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Cell Length, Nucleoid Separation, and Cell Division of Rod-Shaped and Spherical Cells of *Escherichia coli*

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By comparing the dimensions and DNA contents of normal rod-shaped *Escherichia coli* with those of mutants that grow as spheres or ellipsoids, we have determined that two parameters remain unchanged: the DNA/mass ratio and the average cell length (diameter, for spherical cells). In consequence, the average volumes and DNA contents of the spherical mutant cells are about four to six times greater than those of rod-shaped cells growing at a similar rate. In addition, it was found that cells of both rod and sphere forms had approximately the same number of nucleoids (as seen when the DNA was condensed after inhibition of protein synthesis). The nucleoids of the spherical cells therefore consist of four to six completed chromosomes each (polytene nucleoids). We suggest that the attainment of a minimum cell length is necessary for nucleoid separation after chromosome replication and that such a separation is itself a prerequisite for septum formation.

The average dimensions of cells of *Escherichia coli* and other bacteria change markedly with changes in growth rate (20), but it has been noted that two parameters appear to remain relatively constant throughout: the ratio of cell mass to number of copies of the chromosome origin at the time of initiation of DNA replication (M_i; 5) and the length of the rod-shaped cells at the time when they become committed to septation and cell division ($2L_u$; 6, 8–10). These correlations between overall cell dimensions and the onset of the events of the cell cycle were made by measuring cells from populations of normal rod-shaped cells growing at different rates in different media. The importance of these particular cellular dimensions in the control of DNA replication and division was confirmed by experiments in which DNA replication was uncoupled from cell growth (8) and by experiments in which the kinetics of replication and division were measured during changes in growth rate (6). We therefore asked what would happen if cells were to lose their characteristic rod shape and take some other form. Would cells of aberrant shape and size still retain the same initiation mass and cell length at commitment to septation, or would the behavior of such cells reveal some hitherto unsuspected constancy underlying the growth and division of cells of all shapes and sizes?

We chose to study certain temperature-sensitive mutants [*rodA*(Ts) and *pbpA*(Ts) mutants (12, 17, 22)] that grow as rods at 30°C and as cocci at 42°C. These mutants are able to grow and divide at near-normal rates in their coccal form and therefore provide an ideal test system. We found that the mutant cells did indeed behave as if the same two fundamental constants had remained unchanged; in consequence, the cells in their coccal form had an average diameter which was close to the length of rod-shaped cells growing at a similar rate and also had a similar DNA/mass ratio. As a result, the coccal cells were, on average, about four to six times greater in volume and DNA content than equivalent rod-shaped cells.

In addition, we examined the growth and form of a mutant with a reduced amount of RodA protein such that it grows at all temperatures in the form of ellipsoids, i.e., intermediate in shape between rods and cocci. In accord with our hypothesis, these cells also had the same average length as did the normal rod-shaped cells of the parental strain but had a 2.5-fold-greater average cell volume.

There are various models to explain how cells initiate DNA replication at a constant ratio of mass (or volume) to origins (5, 19, 21, 23), but it has always been difficult to understand how cell length could determine the time of onset of cell division. It has recently been suggested that the localization and timing of septum formation depends on the location of chromosomal DNA within the cell (11, 24). Specifically, we have proposed that septa form in the spaces between nucleoids and that the regular spatial separation of newly replicated sister chromosomes into nuclear bodies is the fundamental process underlying the regular timing and localization of cell division (11). If this suggestion were correct, then we would predict that the coccal forms of *E. coli* would have the same number of nuclear bodies per cell as the rods, because according to our model a septum should be formed between each separate nucleoid pair. We found in this study that although the DNA of coccal forms appears to be dispersed around the cell periphery when the cells are actively growing (as reported by others [12]), when the DNA is caused to condense by inhibiting protein synthesis (6, 13–15, 25), then we see only one or two nucleoids per cell, just as in the smaller rod-shaped cells. The nucleoids in the cocci are, however, much larger, in accord with the larger measured amount of DNA that they possess, and must each contain between four and six replicated chromosomes (i.e., chromosome termini). This otherwise surprising observation is nevertheless in accord with our suggestion that separation of replicated chromosomes into spatially distinct nuclear bodies takes place only when there is sufficient linear distance within the cell. This, in turn, would explain why the number of divisions per chromosome copy is dramatically reduced in cocci and why their average diameter should be close to the average length of corresponding rod-shaped cells.

MATERIALS AND METHODS

Bacterial strains. *E. coli* K-12 strain AT1325 *lip* is the parental strain of SP4505 (*lip*$^+$ *pbpA*$^+$ *rodA*$^+$), SP4500 [*lip*$^+$ *pbpA*(Ts)], SP5211 [*lip*$^+$ *rodA*(Ts)] (22), and KEN222. KEN222 was produced by cotransduction of *lip*$^+$ and the

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"sui" allele of *rodA* into strain AT1325. The "sui" allele of *rodA* (3) has recently been shown to be the result of a mutation from a glutamine codon to a stop triplet (amber) in the beginning of the *rodA* coding frame (A. Takasuga, K. J. Begg, W. D. Donachie, T. Ohta, and H. Matsuzawa, Abstr. 1988 Annu. Meet. Jpn. Biochem. Soc.). Because KEN222 carries an amber suppressor (*supE*) that substitutes glutamine at amber codons with low efficiency, this strain effectively produces a low level of normal RodA protein. In consequence, KEN222 grows as ellipsoidal cells at all temperatures (3). Strain B/rA was used as a reference strain to calculate the number of chromosomes per nucleoid in rod-shaped cells.

Culture conditions. Bacteria were grown in Oxoid nutrient broth no. 2 (unless otherwise stated) with vigorous shaking at an appropriate temperature (30, 37, or 42°C). All measurements were made on cultures of low density (less than an optical density at 540 nm [OD_{540}] of 0.2) that had been growing at low density for several generations.

Measurement of cell parameters. (i) Average cell mass. Samples of 0.2 ml were taken at intervals from exponentially growing cultures and added to 0.2-ml samples of 20% Formalin in saline. At the same time, the OD_{540} of a sample of unfixed cells was measured, using a 1-cm light path. Cell concentration was estimated on the fixed samples as particles per milliliter, using a model ZB Coulter Electronic Particle Counter. Average cell mass was taken to be proportional to OD/(particles per milliliter).

(ii) Median cell volume. A Coulter Channelyser was used to determine median cell volume, using the formaldehyde-fixed samples. Previous work has shown that median cell volume is proportional to mean cell volume for such samples (1).

(iii) Average cell length. Measurements were made on photographs of fixed cells on thin layers of agar, using phase contrast as previously described (7).

(iv) DNA content. Total DNA in samples of exponential cultures of known OD were estimated by fluorimetry after DAPI (4,6-diamidino-2-phenylindole) treatment as described by Legros and Kepes (16).

(v) Nucleoid staining. The method of S. Hiraga (10a and personal communication) was used for nucleoid staining. Chloramphenicol-treated cells (200 μg/ml for 10 min) were washed in saline, dried on glass slides, and fixed with methanol. Poly-L-lysine (5 μg/ml) was dried onto the surface of the smears, and then 10 μl of DAPI (5 μg/ml in saline) was added immediately before a cover slip was applied. Preparations were examined and photographed by using simultaneous phase contrast and fluorescence in a Leitz Metallux II microscope.

RESULTS

Comparison of cell lengths and volumes in rod-shaped and coccal forms. Figure 1 shows median cell volume in cultures of SP4504, SP4500, and SP5211 after a shift from 30 to 42°C. After an initial rise in median cell volume due to the temporary inhibition of cell division during the heat shock response (9), the rod-shaped strain (SP4504) reached a new, lower cell volume. (Other strains of *E. coli* K-12 behave similarly after a shift to 42°C [unpublished observations].) In contrast, both mutant strains showed a continuous increase in median cell volumes over the initial 3 h or so at 42°C. The final values for both mutants at 42°C (growing as cocci) were about 4.5 times higher than for the isogenic rod-shaped cells. To achieve balanced growth and constant cell parameters

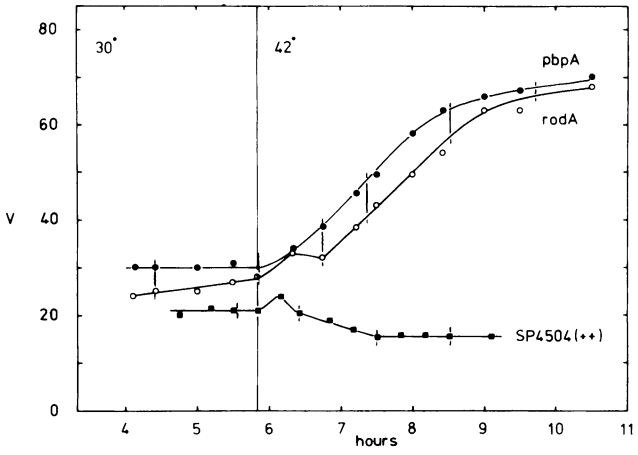

FIG. 1. Change in median cell volume accompanying change in cell shape from rod to coccus. Cultures of SP4500 [*pbpA*(Ts)], SP5211 [*rodA*(ts)], and the isogenic parental strain SP4504 (*rodA*$^+$ *pbpA*$^+$) in balanced growth at 30°C were shifted to 42°C (vertical line). Cultures were diluted periodically (thin vertical lines) 1/3 with prewarmed medium to keep the cell density low (less than OD of 0.2). The generation times at 30°C were 53 min for the mutants and 45 min for the parent. The final generation times at 42°C were 47.5, 42.5, and 38 min, respectively.

with these strains, it is necessary to maintain the cultures at low cell densities. In the experiment shown in Fig. 1, this was ensured by periodic dilution (1/3) with fresh, prewarmed medium. (If the OD is allowed to rise beyond about 0.2, then growth rate, and therefore cell size [5], falls progressively. This is seen in rich medium for both rod and coccal cells but is much more marked in the latter. We assume that this is the result of some environmental constraint, such as oxygen limitation, which is more pronounced in cells with a reduced surface-to-volume ratio [2].) Figure 2 shows the distribution of cell volumes and cell lengths in the *rodA*(Ts) and *rodA*$^+$ cultures. [The *pbpA*(Ts) measurements were similar to those for *rodA*(Ts); data not shown]. In the case of spherical cells, cell diameter was measured. However, most of the coccal cells show the beginnings of constriction, and the longer axis was measured in such cells. It can be seen that the distributions of cell lengths, unlike cell volumes, were almost identical. Table 1 gives the values for mean cell length and median cell volume for *rodA*, *pbpA*, and isogenic *rodA*$^+$ *pbpA*$^+$ cells growing at 42°C. Because these parameters vary strongly with growth rate (9), we show also the ratio of these values after correction for the observed differences in growth rates between the cultures. To do this, median cell volume was assumed to be proportional to $2^{(60/T)}$, where T is the doubling time, and mean cell length was taken to be proportional to $2^{(20/T)}$ (6). It is clear that cell length changed very little between rod-shaped and coccal forms but that cell volume changed greatly.

Because these experiments involved temperature shifts and because we had to make corrections in our expectations to allow for the differences in growth rate, we thought it important to look for another strain that had altered shape but was temperature independent. We found that strain KEN222 answered this description. This strain, which is also derived from strain AT1325 described above, carries an amber mutation near the beginning of the *rodA* coding sequence. Because this strain carries an amber suppressor (*supE*), this mutation is not lethal and the cells grow as ellipsoidal forms (3). This is almost certainly the result of the

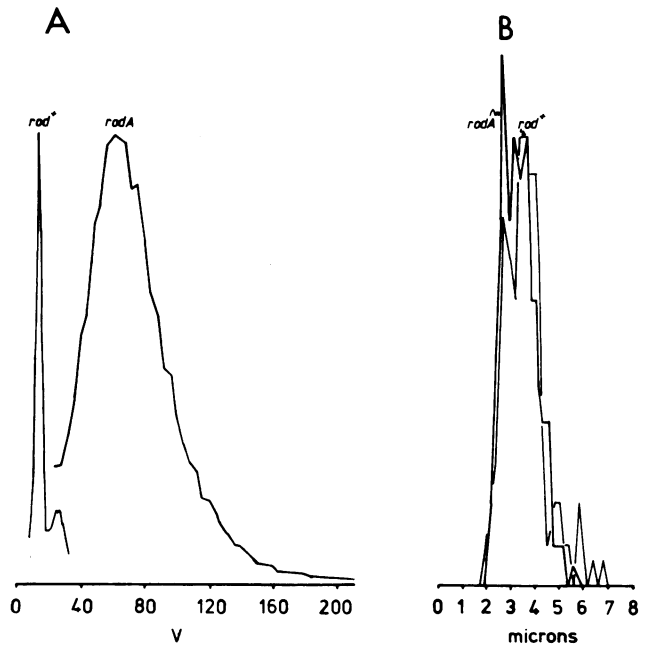

FIG. 2. Distribution of cell volumes (A) and lengths (B) of SP5211 [*rodA*(ts)] and SP4504 (*rodA*$^+$ *pbpA*$^+$) for cultures after prolonged growth at 42°C (see Fig. 1).

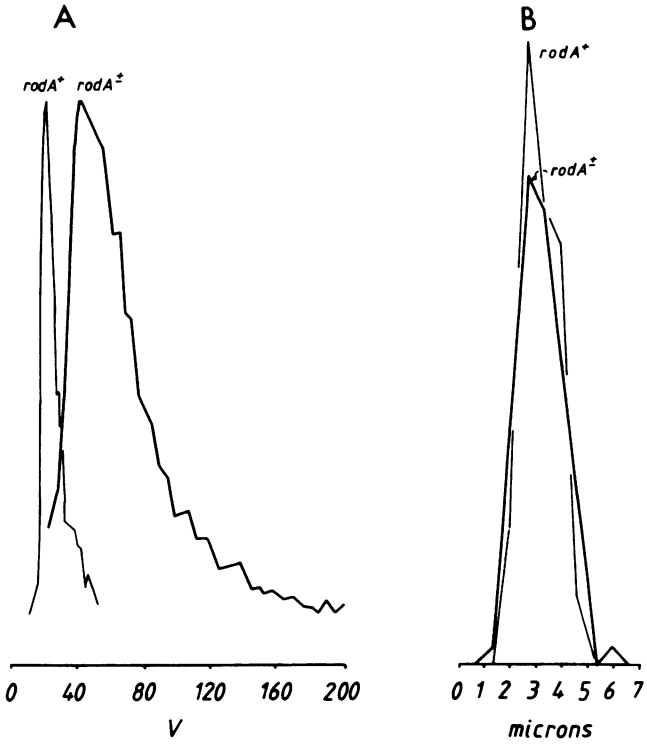

FIG. 3. Distribution of cell volumes (A) and lengths (B) for cultures of KEN222 [*rodA*(Am) *supE*; labeled *rodA*$^\pm$] and SP4504 (*rodA*$^+$) in balanced growth at 37°C.

production of a suboptimal level of wild-type RodA protein, because the action of the suppressing tRNA is to substitute the normal wild-type amino acid (glutamine) at the mutant codon (Takasuga et al., Abstr. 1988 Annu. Meet. Jpn. Biochem. Soc.). KEN222 and its isogenic *rodA*$^+$ strain (SP4504) grew at almost identical rates at 37°C and had identical length distributions (Fig. 3). The mutant cells, however, had a median volume 2.5-fold greater than that of the normal rods (Table 1). This result is in accord with the shape of the cells, which is intermediate between the shapes of normal rods and cocci (3).

DNA contents of rod and coccal forms. The method of Legros and Kepès (16) was found by us to give a linear proportionality between fluorescence and DNA content of whole-cell samples (data not shown). Accordingly, we used this method to estimate the relative DNA contents of SP4504, SP4500, and SP5211 during steady-state growth at 42°C. The DNA/mass (OD) ratios were closely similar in all strains despite the differences in cell size and shape (Table 2). The DNA/mass ratios in these strains were also closely similar to that for strain B/rA after correction for differences in growth rate (data not shown).

Nucleoids in *E. coli* B/r. Fluorescence microscopy of cells stained with DAPI (10a) or Hoechst 33258 (11) clearly shows the location of chromosomal DNA within cells. In preparations made from actively growing cells, the nucleoids are more or less dispersed (depending on strain and growth conditions) and have complicated shapes (see reference 13 for a review of nuclear morphology). When protein synthesis is inhibited, however, the DNA rapidly condenses into tightly packed bodies with well-defined shape and position in the cell (6, 13–15, 25). We find that 5 min in the presence of 200 μg of chloramphenicol per ml is sufficient to give good chromosome condensation (other methods of blocking protein synthesis, such as amino acid starvation, give the same effect but are less convenient to use). The cells are then stained in the usual way. Figure 4 shows B/r cells treated in this way.

We first set out to determine the correspondence between the number of such nucleoids per cell and the number of

TABLE 1. Lengths and volumes of rod-shaped and coccal cells[a]

Strain	Mutation	Temp (°C)	*T* (min)	*L*(obs) (μm)	*L*(calc) (μm)	*V*(obs)	*V*(calc)	*L*(obs)/*L*(calc)	*V*(obs)/*V*(calc)
SP4504		42	38	3.53		15.5			
SP4500	*pbpA*(Ts)	42	47.5	3.02	3.29	70	12.5	0.92	5.6
SP5211	*rodA*(Ts)	42	42.5	3.32	3.40	68	13.8	0.98	4.9
SP4504		37	45	3.17		23			
KEN222	*rodA*(Am) *supE*	37	43	3.17	3.20	60	24	1.01	2.5

[a] Cultures were grown and measured as described in the text. *T*, OD doubling time. *L*(obs), Mean cell length, measured on micrographs. *L*(calc), Mean cell length for a population of cells in balanced growth, calculated from the formula $L = (L_u/\mathrm{Tn2}) \cdot 2^{(20/T)}$ (6, 9), using the measured value for the rod-shaped cells of SP4504 at the corresponding temperature to estimate L_u (unit length [6, 7, 9]). *V*(obs), Median cell volume measured electronically (arbitrary units). *V*(calc), Mean volume for rod-shaped cells at the same growth rate, calculated from the formula $V = (2V_u/\ln 2) \cdot 2^{(60/T)}$ (5, 6, 9), using the measured value for SP4504 cells at the same temperature to estimate V_u (unit volume [6, 9]).

TABLE 2. DNA contents and cell volumes of rod-shaped and coccal cells[a]

Strain	Mutation	Temp (°C)	T (min)	V(obs)	M(obs)	V(obs)/ V(calc)	DNA/ cell	DNA/ OD
SP4504		30	42	13	2.8		17	13
SP4504		42	23	12	2.4		16	14
SP4504		42	23	13	3.0		18	14
SP4500	*pbpA*(Ts)	30	37	19	4.2	1.3	26	14
SP4500	*pbpA*(Ts)	42	30	49	8.1	5.7	58	12
SP4500	*pbpA*(Ts)	42	30	46	10.1	5.4	61	13
SP5211	*rodA*(Ts)	30	37	17	3.5	1.1	24	14
SP5211	*rodA*Ts)	42	26	40	6.6	3.8	50	12
SP5211	*rodA*(Ts)	42	26	46	10.8	4.4	54	12

[a] Cultures were grown in L broth and median cell volumes were estimated by using a Coulter Channelyser different from that used to obtain the results shown in Table 1, the absolute values are not directly comparable. M(obs), Ratio of OD_{600} to cell number per milliliter (arbitrary units). DNA was estimated by the fluorescence method (16), and the values for DNA/cell and DNA/OD are given in arbitrary units.

TABLE 3. Relationship between nucleoids per cell and calculated numbers of chromosome termini per cell for strain B/r in different growth media[a]

Medium	T (min)	V(obs)	V(calc)	N(obs)	Ter(calc)	N'(obs)
L broth	26	31	(31)	2.03 (134)	1.8	1.83 (117)
Super	33	25	22	1.79 (142)	1.6	1.66 (132)
Glucose	49	13	14	1.38 (178)	1.4	1.35 (176)
Glycerol	69	9	11	1.29 (160)	1.25	1.27 (158)

[a] Growth media were L broth or VB salts supplemented with glycerol, glucose, or glucose plus Casamino Acids (Difco Laboratories) plus tryptophan plus adenine plus cytosine plus uracil (Super), as previously described (8). *T*, OD doubling time at 37°C. *V*(obs), Median cell volume (arbitrary units) measured electronically. *V*(calc), Mean cell volume calculated as in Table 1, using the observed median cell volume in L broth as a reference point to give $V_u = 4.3$ U for this strain and temperature. *N*(obs), Mean number of nucleoids per cell (see Materials and Methods). Ter(calc), Mean number of chromosome termini (i.e., completely replicated chromosomes) per cell for an asynchronous population of cells, calculated according to the formula $\text{Ter} = 2^{(22/T)}$. This uses the value of 22 min estimated as the interval between completion of chromosome replication and completion of cell division for this strain and these growth conditions (4). Because the theoretical calculation of Ter assumes that every cell in the population divides exactly 22 min after termination, whereas the real population of cells includes a proportion that have failed to divide on time (i.e., contain three or four nucleoids), we have calculated a second estimate, *N'*, of the average number of nucleoids per cell in that proportion of the population which has either one or two nucleoids per cell. This correction makes the greatest difference to the faster-growing cells, which appear to contain a higher proportion of late-dividing cells. The number of cells in each sample is shown in parentheses.

chromosomes per cell. To do this, we used strain B/rA because this strain has been extensively studied and the relationship between growth rate and the state of chromosome replication is well known (4). Cells were grown for several generations at 37°C in each of four media (Table 3) until balanced growth (defined as constant median cell volume and doubling time) was attained. Chloramphenicol (200 μg/ml) was added, and the cultures were shifted to 42°C. This treatment immediately blocks further cell division (unpublished observations) and causes rapid condensation of the dispersed chromosomal DNA into discrete nucleoids, consisting of DNA tightly packed around a core of ribosomes (13–15, 25). After 10 min, the cells were fixed and stained with DAPI (see Materials and Methods). The average number of nucleoids per cell was easily estimated by the use of mixed fluorescence and phase microscopy as described by Hiraga (see Materials and Methods). There was a good correspondence between the observed average number of nucleoids per cell and the calculated average number of chromosomal termini (i.e., completely replicated chromosomes) per cell.

Nucleoids in SP strains in rod and coccal forms. The rod-shaped cells of strain SP4504 growing with a generation time of 38 min in Oxoid broth at 42°C (Table 1) had an average of 1.2 nucleoids per cell. (In the same medium at

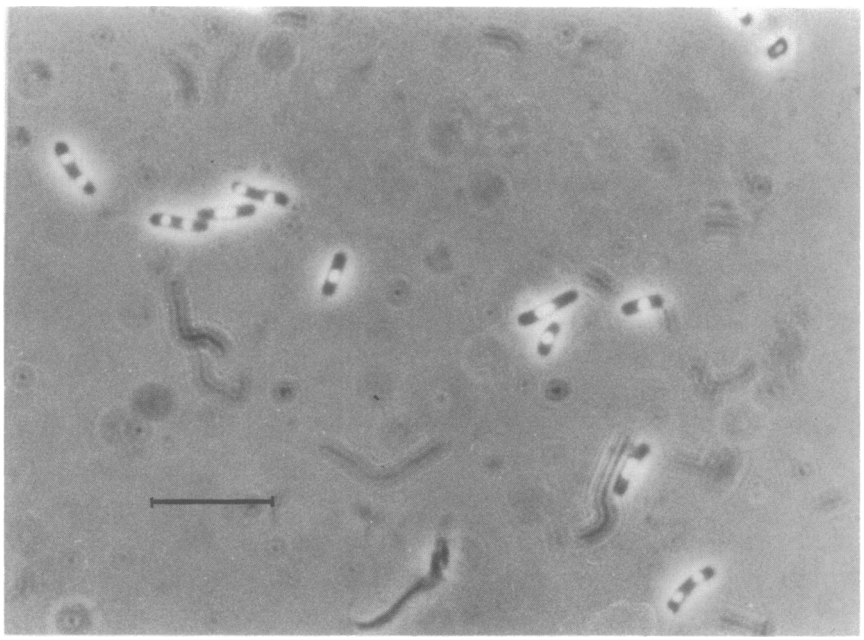

FIG. 4. Nucleoids in *E. coli* B/r. Cells were treated with chloramphenicol for 10 min at 42°C to bring about chromosome condensation in the absence of cell division and then fixed and stained with DAPI (see Materials and Methods). They were photographed by using a mixture of phase-contrast illumination (to show the cells) and fluorescence (to show the nucleoids). Bar, 10 μm.

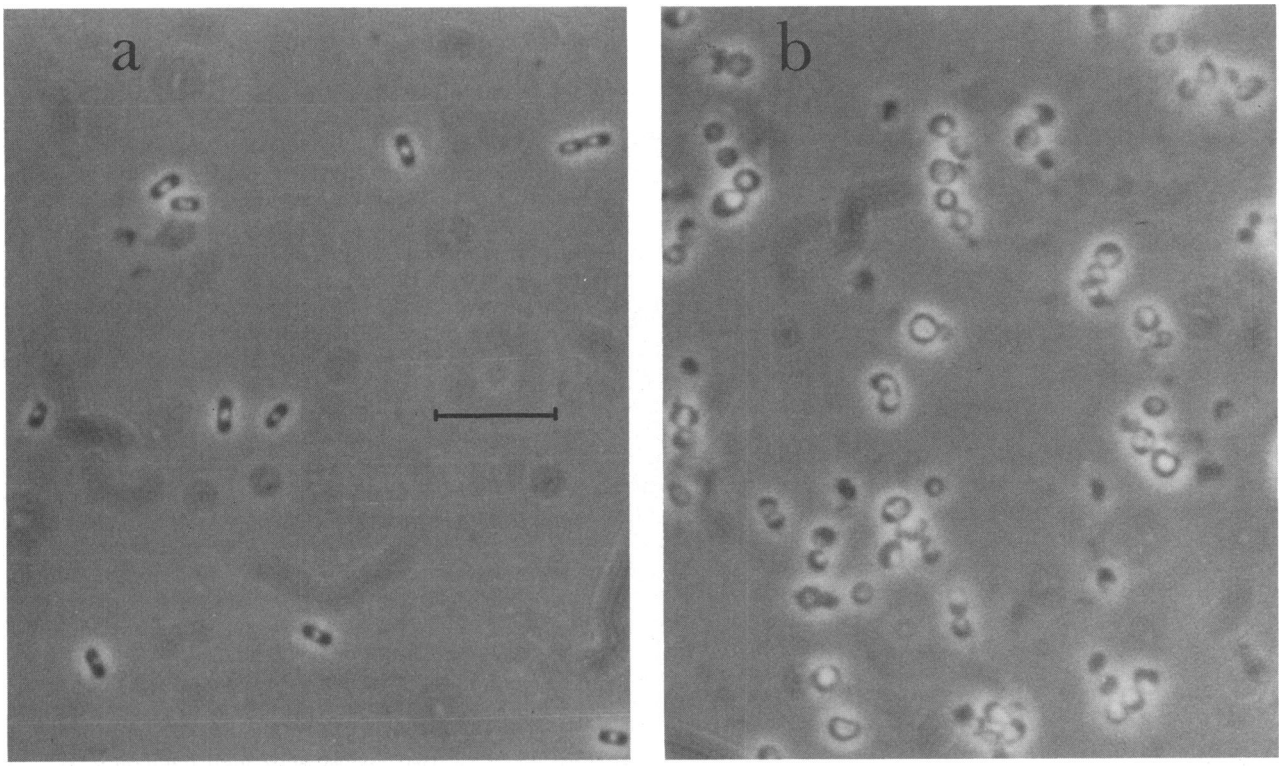

FIG. 5. Nucleoids in strain SP4504 (a; rods) and SP4500 (b; cocci) from cultures maintained at 42°C. Bar, 10 μm.

37°C, this strain had a generation time of 56 min. If *D*, the interval between termination and division, was 22 min as in B/rA, then the calculated average number of chromosome termini per cell in this medium would be 1.3. Therefore, this strain, like B/rA, also has a number of nucleoids that is close to the number of chromosome termini per cell. For our present purpose, it is not necessary to be more precise than that. This purpose is to compare nucleoid number in rod-shaped and coccal forms of isogenic strains that are growing in the same medium at similar rates at the same temperature and have similar length distributions but very different volumes and DNA contents.)

When coccal cells of the mutant strains grown at 42°C were stained with DAPI without prior chloramphenicol treatment, DNA was seen to be dispersed around the periphery of the cells. This result is in accord with earlier reports on DNA distribution as seen by electron microscopy on thin sections of such cells (12). However, after 5 min in the presence of chloramphenicol, the DNA condensed into large nucleoids (Fig. 5b). The average number of nucleoids per cell was about 1.3 for SP4500 cells. This number is hard to estimate very precisely (mainly for geometrical reasons), but it is clearly quite different from the predicted number of chromosome termini per cell calculated from the observed DNA content of these cells (4.8 to 7.2 termini per cell). (This result may be contrasted with that observed when rod-shaped cells of normal length are compared with cells blocked only in cell division. The blocked cells grow into long rods [filaments] with increased volumes [as great as or much greater than the volumes of the cocci studied in this investigation] but with the predicted number of separated nucleoids for their volumes and DNA contents.)

The ellipsoidal cells of strain KEN222 also had a low average number of nucleoids per cell. This number has not been determined precisely but is much less than 2.

DISCUSSION

Certain mutations in the *rodA* or *pbpA* gene cause *E. coli* cells to assume a near-spherical shape rather than the normal rod form during growth at 42°C (22). These coccal mutants are able to grow and divide at nearly the same rate as isogenic rod forms, provided they are grown at low density in rich medium. In rod-shaped cells, a septum is formed for every pair of completed chromosomes (4), but the measurements reported here show that in the coccal form there is only one division for every four to six chromosomes. Thus, a normal rule for cell division appears to be broken in cocci. However, further investigation of the coccal mutants has revealed other constancies that are common to dividing cells of both rod and coccal forms. We find that the average length distribution of cells is largely unchanged after conversion from rod to coccus. This implies that the lengths of cells at the time of onset of septation must also be similar. This finding therefore extends the previous discovery (8, 10) that rod-shaped cells of a wide range of volumes and growing at a wide range of rates initiate septation at much the same length.

We have recently suggested (as the result of a study of division in a mutant that was able to replicate but not separate its chromosomes [11]) that septa are unable to form within a certain minimum distance of a nucleoid. According to this idea, septa could form only after the separation of sister nucleoids to their new positions in the centers of the future sister cells. Only then is there the required space for septation (Fig. 6). To extend this idea to coccal cells, we now

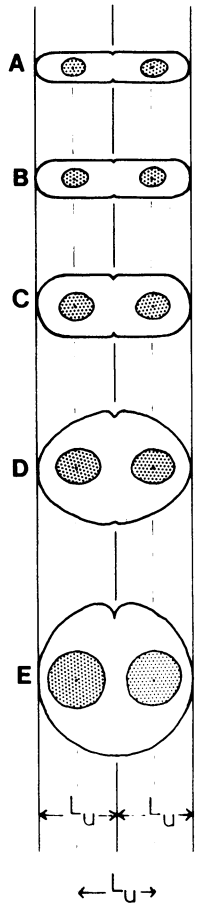

FIG. 6. Illustration of the dimensions of cells and the numbers and locations of nucleoids in cells of different shapes at the time of nucleoid partition and commitment to septation, according to the model proposed here. (A to C) Normal cells (rods) from populations in balanced growth at three generation times: infinitely long (A), 60 min (B), and 20 min (C). (D) Cell growing as an ellipsoid (e.g., RodA$^{\pm}$). E, Cell growing as a perfect coccus (e.g., RodA^{-} or PBP2^{-}). The scale is given in terms of a hypothetical unit length (L_u), which may be defined either as the minimum length of a newborn cell (in a population approaching zero growth rate [6, 8]) or as the minimum distance between nucleoid centers. Relative cell volumes (from top to bottom): 1.0, 1.6, 3.7, 9.7, and 18.8.

propose that nucleoid separation requires both the replication of the chromosome terminus and the achievement of a minimum linear distance within the cell. In rod-shaped cells in which the total condensed nuclear mass occupies about one-third of the cell length (unpublished measurements) and occupies most of the width of the segment of the cell in which it lies, the required space for separation of sister nucleoids is provided by growth to a certain length (further evidence in favor of this hypothesis will be presented elsewhere). Let us now consider the geometry of a (hypothetically perfect) spherical cell containing a single unreplicated chromosome. The measurements that we have made allow us to assume that such a cell will initiate new rounds of chromosome replication whenever the ratio of cell mass (proportional to volume) to number of chromosome origins reaches the same value (M_i [5]) as in rod-shaped cells. However, the geometry of a growing sphere ensures that when replication has been completed, there is no space for nucleoid separation (Fig. 6). Assuming that the ratio of length to width for rod-shaped cells is about 3 (on average), then we can see that a spherical cell will need to reach a volume of about six times that of a rod in order to have the same linear dimension and to do so before nucleoid separation can take place. However, the coupling between cell volume and initiation of chromosome replication (5) will also ensure that a cell of this volume has six times as many chromosomes as a rod of the same overall length. There is nevertheless space within this cell for the separation of only two nucleoids. Our observations on stained cells with condensed DNA suggest that two nucleoids are indeed formed at about this length but that each must contain at least three chromosomes. Only at this stage can cell division be completed.

Our measurements on the ellipsoidal cell population of the KEN222 strain show that these cells also have the same distribution of cell lengths as do isogenic rod-shaped cell populations growing at the same rate and that their cell volumes are intermediate between those of rods and coccal forms. The sizes of their nucleoids are also intermediate between those of the two extreme forms, but the average numbers per cell are similar in all strains with the same growth rates. These measurements are an important confirmation of the generality of our conclusions.

We would like to emphasize that we do not think that the above model is yet completely satisfactory. For example, it is clear from Fig. 5 that coccal cells may start to constrict before there are two visibly separated nucleoids. We must therefore say that it is the completion of septation which is occluded by the presence of an undivided nucleoid rather than the initiation of a septum. Furthermore, uncondensed chromosomal DNA is arranged around the periphery of cocci (perhaps because it is held there by the liaison between the cell membrane and the nascent polypeptide chains of transmembrane proteins). Thus, our model of septal occlusion must be assumed to refer to the position of some sort of nuclear attachment point, the existence of which can be inferred only when the other links between DNA and cell membrane have been loosened by the inhibition of protein synthesis. Nevertheless, we think that the model is worth presenting at this time because it does provide a different way of looking at the cell cycle and because it does offer at least a partial explanation for a wide variety of observations (i.e., the localization and timing of division in cells of all sizes and shapes and in mutants with defects in chromosome partition). We are also aware that the model raises important questions about the mechanism of nucleoid separation itself. These will be addressed in a separate publication (K. J. Begg and W. D. Donachie, J. Bacteriol., in press).

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