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Chromosome Replication Rate and Cell Shape in *Escherichia coli*: Lack of Coupling

ARIEH ZARITSKY[1]* AND CONRAD L. WOLDRINGH[2]

Department of Biology, Ben Gurion University of the Negev, Beer Sheva, Israel,[1] and Department of Electron Microscopy and Molecular Cytology, Universiteit van Amsterdam, 1018 TV Amsterdam, The Netherlands[2]

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The dimensions of Rep$^-$ cells of *Escherichia coli* K-12 were measured and compared with those of their Rep$^+$ isogenic cells (both Thy$^-$). Rep$^-$ cells cultivated identically were longer (but not wider), even though both strains were wider when the rate of chromosome replication was slowed down by lowering the thymine concentration supplied. This eliminates the possibility that cell shape is determined by this rate. Simulating Thy$^+$ phenotype by adding deoxyguanosine resulted in shorter Rep$^-$ cells when growth was faster. This excludes a simple relationship between cell elongation and growth rate, but is consistent with a linear proportionality between the rates of surface synthesis and growth. Thymine limitation of fast-growing Thy$^-$ *E. coli* K-12 cells is shown to result in loss of their uniform shape and production of bizarre morphologies, apparently due to imbalanced synthesis of wall components.

The bacterial chromosome initiates replication when the cell reaches a certain mass (or volume; 5, 28) and terminates a constant time (*C*; 3) later; cell mass accumulates exponentially (8). The observation (20, 40) that Thy$^-$ cells of *Escherichia coli* supported by lower thymine concentrations are thicker, together with the view that bacterial length extension is directly coupled to chromosome replication and segregation (6, 15), led to the proposal (27, 40) that a cell grows by elongation at discrete zones, equivalent in number to the number of completed chromosomes, and at a rate (per zone) proportional to the growth rate. Both faster growth and slower replication thus result in a higher mass-length ratio, and the cell accommodates this "extra" mass by increasing its girth (26, 27, 36, 40).

The above argument assumes that thymine is required for DNA synthesis only. However, there is some evidence (14, 21, 22, 24) that deoxythymidine diphosphate rhamnose (TDPR) is involved in bacterial envelope synthesis (20; R. H. Pritchard, personal communication). When lower thymine concentrations are used for cultivation (14), the intracellular concentration of TDPR is likely to be reduced together with the reduction in the pools of the immediate DNA precursors (1). Thus, if TDPR is rate limiting for envelope synthesis under these conditions, as are the deoxythymidine phosphates (1) in chromosome replication, then both processes can be expected to slow down proportionately (20). The resulting envelope-mass ratio would then decrease with the DNA-mass ratio (27, 40).

The dimensions of temperature-sensitive DNA initiation mutant cells growing at intermediate temperatures, reported in the accompanying paper (29), demonstrate conditions in which reduction in DNA concentration was not associated with an increased cell diameter. These conditions, however, seem not to alter the replication time of the chromosome (29); hence, the proposal (36) that the change in cell diameter is related to a corresponding change in the number of replication positions has not been excluded.

The isolation of the *rep* mutation in *E. coli* K-12 (4) opened up a simple way to test this hypothesis. The extensive studies of Lane and Denhardt (17, 18) demonstrate a longer replication time (*C*) in Rep$^-$ cells than in isogenic Rep$^+$ cultivated identically. As expected, the Rep$^-$ cells are larger than their Rep$^+$ counterparts grown similarly in a variety of media (17). The increased size of Rep$^-$ cells should be expressed mainly in their thickening (as occurs in thymine-limited cultures; 20, 40) if the diameter is determined by the number of replication positions (36).

Here we attempt to resolve this question, by measuring the cellular dimensions of a *rep* mutant of *E. coli* K-12 and of its isogenic Rep$^+$ strain, and conclude that the kinetics of chromosome replication do not determine cell diameter.

MATERIALS AND METHODS

E. coli K-12 *thr leu thy drm* (inferred from lack of sensitivity to deoxyguanosine; 27) and its *rep-3* derivative (4; kindly provided by H. E. D. Lane) were cultivated in AB salts solution (11) supplemented with 0.4% glucose as the sole carbon source, threonine and leucine (50 μg/ml each), and the indicated concentrations of thymine. Casamino Acids (Sigma; 1%) was added for shorter doubling time (τ) and deoxyguanosine (Sigma; 100 μg/ml) for faster replication velocity (1, 37, 39). The cultures were vigorously aerated at 37°C in a reciprocating water-bath shaker and diluted as necessary for maintaining them in a steady state of exponential growth (40), indicated by a constant length distribution (35).

Samples were fixed either by 0.25% formaldehyde for absorbance measurements (Gilford microsample spectrophotometer, at 450-nm wavelength) and for titration (Coulter Counter, model ZB) or by osmium tetroxide for electron microscopy. Electron micrographs of cells, air-dried by a modification (35) of the agar-filtration technique (16), were projected on a screen at a final magnification of between 10,000 and 15,000.

Relative amounts of DNA were determined by incorporation of [^{14}C]thymine (0.02 μCi/μg) into cold trichloroacetic acid-insoluble material (39, 40).

RESULTS

Shape changes with replication time. *E. coli* K-12 and its isogenic *rep-3* derivative were grown in glucose minimal medium supplemented with 5 μg of thymine per ml, with and without deoxyguanosine. Pictures of each of the four cultures, after reaching steady-state growth conditions, are shown in Fig. 1. It seems obvious at first sight that both the *rep* mutation and a limiting thymine concentration modify cell shape. The quantitative analysis is presented as length distributions in Fig. 2; the average dimensions are summarized in Table 1. The results confirm previous observations on other Thy$^-$ strains (20, 40) that as chromosome replication slows down under reduced thymine concentration (1, 39), the cell shape factor (36) decreases, essentially by increasing diameter. However, a reduction in the replication rate due to the presence of the *rep* mutation results in a somewhat increased shape factor, mainly through increased length. This observation seems to contradict the simple relationship proposed by Zaritsky and Pritchard (40) between the number of cell elongation zones and chromosomal termini, provided *rep* does not influence cell cycle parameters other than *C*.

Shape changes with doubling time. The study of cell dimensions at different steady-state growth conditions, which has proved fruitful before (7, 10, 17–20, 32, 33, 35, 40), was exploited again, and the same strains were cultivated in glucose–Casamino Acids-supplemented medium to obtain shorter τ. Steady-state conditions could be reached only when deoxyguanosine was added (38), and the cells looked as shown in Fig. 3. Further experiments are summarized in Table 1. The most striking observation was the de-

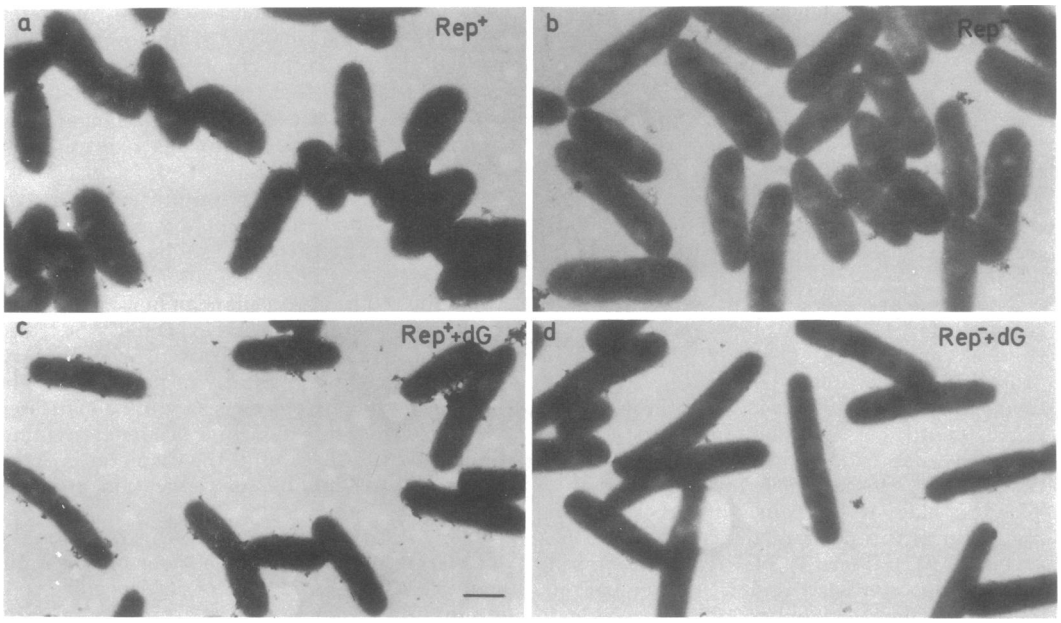

FIG. 1. *Electron micrographs of agar-filtered E. coli K-12 cells grown exponentially in glucose-minimal salts medium, supplemented with 5 μg of thymine per ml with (c, d) or without (a, b) deoxyguanosine. (a) and (c) Rep$^+$; (b) and (d) Rep$^-$. Magnification bar is 1 μm.*

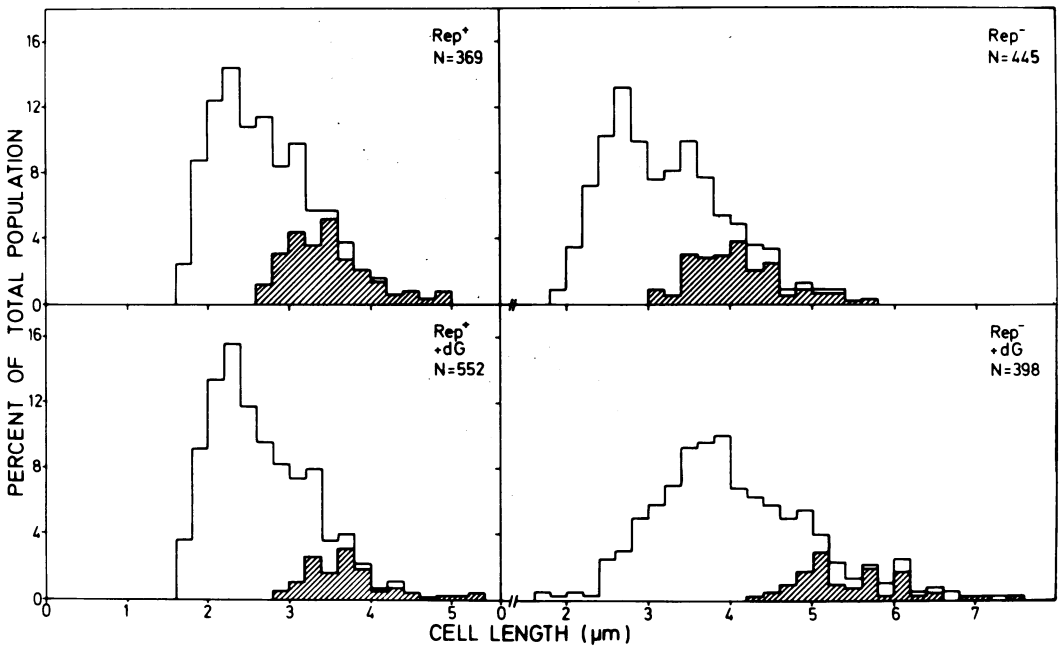

FIG. 2. *Length distributions of cells and of constricted cells (hatched area) from the same cultures as in Fig. 1. N is the total number of cells measured.*

TABLE 1. *Dimensions and shape of E. coli K-12 at various conditions*

Media	Rep	Thymine concn[a] (μg/ml)	τ (min)	C[b] (min)	Avg cell length ($\bar{L}$) (μm)	Avg cell diam ($2\bar{R}$) (μm)	Shape factor ($a = \bar{L}/2\bar{R}$)
Glucose	+	5	51	62	2.68	0.97	2.76
	+	5 + dG	57	41	2.62	0.68	3.83
	−	5	54	127	3.19	1.04	3.07
	−	5 + dG	60	105	4.00	0.73	5.47
Glucose + Casamino Acids	+	3 + dG	31	41	2.79	0.98	2.86
	−	5 + dG	38	79	3.70	1.03	3.59

[a] +dG, Deoxyguanosine was added to medium.

[b] Values of *C* were calculated from relative DNA-mass ratios (38, 39) normalized to give 41 min for Rep+ in deoxyguanosine.

crease in the average length of the Rep⁻ cell compared to that in glucose minimal medium. This excludes the elongation model (6, 7, 10, 40) because it results in a time before division that is negative (*d*; 10) for the presumed doubling in cell elongation rate (Table 2). It is gratifying that the estimated *d* for surface extension (27, 40) was similar in both strains (45 to 46 min; Table 2) and approached the estimated value in an *E. coli* B/r (strain H266; 49 min; R. F. Rosenberger, N. B. Grover, A. Zaritsky, and C. L. Woldringh, J. Theor. Biol., in press).

Shape distortion. Thymine limitation of fast-growing *E. coli* 15T⁻ cells causes them to lose their division control, resulting in a gradual increase in average cell size (40). As demonstrated recently (38), such cells change their otherwise uniform shape and give rise to many monsters, a significant number of which are branched. It was therefore important to find that this phenomenon had nothing to do with the genetic background specifying strain 15; essentially identical cell shapes were observed when the present K-12 strain was cultivated under thymine limitation (without deoxyguanosine) in glucose–Casamino Acids-supplemented medium (Fig. 4) irrespective of the presence of the *rep* gene. The higher proportion of branched cells (some 5 to 10%) in these populations can be attributed to the faster growth conditions employed here.

DISCUSSION

The apparent connection inferred between bacterial cell shape and chromosome replication rate (27, 36, 40) is demonstrated here to be

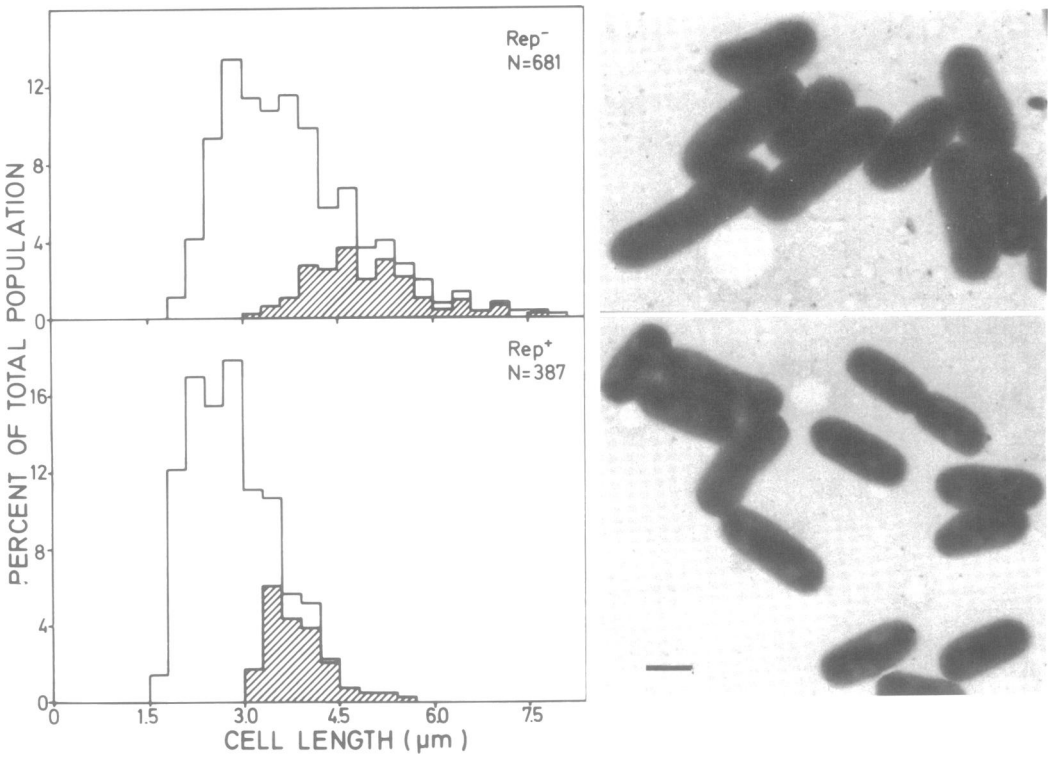

FIG. 3. *Electron micrographs and length distributions of agar-filtered E. coli K-12 cells grown exponentially as in Fig. 1 and 2, supplemented with Casamino Acids and deoxyguanosine. Magnification bar is 1 μm. N is the total number of cells measured.*

TABLE 2. *Time before division (d) when cell growth rate doubles*[a]

Rep	*d* (min)	
	Elongation	Surface growth
+	6.1	44.8
−	−11.8	45.8

[a] Values for elongation were derived from the equation: $d = \tau_1\tau_2(\log \bar{L}_1 - \log \bar{L}_2)/(\tau_2 - \tau_1)\log 2$ (10, 40), where $\bar{L}_1$, $\bar{L}_2$ are average cell lengths when growing with τ_1, τ_2, respectively. Values for surface growth were derived similarly, but with average surface area [$\bar{S} = 2\pi\bar{R}(\bar{R} + \bar{L})$; Rosenberger et al., J. Theor. Biol., in press] replacing $\bar{L}$.

fortuitous, using a mutant that is affected in its replication machinery (4, 17, 18, 34). The independent effect on *C* of limiting thymine and the presence of *rep* (Table 1; 18) is an indication that thymine metabolism is not altered in the mutant strain.

Thymine is not a normal intermediate in DNA synthesis except in the presence of deoxyribonucleotides or a *thy* mutation (see, e.g., 27). This could be the reason why *E. coli* and *Bacillus subtilis* seem not to have evolved an active transport system for this base (31). Consequently the intracellular concentrations of thymine and its metabolites are low, resulting in slower rates of the reactions for which these serve as substrates. The possibility of simulating conditions of thymine prototrophy in *drm* mutants of Thy⁻ strains by adding deoxyguanosine to the growth medium (1, 37, 39) permits studying the proposed link between C/τ and cell shape (36) without the complication of limiting thymine.

In spite of the similar effect on *C* and on cell size of the *rep* mutation in response to reduction in the supply of thymine, the two treatments resulted in opposite responses of cell dimensions (Table 1). As intuitively expected, the increased size of the *rep*-containing cell was expressed by its becoming longer (due to an extended period of elongation when divisions are delayed). The marked thickening of thymine-limited cells, which has previously been interpreted as resulting from a reduced differential rate of synthesis of envelope components over total dry weight (inferred from the lower surface-mass ratio; 27, 40), does not seem to be caused by delaying termination of chromosome replication (27, 40) or by lengthening of *C* (36). It may be another consequence of limiting thymine metabolites affecting cell envelope synthesis directly.

TDPR is utilized in the synthesis of cell wall

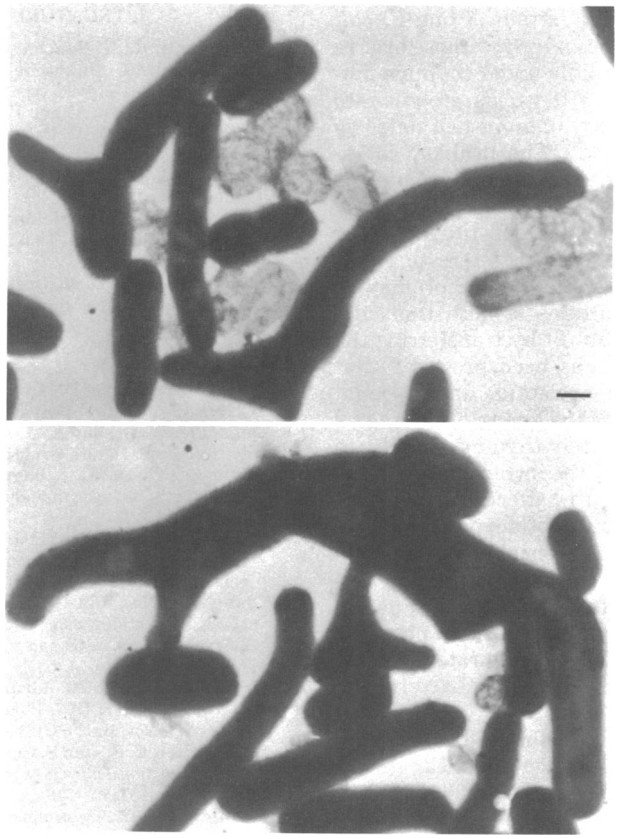

FIG. 4. *Electron micrographs of agar-filtered E. coli cells (Rep⁺) grown exponentially in glucose-minimal salts medium supplemented with 3 μg of thymine per ml and Casamino Acids. Magnification bar is 1 μm.*

lipopolysaccharide (22, 24). Mutations in genes coding for enzymes involved in TDPR synthesis are pleiotropic; they alter the lipopolysaccharide layer in a way similar to uridine diphosphate glucose-negative mutations and influence the intracellular concentration of dTTP (22). A recent report by Ohkawa (23) describes a class of mutants (Ter-21) which shows a spherical or ellipsoidal form after UV irradiation, rather than the usual filamentation expressed by their parent strain. This phenomenon has been explained (23) by the apparent fragile cell wall synthesized in the mutant (due to deficient concentration of TDPR following UV irradiation), in accord also with the prolate shape of thymine-limited Thy⁻ cells (Fig. 1; 40) and, perhaps, with the phenomenon of branching observed at fast growth under these conditions (Fig. 4; 38). The typical fields shown in Fig. 4 also include a few lysed cells, not usually found otherwise. Light microscopy of living or of OsO_4-fixed samples failed to show any lysis; this must therefore occur during cell drying in preparation for electron microscopy.

The apparent imbalanced synthesis of envelope components referred to here (and see 22) should now be compared with the recent studies of I. B. Holland and A. Boyd (personal communication); they did not find any difference in the relative rate of either outer or inner membrane protein synthesis versus total proteins in *E. coli* B/r when Thy⁻ cells were thymine limited to different extents. Thus the synthesis of the majority of envelope proteins is not directly linked to surface growth in this strain. This observation is consistent with the view that the outer membrane proteins do not play a key role in bacterial morphogenesis since mutants of *E. coli* K-12 lacking all "major" proteins of the outer cell envelope membrane still display cylindrical shape (13).

We are therefore inclined to explain the shape changes observed under thymine limitation as resulting from reduced differential rate of at least one envelope component, the synthesis of which involves TDPR, accompanied by weakening of this layer in some strains of *E. coli* (K-12 here; 15 in reference 38). (Since the genetic background seems to be important in this connection [2, 23, 30], it appears worth noting again

[see also 38] that a Thy⁻ strain of one *E. coli* B/r [LEB-16; 19, 20] does not show these bizarre forms when growing rapidly under thymine limitation, but rather slows down its growth rate [Woldringh and Zaritsky, unpublished data] as though straight-jacketed.) The primary candidate for such a component is the O side-chain of the lipopolysaccharide (10, 21), but it is not easy to imagine how this minor constituent could modify cell shape by itself. It is interesting that the same lipid (a C_{55}-isoprenoid alcohol) may be involved in the biosynthesis of both this side-chain and peptidoglycan (9) by transferring the appropriate sugars. It has been proposed that the presence of some nucleotide sugars could activate the enzymes transferring other sugars (21). Thus, restricting the thymine supply to Thy⁻ strains could limit the intracellular TDPR concentration and, in turn, affect the sugar nucleotides necessary for peptidoglycan biosynthesis to a degree that influences cell shape. It is unlikely, however, that this mechanism operates during nutritional shifts of Thy⁺ strains; hence, we are forced to return to the question posed by the correlation between growth rate and cell width in gram-negative bacilliform bacteria (10, 26, 27, 32, 33, 40).

One possible positive conclusion from the data presented here is that if the view of linear growth limited by the number of zones (6, 26, 27, 40) is correct, this number doubles some 45 min ($=d$) before division (Table 2), in close agreement with the estimated value of d in *E. coli* B/r strain H266 for surface growth model (49 min; Rosenberger et al., J. Theor. Biol., in press; C. L. Woldringh, N. B. Grover, R. F. Rosenberger, and A. Zaritsky, submitted for publication). The notion of a "unit cell length" (6), predicting a d value of 15 to 20 min (7, 10), should therefore be abandoned, to be replaced perhaps by a "unit cell surface." The estimated d of 45 min does not necessarily contradict the possibility that the presumed doubling in number of surface growth zones corresponds with the time when a specific segment of the chromosome is replicated (27), because the exact way D (3) changes with C (17, 19, 40) may vary among different strains and growth conditions. These and other cell cycle parameters in the present strains are under careful study now.

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