accumulation of "initiation potential" during the period of cell growth without DNA synthesis will allow the cells to quickly restore their normal DNA/mass ratio when DNA synthesis is allowed to restart (Donachie, 1968; Donachie et al., 1968; Donachie, 1969). Would these new copies of the chromosome be relocated at normal positions within the cells?

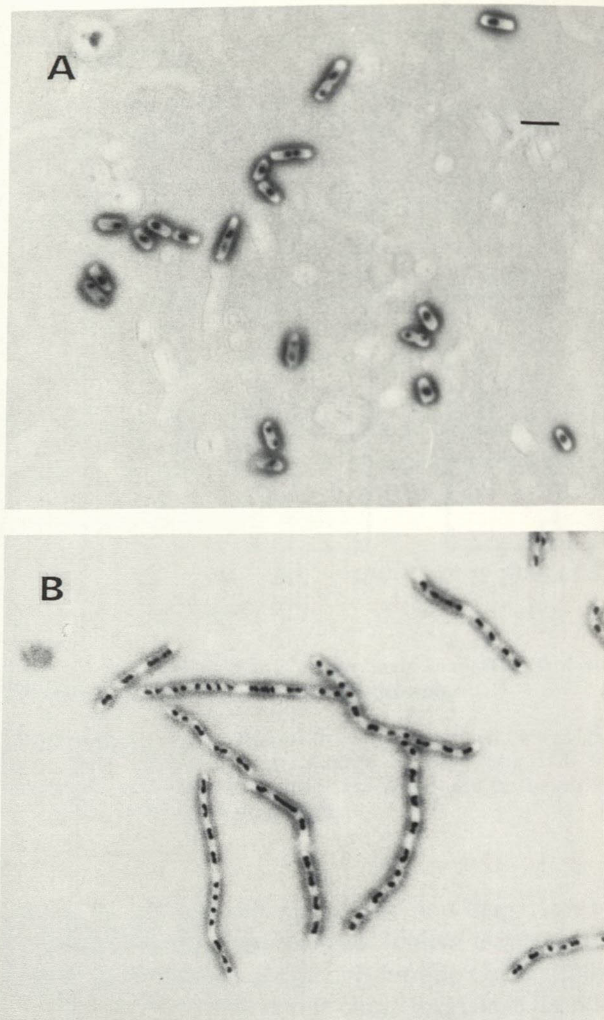

Figure 4. Effect of blocking cell division without interrupting DNA synthesis.

Nucleoids in cells of (A) AB2497 (*thyA*) growing at 37°C in supplemented minimal medium (see Materials and Methods) (+ thymine) and (B) TOE13 (*ftsA*13 (ts) *thyA*) growing at 42°C in the same medium (+ thymine). In this reverse contrast print, DAPI-stained nucleoids are dark and cell bodies are light. Scale bar, 5 μm.

Figure 4 shows that nucleoids are partitioned normally in long cells that have formed during a block to cell division without interruption of DNA synthesis. (In this example, cell division was blocked by a shift to 42°C in the *ftsA*12 mutant.) Figure 5 shows that, in complete contrast, chromosome partition does not take place in cells that have previously undergone a temporary period of growth without DNA synthesis, even though the normal DNA/mass content of the cells has been restored. In this experiment, cells of a *thyA* strain (AB2497, the parent strain of the *ftsA* mutant used to produce the cells in Fig. 4) were grown for one mass doubling time without thymine before thymine was restored and DNA synthesis restarted. As shown

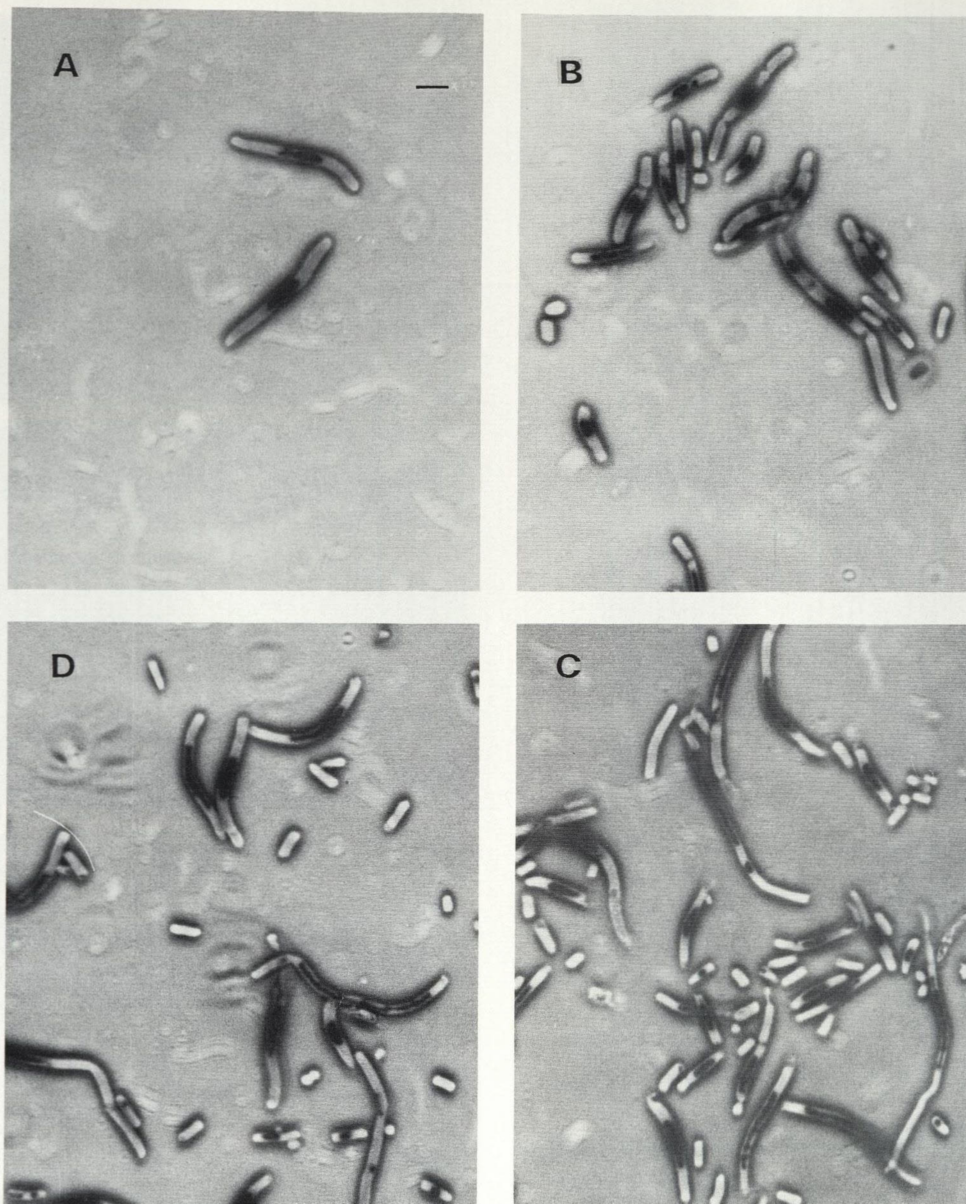

Figure 5. Nucleoids in cells recovering from a period of growth without DNA replication.

Cells of AB2497 (*thyA*) growing in supplemented minimal medium containing thymine at 37°C (see Fig. 4a) were washed and resuspended in fresh medium without thymine and grown for one mass doubling. Thymine was then added and the cells were allowed to continue growing. Cells are shown at 45 (**A**), 60 (**B**), 90 (**C**) and 135 (**D**) min after the readdition of thymine. Scale bar, 5 μm.

in Fig. 6, cell division stopped soon after thymine removal, leaving a population of mostly uninucleate cells (Donachie and Begg, 1989b; Hiraga et al., 1990) that were prevented from further division by the induction of the SOS-dependent division inhibitor SfiA (see Huisman and D'Ari, 1981). Division remained blocked for an initial period after readdition of thymine (at 100 min in Fig. 6) until the DNA/mass ratio was restored (Donachie et al., 1968; Donachie, 1969) and the SfiA protein degraded (Huisman and D'Ari 1981). Figure 5A shows cells at this time (145 min in Fig. 6). All the DNA is found as a single large mass in the cell center. (Contrast the cells in Fig. 4B, which have similar lengths and DNA contents.)

As previously reported, cell division resumes about 40 to 50 min after restoration of thymine, when all blocked rounds of DNA replication have been completed and the normal DNA content of the cells restored (Donachie et al., 1968; Donachie, 1969; Jones and Donachie, 1973). However, it was not known at that time that DNA partition had failed to take place in such cells and it was therefore not known that, as

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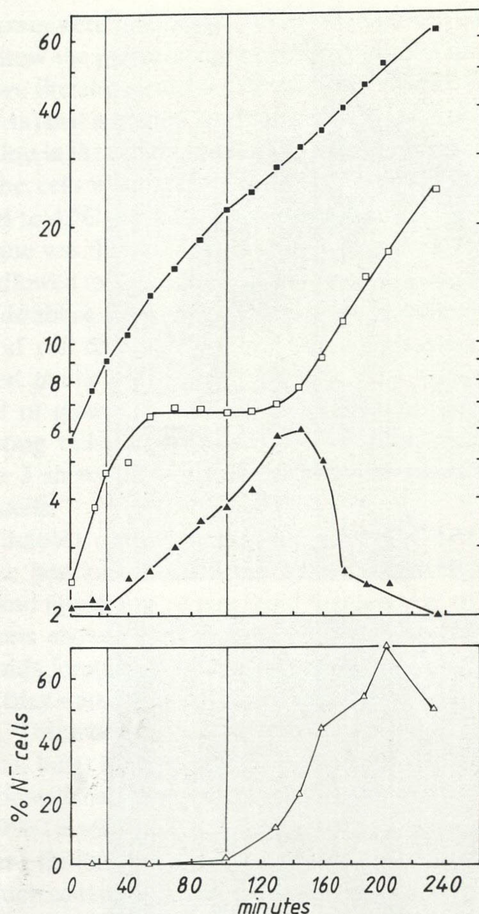

Figure 6. Kinetics of cell growth and division in the experiment described in Fig. 5.

■, OD; □, cells/ml; ▲, median cell volume. (The units are arbitrary.) The bottom panel shows frequency of anucleate (N^-) cells.

shown in Fig. 5, the first wave of cell division produces mainly anucleate daughter cells. This is because septa form almost exclusively in DNA-free areas of the cell, as previously reported in mutant cells in which chromosome separation had failed to take place normally (Donachie, 1979; Fairweather et al., 1980; Donachie, 1981; Donachie et al., 1984; Woldringh et al., 1985; Hussain et al., 1987a). Figure 6 shows that the proportion of anucleate (N^-) cells reached as much as 70% of total cells at 100 min after restoration of DNA replication.

As shown in Fig. 5, C and D, the central mass of DNA continued to enlarge as DNA synthesis continued during this period of resumed cell division, but nucleated (N^+) daughter cells began to be produced in increasing numbers as individual nucleoids separated from the ends of the main nuclear body and septa formed between them to produce nucleated progeny cells. This explains why it is that a transient period of inhibition of DNA synthesis is not a lethal event for a population of cells (Donachie and Hobbs, 1967). Partition in the progeny of these normally sized nucleated cells is presumably perfectly normal thereafter.

Repartition of Nucleoids Following a Period of Growth Without DNA Synthesis

In the preceding experiment, the formation of N^- cells from the ends of the recovering long cells quickly reduced the length of the "mother" nucleated cells, while the subsequent separation of "wandering" individual nucleoids into new N^+ progeny cells resulted in the culture soon being overgrown by normal cells. To see whether repartition of chromosomes could eventually take place, given sufficient time, we therefore carried out a similar experiment using an *ftsA*12 *thyA* strain (TKF12) in which cell division was blocked throughout the experiment. As shown in Fig. 7, cells were transferred to medium without thymine at 42°C for 1 h, after which thymine was restored and the cells were allowed to continue growing at 42°C. No division took place over this time and the average cell volume (and length) eventually reached 14 times that of the original cells that had been growing with thymine at 30°C. During this period DNA synthesis continued and individual nucleoids gradually spread through the growing, elongating cells until, by about 140 min after resumption of DNA synthesis, nucleoids were distributed irregularly throughout the filamentous cells (Fig. 7). The process of repartition appeared to be by gradual dispersal of individual masses of DNA from an area of high concentration at the cell center out to the cell poles (Fig. 7). As a result, regular spacing of nucleoids in the filaments was not achieved, in contrast to filaments in which DNA replication took place throughout cell elongation (Fig. 4).

Reduction in the Rate of DNA Synthesis Relative to the Rate of Cell Elongation Also Prevents Normal Chromosome Positioning

When the *dnaE*486 *thyA* mutant was grown at 42°C in the presence of thymine, DNA synthesis was not completely inhibited but continued at about one-half of the rate of cell mass increase (Fig. 8). The *dnaE* gene codes for the α subunit of DNA polymerase III (Bachmann, 1983), and the *dnaE*486 mutation appears to code for a mutant subunit that retains some activity at 42°C (although not enough to allow colony formation on plates at 42°C, for reasons which will become obvious). As shown in Fig. 8, cell growth continued for a long time at 42°C, while DNA synthesis and cell division continued at about half that rate. As shown in Fig. 9, the nuclear bodies enlarged in these cells but failed to partition into spatially separated nucleoids, just as in the case of cells that were rapidly synthesizing DNA after a transient complete block to DNA replication. Once again, cell division occurred exclusively in

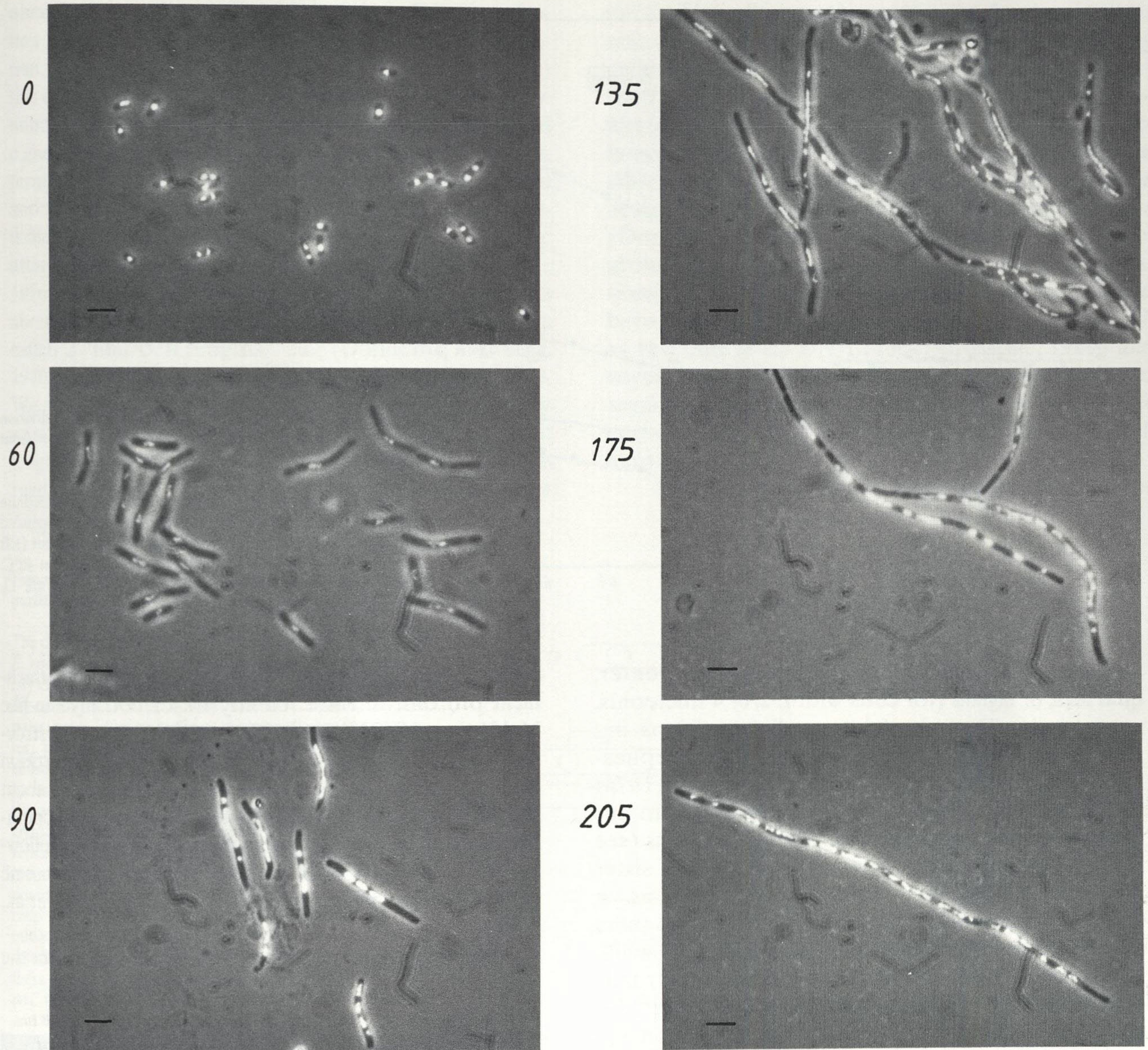

Figure 7. Recovery of nucleoid content and distribution after transient inhibition of DNA synthesis.

Cells of TKF12 [*ftsA*12 (ts) *thyA*] were grown in supplemented minimal medium (+ thymine) at 30°C. At 0 min the cells were washed and resuspended in fresh medium without thymine and growth was continued at 42°C. At 60 min, thymine was restored and growth was continued at 42°C. DAPI-stained cells are shown at 0, 60, 90, 135, 175, and 205 min.

the DNA-free ends of the growing cells to produce N^- daughter cells. The inability of *dnaE*486 cells to form viable colonies on agar at 42°C is therefore explained.

The partition mechanism for replicated genomes therefore fails, not only when DNA synthesis has been completely blocked and then allowed to resume, but also when the rate of DNA replication lags behind the rate of cell elongation. A possible explanation for this will be discussed below, but here we should point out that cells carrying this allele of *dnaE* mimic the behavior of the various "Par" mutants of *E. coli* that have previously been described (Hirota et al., 1968, 1971; Orr et al., 1979; Fairweather et al., 1980; Donachie et al., 1984; Norris et al., 1986; Hussain et al., 1987a,b; Kato et al., 1988, 1989), many of which are probably partially blocked in DNA synthesis (Norris et al., 1986). Norris et al. (1986) previously recognized the possibility that "perturbed DNA replication" might itself be a cause of mis-segregation of nucleoids.

CONCLUSIONS

The first set of observations reported in this paper suggests that nucleoids are fixed within the cell at

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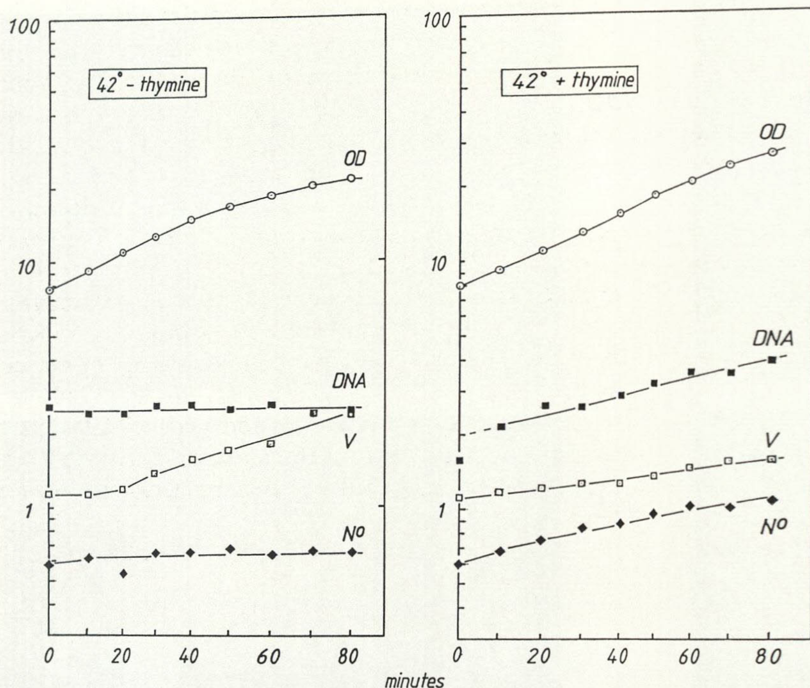

Figure 8. Growth, DNA synthesis, and cell division in a *dnaE*486 *thyA* mutant at 42°C with or without thymine.

Cells were grown in supplemented minimal medium (+ thymine) at 30°C, then washed and resuspended in fresh medium with (right panel) or without (left panel) thymine and growth was continued at 42°C. Symbols: (○, OD; ■, DNA/ml; ◆, cells/ml; □, median cell volume.)

positions corresponding approximately to the center, quarters, or eighths (for cells with 1, 2, or 4 nucleoids, respectively). The lack of intermediate positions implies that nucleoids "jump" apart rapidly after replication of the terminus, as first reported by Sargent (1974) for *Bacillus subtilis* cells. This is best illustrated in our work by the positions of nucleoids in *ftsA* filaments (see Fig. 4B). In these long cells, separation of pairs of sister nucleoids is not always completely synchronous in different sections of the same cell. In one section pairs may be still attached together (dumbell shapes) while in another part of the cell they may have already separated. The spacing between the centers of connected pairs is, on average, half of that between the centers of separate nucleoids in the same cell. Thus the nuclear centers must move apart immediately after separation. This rapid movement in turn suggests that there may be a "mitotic-like" mechanism that can rapidly separate sister chromosomes by a fixed distance.

The minimum requirements for the prokaryotic chromosomal partition system are, therefore, as follows: (i) A protein or proteins to attach the chromosome to the cell envelope (perhaps involving a specific chromosomal "par" site; or a "lamin-like" function); (ii) a release system to detach the chromosomes from the envelope after replication; and (iii) a "partition-mechanism" to separate sister chromosomes within the cell. None of these components have yet been identified, but candidates include the TolC (MukA) membrane protein, the absence of which is correlated with the production of a much higher than normal proportion of anucleate cells (Hiraga et al., 1989) and which could therefore be the proposed membrane-attachment protein, and the recently described myosin-like MukB protein (Hiraga, 1990), which, from its structure, might be able to act as part of the partition mechanism. However, all we yet know for certain about the partition system is that it cannot come into operation without a short period of protein synthesis following the completion of each round of chromosome replication (Donachie and Begg, 1989b; Hiraga et al., 1990).

We have shown here that treatments that alter the

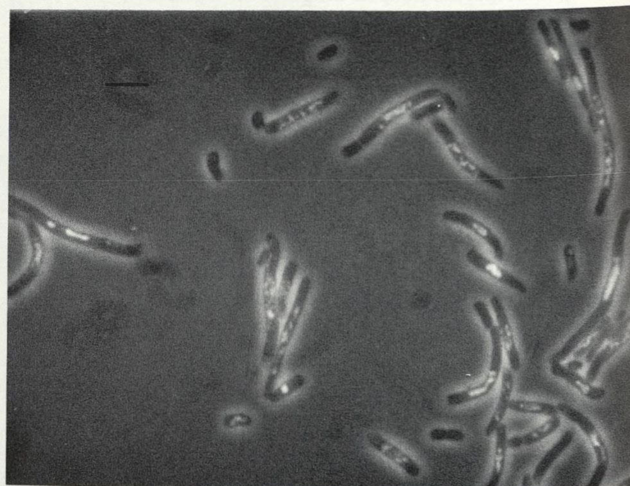

Figure 9. DAPI-stained cells of the *dnaE*486 *thyA* mutant growing at 42°C in the presence of thymine (experiment shown in Fig. 8).

Nucleoids have failed to separate normally and anucleate cells have been produced. Scale bar, 5 μm.

normal temporal coordination between DNA replication and cell growth can prevent the normal localization of chromosomes within the cell. We suggest that this could be the result of the normal operation of the minimal partition system outlined above. This would come about as follows. During undisturbed growth, termination of each round of chromosome replication and subsequent spatial separation of sister chromosomes (i.e., nucleoid partition) coincides with the attainment of a specific cell length (Donachie et al., 1976; Grover et al., 1977) (see Fig. 10). This length is about 2.8 μm (depending on strain) and has been called 2 "unit cell lengths," $2{\cdot}L_u$ (Donachie and Begg, 1970; 1989a,b; Donachie et al., 1976; Donachie, 1981). We propose that the partition mechanism separates nucleoid centers at this stage by approximately 1 unit length (1.4 μm), after which the separated nucleoids reattach to the cell envelope. This will place sister nucleoids in the approximate centers of each new sister cell. If, however, termination is delayed in growing cells (as in the experiments described in this paper) then cells will be longer than $2{\cdot}L_u$ at the time of nucleoid separation, but the separation distance between nucleoids will be unchanged ($1{\cdot}L_u$). In cases in which termination has been delayed without alteration of the normal coupling between initiation and cell mass (Donachie, 1968), as, for example, after a period of growth without DNA replication, two sets of terminations will take place in rapid succession (the interval between terminations depending on the age of the cell at the time of the DNA block) (Donachie, 1969) and several nucleoids will be formed and will attempt to separate while they are in the same part of the cell (the center). The result will be four nucleoids in the cell center.

Because nucleoids are large bodies, the presence

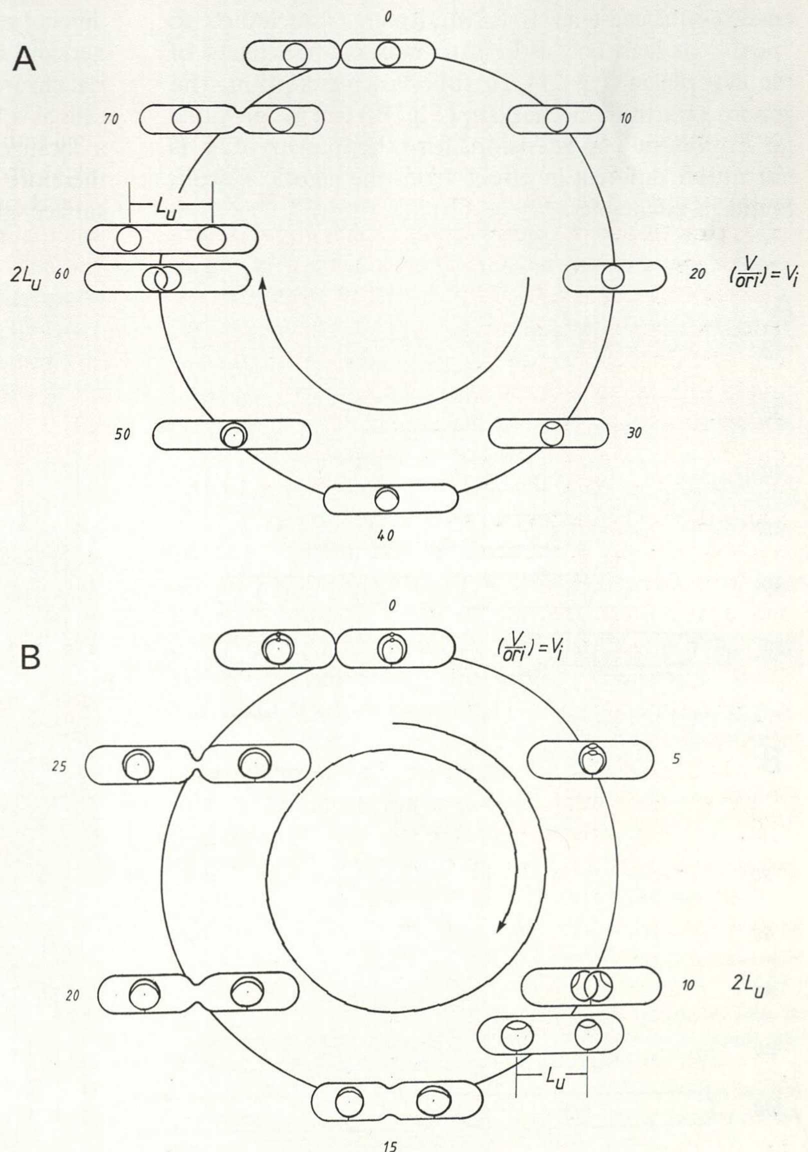

Figure 10. Model of a "mitotic-like" chromosome partition mechanism for *E. coli*.

The diagram shows the growth and division cycles of *E. coli* cells at 37°C in two different growth media. (**A**) Generation time, 80 min. (**B**) Generation time, 30 min. The cells (cylinders with hemispherical poles) grow by elongation, without change in diameter, provided they are in balanced growth at a constant growth rate. However, average cell volume increases as a function of growth rate, such that both the average cell length and average cell width increase but the ratio of average length to width remains the same. Under all conditions, initiation of rounds of chromosome replication takes place at doublings of a fixed "unit volume," such that the ratio of cell volume to number of copies of the initiation site (*oriC*) equals a constant, the "initiation volume," V_i. The time taken for pairs of replication forks to travel around the circular chromosome from *oriC* to the terminus on the opposite side is 40 min, and this is also independent of the growth rate of the cells. Consequently, new rounds of replication will begin before the previous one has been completed in cells with generation times less than 40 min. The time of initiation and completion of each round of chromosome replication is shown as the beginning and end points of the inner arc or spiral in the two cycles. Chromosomes are drawn diagrammatically as circles with a diameter approximately equal to that of nucleoids (i.e., the folded chromosome): the true circumference of an unfolded chromosomal circle is about 1,000 times that of the length of the cell. The course of replication is shown at intervals. At the completion of each round of replication (i.e., when pairs of replication forks meet at the terminus) cells will have reached a constant length ($2{\cdot}L_u$, or two "unit lengths," about 2.8 μm: Donachie et al., 1976) independent of the growth rate of the cells. It is proposed that sister chromosomes are then moved rapidly apart by half this distance (i.e., one unit length, $1{\cdot}L_u$). During further cell elongation it is assumed that the chromosomes (nucleoids) remain in these same positions relative to one another and to the cell poles. The calculations of cell dimensions and growth were made according to the model of an "ideal" cell of *E. coli*, as described previously (Donachie, 1981).

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of several of them at spacings of less than $1 \cdot L_u$ might give the appearance of a continuous mass, as in the cells shown here (Figs. 5, 7, and 9). Subsequent rounds of DNA replication would produce more nucleoids in the central region of the elongated cell, and the filling of this region with DNA would cause newly formed nucleoids to be displaced out (by $1 \cdot L_u$) from the outer edges of the mass of nucleoids. Simple lack of space for large nucleoids in the relatively narrow cellular tube would result in single nucleoids and groups of nucleoids being displaced from the initial mass into the anucleate portions of the elongating cells (in situations in which cell division was blocked) or being incorporated into newly formed daughter cells (in situations in which cell division was not blocked). In this "minimal" model we do not, therefore, envisage any specific mechanism for exactly locating nucleoids within cells, only a mechanism for separating sister nucleoids by an approximately constant distance. When nucleoids are formed abnormally close together, physical interference would cause newly formed pairs of nucleoids to "push" at least one sister into nucleoid-free parts of the cytoplasm (Fig. 11). In this basic description, the genome partition mechanism (Fig. 10) that we propose for *E. coli* and, by extension, for other prokaryotes, is not much different in effect from the mitotic system found in eukaryotes.

It should be noted that the proposed partition mechanism for prokaryotic genomes does not require the preformation of specific chromosome attachment sites in the cell envelope. In our minimalist model, nucleoids simply attach wherever they find themselves after separation by a fixed distance. This might be the explanation for the rather wide distribution of nucleoid positions in individual cells (Figs. 2 and 3). However, our observations do not rule out the possibility that such preformed attachment sites, regularly spaced along the length of the cell (like the "periseptal annuli" described by MacAlister et al., 1983; Cook et al., 1987) do arise during growth in the absence of DNA replication. Our model could then be modified to allow newly separated nucleoids to attach at the nearest available preformed sites. Distant sites (e.g., beyond the central nucleoid mass in elongated cells recovering from a period of growth without DNA replication) would be filled only slowly, erratically, or even not at all (as in the very long cells shown in Fig. 7). Our present model differs fundamentally from the model of Jacob, Brenner, and Cuzin (1963), who first suggested that bacterial chromosomes might be partitioned into daughter cells as a result of being attached to the outer edges of a localized central zone of membrane growth and therefore being continuously dragged apart by cell surface growth. Instead, our model for prokaryotes

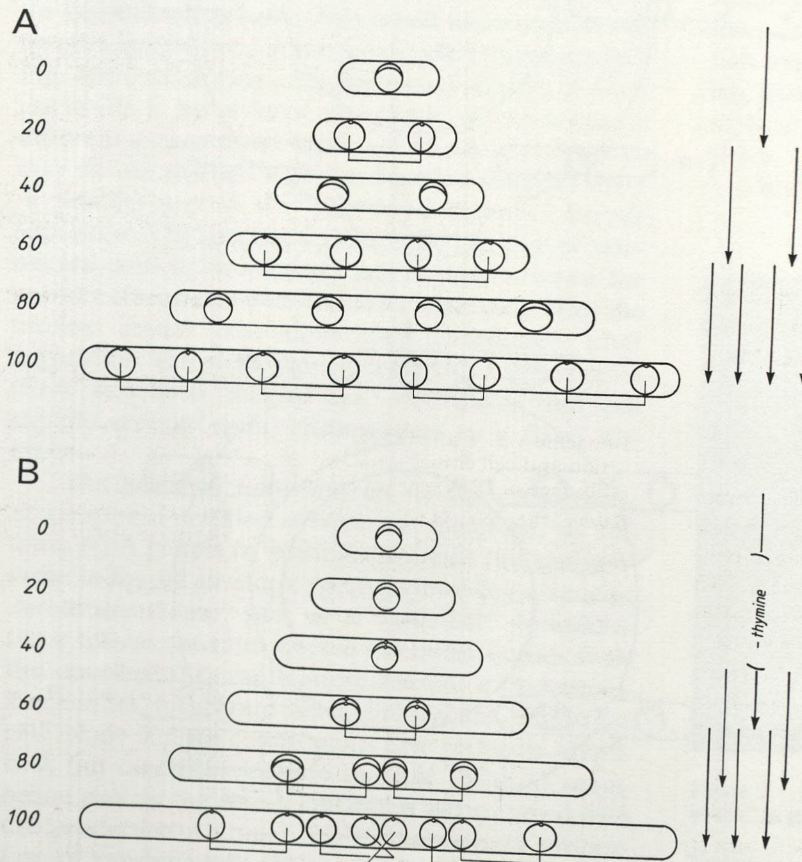

Figure 11. Prediction of the locations of chromosomes in growing, nondividing cells according to the "mitotic" model.

The diagrams show the proportions of the cells at 20-min intervals following the block to cell division, assuming a volume doubling time of 40 min (Donachie, 1981). The locations and state of replication of the chromosomes are also shown, as in Fig. 10. (The figure shows the fate of a "newborn" cell under these conditions. Cells at different stages of the cycle would behave similarly.) **(A)** Cell division alone is blocked and chromosome replication goes on normally. Sister chromosomes are separated by the fixed distance L_u at the completion of each round of replication and so come to be evenly distributed throughout the cell. **(B)** The conditions are identical to those in (A), except that DNA replication is prevented (by thymine starvation) during the first doubling in cell volume, and then allowed to resume. Initiations of new rounds of replication take place at the same cell volumes as before and sister chromosome separation takes place by the same distance, L_u, at the completion of each round of replication. However, the temporary inhibition of DNA replication ensures that the chromosomes (and therefore nucleoids) will no longer come to be evenly spaced within the cell and, instead, will be crowded together in the central section so as form an apparently single mass of DNA with outlying single nucleoids. The model, as drawn, assumes absolute maintenance of relative chromosome positions during cell elongation and also assumes that sisters are moved apart by exactly L_u (1.4 μm). Small variations in these parameters lead to much more irregular positioning of nuclear masses.

resembles the mitotic process of eukaryotes: there, also, chromosomes are attached to the (nuclear) membrane (by lamins), are detached after replication, and sister chromosomes are separated by a fixed distance (by the mitotic mechanism) before becoming reattached to the membrane. In cells such as those of the fission yeast, *Schizosaccharomyces pombe,* the nuclear membrane does not break down during this process and instead divides into two nuclei after separation of sister genomes. Our model for *E. coli* is therefore formally similar to the nuclear cycle of *S. pombe.*

In summary, our experiments show that disruption of the normal coordination between chromosome replication and cell elongation results in abnormal localization of DNA within the cell. We suggest that this may be the consequence of a "mitotic-like" mechanism in *E. coli* that rapidly separates sister chromosomes by a fixed distance after replication, after which they reattach to the cell envelope. In this respect, genome partition in prokaryotes may resemble that in eukaryotes, and it would be interesting to see whether experiments similar to ours would cause mislocation of nuclei in a similarly shaped eukaryotic cell, such as the rod-shaped yeast *S. pombe.*

MATERIALS AND METHODS

Strains

Escherichia coli K-12 AB2497 (*leu thr proA his argE lac gal ara xyl mtl thi tsx rpsL thyA*), TKF12 *ftsA*12 and TOE13 *ftsA*13 (derived from AB2497), and CGSC 509 (*leu thr thi met thyA deo lacY rpsL tonA supE*44 *dnaE*486) were used in these experiments.

Media and Growth Conditions

Cells were grown with vigorous shaking at the indicated temperatures in minimal medium (Vogel-Bonner salts with glucose, casamino acids, tryptophan, adenine, uracil, and thymine as required at 50 μg/ml) or in Oxoid nutrient broth No. 2 plus thymine (50 μg/ml).

Visualization of Nucleoids

Nucleoids were condensed by the addition of chloramphenicol (200 μg/ml) for 5 min at 42°C (to block cell division during chromosome condensation) (Donachie and Begg, 1989a,b), and samples were fixed and stained with DAPI (4,6-diamidino-2-phenylindole) for viewing and photography under mixed phase and fluorescence illumination with a Leitz Metallux II microscope (Hiraga et al., 1989; Donachie and Begg, 1989a,b). All measurements of nucleoid positions and cell lengths were made from prints of these photographs.

Measurement of Cell Parameters

Cell growth in mass was followed as optical density (OD) at 540 nm in a 1-cm light path. Cell numbers and median cell volumes were measured on fixed samples by means of a Coulter Electronic Particle Counter and Channelyser, as previously described (Begg and Donachie, 1978). Total DNA was measured by using DAPI, as already described (Donachie and Begg, 1989a).

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