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Conformation and Segregation of Nucleoids Accompanying Cell Length Extension After Completion of a Single Round of DNA Replication in Germinated and Outgrowing *Bacillus subtilis* Spores

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When germinating spores of the temperature-sensitive DNA initiation mutant of *Bacillus subtilis* TsB134 are shifted to the restrictive temperature at a time such that just one or two rounds of replication are accomplished, the completed, nonreplicating nucleoids that form eventually adopt a doublet conformation. This conformation has now been observed after fixation by glutaraldehyde or osmium tetroxide, as well as by Formalin as found previously. The doublet was observed in media of different degrees of richness and under both light and electron microscopes. Electron micrographs of serial sections through the doublet were consistent with its formation by the gradual pulling apart of a single mass of DNA into two lobes. A systematic study was made of the effect of the time of shifting from the permissive to the restrictive temperature and of the restrictive temperature used on the number of nucleoids segregating within the outgrowing rod. It was established that the doublet nucleoid behaved as a single unit in replication control and segregation in both rich and poor media. Measurement of the relative position of the two segregating nucleoids within the outgrowing rod after completion of just one round of replication yielded quantitative information on the segregation and cell length extension processes. Segregation was accompanied by cell length extension at approximately equal rates on both sides of each nucleoid. Furthermore, the data were consistent with an exponential increase in such an extension with time over the early and major portion of the period studied, but it was not possible to rule out other models of length extension.

Germinated and outgrowing spores of temperature-sensitive *dna* initiation mutants of *Bacillus subtilis* provide a very useful system for studying certain aspects of the replication and division cycle in bacteria (3, 11). When the spores are germinated at the permissive temperature and shifted at the appropriate time to the restrictive temperature, only a single round of DNA replication is initiated. The round proceeds rapidly to completion and is followed by the formation of a division septum between the two segregating daughter nucleoids.

An unexpected finding was obtained by staining Formalin-fixed preparations with acridine orange: each of the segregating nucleoids eventually adopted a doublet conformation (12). The components of the doublet remained together during prolonged cell extension, and there was no evidence to suggest that the doublet represented more than one chromosome. Recently it was reported that Formalin is unable to fix the nucleoid of exponentially growing *Streptococcus faecalis* in its dispersed configuration, but that glutaraldehyde can (6). In this paper, we have extended the range of fixatives, growth media, and methods of observation, including electron microscopy, to confirm the validity of the doublet conformation finding. Also, further experiments were performed to establish more firmly that the doublet nucleoid behaves as a single unit in replication control and segregation. Finally, the rate of length extension of the outgrowing rod and the relative positions of the nucleoids within it have been examined for information on the length extension and segregation processes.

MATERIALS AND METHODS

Bacterial strains, spore preparation, and growth conditions. *B. subtilis* 168 *trpC2 dnaB134*(Ts) *thyA thyB*, obtained from N. Mendelson and referred to as TsB134, was used in all experiments. Spores were prepared as described previously (2) except for the use of 30°C as the growth temperature. Spores (10^8/ml) were germinated at 34°C either in minimal medium (3) plus thymine (20 μg/ml) or in Penassay broth (Difco Laboratories) plus thymine (20 μg/ml). Temperature

shifts were accomplished by pouring a germinated culture into an equal volume of the same medium at 45 or 47°C.

Glutaraldehyde fixation, staining, and light microscopy. Glutaraldehyde (70%; stored in vials under nitrogen) was diluted to 10% in water just before each experiment. At appropriate times samples were added to an equal volume of diluted glutaraldehyde and incubated with shaking at 34°C for 1 h. The fixed samples were stored in the refrigerator. Acridine orange staining and fluorescence microscopy were carried out exactly as described before (12).

Electron microscopy. Samples were prepared for electron microscopy by the method of Whitehouse et al. (20), with modifications as described previously (4). The overall procedure uses prefixation with osmium tetroxide. Sections of the embedded material were cut on an LKB Ultramicrotome III with a diamond knife. Ribbons up to 40 sections long and approximately 30 nm thick were transferred to copper grids. After further staining with lead citrate, the sections were observed in a Philips 201 electron microscope. Approximately 19 sections were needed to capture the entire 0.64-μm-diameter cell. Three-dimensional cardboard models of nucleoids were constructed from prints at a magnification of ×65,000.

Measurement of nucleoid positions and cell lengths. Color transparencies (Kodak Ektachrome 200) were projected onto a screen to give an overall magnification of ×10,000. Measurements were made directly on the projected image with dividers, from the tip of one pole of a cell to the central region of the closer nucleoid. For a doublet nucleoid, the center was taken as halfway between the individual components. The nucleoids and the outline of the cells were very clear and better than indicated in the black- and -white photographs (e.g., Fig. 4). For each sample, taken at a fixed time, 100 "full" cells (i.e., 200 "half" cells) were measured.

RESULTS

Doublet nucleoid under a variety of conditions. The findings of Daneo-Moore et al. (6) raise the possibility that Formalin fixes DNA fairly slowly. Therefore the well-defined doublet nucleoids observed previously (12) could have resulted from the condensation of a more dispersed structure during slow fixing. The faster fixative, glutaraldehyde, was therefore used at a final concentration of 5%. When germinated and outgrowing TsB134 spores were allowed to undergo a single round of replication after a shift from 34 to 47°C at 80 min in minimal medium, doublet nucleoids were observed at as high a frequency (>80%) as before. In fact, the nucleoids stained better with acridine orange after glutaraldehyde fixation (some examples of doublet nucleoids fixed with glutaraldehyde can be seen in Fig. 4), and advantage was taken of this to analyze the nucleoid segregation process in some detail (see below). Also, in a small proportion (~5%) of the nucleoids fixed with glutaraldehyde, one of the components of the doublet appeared to dissociate further into smaller components.

Experiments along similar lines were performed with a much richer medium, Penassay broth, and doublets were again found at a high frequency (>80%). The frequency of doublet nucleoids, however, decreased drastically (to less than 20%) whenever the time of the temperature shift was such that three successive rounds of replication could be initiated and completed.

Electron microscope studies. Spores of TsB134 were germinated at 34°C in minimal medium and shifted to 47°C at 80 min. At 180 min, sufficient time to allow clear resolution of the doublet conformation in most of the nucleoids by light microscopy, the culture was fixed with osmium tetroxide and processed for electron microscopy as described above. Figure 1a shows a section through a rod that developed from a single spore. Note that each half cell (or daughter cell; see Fig. 4) shows two areas of highly condensed DNA lying very close to one another. There is also a large mesosome present on each side of the central division septum. Serial sections of 22 nucleoids were examined (examples are shown in Fig. 1b–d) and, in some cases, three-dimensional models were constructed (Fig. 2). In 14 cases, the nucleoid consisted of two major masses of DNA, which confirmed the earlier finding by light microscopy. In eight of these cases, the two masses were connected by a thick (Fig. 1b, 1c, 2a) or thin (Fig. 1d, 2b, 2c) bridge of DNA. In three cases, the masses came very close to one another but no connection was visible (Fig. 2d). In the other three cases, the masses of DNA were further apart (Fig. 1e), but there may well have been thin connecting strands of DNA that were not visible. Most of the remaining eight nucleoids examined showed a single major mass of DNA. It should be noted that, in some cases, additional minor masses of DNA were connected to the larger ones (Fig. 1d, 2c).

The types of nucleoid conformation seen under the electron microscope are consistent with a single mass of DNA resolving into a doublet structure by gradually pulling apart into two regions of roughly equal size. The fact that a connecting bridge was frequently seen when the two masses were well separated is consistent with the idea that the nucleoid is composed of a single chromosome, but does not prove it. Osmium tetroxide, as used in the electron microscopy, appears to be a slower fixative than glutaraldehyde (6), but there is no reason to believe that its use would give rise to artifactual connecting bridges.

Doublet nucleoid as a single unit of replication and segregation. It is clear that the completed nucleoids obtained after one or two rounds of replication following spore germination readily adopt a doublet conformation. It was reported

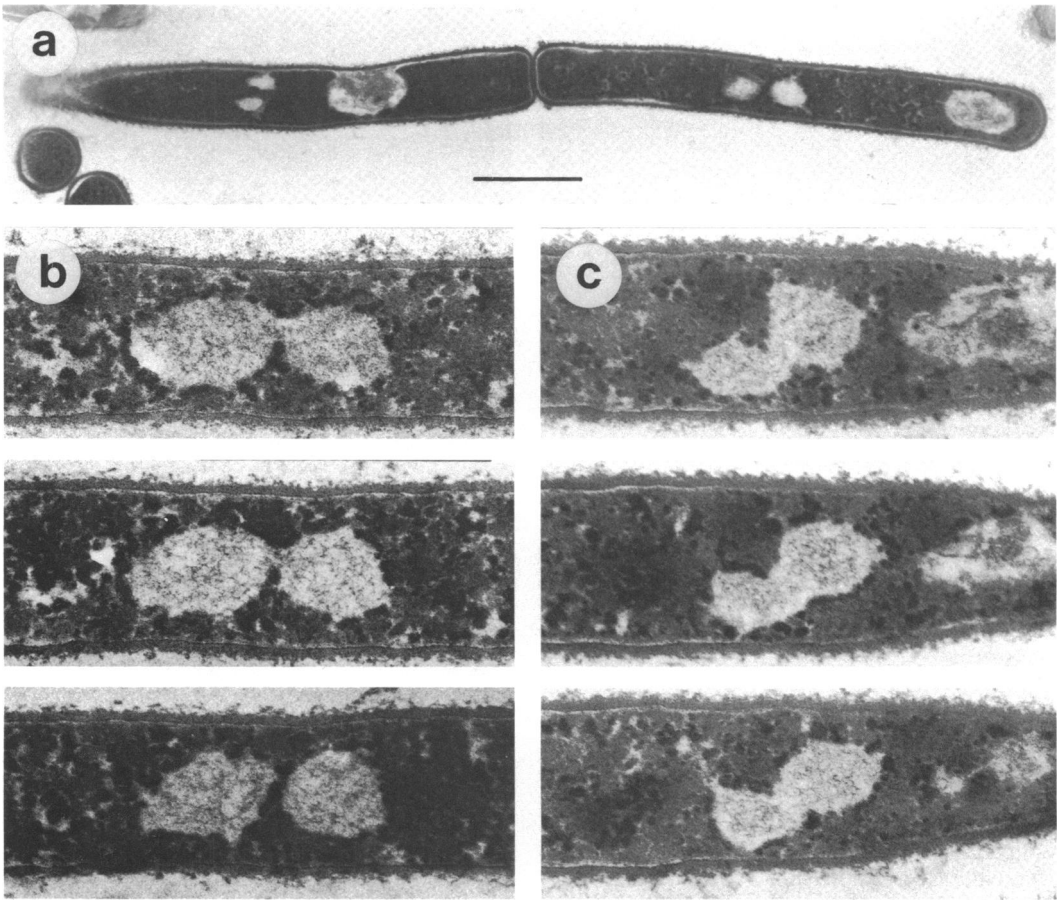
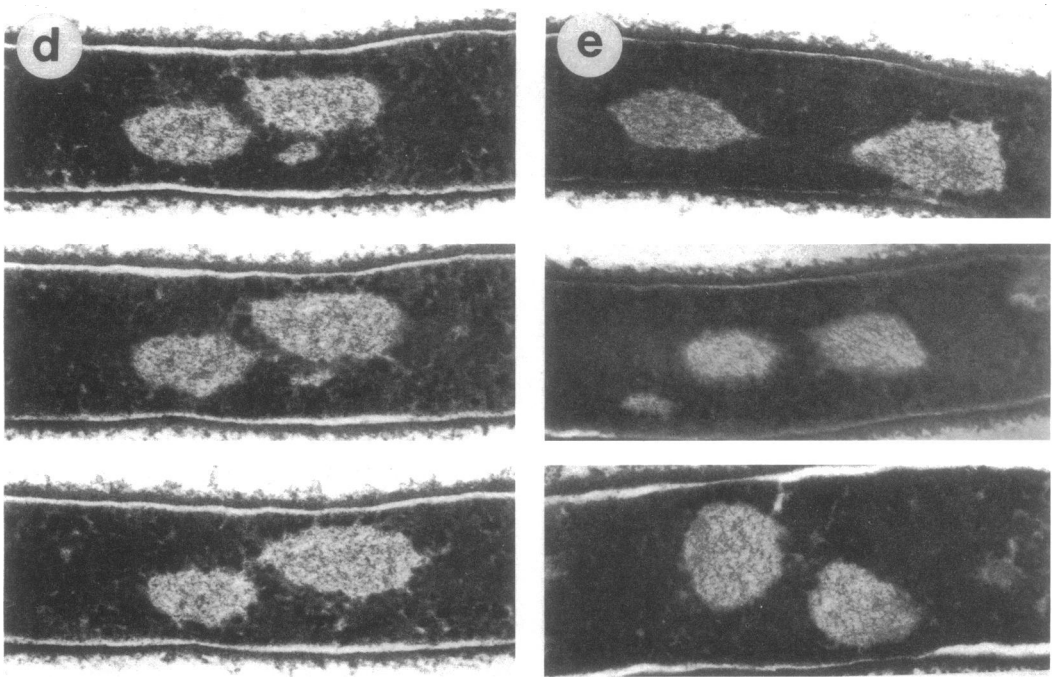


FIG. 1. Electron micrographs showing typical examples of doublet nucleoids. TsB134 spores were germinated in minimal medium at 34°C and shifted to 47°C at 80 min. (a) Most of an outgrowing rod (two half cells) that developed from a single spore. A central septum is clearly visible, as well as a nucleoid and mesosome in each half cell. The nucleoid in the half cell on the right consisted of two masses of DNA with a discrete connection between them. This is clear from (b), in which serial sections in the region of the connecting bridge of this nucleoid are shown. Examination of the nucleoid in the left half cell of (a) in serial section showed that the two separated areas of DNA did not extend very far but soon merged into a single mass. (c and d) Three serial sections, in each case, of other doublet nucleoids in the vicinity of a connecting bridge. (e) Thin sections from three separate doublet nucleoids that did not have a visible connecting bridge. Bar = 1 μm in each case.

recently that nucleoids isolated from exponentially growing *Escherichia coli* consist of two masses of DNA, each representing a partially replicated chromosome and connected by protein (18). Furthermore, evidence has been found for the presence of two identical chromosomes in swarmer cells of *Caulobacter crescentus*. When progeny daughter cells are formed after DNA replication, two chromosomes segregate into each daughter cell (9).

In earlier experiments, we could not obtain any evidence to suggest that the components of the completed doublet observed here represent individual chromosomes (12). The components of the doublet would not partition into separate cells, even when DNA-less cells were forming, and the doublet structure was not formed if the nucleoid was allowed to proceed partway into a subsequent round of replication. Also, the segregation pattern of the nucleoids (number and conformation), under conditions in which both the restrictive temperature and the time of the shift were varied, was consistent with the doublet nucleoid representing a single chromosome. This latter type of experiment was extended in a more systematic approach to include media of different degrees of richness so as to establish more definitively that the doublet nucleoid behaves as a single unit in replication control and segregation.



When TsB134 spores were germinated in the minimal medium at 34°C and shifted at 80 min to 47°C to allow initiation of a single round of replication, two nucleoids (usually doublet) eventually segregated (12; Table 1). The one-nucleoid class resulted from spores that did not initiate replication by 80 min. Second initiations occurred when the temperature shift took place after 80 min. When the shift was made at 100 or 120 min, the new major class contained four nucleoids (Table 1). The three-nucleoid class remained small. Thus, within the rod growing out from a single spore, both first-generation nucleoids initiated a second round of replication at approximately the same time, and each gave rise to two new nucleoids. It is significant that the same result was achieved when Penassay broth was used. This richer medium induces early dichotomous replication after the germination of *B. subtilis* spores (15). This means that the second initiations, in the case of the 80-min shift, would have occurred well before the first

TABLE 1. Nucleoid segregation patterns following initiation and completion of one or two rounds of replication after germination of TsB134 spores

Medium	Restrictive temp	Time of temp shift (min)	% of rods with indicated no. of nucleoids[a]				
			1	2	3	4	Other
Minimal medium	47°C	80	16	81	2	0	1
		100	2	77	5	15	1
		120	1	23	9	66	1
Penassay broth	47°C	40	42	55	3	0	0
		60	6	86	6	2	0
		80	5	24	7	62	2
Minimal medium	45°C	80	7	73	18	2	0
Penassay broth	45°C	60	2	75	22	1	0

[a] The nucleoid segregation patterns were scored on samples fixed at 200 min (minimal medium) or 150 min (Penassay broth). No distinction was made between nucleoids that were doublet and those that were not. At least 100 rods were scored in each case.

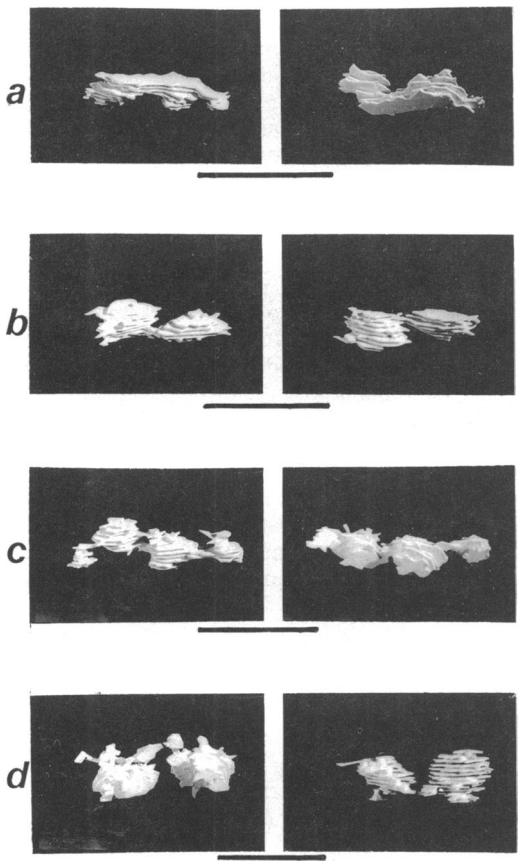


FIG. 2. Three-dimensional models (two views of each) of four doublet nucleoids, constructed from electron micrographs of serial sections of the type shown in Fig. 1. (b) Model of the nucleoid shown in Fig. 1d. (d) The two masses of DNA shown come very close to one another, but a connection is not visible. Bar = 1 μm in each case.

rounds terminated (Fig. 3a). The three-nucleoid class was again small. Thus, a first-generation nucleoid origin rarely initiates a second round of replication significantly ahead of its sister when it is allowed to replicate at the permissive temperature.

Whereas 47°C effectively blocks second initiations in germinating TsB134 spores, 45°C allows some to occur (11). Evidently, at 45°C there is a low probability that the *dnaB* product will be in its active conformation for sufficient time to successfully effect some initiations within the time available. In these circumstances the first-generation nucleoids might be expected to behave independently of one another in the timing of subsequent initiations. This would account for the emergence of the three-nucleoid class as a prominent one when the shift is made to 45°C at 80 min in the minimal medium (Table 1). Even under these conditions, it appears that the components of the doublet always replicated together as a unit, i.e., one of the components of the doublet did not replicate independently of the other to give rise to triplet nucleoids. That the same pattern of segregation, with the three-nucleoid class becoming prominent, was found when a shift to 45°C was made in Penassay broth is of major significance. This means that even under circumstances that promote dichotomous replication, the origin region of each partially replicated nucleoid behaves as a unit independent of its sister origin. And, again, each successful initiation gives rise to an additional segregating unit. It is easy to explain these findings if each completed nucleoid were originally a single chromosome and re-initiation occurred on only one of the two replicated arms of the partially completed chromosome, as shown in Fig. 3b.

Nucleoid segregation and cell length extension. It is generally considered that nucleoid segregation is achieved by surface extension between membrane or cell wall sites to which the nucleoids are attached (17). It was therefore of considerable interest to establish the relationship between the processes of nucleoid segregation and cell length extension. Mendelson (13) took advantage of the outgrowing TsB134 spore system to obtain valuable quantitative data in this area with an autoradiographic approach. However, the experimental conditions did not provide synchrony in the timing of these processes, nor was it clear what was happening with the initiation and completion of chromosome

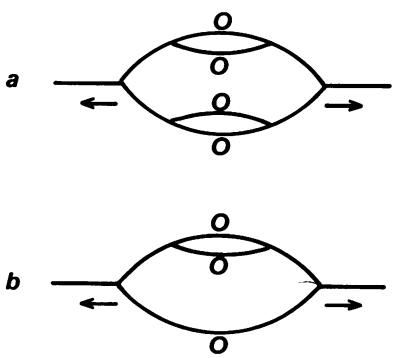


FIG. 3. Diagrammatic representation of the origin region of reinitiated chromosomes. The arrows show the direction of movement of the two primary replication forks. (a) Both arms of the partially replicated chromosome have reinitiated to give a total of four origins (symmetric reinitiation). (b) Only one arm has reinitiated to yield a total of three origins (asymmetric reinitiation).

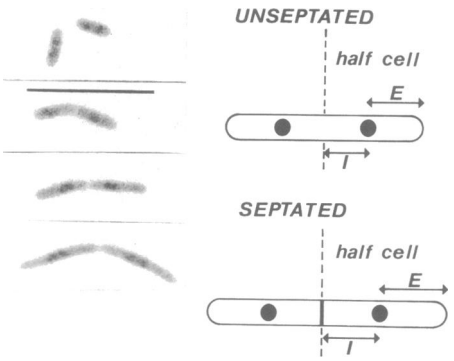


FIG. 4. Nucleoid segregation and cell extension after completion of a single round of replication following spore germination. TsB134 spores were germinated in minimal medium at 34°C and shifted to 47°C at 80 min. The left panel shows selected examples of glutaraldehyde-fixed and acridine orange-stained rods as they septated and elongated over the 120- to 170-min period. Measurement of the parameters *E* and *I* (right panel) were made on images projected from the colored slides, as described in the text. This procedure afforded optimal precision and definition of nucleoids and cells. In the drawing, the nucleoid (doublet or single) is shown as a single unit. See the text for the definition of nucleoid positions.

replication. We extended Mendelson's experiment by examining the segregation and extension processes in a better-defined and synchronous situation. Nucleoids were identified by acridine orange staining.

Figure 4 (left panel) shows examples of germinated and outgrowing TsB134 spores elongating after the completion of a single round of replication in minimal medium at 47°C. The system is highly synchronous (11). The segregation of daughter nucleoids became obvious at 120 min, i.e., soon after the round would have terminated. Acridine orange staining allowed measurement of the segregation at a relatively early stage (Fig. 4, uppermost two rods). The nucleoids continued to move apart, and a central division septum appeared, on the average, at 140 min. Each nucleoid gradually adopted a doublet conformation, and at 170 min DNA-less cells began to form (15% of the population). The relative positions of the nucleoids and septum within the extending rod or cell were measured over the 120- to 170-min period, i.e., after completion of the round and before the onset of large amounts of DNA-less cell formation. Cells in which chromosome replication had not initiated and which contained only one nucleoid were not analyzed.

The measurements are expressed in terms of two parameters, *E* and *I*. *E* is the distance between the pole of the cell and the center of the nucleoid. *I*, for an unseptated cell, is half the distance between the nucleoids; for a septated cell, *I* is the distance between the septum and the nucleoid. The technique of measurement (see above) has a resolution of ±0.1 μm.

The average values of *E* and *I* for each of the six samples (10-min intervals) are shown in Fig. 5. The standard error was ≤0.05 μm (200 half cells measured in each case). For any sample, the average value of *E* was consistently higher than that for *I*, and the difference remained constant. When *E* was plotted against *I*, the points fit a straight line (Fig. 5). The line of best fit obtained from linear regression had the equation $E = I + 0.28$ μm. Hence, $\Delta E/\Delta I = 1$, or any increase in *E* is accompanied by an equal in-

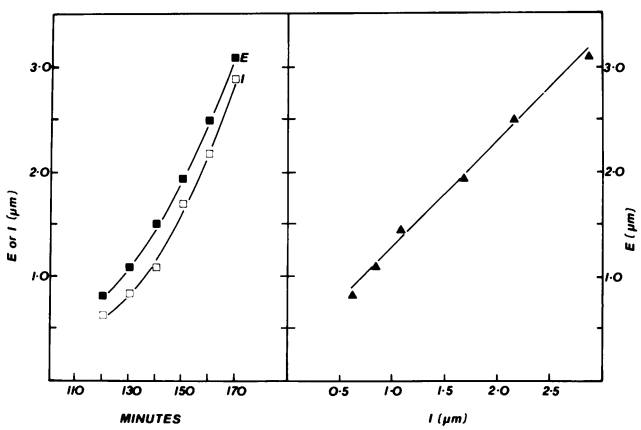


FIG. 5. Nucleoid position in the elongating half cell after completion of a single round of replication following spore germination. The left panel shows the average values of *E* and *I* (see the legend to Fig. 4 for experimental details) measured on samples taken at various times over the 120- to 170-min period. The right panel describes the relationship between these average values of *E* and *I*. The line of best fit was obtained from linear regression analysis.

crease in I. Thus, growth occurs in the region between the nucleoids and in the region between the nucleoid and the pole of the cell. It is likely that the constant of 0.28 μm in the equation represents the contribution of the hemispherical polar cap (see below). In such a situation, $E + I - 0.28$ μm would represent the length of the actively growing region of the half cell. Direct examination of the position of the nucleoid within the half cells of the 160-min sample of Fig. 5 showed that in 89% of the cases it was located within the central one-third of this growing region. A plot of $E + I - 0.28$ μm versus time is shown in Fig. 6. The length of this actively growing region appeared to increase in an exponential manner until 150 min, which covered the period of formation of the central division septum; it was then no longer exponential. The optical density of cultures germinated under identical conditions followed a similar time course (Fig. 6). It seems that as long as the turbidity of the culture increases exponentially, so does the length of the growing region of the half cell. However, the present data cannot rule out the possibility that the rate of cell length extension is linear, with a doubling in rate at about 140 min.

In the experiments just described, rods containing DNA-less cells (significant in the 170-min sample) were excluded from the analysis. The experiments were repeated at half the initial spore concentration, and the same results, except for a slightly lower rate of cell length extension, were obtained. The value for $E - I$ was 0.24 μm, and there was no significant difference if the DNA-less cells were included in or excluded from the analysis.

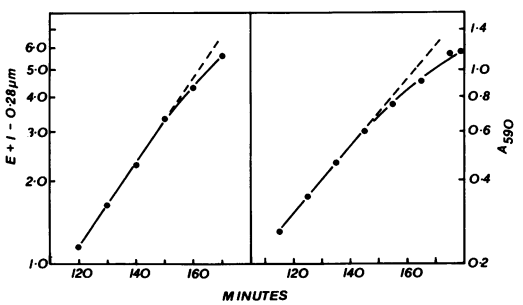


FIG. 6. Cell length extension after completion of a single round of replication following spore germination. See the legend to Fig. 4 for experimental conditions. The left panel shows the increase in length of the actively growing region of the half cell, $E + I - 0.28$ μm, calculated from the data shown in Fig. 5. The right panel shows the increase in absorbancy at 590 nm (A_{590}) of a separate culture of germinated spores growing out under conditions identical to those used for collecting the data shown in Fig. 5.

DISCUSSION

The completed *B. subtilis* nucleoid, obtained after one or two rounds of DNA replication following spore germination and outgrowth, adopts a doublet conformation, as reported previously (12). We have now shown that the doublet is formed in media of different degrees of richness and after fixation in various ways (Formalin, glutaraldehyde, or osmium tetroxide) and that it can be observed under both light and electron microscopes. The electron micrographs show that the two masses of DNA which constitute the doublet are formed by the gradual pulling apart of a single mass into two lobes.

Regardless of whether the individual lobes (or components) represent separate chromosomes, it is clear that the nucleoid as a whole behaves as a single unit in replication control and segregation. That is, the doublet nucleoid, after initiation and completion of a further round of replication, gives rise to two new doublets. However, it is certainly easier to explain the generation of three nucleoids in Penassay broth after a shift to 45°C after the initiation of just one round of replication if the original spore nucleoid (which also can exist as a doublet) were a single chromosome, as has already been suggested (12). Such conditions would give rise to an asymmetrically reinitiated chromosome (see Fig. 3b) which, upon the completion of all initiated rounds, would yield three chromosomes. The asymmetrical reinitiation of chromosome replication in *E. coli* and *B. subtilis* has been reported before (19, 22).

Daneo-Moore et al. (6) showed that the dispersed configuration of the exponential-phase nucleoid of *S. faecalis* can be preserved by fixation with glutaraldehyde. In the present experiments with *B. subtilis*, glutaraldehyde fixation yielded a relatively condensed nucleoid, but under the conditions used here, it was complete and nonreplicating and was probably analogous to the stationary-phase situation in *S. faecalis* in which more highly condensed nucleoids are seen. Certainly when the *B. subtilis* nucleoid, which normally forms a doublet in its completed state, was allowed to proceed partway into a further round of replication, it was considerably more diffuse, even after fixation with Formalin (12). It is possible that the doublet nucleoid results from the condensation of a more diffuse structure. Because it is completed and nonreplicating, it may be more prone to condensation.

We quantified the processes of cell growth and nucleoid segregation in *B. subtilis*. Our findings are in agreement with, and extend those previously reported by, Mendelson (13, 14). The system used here was much better defined than that used previously, and measurement was restricted to situations in which DNA-less cell

formation was minimal. Although the data relate to conditions under which rounds of replication have already been completed, they have considerable bearing on the situation in exponentially growing cells. It is clear that segregation of the two completed nucleoids, which proceeds during outgrowth of the spore, is accompanied by cell length extension on both sides of each nucleoid. This is consistent with the model of nuclear segregation proposed by Sargent (16) but not with those of others (5, 10). The average position of each nucleoid was slightly off center within each half cell that formed (Fig. 7). The distance from the outside pole to the center of the nucleoid (frequently doublet) was greater than that from the nucleoid to the central septum (or the center of the unseptated full cell) by 0.24 to 0.28 μm (two experiments). This difference may represent the contribution of the hemispherical polar cap (Fig. 1a). From electron micrographs of sections of 180-min samples, it appeared that there would have been little rounding off of the central septum in the samples used for length measurements. Although the pole of the cell is subject to turnover (8), it does not appear to be responsible for any cell elongation (1, 7). If this is so, then the present data show that the nucleoid tends to occupy a central position in the actively growing region of the half cell (Fig. 7, unshaded area).

In Sargent's model for chromosome segregation (16), it is proposed that after the termination of replication each daughter nucleoid undergoes a discrete "jump" to a new position at which growth zones develop and then extend at a constant or linear rate (assuming a constant cell diameter). The data obtained in this study indicated nothing about this proposed jump in the earliest stage of segregation. However, the data were consistent with an exponential increase in length of the region of active growth (Fig. 7), at least until the half cell reached 3.6 μm in length.

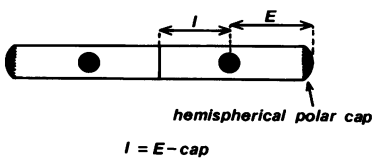


FIG. 7. Diagrammatic representation of a rod containing two half cells separated by a central septum. The hemispherical polar caps are shaded; such caps have not yet started to form at the site of the central septum. The nucleoid is positioned at the midpoint of the unshaded region of each half cell so that $I = E -$ cap. The unshaded region is considered to represent the actively growing region of the rod as it develops from a germinated spore under the conditions described in the legend to Fig. 4.

Thus, there could be a continuous doubling of sites of cell extension. The fall-off from exponential length extension after 150 min (Fig. 5) was possibly the result of the very large cell mass/DNA ratio that was attained. Although the data presented here cannot rule out the possibility that cell length extension is linear, with a doubling in linear rate at 140 min, such a situation would mean that the change in rate would occur at least 20 min after chromosome segregation.

One final comment about nucleoid segregation and chromosome attachment to the membrane is pertinent. Specifically, the interaction of the chromosome origin with the membrane has been proposed as a likely means by which chromosome segregation is achieved (17). It has been shown recently that such interaction is rapidly and significantly reduced when *dnaB* mutants are shifted to the restrictive temperature (21). However, it is clear from the present work that ordered segregation of nucleoids proceeds under such conditions. It would be of considerable interest to know the exact status of the chromosome origin-membrane interaction under the conditions of spore outgrowth used here and where the *dnaB* product is inactive in the initiation of rounds of replication.

ACKNOWLEDGMENTS

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LITERATURE CITED

1. **Archibald, A. R., and H. E. Coapes.** 1976. Bacteriophage SP50 as a marker for cell wall growth in *Bacillus subtilis*. J. Bacteriol. **125**:1195–1206.
2. **Callister, H., and R. G. Wake.** 1974. Completed chromosomes in thymine-requiring *Bacillus subtilis* spores. J. Bacteriol. **120**:579–582.
3. **Callister, H., and R. G. Wake.** 1977. Completion of the replication and division cycle in temperature-sensitive DNA initiation mutants of *Bacillus subtilis* 168 at the nonpermissive temperature. J. Mol. Biol. **117**:71–84.
4. **Callister, H., and R. G. Wake.** 1981. Characterization and mapping of temperature-sensitive division initiation mutations of *Bacillus subtilis*. J. Bacteriol. **145**:1042–1051.
5. **Clark, D. J.** 1968. The regulation of DNA replication and cell division in *E. coli* B/r. Cold Spring Harbor Symp. Quant. Biol. **33**:823–838.
6. **Daneo-Moore, L., D. Dicker, and M. Higgins.** 1980. Structure of the nucleoid in cells of *Streptococcus faecalis*. J. Bacteriol. **141**:928–937.
7. **De Chastellier, C., C. Frehel, and A. Ryter.** 1975. Cell wall growth of *Bacillus megaterium*: cytoplasmic radioactivity after pulse-labeling with tritiated diaminopimelic acid. J. Bacteriol. **123**:1197–1207.
8. **Fan, D. P., B. E. Beckman, and M. M. Beckman.** 1974. Cell wall turnover at the hemispherical caps of *Bacillus subtilis*. J. Bacteriol. **117**:1330–1334.
9. **Iba, H., and Y. Okada.** 1980. Chromosome segregation in an asymmetrically dividing bacterium, *Caulobacter crescentus*. J. Mol. Biol. **139**:733–739.
10. **Jacob, F., S. Brenner, and F. Cuzin.** 1963. On the regulation of DNA replication in bacteria. Cold Spring Harbor Symp. Quant. Biol. **28**:329–347.

11. **McGinness, T., and R. G. Wake.** 1979. Division septation in the absence of chromosome termination of *Bacillus subtilis*. J. Mol. Biol. **134**:251–264.
12. **McGinness, T., and R. G. Wake.** 1979. Completed *Bacillus subtilis* nucleoid as a doublet structure. J. Bacteriol. **140**:730–733.
13. **Mendelson, N. H.** 1968. Can defective segregation prevent initiation? Cold Spring Harbor Symp. Quant. Biol. **33**:313–316.
14. **Mendelson, N. H.** 1968. Nuclear segregation without DNA replication in *Bacillus subtilis*. Biochim. Biophys. Acta **190**:132–138.
15. **Oishi, M., A. Oishi, and N. Sueoka.** 1966. Location of genetic loci of soluble RNA on *Bacillus subtilis* chromosome. Proc. Natl. Acad. Sci. U.S.A. **55**:1095–1103.
16. **Sargent, M. G.** 1974. Nuclear segregation in *Bacillus subtilis*. Nature (London) **250**:252–254.
17. **Sargent, M. G.** 1979. Surface extension and the cell cycle in prokaryotes. Adv. Microbiol. **18**:105–176.
18. **Van Ness, J., and D. E. Pettijohn.** 1979. A simple autoradiographic method for investigating long range chromosome substructure: size and number of DNA molecules in isolated nucleoids of *Escherichia coli*. J. Mol. Biol. **129**:501–508.
19. **Wake, R. G.** 1972. Visualization of reinitiated chromosomes in *Bacillus subtilis*. J. Mol. Biol. **68**:501–509.
20. **Whitehouse, R. L. S., J. C. Benichou, and A. Ryter.** 1977. Procedure for the longitudinal orientation of rodshaped bacteria and the production of a high cell density of procaryotic and eucaryotic cells in thin sections for electron microscopy. Biol. Cell. **30**:155–158.
21. **Winston, S., and N. Sueoka.** 1980. DNA–membrane association is necessary for initiation of chromosomal and plasmid replication in *Bacillus subtilis*. Proc. Natl. Acad. Sci. U.S.A. **77**:2834–2838.
22. **Worcel, A.** 1970. Induction of chromosome re-initiations in a thermosensitive DNA mutant of *Escherichia coli*. J. Mol. Biol. **52**:371–386.