

## Nucleoid Partitioning and the Division Plane in *Escherichia coli*

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*Escherichia coli* nucleoids were visualized after the DNA of OsO<sub>4</sub>-fixed but hydrated cells was stained with the fluorochrome DAPI (4',6-diamidino-2-phenylindole dihydrochloride hydrate). In slowly growing cells, the nucleoids are rod shaped and seem to move along the major cell axis, whereas in rapidly growing, wider cells they consist of two- to four-lobed structures that often appear to advance along axes lying perpendicular or oblique to the major axis of the cell. To test the idea that the increase in cell diameter following nutritional shift-up is caused by the increased amount of DNA in the nucleoid, the cells were subjected to DNA synthesis inhibition. In the absence of DNA replication, the nucleoids continued to move in the growing filaments and were pulled apart into small domains along the length of the cell. When these cells were then transferred to a richer medium, their diameters increased, especially in the region enclosing the nucleoid. It thus appears that the nucleoid motive force does not depend on DNA synthesis and that cell diameter is determined not by the amount of DNA per chromosome but rather by the synthetic activity surrounding the nucleoid. Under the non-steady-state but balanced growth conditions induced by thymine limitation, nucleoids become separated into small lobules, often lying in asymmetric configurations along the cell periphery, and oblique and asymmetric division planes occur in more than half of the constricting cells. We suggest that such irregular DNA movement affects both the angle of the division plane and its position.

A bacterial cell maintains its DNA in a central space called the nucleoid. In living cells, this domain can be visualized either by phase contrast in the presence of gelatin (25) or by fluorescence microscopy with a fluorochrome (13). Fixing the cells with osmium tetroxide results in nucleoids that are more compact (5, 39) but that still retain characteristic shapes which seem to reflect the replication and segregation states of the chromosome (see also references 7, 16, and 32). These distinct nucleoid shapes indicate that the DNA is not dispersed randomly throughout the cytoplasm but that most of it is confined to a region separated from the cytoplasmic phase by some unknown chemical property (38). This phenomenon does not preclude the presence throughout the cytoplasm of short loops of DNA that extend towards the plasma membrane and are actively involved in transcription and translation (14, 33). That such connections are indeed effective in shaping the nucleoid is suggested by the old observation that treatment of cells with chloramphenicol causes the nucleoid to round off (21).

Several models for the various mechanisms and forces involved in the movement and positioning of the chromosomes within the cell have been advanced. The classical replicon hypothesis (20) considered envelope growth between postulated sites of chromosomal attachment to the plasma membrane. However, the rigid peptidoglycan layer has been shown to be synthesized in a dispersed mode (44) rather than in a central zone, as would be necessary for the outward growth of any DNA attachment points. Two alternative models for the motive force in DNA partitioning have recently been put

forward, one of them based on the formation of ribosome assembly compartments produced by the major clusters of ribosomal genes that flank the *oriC* region at 72 and 88 min (47) and the other based on the existence of force-generating proteins (18, 19).

Whatever the mechanism for DNA movement, it must result in the separation of the replicated chromosomes along the major axis of the cell and in the formation of a division plane perpendicular to that axis. It is not known whether DNA movement is driven by the replication process itself (6), by growth of the cytoplasm (47), or by a separate mechanism involving cytoskeleton-like components (18, 19), nor is it known whether the direction of the movement remains constant during the cell cycle, whether its orientation is influenced by the shape of the cell, or, conversely, whether DNA movement plays a role in the pattern of envelope growth and the determination of diameter and shape.

In most strains, slowly growing cells have small diameters (34, 42, 48, 49, 51) and contain only two segregating chromosomes. Nucleoids in short, newborn cells look like simple rods, but in longer cells nearing termination, they occasionally assume a dumbbell shape. Under conditions of rapid growth, however, when replication is multiforked (7, 31, 34) and the cells are wider (34, 42, 48, 49, 51, 53), four origins segregate simultaneously and nucleoids show more complex shapes that take up more space along the minor axis of the cell. The mechanism responsible for this increase in diameter has remained a mystery because under all steady-state growth conditions, such cells augment their volume essentially by elongation (24, 37).

It has previously been proposed that cell diameter could be actively determined by the amount of DNA packed in an individual chromosome (46). This idea was based on the observation that the rate of peptidoglycan synthesis was lower in the region surrounding the nucleoid than elsewhere (28). Under conditions of fast growth (in rich media) or slow

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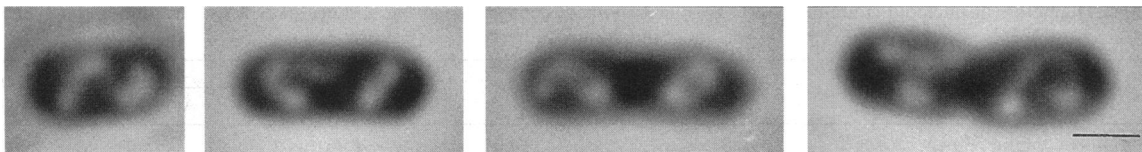


FIG. 1. Fluorescence micrographs of *E. coli* MC4100 cells grown in Casamino Acids medium (doubling time, 27 min), fixed with 0.1% OsO<sub>4</sub>, and stained with DAPI. Note the two- and three- to four-lobed nucleoids. The appearance of such structures in any particular case depends on the actual plane of focus and angle of view. Although symmetrically positioned nucleoids are expected to induce a perpendicular division plane (third panel from left), nucleoids can have changed their shape and position at the time of visible constriction (rightmost panel). Bar, 1  $\mu$ m.

replication (a *thy* mutant under limiting thymine concentrations), elongated nucleoids were frequently observed to lie at acute angles to the major axis of cells with increased diameters (53). This phenomenon can also be seen in the time-lapse micrographs of Mason and Powelson (25) and of Gumpert (16). Such observations raise the questions of whether the motive force in DNA movement could involve the replication process itself and whether it could play an active role in determining cell diameter. In the present article, we examine the nature of this motive force and its possible influence on cell diameter and on the orientation of the division plane.

## MATERIALS AND METHODS

**Bacterial strains.** The following *Escherichia coli* K-12 strains were used: MC4100 [*F*<sup>-</sup> *araD139*  $\Delta$ (*argF-lac*)*UI169 deoC1 ffb5301 lysA ptsF25 rbsR relA1 rpsL150*] (strain LMC500 [36]), its isogenic derivative *dnaX*(Ts) (strain LMC 1012 [27]), and CR34 (*thr-1 leuB6 thyA*) (52). In addition, several *E. coli* B/r strains were used: H266 (37), K *thy* (laboratory strain LMC 218), and A (ATCC 12407 [9]).

**Media and growth measurements.** The cultures were grown at 37°C in either 1% tryptone plus 0.5% yeast extract or in minimal medium containing 6.33 g of K<sub>2</sub>HPO<sub>4</sub> · 3H<sub>2</sub>O, 2.95 g of KH<sub>2</sub>PO<sub>4</sub>, 1.05 g of (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 0.1 g of MgSO<sub>4</sub> · 7H<sub>2</sub>O, 0.3 mg of FeSO<sub>4</sub> · 7H<sub>2</sub>O, 7.1 mg of Ca(NO<sub>3</sub>)<sub>2</sub> · 4H<sub>2</sub>O, and 5 g of glucose per liter, supplemented with 1% Casamino Acids (Sigma) for nutritional shift-up. NaCl at 0.1 M was added to bring the osmolality to 300 mosM (Micro-Osmometer; Advanced Instruments, Needham Heights, Mass.). Required amino acids were provided at 50  $\mu$ g/ml each, and thiamine was provided at 1  $\mu$ g/ml. Thymine was supplied to *thy* strains at the concentrations indicated below, augmented by 100  $\mu$ g of deoxyguanosine per ml in the case of CR34 in order to obtain high replication rates (52). Cells were cultured for at least 10 doubling times with periodic dilutions to obtain steady-state growth. The *A*<sub>450</sub> was measured with a Gilford (Oberlin, Ohio) microsample spectrophotometer, and cell concentrations were measured with an electronic particle counter (orifice diameter, 30  $\mu$ m).

**Nutritional shift-down and thymine step-up.** In order to obtain short, wide, rod-shaped cells, a steady-state culture of strain CR34 in glucose (0.1%) minimal medium with 0.5  $\mu$ g of thymine per ml was simultaneously shifted down and stepped up (51) by the addition of 1%  $\alpha$ -methyl glucoside (50) and 20  $\mu$ g of thymine per ml (51, 53). Rapidly growing cells with long replication times (31) were thus transferred instantaneously to conditions of slower growth and shorter replication times.

**Fluorescence microscopy.** For cell immobilization, a 50- $\mu$ l drop of 2% agarose mixed with growth medium at 46°C was spread under a coverslip coated with silicon grease. After gelation, the coverslip was removed and 3  $\mu$ l of a concentrated cell suspension was spread over the smooth surface under a second coverslip and sealed with 2% agarose. The preparation

was illuminated at 330 to 380 nm in an Olympus BH-2 microscope and photographed with 100ASA Tmax Kodak film, with an SPlan 100PL 1.25 oil immersion lens, a 3.3 $\times$  photo-ocular, and an emission filter of 420 nm. Where indicated, photographs were taken with a cooled charge-coupled device camera (Astromed model TE3W, Cambridge, United Kingdom) and a 6.7 $\times$  photo-ocular. The images were processed by the image analysis program SCILIMAGE (40). Magnifications were determined by means of a micrometer slide.

**Preparation and staining of cells.** Cells were fixed by adding OsO<sub>4</sub> (final concentration, 0.1%) directly to the growth medium. The fluorochrome DAPI (4',6-diamidino-2-phenylindole dihydrochloride hydrate) (Sigma) was then added to a final concentration of 0.2 to 0.4  $\mu$ g/ml, and the cells were concentrated by centrifugation (Eppendorf, 10 s at 10,000  $\times$  g) and prepared without further washing or drying. This method of DAPI staining readily visualizes nucleoids in the fluorescence microscope (2, 19, 35, 36, 54) while avoiding the extensive volume shrinkage (30%) associated with dehydration during preparation for electron microscopy (42, 47). Observation of hydrated cells in the fluorescence microscope is capable of providing an overall impression of the three-dimensional nucleoid organization in a large number of cells. (Real three-dimensional nucleoid images can be obtained by confocal scanning light microscopy [13, 39], but the sample sizes are still too small to permit reliable inferences to be drawn.)

**Electron microscopy.** Cells were prepared for electron microscopy as previously described (42).

## RESULTS

**Nucleoid shape and division plane during balanced growth.** It is characteristic of the fluorescent images of nucleoids in slowly growing cells that they seem to expand lengthwise, in pace with the elongating cell (41). By contrast, nucleoids in rapidly growing cells show recurrent appearances of two- to four-lobed structures that often lie in planes perpendicular or oblique to the major axis of the cell. In longer cells and in constricting cells, these structures appear to be oriented symmetrically around the major axis. Furthermore, the nucleoids in many dividing cells attain configurations of lobules that are comparable on either side of the division plane. A sequence selected from a fast-growing population of 230 DAPI-stained MC4100 cells (Fig. 1) illustrates these phenomena only partially, because the appearance of the nucleoids changes with the plane of focus. We believe that the apparent variation in the nucleoid shape and in the brightness of some of its lobules results from viewing the same three- or four-lobed structures from different angles. The micrographs suggest that the mechanism of nucleoid movement can also act in oblique directions once more space is available along the minor axis of the cell. We propose that the motive force shaping the lobular nucleoids depends either on the increased rate of cytoplasmic growth or on the more complicated mode of replication by

TABLE 1. Diameters at nucleoid-containing and subpolar regions of cells grown in the absence of DNA replication and subjected to a nutritional shift-up

Strain	Growth condition(s) <sup>a</sup>	Doubling time (min)	DNA synthesis	Diam (μm) <sup>b</sup>		Corresponding figure
				At nucleoid	At pole <sup>c</sup>	
<i>dnaX</i> (Ts)	Gluc, 30°C	87	+	1.06	0.95	2B
	Gluc, 42°C	58	–	1.03	0.90	2C
	Shift-up	22	–	1.33	1.02	2D
	TY, 30°C <sup>d</sup>	26	+	1.32	1.11	
B/r <i>K thy</i>	Gluc + CAM	63	+	0.79	0.66	3B
	No CAM, no thymine	75	–	0.94	0.71	3C
	Shift-up	30	–	1.27	0.91	3D

<sup>a</sup> Gluc, glucose-containing medium; TY, tryptone-yeast extract medium; CAM, chloramphenicol.

<sup>b</sup> At least 60 cells per sample; coefficient of variation, 4 to 6%.

<sup>c</sup> About 1 diameter from cell tip.

<sup>d</sup> Steady-state growth.

multiple forks and that symmetrically positioned nucleoids induce a perpendicular division plane.

**Nature of the motive force.** To test whether nucleoid movement depends on replication, two experiments in which DNA synthesis but not cell growth was inhibited were performed. Under these conditions, the cells were also subjected to a nutritional shift-up, to determine whether cell diameter would increase as it does during the usual shift-up experiment with replicating cells (34, 43).

In the first experiment (Fig. 2), an *E. coli dnaX*(Ts) culture was transferred to the nonpermissive temperature (42°C), which, as has been shown previously, completely inhibits DNA synthesis within 20 min and causes filamentation due to the SOS response (27). After 30 min, an aliquot was shifted up to a rich medium (Fig. 2A; Table 1). In the relatively slowly growing cells (Fig. 2B), the nucleoids looked like simple ellipsoids. When replication was inhibited (Fig. 2C), however, the nucleoids no longer retained this confined organization but separated into small domains. The cells responded to the nutritional shift-up by increasing their diameter, particularly at the site of the nucleoids (from about 1.0 to 1.3 μm; Table 1), resulting in short filaments with a tapered appearance (Fig. 2D). Thus, the separate domains of the existing nucleoid move within the growing cell in the absence of DNA replication while the nucleoids continue to exert an effect on envelope synthesis, causing a local increase in diameter (30%; Table 1).

In this experiment, the cells were still in the process of DNA replication and partitioning at the time of the temperature shift. A second experiment was therefore carried out with *E. coli* B/r *K thy* cells containing nonreplicating chromosomes obtained by treatment with 300 μg of chloramphenicol per ml, which allows DNA synthesis to continue while preventing initiation of new rounds of replication (10). After 1 h of incubation, the inhibitor was removed by filtration and the cells were suspended in the same medium but without the required thymine, thus allowing protein synthesis to resume (Fig. 3A, line c) while inhibiting DNA synthesis and initiation of new rounds of chromosome replication.

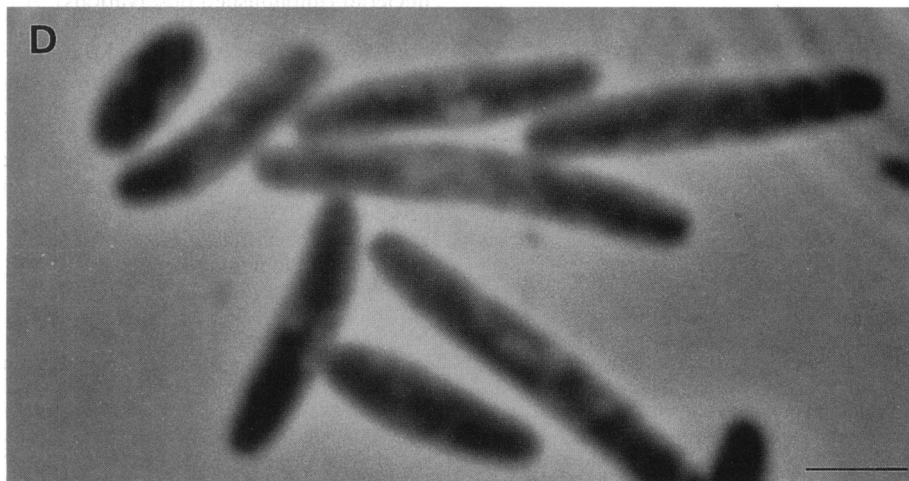
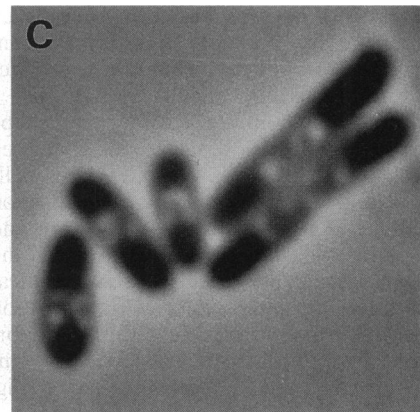
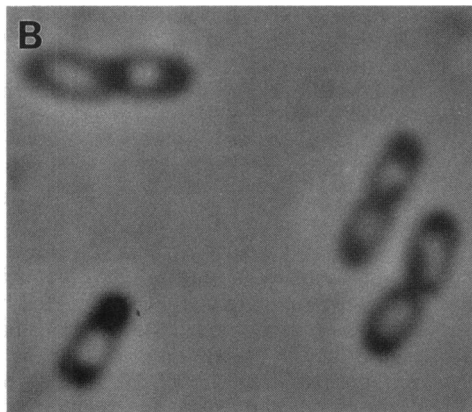
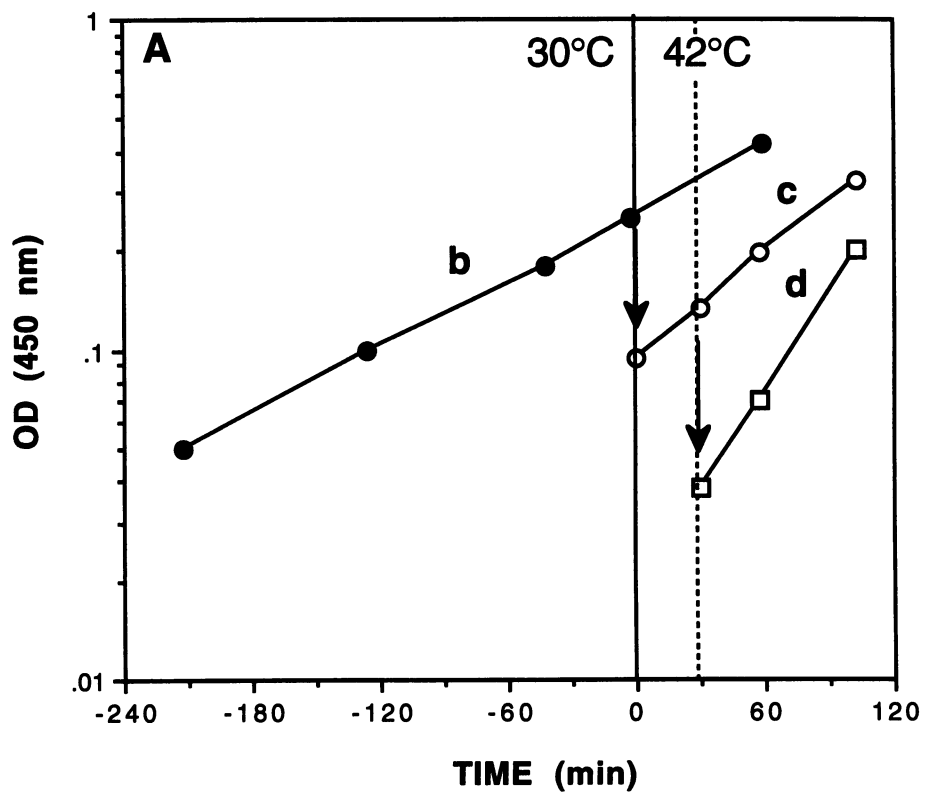
The chloramphenicol treatment resulted in a homogeneous

population of cells with either one or two ellipsoidal nucleoids (Fig. 3B) and relatively small diameters (Table 1). Upon resumption of growth in the absence of DNA synthesis (Fig. 3C), the nucleoids remained compact for about 1 h (33% mass increase) but then spread out into small regions along the periphery of the central part of the cell, as seen after 180 min (250% mass increase). When the cells were permitted to resume growth at a higher rate (shift-up), the nonreplicating nucleoids spread even further, over large distances, almost obliterating the fluorescence of the DAPI-stained nucleoids (Fig. 3D). Here, too, the mean cell diameter at the site of the nucleoids increased more (from 0.8 to 1.3 μm; Table 1) than that near the poles (from 0.7 to 0.9 μm; Table 1), suggesting an effect of the nucleoid on cell diameter.

A comparison of the micrographs in Fig. 2 and 3 (compare panels C with panels D) suggests that the nonreplicating nucleoids could not keep pace with the elongating cells. Consequently, DNA-less cell poles that were pinched off as DNA-less cells developed (already visible in Fig. 3D) upon entry into the stationary phase and recovery from the SOS response (27). Similar pictures (not shown) were obtained during growth of a *dnaA* mutant at nonpermissive temperatures (27). Such fragmented nucleoids have in the past (26) been observed to contract back to confined shapes when the short filaments were treated with rifampin (120 μg/ml for 15 min). Fragmentation thus appears to reflect not DNA breakdown but rather the movement and spreading of the intact nonreplicating nucleoid, quite likely by the protein synthesizing system.

**Nucleoid shape and division plane during non-steady-state, balanced growth.** Although the amount of DNA may have no direct influence on cell diameter, as suggested above and previously (46), the observations nevertheless imply that its presence can affect the cell diameter and, therefore, perhaps also the positioning of the division plane. To test the idea that the direction of nucleoid movement could influence the plane of division, it is necessary to find an experimental regimen in which complex replicating nucleoids develop without the increased synthetic activity and without the inhibition of cell

FIG. 2. (A) Growth curves of *E. coli* MC4100 *dnaX*(Ts) cultured in glucose minimal medium at 30°C (line b) and shifted at time zero to 42°C to block DNA synthesis (line c). After 30 min, the culture was shifted up by dilution into tryptone-yeast extract medium at 42°C (line d). OD, optical density. (B to D) Fluorescence micrographs of OsO<sub>4</sub>-fixed, DAPI-stained cells, corresponding to the last points of lines b, c, and d, respectively. Note nucleoids spreading into small domains (C). In certain cells (D), as many as 10 separate lobules can be counted, probably belonging to a single, intact chromosome. Note also a localized increase in cell diameter and tapering of cell poles. See Table 1 for doubling times and cell diameters. Bar, 2 μm.



division associated with nutritional shift-up. Such conditions are obtained with thymine-requiring strains, in which the rate of chromosome replication depends on the exogenous thymine concentration (31). In contrast to thymine starvation, in which DNA synthesis is blocked completely, thereby inducing the SOS response (compare Fig. 2 and 3) and causing unbalanced growth culminating in cell death (8), thymine limitation allows cells to continue to grow in a balanced manner at the same growth rate (31). Such cells contain multiforked chromosomes and are larger, just as wild-type cells are under fast-growth conditions (51). They are also wider at lower thymine concentrations, as expected if the larger nucleoids are to be accommodated by an increase in cell diameter.

The cells in Fig. 4A were grown in glucose and Casamino Acids medium at a high thymine concentration (20  $\mu\text{g/ml}$ , with deoxyguanosine) and display the nucleoid shapes characteristic of fast-growing cells (Fig. 1). After 13 generations of non-steady-state growth at a limiting thymine concentration (2.5  $\mu\text{g/ml}$ , without deoxyguanosine), the cells are longer and wider (33% diameter increase). Now the nucleoids usually consist of four to eight or more small lobules, arranged in complex configurations often lying along the periphery of the cell (Fig. 4B). Treatment of these cells with chloramphenicol (300  $\mu\text{g/ml}$ ) revealed (Fig. 4C) that such extended complexes are still able to contract into one or two, and in longer cells four, spherical nucleoids often lying asymmetrically within the cells.

More than half the constricting cells grown at the low thymine concentration (Fig. 4B) exhibited asymmetric invaginations in nucleoid-free regions or oblique constrictions, causing the cell halves to lie at acute angles to each other. Probably as a result of such abnormal divisions, most cells contained a polar tip that was flattened or bent and sometimes even broadened into a branch. We contend that the aberrant constrictions are induced by nucleoids which, in these broader cells, have more freedom to move in irregular directions.

As a further test of the possible influence of nucleoid shape and movement on the plane of division, the frequency of cell division was increased by subjecting *E. coli* CR34 *thy* cells growing in glucose medium at a low thymine concentration (0.5  $\mu\text{g/ml}$ ) to a simultaneous nutritional shift-down and thymine step-up (see Materials and Methods). During the transition period, the increased rate of chromosome replication resulted in more frequent initiations of cell constriction despite the lower rate of increase in volume. A large proportion (40%) of the constricting cells again displayed aberrant shapes caused by asymmetric and oblique constrictions (Fig. 4D), and 10% of the constricting cells showed branching.

## DISCUSSION

The questions whether the motive force in nucleoid movement could be affected by DNA replication and whether it could play an active role in determining cell diameter (and thus shape) were examined in experiments in which DNA and protein synthesis were inhibited and stimulated, respectively. The results (Fig. 2 and 3) clearly indicate that nucleoid movement is not influenced by the DNA replication process itself.

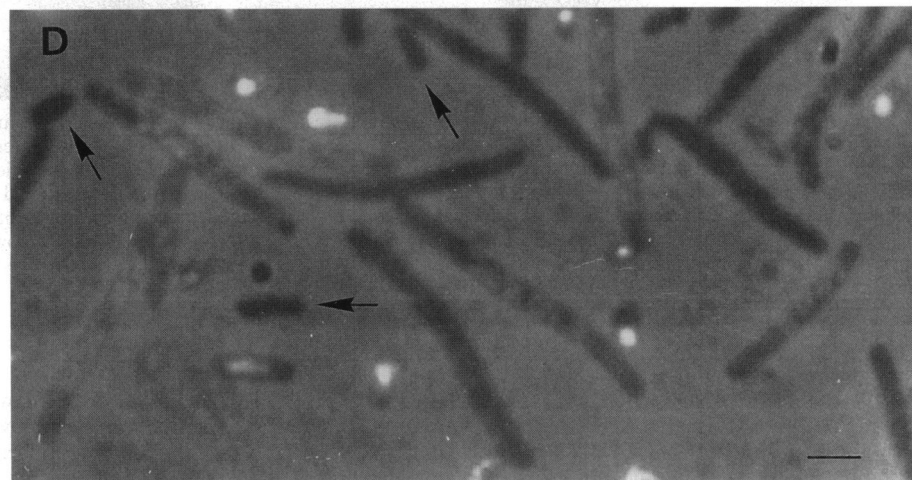
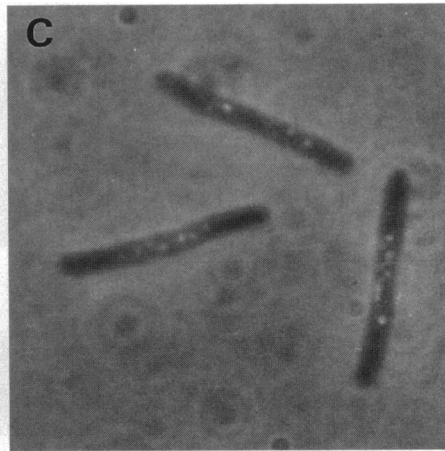
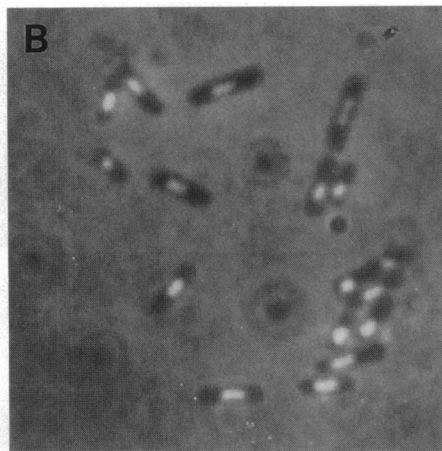
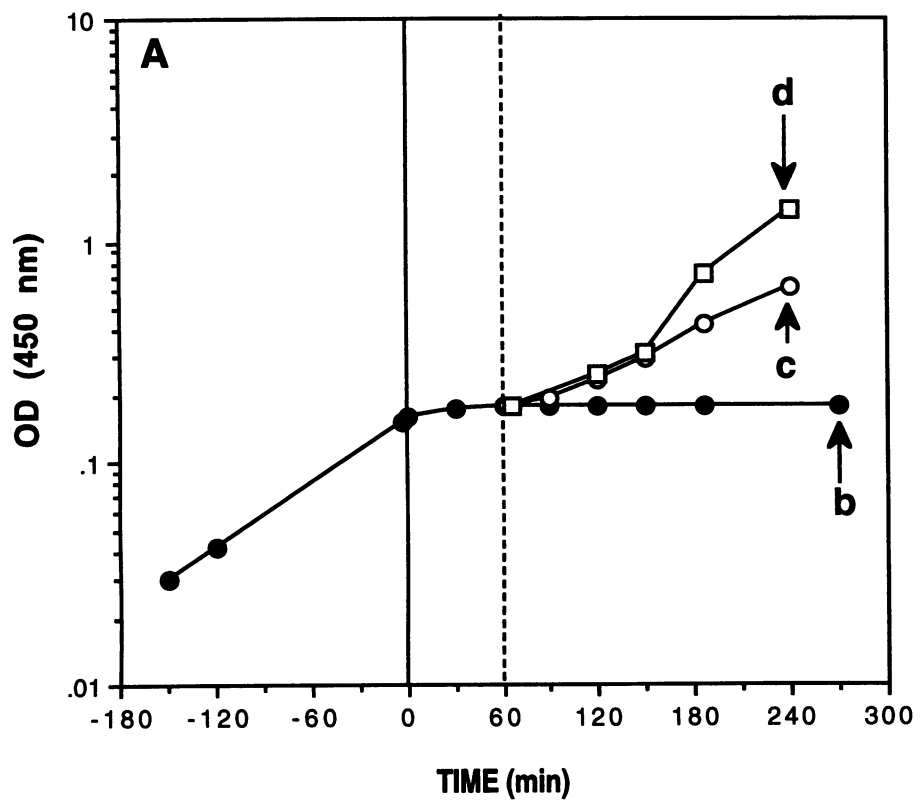
Our observations (Fig. 2 and 3 and Table 1) demonstrate further that, following a nutritional shift-up, cells increase their diameter even in the absence of DNA synthesis. This increase, however, seems out of balance, occurring mostly in the regions surrounding the nucleoids. Such results suggest that even a nonreplicating nucleoid has some influence on the pattern of envelope growth. This is consistent with the so-called nucleoid occlusion model (27, 45, 46), which was based on autoradiography demonstrating that the rate of peptidoglycan synthesis is suppressed in the vicinity of the nucleoid (28). From those observations, it was inferred that envelope growth slows down in the region of the nucleoid because of its activity in the transcription and translation processes. If that activity were related primarily to the expression of rRNA genes and the process of ribosome assembly, then induction of multifork replication at a constant growth rate would also affect envelope growth. And indeed it does: when a thymine-requiring strain was grown at a low thymine concentration (Fig. 4B), a condition characterized by complex replicating nucleoids but an unchanged mass doubling time (31), the diameter of the cells was found to increase by over 30% despite the attendant drop in overall DNA synthesis.

The nucleoid occlusion model (45, 46) predicts that inhibition of peptidoglycan synthesis can be relieved only in DNA-free zones, either between partitioned chromosomes or at the cell poles. Similarly, the surface stress model (22) predicts that it is the local activation of surface synthesis which results in a decrease in cell diameter (invagination) and that the cell would bulge where surface growth is slowed. During the normal cell cycle, in which the replicating DNA is partitioned regularly, any differential synthesis along the cell surface caused by the nucleoid occlusion effect is smoothed out. There can be no smoothing in the SOS filaments of Fig. 2 and 3, however, so it may be the nucleoid effect that causes the cells to taper. The tapered cells observed previously during the length overshoot following nutritional shift-up (43) could be explained in a similar way.

Three possible mechanisms for nucleoid movement, not mutually exclusive, have appeared in the literature. The first, proposed by Cavalier-Smith (6), assumes that the two sister origins are actively transported by the action of DNA helicases and by the supercoiling tension exerted by DNA gyrase. That supercoiling could constitute a force capable of moving DNA is confirmed by the observation that the addition of high salt concentrations (200 to 300 mM NaCl) to the growth medium increases plasmid DNA supercoiling (17) and confines the nucleoid (unpublished observations). A role for the supercoiling domains in the vicinity of the origins is also envisioned in the recent models for nucleoid formation advanced by Løbner-Olesen and Kuempel (23) and by Gumpert (16).

The second model conceives of the DNA as moving over cytoplasmic fibers, with the help of force-generating enzymes like the recently described protein MukB, in a mitosis-type process (18, 19). The observation that cell division gene products such as FtsA (15) and FtsZ (3) may form self-assembled structures lends support to the possibility of internal cytoskeletal elements in bacteria.

FIG. 3. (A) Growth curves of *E. coli* B/r K *thy* cultured in glucose minimal medium containing 20  $\mu\text{g}$  of thymine per ml at 37°C. At time zero, chloramphenicol was added at a concentration of 300  $\mu\text{g/ml}$  (line b). After 30 min, an aliquot was filtered (Millipore; 0.22- $\mu\text{m}$  pore size) and the cells were resuspended in the same medium but without thymine, either with (line d) or without (line c) 1% Casamino Acids. OD, optical density. (B to D) Fluorescence micrographs of  $\text{OsO}_4$ -fixed and DAPI-stained cells corresponding to the last points of lines b, c, and d, respectively. Note how the compact nucleoids (B) become spread out into small domains (C and D) and how the cell diameter increases (D). Arrows indicate DNA-less cells. Photographs were taken with a cooled CCD camera. See Table 1 for doubling times and diameters. Bar, 2  $\mu\text{m}$ .





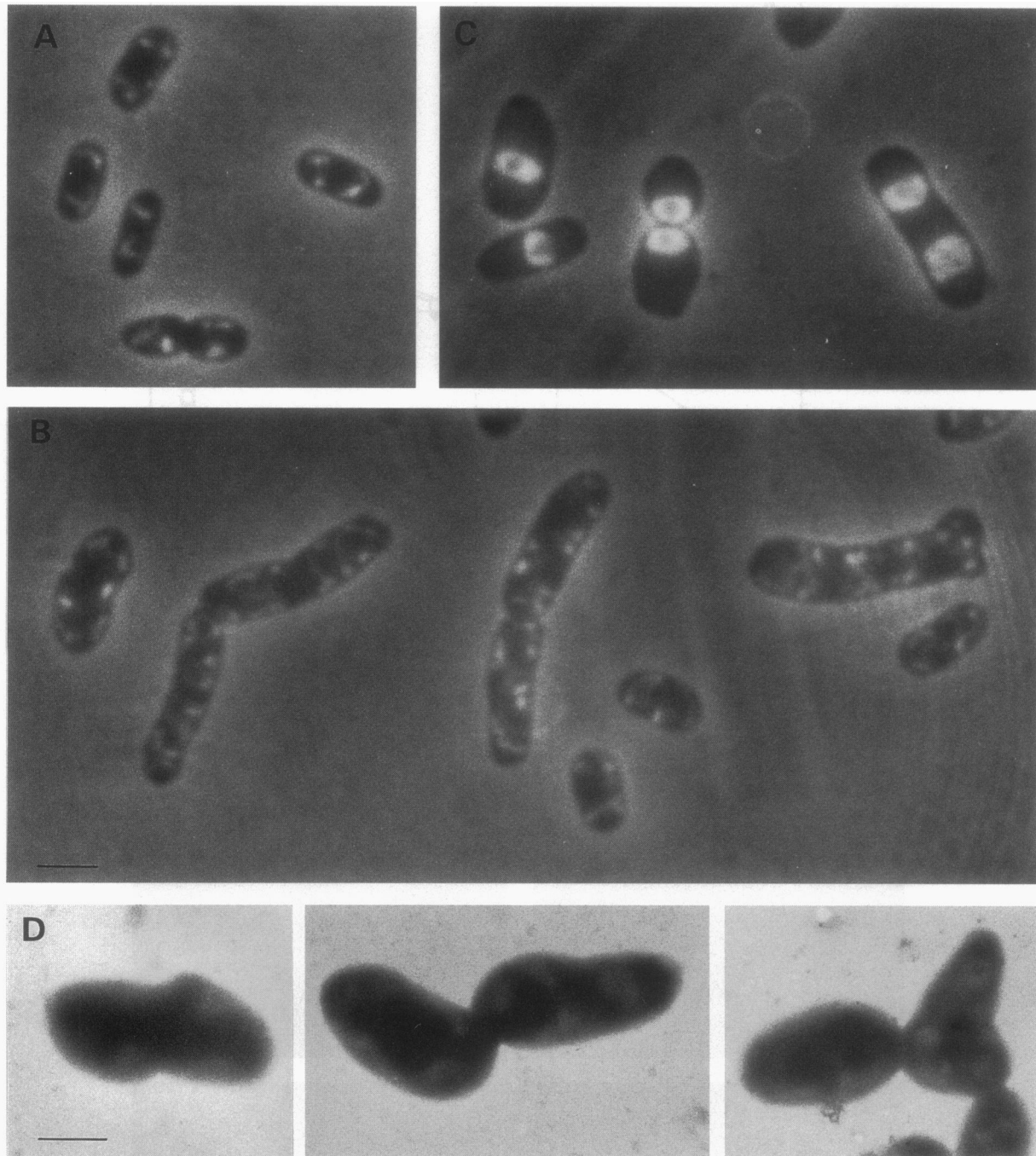


FIG. 4. (A) *E. coli* CR34 *thy* grown in glucose Casamino Acids medium at a high thymine concentration (20  $\mu\text{g/ml}$ ) augmented by 100  $\mu\text{g}$  of deoxyguanosine per ml (doubling time, 30 min). Compare the nucleoid configuration with that in Fig. 1. (B) Cells shifted (stepped down) to a limiting concentration of 2.5  $\mu\text{g}$  of thymine per ml and grown under non-steady-state, balanced conditions for 13 generations. Note the irregular configuration of nucleoid lobules, asymmetric invaginations causing cell halves to lie at an angle to each other, and flattened or broadened cell tips. (C) Cells grown for 10 generations as for panel B and treated with 300  $\mu\text{g}$  of chloramphenicol per ml for 90 min. Note contraction of lobules into spherical nucleoids. Cells in panels A through C were visualized by fluorescence microscopy. Bar, 2  $\mu\text{m}$ . (D) Cells grown in glucose minimal medium with 0.5  $\mu\text{g}$  of thymine per ml which were simultaneously shifted down and stepped up (see Materials and Methods). Cells were visualized by whole-mount electron microscopy (42). Note asymmetric invaginations and branching. Bar, 1  $\mu\text{m}$ .

The third mechanism (47) envisages two microcompartments forming near *oriC* around the duplicated ribosomal genes from each daughter strand. Here, the first loops of replicated DNA are assumed to move apart by expansion of the two ribosomal assembly centers, which could be regarded

as nucleolar-like compartments. An axis for partitioning can then be thought of as developing between the ribosomal microcompartments themselves. Such movement will continue independently of DNA replication, in accord with the present results (Fig. 2 and 3).

Our observations of continued nucleoid movement in the absence of DNA replication (Fig. 2 and 3) conform to the cytological findings (40a, 41) that nucleoids move gradually and continuously through the cell. Others have proposed that after termination, nucleoids move apart rapidly to assume their new positions in the prospective daughter cells (2, 16, 18, 19, 54), just as in eukaryotic mitosis, by a mechanism in which *oriC* (11, 29) or *ter* takes the lead in segregation. One should, however, bear in mind the well-documented fact (12, 41) that structural separation of daughter nucleoids immediately follows the termination of chromosome replication, which implies that partitioning must have occurred concomitantly with replication.

Many mutants with defects in peptidoglycan synthesis (*pbpA*) or in the division machinery (*ftsZ*, *pbpB*) display aberrant positioning of constrictions or pole formation and oblique division planes. The observations of Bi and Lutkenhaus (4) have shown that the latter may develop from disturbances in the assembly of the mutated FtsZ protein into a ring-like structure. Here, we report that *thy* mutants with no apparent defects in surface growth or in division can be induced to exhibit similar aberrations simply by being grown under balanced but non-steady-state conditions. We propose that such conditions could first cause abnormal movement of the nucleoids (due to induction of multifork replication) and then induce formation of a deviant FtsZ ring and division plane.

The phenomenon of branching (Fig. 4) has been seen before (52) but was not understood at the time. To the best of our knowledge, such a phenomenon has never been found in slowly growing cells with small diameters, nor have mutants displaying that phenotype been described, which suggests that it is induced by rapid, unbalanced growth (Fig. 4). This also applies to the observations of Åkerlund et al. (1), although in their experience the branching was restricted to growth in Casamino Acids medium supplemented with acetate and was absent in broth cultures.

A relationship between cell shape, direction of nucleoid movement, and position of the division plane has also been reported for plant cells. In those cells, the spindle axis in a small proportion of the cells, those that have not attained sufficient height in the longitudinal direction of the cell column, is forced to rotate in order to ensure the proper separation of the chromatids; this diagonal spindle orientation is thought to necessitate the formation of an oblique division plane (30).

Further characterization of the nature of bacterial partitioning and the causal relationship between nucleoid movement and cell shape must await a genetic identification of centromere-like DNA sequences and a characterization of the forces acting on the chromosomal DNA.

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#### REFERENCES

1. Åkerlund, T., K. Nordström, and R. Bernander. 1993. Branched *Escherichia coli* cells. *Mol. Microbiol.* **10**:849–858.

2. Begg, K., and W. D. Donachie. 1991. Experiments on chromosome separation and positioning in *Escherichia coli*. *New Biol.* **3**:475–486.
3. Bi, E., and J. Lutkenhaus. 1991. FtsZ ring structure associated with division in *Escherichia coli*. *Nature (London)* **354**:161–164.
4. Bi, E., and J. Lutkenhaus. 1992. Isolation and characterization of *ftsZ* alleles that affect septal morphology. *J. Bacteriol.* **174**:5414–5423.
5. Bohrmann, B., W. Villiger, R. Johansen, and E. Kellenberger. 1991. Coralline shape of the bacterial nucleoid after cryofixation. *J. Bacteriol.* **173**:3149–3158.
6. Cavalier-Smith, T. 1987. Bacterial DNA segregation: its motors and positional control. *J. Theor. Biol.* **127**:361–372.
7. Chai, N.-C., and K. G. Lark. 1970. Cytological studies of deoxyribonucleic acid replication in *Escherichia coli* 15T<sup>-</sup>: replication at slow growth rates and after a shift-up into rich medium. *J. Bacteriol.* **104**:401–409.
8. Cohen, S. S., and H. D. Barner. 1954. Studies on unbalanced growth in *Escherichia coli*. *Proc. Natl. Acad. Sci. USA* **40**:885–893.
9. Cooper, S., and C. E. Helmstetter. 1968. Chromosome replication and the division cycle of *Escherichia coli* B/r. *J. Mol. Biol.* **31**:519–540.
10. Cooper, S., and M. Wuesthoff. 1971. Comment on the use of chloramphenicol to study the initiation of DNA synthesis. *J. Bacteriol.* **106**:709–711.
11. Dingman, C. W. 1974. Bidirectional chromosome replication: some topological considerations. *J. Theor. Biol.* **43**:187–195.
12. Donachie, W. D., and K. J. Begg. 1989. Chromosome partition in *Escherichia coli* requires postreplication protein synthesis. *J. Bacteriol.* **171**:5405–5409.
13. Drlica, K. 1991. Bacterial chromosome. *Encycl. Microbiol.* **1**:517–527.
14. Dürrenberger, M., M.-A. Bjornsti, T. Uetz, J. A. Hobot, and E. Kellenberger. 1988. The intracellular location of the histonelike protein HU in *Escherichia coli*. *J. Bacteriol.* **170**:4757–4768.
15. Gayda, R. C., M. C. Henk, and D. Leong. 1992. C-shaped cells caused by expression of an *ftsA* mutation in *Escherichia coli*. *J. Bacteriol.* **174**:5362–5370.
16. Gumpert, J. 1993. Cellular growth without a murein sacculus—the nucleoid-associated compartmentation concept, p. 453–463. *In* M. A. de Pedro et al. (ed.), *Bacterial growth and lysis*. Plenum Press, New York.
17. Higgins, C. F., C. J. Dorman, D. A. Stirling, L. Waddell, I. R. Booth, G. May, and E. Bremer. 1988. A physiological role for DNA supercoiling in the osmotic regulation of gene expression in *Salmonella typhimurium* and *Escherichia coli*. *Cell* **52**:569–584.
18. Hiraga, S. 1992. Chromosome and plasmid partition in *Escherichia coli*. *Annu. Rev. Biochem.* **61**:283–306.
19. Hiraga, S., T. Ogura, H. Niki, C. Ichinose, and H. Mori. 1990. Positioning of replicated chromosomes in *Escherichia coli*. *J. Bacteriol.* **172**:31–39.
20. Jacob, F., S. Brenner, and F. Cuzin. 1963. On the regulation of DNA replication in bacteria. *Cold Spring Harbor Symp. Quant. Biol.* **28**:329–348.
21. Kellenberger, E. 1990. Intracellular organization of the bacterial genome, p. 173–186. *In* K. Drlica and M. Riley (ed.), *The bacterial chromosome*. American Society for Microbiology, Washington, D.C.
22. Koch, A. L. 1985. How bacteria grow and divide in spite of internal hydrostatic pressure. *Can. J. Microbiol.* **31**:1071–1084.
23. Løbner-Olesen, A., and P. L. Kuempel. 1992. Chromosome partitioning in *Escherichia coli*. *J. Bacteriol.* **174**:7883–7889.
24. Marr, A. G., R. J. Harvey, and W. C. Trentini. 1966. Growth and division of *Escherichia coli*. *J. Bacteriol.* **91**:2388–2389.
25. Mason, D. J., and D. M. Powelson. 1958. Nuclear division as observed in live bacteria by a new technique. *J. Bacteriol.* **71**:474–479.
26. Mulder, E. 1991. Toporegulation of division in *Escherichia coli*. The nucleoid occlusion model. Ph.D. thesis. University of Amsterdam, Amsterdam, The Netherlands.
27. Mulder, E., and C. L. Woldringh. 1989. Actively replicating nucleoids influence positioning of division sites in *Escherichia coli* filaments forming cells lacking DNA. *J. Bacteriol.* **171**:4303–4314.



28. **Mulder, E., and C. L. Woldringh.** 1991. Autoradiographic analysis of diaminopimelic acid incorporation in filamentous cells of *Escherichia coli*: repression of peptidoglycan synthesis around the nucleoid. *J. Bacteriol.* **173**:4751–4756.
29. **Ogden, G. B., M. J. Pratt, and M. Schaechter.** 1988. The replicative origin of the *Escherichia coli* chromosome binds to cell membranes only when hemimethylated. *Cell* **54**:127–135.
30. **Oud, J. L., and N. Nanninga.** 1992. Cell shape, chromosome orientation and the position of the plane of division in *Vicia faba* root cells. *J. Cell Sci.* **103**:847–855.
31. **Pritchard, R. H., and A. Zaritsky.** 1970. Effect of thymine concentration on the replication velocity of DNA in a thymineless mutant of *Escherichia coli*. *Nature (London)* **226**:126–131.
32. **Robinow, C. F.** 1960. Outline of the visible organization of bacteria, p. 45–108. *In* J. Brachet and A. E. Mirsky (ed.), *The cell*, vol. IV. Academic Press, New York.
33. **Ryter, A., and A. Chang.** 1975. Localization of transcribing genes in the bacterial cell by means of high resolution autoradiography. *J. Mol. Biol.* **98**:797–810.
34. **Schaechter, M., O. Maaløe, and N. O. Kjeldgaard.** 1958. Dependency on medium and temperature of cell size and chemical composition during balanced growth of *Salmonella typhimurium*. *J. Gen. Microbiol.* **19**:592–606.
35. **Schmid, M. B.** 1990. A locus affecting nucleoid segregation in *Salmonella typhimurium*. *J. Bacteriol.* **172**:5416–5424.
36. **Taschner, P. E. M., J. G. J. Verest, and C. L. Woldringh.** 1987. Genetic and morphological characterization of *ftsB* and *nrdB* mutants of *Escherichia coli*. *J. Bacteriol.* **169**:19–25.
37. **Trueba, F. J., and C. L. Woldringh.** 1980. Changes in cell diameter during the division cycle of *Escherichia coli*. *J. Bacteriol.* **142**:869–878.
38. **Valkenburg, J. A. C., and C. L. Woldringh.** 1984. Phase separation between nucleoid and cytoplasm in *Escherichia coli* as defined by immersive refractometry. *J. Bacteriol.* **160**:1151–1157.
39. **Valkenburg, J. A. C., C. L. Woldringh, G. J. Brakenhoff, H. T. M. van der Voort, and N. Nanninga.** 1985. Confocal scanning light microscopy of the *Escherichia coli* nucleoid: comparison with phase-contrast and electron microscope images. *J. Bacteriol.* **161**:478–483.
40. **Van der Voort, H. T. M., H. J. Noordmans, J. M. Messerli, and A. W. M. Smeulders.** 1993. Physically realistic volume visualization for interactive analysis. *Bioimaging* **1**:20–29.
- 40a. **Van Helvoort, J. M. L. M., and C. L. Woldringh.** 1994. Nucleoid partitioning in *Escherichia coli* during steady state growth and upon recovery from chloramphenicol treatment. *Mol. Microbiol.* **13**:577–583.
41. **Woldringh, C. L.** 1976. Morphological analysis of nuclear separation and cell division during the life cycle of *Escherichia coli*. *J. Bacteriol.* **125**:248–257.
42. **Woldringh, C. L., M. A. de Jong, W. van den Berg, and L. Koppes.** 1977. Morphological analysis of the division cycle of two *Escherichia coli* substrains during slow growth. *J. Bacteriol.* **131**:270–279.
43. **Woldringh, C. L., N. B. Grover, R. F. Rosenberger, and A. Zaritsky.** 1980. Dimensional rearrangement of rod-shaped bacteria following nutritional shift-up. II. Experiments with *Escherichia coli* B/r. *J. Theor. Biol.* **86**:441–454.
44. **Woldringh, C. L., P. Huls, E. Pas, G. J. Brakenhoff, and N. Nanninga.** 1987. Topography of peptidoglycan synthesis during elongation and polar cap formation in a cell division mutant of *Escherichia coli* MC4100. *J. Gen. Microbiol.* **133**:575–586.
45. **Woldringh, C. L., E. Mulder, P. G. Huls, and N. Vischer.** 1991. Toporegulation of bacterial division according to the nucleoid occlusion model. *Res. Microbiol.* **142**:309–320.
46. **Woldringh, C. L., E. Mulder, J. A. C. Valkenburg, F. B. Wientjes, A. Zaritsky, and N. Nanninga.** 1990. Role of nucleoid in toporegulation of division. *Res. Microbiol.* **141**:39–49.
47. **Woldringh, C. L., and N. Nanninga.** 1985. Structure of nucleoid and cytoplasm in the intact cell, p. 161–197. *In* N. Nanninga (ed.), *Molecular cytology of Escherichia coli*. Academic Press, New York.
48. **Zaritsky, A.** 1975. On dimensional determination of rod-shaped bacteria. *J. Theor. Biol.* **54**:243–248.
49. **Zaritsky, A., N. B. Grover, J. Naaman, C. L. Woldringh, and R. F. Rosenberger.** 1982. Growth and form in bacteria. *Comments Mol. Cell. Biophys.* **1**:237–260.
50. **Zaritsky, A., and C. E. Helmstetter.** 1992. Rate maintenance of cell division in *Escherichia coli*: analysis of a simple nutritional downshift. *J. Bacteriol.* **174**:8152–8155.
51. **Zaritsky, A., and R. H. Pritchard.** 1973. Changes in cell size and shape associated with changes in the replication time of the chromosome of *Escherichia coli*. *J. Bacteriol.* **114**:824–837.
52. **Zaritsky, A., and C. L. Woldringh.** 1978. Chromosome replication rate and cell shape in *Escherichia coli*: lack of coupling. *J. Bacteriol.* **135**:581–587.
53. **Zaritsky, A., and C. L. Woldringh.** 1987. Similarity of dimensional rearrangements in *Escherichia coli* cells responding to thymine limitation (step-down) and to nutritional shift-up, p. 78–80. *In* M. Vicente et al. (ed.), *Molecular basis of bacterial growth and division*. EMBO Workshop, Segovia, Spain.
54. **Zyskind, J. W., A. L. Svitil, W. B. Stine, M. C. Biery, and D. W. Smith.** 1992. RecA protein of *Escherichia coli* and chromosome positioning. *Mol. Microbiol.* **6**:2525–2537.