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Cytological Studies of Deoxyribonucleic Acid Replication in *Escherichia coli* 15T⁻: Replication at Slow Growth Rates and After a Shift-Up into Rich Medium

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We examined the gross nuclear morphology of *Escherichia coli* 15T⁻ grown in different media with doubling times ranging from 22 to 270 min. In slowly growing cells, deoxyribonucleic acid synthesis was measured by autoradiography and shown to occur with greatest probability during the first two-thirds of the division cycle. In such cells, segregation occurred later, at the end of the division cycle rather than at the end of deoxyribonucleic acid replication. Nuclear regions in L-broth cells (22-min doubling time) cannot correspond to separate chromosomes but probably represent regions of replication activity. Segregation of template nucleotide strands was measured after a shift-up from proline M9 or glucose M9 media into L broth. A model is presented to account for the pattern of segregation observed.

In bacteria, the amount of deoxyribonucleic acid (DNA) per cell and the rate at which it is synthesized varies with changes in the growth conditions. At rapid growth rates in rich medium, bacteria contain several chromosomes per cell (5, 7, 12, 19) and each replicating chromosome may contain several replication forks (1, 2, 8, 22). At slow growth rates in poor medium, each cell contains only one replicating chromosome and its replication occurs during a fraction of the cell division cycle (1, 2, 8, 11).

The products of replication, the daughter chromosomes, eventually are segregated into separate cells (4, 12, 14). This separation occurs under all conditions of growth even in cells in which several chromosomes are replicating simultaneously (12, 14). Attempts to understand the mechanism of segregation have utilized cytological studies of bacteria which grow in chains (6, 16, 18). In such studies the position of radioactive DNA in multinucleate chains has been noted and related to random or nonrandom segregation mechanisms.

In the present study, we used autoradiography in the electron microscope to obtain better resolution for the location of radioactive DNA. We examined slowly growing cultures of *Escherichia coli* 15T⁻ to determine the period in the division cycle during which DNA is synthesized and during which it segregates. We also studied the segregation of labeled DNA under conditions in which radioactive bacteria are allowed to become multinucleate after a shift-up into rich medium.

From the distribution of radioactivity in such bacteria, it is possible to observe the order of segregation which is occurring.

MATERIALS AND METHODS

Bacterial strain and growth. *E. coli* 15T⁻ (555-7) was described in detail previously (11). It was grown in M9 medium (10) with different carbon sources (11), supplemented with the required amino acids, arginine (100 μg/ml), methionine (20 μg/ml), and tryptophan (20 μg/ml). Thymine was present at a concentration of 4 μg/ml for normal growth and 2 μg/ml during labeling of the cells with radioactive thymine.

The carbon sources were used in the M9 medium in the following concentrations: glucose (0.4%), succinate (0.9%), aspartate (0.1%), proline (0.2%), and acetate (0.5%).

L broth consisted of Difco Yeast Extract (0.1%), Difco Tryptone (0.5%), NaCl (0.5%), and glucose (0.2%), adjusted to *p*H 7.

All cultures were transferred from one medium to another by collecting cells on membrane filters (Bac-T-Flex B-6, Schleicher & Schuell Co., Keene, N.H.), washing with warm (37 C) M9 buffer, and suspending in the desired medium.

For radioactive labeling, cells grown in different media were washed free of thymine and labeled with radioactive ^{3}H-methyl-labeled thymine (New England

Nuclear Corp., Boston, Mass.) for different periods corresponding to a constant fraction of the generation time. After labeling, cells were washed and transferred into medium free of radioactive materials for further culturing. Samples were taken after zero, one, or two generations of growth measured as a one-, two-, or fourfold increase in cell number. Cell counts were made with a Coulter Counter, model A, by using a 30-μm orifice, a current setting of 3, and a threshold setting which varied from 5 to 10 according to the cell size.

Cells were fixed immediately after sampling, by addition of OsO_4 to 0.1%, and were transferred immediately to M9 buffer (no carbon source) containing 0.1% OsO_4. After 10 min, the cells were collected by filtration and suspended in 1% Tryptone containing 0.5% NaCl. At this step, the titer of the cells was adjusted to between 4×10^8 and 5×10^8/ml; 0.1 ml of this sample was spread on collodion-coated agar plates [described previously as Kellenberger's agar filtration method (17)] and fixed with OsO_4 vapor for 10 min. The agar was then cut into pieces, and the film with the bacteria was floated onto a water surface and picked up on electron microscope grids.

Ilford L-4 emulsion (Ilford Limited, Ilford Essex, London, England) in gel form was melted and diluted 1:3. This was spread over the grids for autoradiography (3). After a sufficient exposure (usually 2 or 3 months), grids were developed in the physical developer of Caro (3). The autoradiographs were examined and pictures were taken with an RCA EMU-2D model electron microscope at 50 kv.

RESULTS

Gross nuclear morphology at different growth rates. The generation time of *E. coli* 15T$^-$ varies with different carbon sources. The growth rate and DNA content of cells growing in six different media are given in Table 1. At doubling times of 120 min or longer, a gap in DNA synthesis is observed, whereas at doubling times of less than 40 min, chromosomes contain more than one replication fork (1, 2).

The nuclear morphology of these cells is quite different (Fig. 1). Small, slowly growing cells, in aspartate, proline, or acetate medium, all have a similar morphology. Their nucleus appears as a single electron-transparent body which occupies the central region of the cell. Differences in the electron density of this single nucleus are apparent in longer cells and indicate that the nuclear material is not uniformly distributed.

All of these cells contain a single replicating chromosome, which replicates during only a portion of the division cycle. Under conditions of more rapid growth, in succinate or glucose medium, cells with two distinct nuclear bodies can be observed. Finally, in L broth, very large cells which divide every 22 min have a complex nuclear pattern characterized by two bi-lobed nuclear regions which are sufficiently separate to appear as four distinct regions.

TABLE 1. *Growth rate, travel time, and DNA content of E. coli 15T$^-$ growing in different media*

Medium	Doubling time	Travel time[a]	DNA content (10^{-14} g/cell)
	min	*min*	
L broth	22	40[b]	2.6[e]
Glucose-M9	40	40[c]	1.4[d]
Succinate-M9	70	55[c]	1.1[d]
Aspartate-M9	120	80[c]	0.7[d]
Proline-M9	180	120[d]	0.51[d]
Acetate-M9	270	180[d]	0.55[d]

[a] The time necessary to replicate the chromosome from origin to terminus is the travel time (1).

[b] The travel time in L broth has not been measured directly. However, there is evidence to indicate that the 15T$^-$ chromosome cannot replicate faster than a travel time of 40 min (1).

[c] Taken from Bird and Lark (1, 2).

[d] Taken from C. Lark (11).

[e] Taken from K. G. Lark et al. (14).

Replication and distribution of DNA within slowly growing cells. When cultures of 15T$^-$ are grown in aspartate medium, cells divide every 2 hr. Under these conditions DNA is synthesized during 80 min of the division cycle (1, 2). We labeled aspartate cultures for 9 min with ^{3}H-thymidine and subsequently examined the distribution of radioactivity within the cell population. This was done by preparing cells for autoradiography in an electron microscope and tabulating the cells according to size and whether or not they were radioactive. A typical field of bacteria can be seen in Fig. 4A. The results from this experiment are shown in Fig. 2. Some nonradioactive cells can be seen among cells of all size categories. However, the highest frequencies of such cells are observed among large cells and very small cells, i.e., among cells prior to or just after division. This is seen more clearly in Fig. 3, where the fraction of cells without label is presented for each size class. Two conclusions can be drawn. (i) In most cells DNA synthesis ceases as cells become larger and resumes shortly after division. (ii) Some cells (30 to 40%) are synthesizing DNA late in the division cycle, i.e., when they are very large.

Similar results (not presented here) were obtained with cultures of 15T$^-$ grown in proline medium.

When aspartate cultures are allowed to incorporate ^{3}H-thymine and are then grown for one or two generations in nonradioactive medium, the labeled chromosomes will be segregated into daughter cells. Figure 4 presents radioautographs of aspartate cells immediately after incorporating ^{3}H-thymine (A) and after two generations of growth in nonradioactive medium (B).

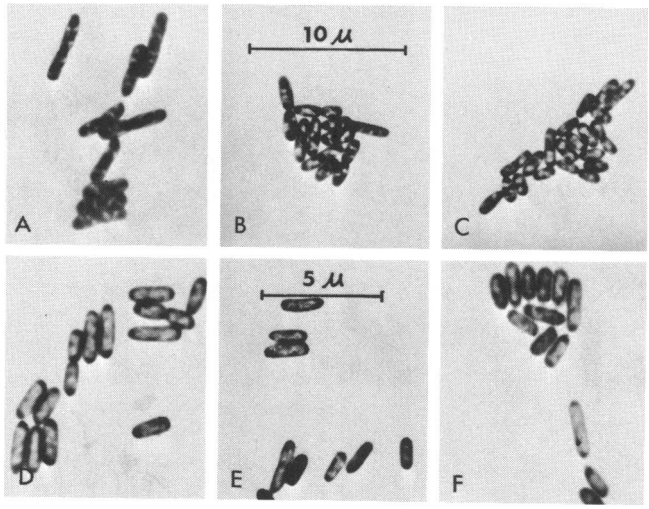

FIG. 1. *Nuclear morphology of Escherichia coli 15T⁻ growing in different media. A, L broth; B, glucose-M9; C, succinate-M9; D, asparate-M9; E, proline-M9; and F, acetate-M9. Cells from exponential cultures were fixed and mounted on collodion membranes. The nuclear regions appear as electron-transparent areas.*

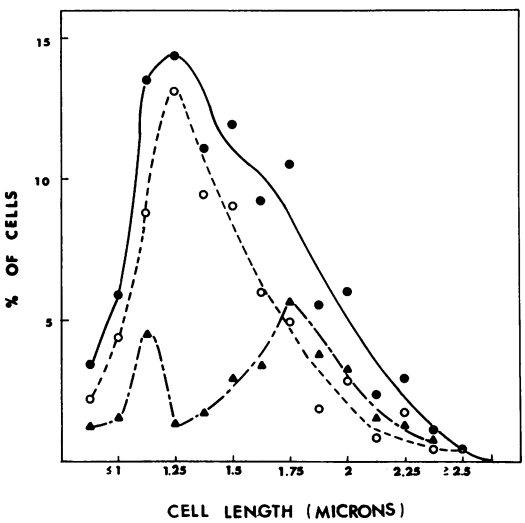

FIG. 2. *Size distribution of radioactive pulse-labeled aspartate cells. Symbols: ●, size distribution of the total cell population; ○, size distribution of radioactive cells; ▲, size distribution of nonradioactive cells. An exponential-phase culture of E. coli 15T⁻ growing in aspartate M9 medium was labeled with ³H-thymine (200 μCi/2 μg/ml) for 9 min. The culture was washed on a membrane filter to remove medium radioactivity, suspended in medium, fixed with OsO_4, and mounted on collodion. Autoradiographs were prepared and developed after 2 months of exposure. Pictures were taken, and the size of individual cells was measured; 981 cells were examined.*

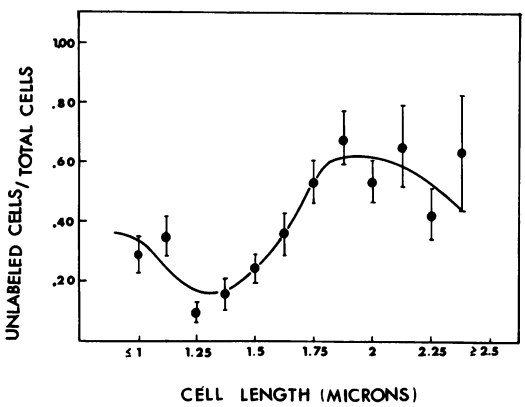

FIG. 3. *Frequency of unlabeled aspartate cells classified according to size. The data in Fig. 2 are graphed to present the fraction of unlabeled cells in each size category. Standard deviations are shown by the error flags.*

After the two generations of growth, the location of radioactivity within the cell serves as an index of segregation. Under these conditions each cell has one replicating chromosome which, if it is radioactive, contains ³H-thymine in only one nucleotide strand and, therefore, in only one of the two daughter chromatids. By observing the location of radioactive label within the cell, the location of the parental DNA template can be established. After replication is completed, the segregation of the daughter chromosomes should place a radioactive chromosome in one end of the cell. In the cells in Fig. 4B, both central and polar labeling is observed.

In Fig. 5, the central or polar location of label is tabulated according to cell size for cells from autoradiographs of the type shown in Fig. 4B.

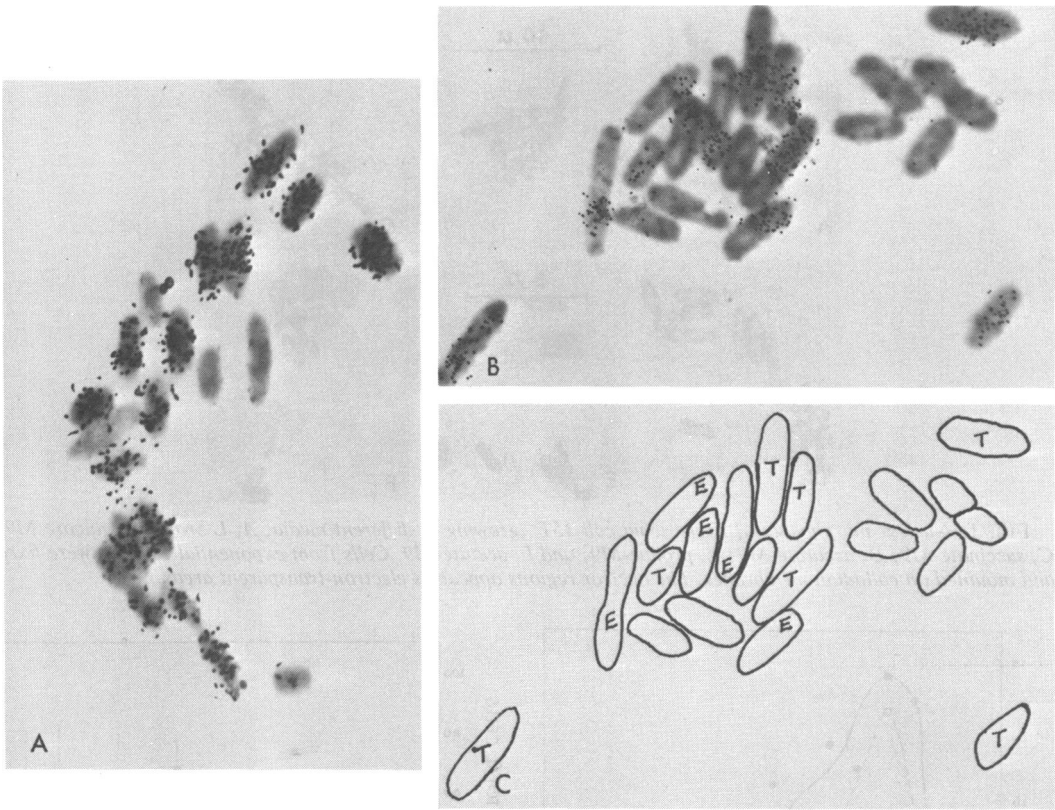

FIG. 4. *Autoradiographs of radioactive aspartate cells prepared immediately after radioactive labeling (A) or after two generations of growth in nonradioactive medium (B). An exponential-phase culture of 15T⁻ growing in aspartate-M9 medium was labeled with radioactive thymine as in the experiment in Fig. 2. After removing the cells from radioactive medium, one sample of the culture (A) was fixed immediately and another (B) was fixed after two generations of growth (fourfold increase in cell titer) in nonradioactive media. Autoradiographs were prepared and developed after 1 to 2 months of exposure. The cells in sample B were classified according to the location of radioactive label: total label (T) or end label (E). An example of this classification is shown C.*

The polar location of radioactivity was found to some extent in all classes of cells, but the majority is in very large cells. From the size of these cells it appears that the polar location of DNA occurs with highest frequency very late in the cell division cycle and, thus, by inference, that segregation occurs late in the cell division cycle.

Segregation of DNA after a shift-up. When aspartate or proline cultures of 15T⁻ are shifted into L broth, cells increase in size with little division for the first 40 to 50 min. This is shown in Fig. 6, in which the cell concentration was measured in a Coulter counter after a shift-up from proline or aspartate medium. Figure 7 presents the change in cytology of such cells with time. It can be seen from a comparison with Fig. 1 that they increase in size and that their nuclear pattern changes progressively to that observed in succinate, glucose, and finally L-broth cells. The transition is complete by 80 min, at which time cell division has been initiated (Fig. 6).

When cells in proline medium are labeled with radioactive thymine, radioactivity is incorporated into a single replicating chromosome in each cell, giving rise to two radioactive daughter chromosomes. If such cells are transferred to L-broth medium, the radioactivity is segregated eventually into daughter cells, each of which may contain four nuclear regions. Examples of such radioactive cells are shown in Fig. 8. Within each cell, two of the four regions are central and two are polar. We tabulated the location of radioactivity in such cells after a shift-up from proline into L-broth medium (Table 2).

At the end of two generations of growth (fourfold increase in cell number) only one-third of the cells contain four distinguishable nuclear regions. The decrease in the percentage of radio-

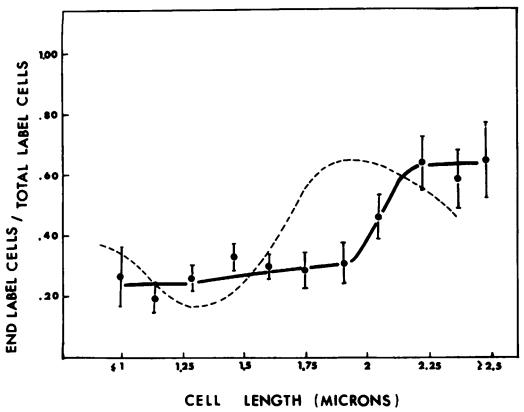

FIG. 5. *Location of radioactive DNA in aspartate cells after two generations of growth in nonradioactive medium. Cells treated as in Fig. 4B were classified according to size and distribution of label. The frequency of end-labeled cells in each size class is graphed. The standard deviations are shown by the error flags; 1,442 labeled cells were examined.*

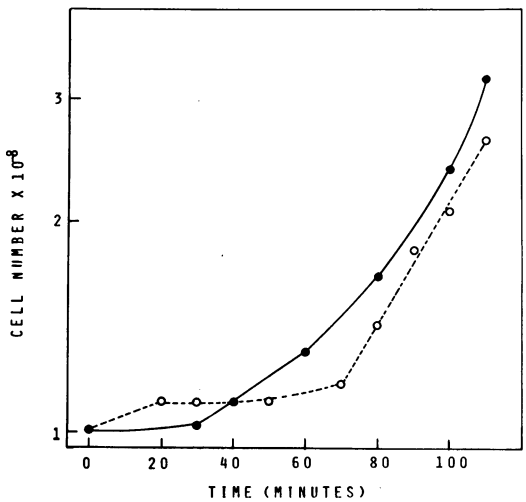

FIG. 6. *Cell division after a shift-up from aspartate-M9 or proline-M9 medium into L broth. Exponential-phase cultures of 15T⁻ in aspartate-M9 medium* (○) *or in proline-M9 medium* (●) *were transferred to L broth, and the number of cells was counted at intervals in a Coulter counter.*

active cells corresponds to the segregation of two labeled units, as would be expected for cells in proline medium if the radioactivity were incorporated into one replicating chromosome.

A survey was made of cells containing radioactive nuclei. At the end of one division (twofold increase in cell number), only 15% of the cells contained two radioactive regions. Thus, despite the increase in number of nuclear bodies per cell, the majority of parental radioactive chromosomes had segregated into separate daughter cells.

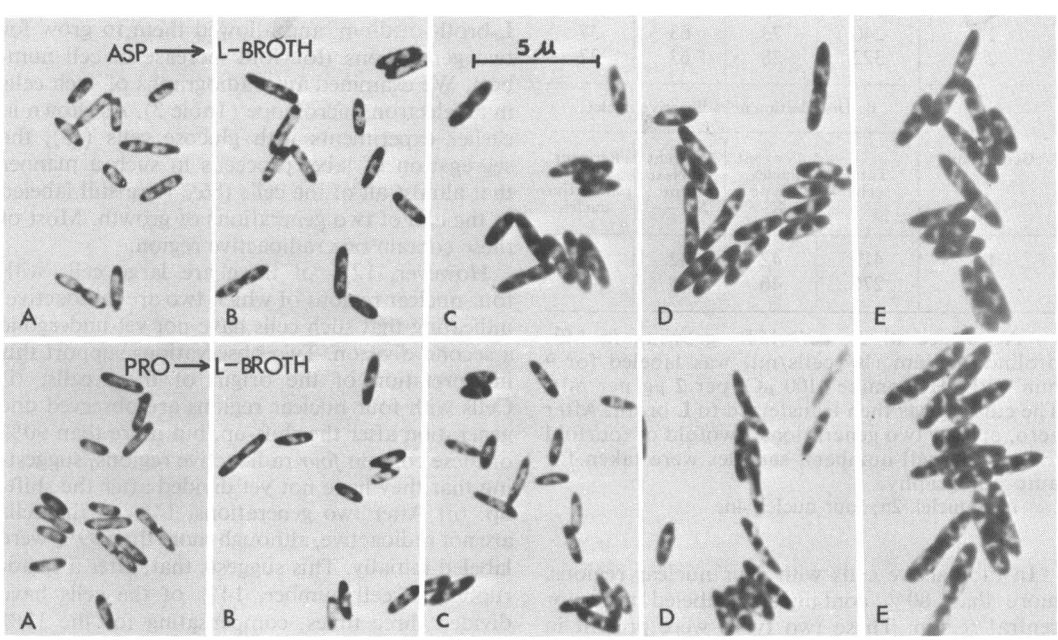

FIG. 7. *Nuclear morphology of cells shifted up from aspartate or proline-M9 media into L broth. Samples to be prepared for electron microscopy as in Fig. 1 were taken at intervals after the shift-up. A, 0 min; B, 20 min; C, 40 min; D, 60 min; E, 100 min.*

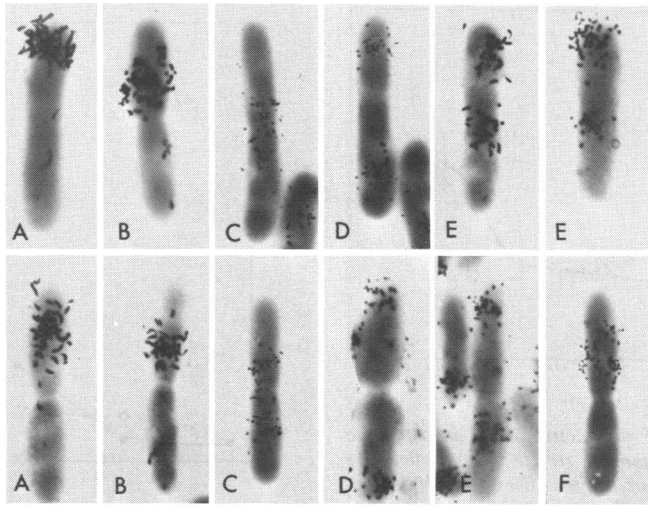

FIG. 8. *Autoradiographs showing the location of radioactive DNA in cells from the experiments in Tables 2 and 3. A, one end region labeled (+ − − −); B, one central region labeled (− + − −); C, both central regions labeled (− + + −); D, both end regions labeled (+ − − +); E, one end and the alternate central region labeled (+ − + −); F, one end and the adjacent central region labeled (+ + − −).*

TABLE 2. *Shift-up of radioactive cells from proline medium into L broth*[a]

Generation	A: Distribution of cell types			
	Total cells	Per cent radioactive	Per cent $2n^b$	Per cent $4n^b$
0	767	73		
1	240	73	63	37
2	322	38	63	37
Generation	**B: Distribution of radioactive nuclei**			
	Total cells	Per cent nuclear type -X--	Per cent nuclear type X---	Per cent with 2 radioactive nuclei
1	419	42	42	16
2	276	46	45	9

[a] A culture of *E. coli* 15T⁻ growing in M9-proline medium (10^8 cells/ml) was labeled for 9 min with ^{3}H-thymine (100 μCi per 2 μg per ml). The culture was then transferred to L broth. After zero, one, or two generations (twofold or fourfold increase in cell number), samples were taken for autoradiography.

[b] Two nuclei, 2n; four nuclei, 4n.

In radioactive cells with four nuclear regions, more than 80% contained a labeled polar or central region. These two types were present in equal amounts and with no indication of a preferential localization of radioactive DNA in either a central or a polar region at the end of two divisions.

Cells of 15T⁻ growing in glucose medium contain, on the average, two replicating chromosomes per cell (12). When such cells are pulse-labeled, radioactivity is incorporated into four nucleotide strands in each cell. We labeled cells in this manner, shifted them into nonradioactive L-broth medium, and allowed them to grow for two generations (fourfold increase in cell number). We examined autoradiographs of such cells in an electron microscope (Table 3). As shown in earlier experiments with glucose cells (13), the segregation of label proceeds in such a manner that almost all of the cells (86%) are still labeled at the end of two generations of growth. Most of these contain one radioactive region.

However, 12% of these are large cells with four nuclear regions of which two are radioactive, indicating that such cells have not yet undergone a second division. Two observations support this interpretation of the origin of these cells. (i) Cells with four nuclear regions are observed one generation after the shift-up, but more than 90% of these contain *four* radioactive regions, suggesting that they have not yet divided after the shift-up. (ii) After two generations, 14% of the cells are not radioactive, although more than 99% were labeled initially. This suggests that, after a quadrupling in cell number, 14% of the cells have divided three times, compensating for the 12% which have not yet divided twice. This heterogeneity in cell division may be the result of the shift-up

TABLE 3. *Shift-up of radioactive cells from glucose medium into L broth*[a]

Generation	Fraction of unlabeled cells
0	4/122
1	1/143
2	38/267

Distribution of two radioactive nuclei in second generation cells[b]

Nuclear type	Per cent
+−+−	69
+−−+	15
−++−	12
++−−	4

[a] A culture of *E. coli* 15T⁻ growing in M9-glucose medium (2×10^8 cells/ml) was labeled for 3 min with ^{3}H-thymine (100 μCi per 2 μg per ml). The culture was then transferred to L broth after zero, one, and two generations (twofold or fourfold increase in cell number), and samples were taken for autoradiography.

[b] L-broth cells with four nuclear bodies (154 cells out of 1,308 examined).

or it may represent the heterogeneity in division times observed normally (15, 20).

The radioactive patterns of cells with four nuclear regions which were observed after two generations are shown in Fig. 8. Table 3 gives the percentage of each cell with a particular distribution of radioactive label. It can be seen that 69% of the cells are of a particular type in which radioactive and nonradioactive nuclear regions alternate. This is far greater than that expected from random distribution, and it indicates a tendency for the progeny DNA of a particular replication cycle to alternate spatially with the product of earlier or later replication cycles. Such a distribution would insure the most rapid segregation of labeled DNA into different daughter cells.

DISCUSSION

In *E. coli* 15T⁻ growing at different rates, characteristic nuclear patterns are observed which range from single, central nuclear bodies in small cells growing in poor medium (acetate, proline, or aspartate carbon sources) to four nuclear regions in large rapidly growing cells in rich medium (such as L broth).

Although these nuclear patterns are characteristic of a given growth rate, small binucleate cells are observed occasionally at slow growth rates and uninucleate large cells are observed in rapidly growing cultures. Such deviations probably represent metabolic deviations of individual

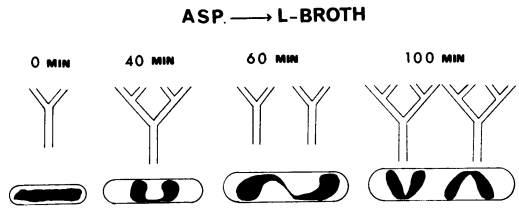

FIG. 9. *Suggested correlation after a shift-up for the change in appearance of the nuclear regions of a cell with the stages of chromosome replication.*

cells which are dividing more or less rapidly than the total population as observed by others (15, 20).

When cultures are shifted up from slow growth medium into L broth, the cells change their nuclear pattern to resemble, first, succinate, then glucose cells, and ultimately L broth cells. Previous studies on such a shift-up (2) demonstrated that, in most cells, a new round of replication is initiated within 40 min after the transfer to rich medium. However, completion of the round in progress is delayed and, in most cells, will finish between 50 and 60 min after the shift. This delay in terminating replication probably is responsible for the delay in cell division seen in Fig. 6, since termination is necessary for separation and segregation of the chromosomes into daughter cells (*see* Fig. 9). Prior to division, multiple nuclear regions become apparent within the cells.

The etiology of these nuclear regions can be clarified to some extent. In slowly growing cells, only one replication fork is present per cell and, even in the longest cells, only one nuclear region can be observed. In more rapidly growing cells (in succinate or glucose medium), two such regions can be observed and two chromosomes may be replicating in each cell. However, in L broth, four nuclear regions are often observed (*see* Fig. 1).

Previous experiments (1, 2, 8) have demonstrated that such rapidly growing cells do not contain four separate chromosomes, but two, which replicate dichotomously. Therefore, it is impossible to equate a nuclear region with the presence of a separate chromosome (i.e., with the finished product of segregation). This concept was originally proposed by Cooper and Helmstetter (5). On the other hand, since these cells contain two chromosomes and each one may have two sets of replication forks (Fig. 9), it seems probable that the nuclear regions observed may be associated with the sites of newly formed replication forks which may occur on the same or on different chromosomes. If this is true, it would indicate that, when replication is ini-

tiated, the process of segregation is also initiated and that segregation may be determined by the location of the replication fork itself. Figure 9 presents the sequence of nuclear patterns in a shift-up and the proposed corresponding replicative state of the chromosome.

Although it is impossible to distinguish replication from segregation of chromosomes by the appearance of separate nuclear bodies, we have observed a situation in which replication and segregation can be studied separately. Under conditions of slow growth in aspartate medium, cells do not synthesize DNA throughout the division cycle (1, 2, 11). Our experiments indicate that DNA synthesis can occur in cells of any size (Fig. 3) and, by inference, in cells at any stage in their division cycle. Thus, replication is not rigorously confined to a particular portion of the cell division cycle. However, DNA replication appears to be least probable during the last 30% of the division cycle, an observation which supports the model of Cooper and Helmstetter (5). Thus, in 60 to 70% of the cell population, DNA synthesis probably begins shortly after division, continues for 80 min, and ceases 30 to 40 min before the end of cell division. In the remainder of the cells, the 80-min period of replication may be shifted to a later stage of the division cycle. The extent of this variation is greater than that observed by Helmstetter et al. (9) in *E. coli* B/r. Their results indicate that almost all of the cells cease DNA synthesis late in the cycle.

Segregation of the replicated chromosome does not occur immediately after the cessation of replication; instead, it occurs just before cell division. When, after radioactive labeling, cells are grown in nonradioactive medium for two generations, radioactivity frequently is located at the end of the very largest cells but infrequently is seen in this position in smaller cells. The rise in frequency of the two curves in Fig. 5 indicates that, whereas replication ceases when cells reach a length longer than 1.7 μm, segregation of radioactive DNA becomes only frequent in cells longer then 2.1 μm. Thus, in this system, replication and segregation appear to be separated in time, affording an opportunity to study the cytology of chromosome segregation in bacteria.

One generation after pulse-labeled proline cells are transferred to L-broth medium, the radioactivity appears in one nuclear region. In cells which contain four nuclear regions, radioactivity is found equally frequently in a polar or in a central position. This is true after the first or the second generation of growth in L-broth medium. At the end of the first generation, division of a cell with four nuclear regions

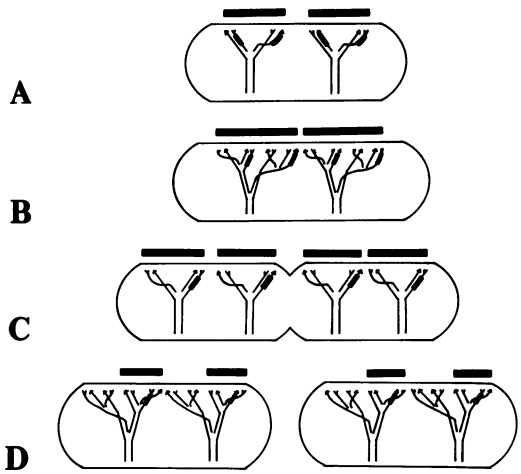

FIG. 10. *Model for the segregation of radioactive nucleotide strands after a shift-up from glucose-M9 medium to L broth. The labeled deoxynucleotide strands are indicated by heavy lines. Each replicating chromosome contains two template strands of opposite polarity (↑ or ↓). With equal probability, the new templates are located all to the left or all to the right of the previously attached templates. A, A cell in glucose-M9 medium; B, the same cell, with two replicating chromosomes each with two sets of replication forks; C, a later stage, before the first division; D, the two daughter cells after the first division—each has two chromosomes and each chromosome contains two sets of replication forks. The bars represent radioactive nuclear regions.*

will yield a cell in which the radioactive region is located at an end (*see* Fig. 10). Nevertheless, after a second generation of growth, radioactivity is found again with equal probability in polar or central regions. It appears, therefore, that segregation in such cells is occurring at random with respect to new growth and septation. This supports the conclusion of Ryter et al. (18) that segregation and septation are randomly oriented in *E. coli*. Their conclusions were based on the frequency of polar radioactive nuclei in strains of *E. coli* K-12 in which growth in nonradioactive medium occurred in long chains.

This conclusion must be qualified, however, by the nonrandom distribution of two radioactive regions among four possible positions in L-broth cells pulse-labeled in glucose medium (*see* Table 3). If these labeled chromosomes were randomly distributed, 50% of the cells would have one polar and one central radioactive region, 25% would have two radioactive polar regions, and 25% would have two radioactive central regions. Instead, the distribution is heavily biased toward cells with one polar and one central radioactive region.

In the discussion which follows, two restrictions must be kept in mind. (i) We have no evi-

dence that the class of cells in Table 3 (12% of the population) are normal, healthy cells, other than the observation that after doubling they contain four radioactive nuclei and after quadrupling they contain two (which implies that they are capable of division). (ii) We do not know whether the segregation pattern observed is unique to the events following a shift-up, or whether it represents a normal pattern.

However, the segregation model in Fig. 10 was suggested by the results in Tables 2 and 3 and may serve as a basis for designing future experiments. In this model (Fig. 10), we assume that each template strand of the DNA molecule is attached to the membrane at a template-specific region [previous evidence from several laboratories (4, 12, 18, 21) indicates an attachment of DNA to the cell membrane] which corresponds to the two nucleotide strands of opposite polarity.

We assume that, next to each specific region, a new region of opposite specificity is formed and that, in any cell, these regions adjoin the original regions from the same side, i.e., either both from the right or both from the left. Thus, ↑ ↓ will become either ↑ ↓ ↓ ↑ or ↓ ↑ ↑ ↓. If this rule is followed, and new templates are attached accordingly, the distribution of chromosomes following a shift-up from aspartate or glucose into L broth will correspond to the labeling patterns observed. This is shown in Fig. 10. In the initiation between A and B, ↑ ↓ becomes ↓ ↑ ↑ ↓ and ↓ ↑ becomes ↑ ↓ ↓ ↑. In the initiation between C and D, ↑ ↓ becomes ↑ ↓ ↓ ↑ and ↓ ↑ becomes ↓ ↑ ↑ ↓.

This segregation model accounts for the alternation of radioactive regions observed in the experiment in Table 3 and for the equal probability of internal or polar labeling observed in Table 2. It assumes that segregation of strands begins when new replication forks are initiated and that the circularity of the chromosome is lost when multiple replication forks are introduced.

In our model, growth toward the left or toward the right could correspond to internal or external growth of multicellular chains of cells and can occur with equal probability at each division. Thus, this model also will satisfy the observations of Ryter (16, 18) which demand that the frequency of the polar location of label in chains of *E. coli* decrease by a factor of two with each generation.

ACKNOWLEDGMENTS

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