JOURNAL OF BACTERIOLOGY, Aug. 1989, p. 4303–4314
0021-9193/89/084303-12$02.00/0

Vol. 171, No. 8

Actively Replicating Nucleoids Influence Positioning of Division Sites in *Escherichia coli* Filaments Forming Cells Lacking DNA

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Received 3 January 1989/Accepted 8 May 1989

The positioning of constrictions in *Escherichia coli* filaments pinching off anucleate cells was analyzed by fluorescence microscopy of *dnaX*(Ts), *dnaX*(Ts) *sfiA*, *dnaA46*(Ts), *gyrA*(Am) *supF*(Ts), and *gyrB*(Ts) mutants. In filaments with actively replicating nucleoids, constrictions were positioned close to the nucleoid, whereas in nonreplicating filaments, positioning of constrictions within the anucleate region was nearly random. We conclude that constriction positioning depends in an unknown way on nucleoid replication activity.

Cell division in *Escherichia coli* involves the tight coordination in time and space of the processes of cell growth, DNA replication, and cell constriction. Concomitant with the increase in cell mass and DNA replication, the daughter chromosomes are segregated into the cell halves. After termination of DNA replication and completion of segregation, the cell is constricted between the nucleoids in the cell center. Constriction requires discontinuation of enzyme activity for cell wall synthesis at the constriction site (1, 21). This may be achieved either by local activation of enzymes or by positioning of specific enzymes at the constriction site. Although termination of DNA replication has been suggested to signal the cell to initiate a constriction (11), the mechanism is still not well understood. So far, factors that determine the positioning of a constriction site have not been identified.

According to the concept of zonal growth in bacterial cells (5, 8), the site of constriction is determined by growth zones that occur in the lateral cell wall. It has also been suggested that "zones of adhesion" between the cell membrane and the peptidoglycan layer may be involved in the positioning of constrictions (16). Both ideas imply that constriction sites are predetermined within the cell envelope in such a way that the specific enzymes for constriction are concentrated at potential division sites, which are placed at regular distances from the cell pole. Experiments with cells carrying the temperature-sensitive DNA initiation mutation *dnaA46*(Ts) have been interpreted in terms of predetermined division sites (2). At the restrictive temperature, the SOS response and the related cell division inhibition are not induced in this mutant (for a review, see reference 12), which thus continues to divide, pinching off DNA-less cells. At 42°C, the *dnaA46*(Ts) mutant was reported to pinch off DNA-less cells of uniform length similar to the newborn cell length (5, 10), suggesting that the cells can measure the distance between constriction and pole.

Contrary to the results of Hirota et al. (5), observations on other DNA-less cell-forming mutants suggest the absence of regularly spaced, predetermined constriction sites in the lateral cell wall. For instance, the *dnaB* mutation, by which DNA replication and constriction initiation are also uncoupled, produces DNA-less cells that are not uniform in size (7). Anucleate cells with a broad range of cell lengths are also pinched off from filaments of the *dnaX*(Ts) mutant recovering from a temperature shift (21). In addition, inhibition of DNA synthesis in *min* mutants, which normally pinch off minicells and short DNA-less cells, causes the formation of DNA-less cells with lengths that vary from 1.2 to 13 μm (9). Finally, *gyrA*(Am) *supF*(Ts) (*parD* [6]) and *gyrB*(Ts) (2) mutants, which show a defect in DNA segregation, pinch off DNA-less cells, which may vary in length from the size of minicells to normal rod size. It has thus been suggested that, in the absence of segregated nucleoids, constrictions are positioned randomly within the nucleoid-free cell ends of the filaments (6, 21).

In this article we present observations which suggest that, in the absence of unsegregated nucleoids, division sites are neither predetermined nor randomly positioned. We show that recovering temperature-sensitive replication mutants and DNA segregation mutants growing at the restrictive temperature, which both contain actively replicating nucleoids, preferentially constrict close to the nucleoids. In dividing filaments that do not replicate their chromosome, positioning of constrictions relative to the nucleoid is close to random.

MATERIALS AND METHODS

Bacterial strains, growth conditions, and replication rate measurements. The *Escherichia coli* strains used in this study are listed in Table 1. The cells were cultured under steady-state conditions at 30 or 37°C in minimal medium (18) supplemented with glucose (0.4%) as the sole carbon source, thiamine (4 μg/ml), and the required amino acids (50 μg/ml). The osmolality of the medium was adjusted to 300 mOsm by the addition of 0.1 M NaCl. Temperature shifts were carried out by diluting the steady-state culture (OD_{450}, 0.2 to 0.3) into warm medium; four times for a shift from 30 to 42°C, and two times for a shift back from 42 to 30°C. During the experiments, the OD_{450} was never allowed to increase above 0.3.

To measure the rate of DNA synthesis in the replication and initiation mutants under the various growth conditions, cells were pulsed every 10 min, in a total volume of 400 μl, with 12 μCi of [^{3}H]thymidine (30 μl) (Amersham Corp.). [^{3}H]thymidine incorporation was stopped after 10 min by the addition of 100 μl of cold 50% trichloroacetic acid (TCA). Samples (100 μl) were put on GF/A glass filter (Whatman) in duplicate. The filters were equilibrated in 10% TCA containing thymine (200 μg/ml), washed once with 5% TCA, twice with acetone, and once with ether, and finally dried. Radio-

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TABLE 1. *E. coli* K-12 strains

Strain	Genotype	Source or reference
LMC500	F⁻ *araD139* Δ(*argF-lac*)*U169 deoC1 flbB5301 lysA ptsF25 rbsR relA1 rpsL150*	Taschner et al. (19)
LMC1011	LMC500 *minB zcf*::Tn*5*	P1(GC7115)×LMC500
LMC1012	LMC500 *dnaX2016*(Ts) *zba*::Tn*10*	P1(LMC722)×LMC500
LMC1013	LMC500 *dnaX2016*(Ts) *sfiA*::Tn*5 zba*::Tn*10 pyrD*	P1(LMC605)×LMC1012
LMC1039	LMC500 *dnaA46*(Ts) *tna*::Tn*10*	P1(LMC1061)×LMC500
LMC1040	LMC1039 *sfiA*::Tn*5*	P1(LMC605)×LMC1039
WM1028	*dnaA46*(Ts) *ilvB lac supF thi*	W. M. Messer
OV6	F⁻ *ara*(Am) *deo galE galU42*(Am) *gyrA*(Am) *his ilv lac-125*(Am) *leu supF-A81*(Ts) *trp*(Am) *tsx*(Am)	Hussain et al. (6)
LE234	F⁻ *argE gyrB*(Ts) *ilv leu metB rpsL supE thi tna xyl*	E. Orr et al. (15)
GC7115	F⁻ λ⁻ *minB1 sfiA85 zcf-289*::Tn*10*	Jaffé et al. (10)
LMC722	AX727 *zba*::Tn*10*	
AX727	*dnaX2016*(Ts) *gal lac rpsL thi*	J. M. Henson et al. (4)
LMC605	LMC500 *pyrD sfiA*::Tn*5*	P1(GC2555)×LMC500
GC2555	F⁻ *pyrD sfiA*::Tn*5*	R. D'Ari
LMC1061	WM1028 *tna*::Tn*10*	P1(WP72)×WM1028
WP72	*arg bglR galE pro rpsL thi tna*::Tn*10*	Schaus et al. (17)

activity on the filters was determined in toluene with 4 g of 2,5-diphenyloxazol per liter in a scintillation counter.

Genetic techniques. Phage P1 *vir*-mediated transduction was carried out as described by Miller (13). Temperature sensitivity of the strains was tested on TY plates without NaCl at 42°C and on TY plates with 0.5% NaCl at 30°C.

Microscopic techniques. The positioning of constrictions relative to nucleoids and cell poles was determined from fluorescence microscope pictures. For fluorescence microscopy, exponentially growing cells (OD_{450} of 0.1 to 0.2) were harvested and fixed in 0.1% OsO_4. The cells were concentrated 20 to 40 times by centrifugation and suspension in veronal-acetate buffer (pH 6) containing 0.12 M NaCl and 0.01 M $MgCl_2$. The nucleoids were specifically stained with fluorochrome 33342 (Hoechst-Roussel Pharmaceuticals Inc.) at a final concentration of 16 μg/ml; 5 μl of the cell suspension was left to dry on a polylysine-coated cover slip (50 μl, 0.01%) and covered with 3% methocel (5 μl). The fluorochrome was irradiated at 365 nm in a fluorescence microscope (Zeiss). Cells with fluorescent nucleoids were photographed with Tmax films (400 ASA; Kodak Ltd.), which were developed with Acu-1 developer (Acufine Inc.).

Distances between nucleoids and constrictions (L_{NC}) or between nucleoids and cell poles (L_{NP}) and between constrictions and cell poles (L_{CP}) and individual cell lengths were measured from fluorescence microscope pictures projected onto a digitizing screen (Summagraphics Co.) at a final magnification of 3,200. The distances were measured by tipping the nucleoid poles, constrictions, and cell poles with an electronic pen (19); different distances were measured in separate runs. Fifty measurements of a nucleoid-cell pole length in one cell (mean, 0.9 μm) gave a coefficient of variation (CV) of 8%. The maximum measurement error was thus estimated at 10%.

RESULTS

Growth and cell division characteristics of DNA-less cell-forming mutants. Mutants that are affected in DNA replication or in the segregation of nucleoids form anucleate cells, either during restrictive growth in the absence of SOS-dependent cell division inhibition or when recovering from SOS-dependent filamentation. To study the positioning of constrictions under these two conditions, we examined the *dnaX*(Ts), *dnaX*(Ts) *sfiA*, and *dnaA46*(Ts) replication mutants, the *gyrA*(Am) *supF*(Ts) and *gyrB*(Ts) segregation mutants and a *minB* mutant (Tables 2 and 3).

The *dnaX*(Ts) replication mutant only pinched off DNA-less cells when recovering from growth at the restrictive temperature. Figure 1A shows the growth and division characteristics after a temperature shift of a steady-state population from 30 to 42°C and back to 30°C. The temperature shift resulted in an immediate increase in growth rate, which from then on decreased steadily. DNA synthesis was

TABLE 2. Constriction efficiency of isogenic *dna* mutants and some *gyr* mutants, expressed as percentages of constricting cells and of DNA-less cells, determined from fluorescence microscope photographs

Genotype of strain	Growth conditions	% Constricting cells[a]				% Anucleate cells
		C_t	C_c	C_p	C_d	
dnaX(Ts) *sfiA*	2 h at 42°C	14	2	12	—[b]	8
dnaX(Ts) *sfiA*	4.5 h at 42°C	13	—	13	—	17
dnaX(Ts)	2 h after shift back to 30°C	77	23	27	27	12
dnaA46(Ts)	2 h at 42°C	3	2	1	—	—
dnaA46(Ts)	4.5 h at 42°C	13	2	11	—	7
dnaA46(Ts)	1.5 h after shift back to 30°C	82	21	63	—	25
gyrA(Am) *supF*(Ts)	3 h at 42°C	15	6	11	—	41
gyrB(Ts)	2 h at 42°C	23	4	19	—	34

[a] C_t, Total percentage of constricting cells; C_c, percentage of centrally constricting cells; C_p, percentage of polarly constricting cells; C_d, percentage of doubly constricting cells (central and polar).

[b] —, None found.

TABLE 3. Positioning of constrictions of anucleate-cell-forming filamentous mutants and lengths of new born cells

Genotype of strain	Growth conditions	Constriction position[a]		L_0 (μm)[a]
		L_{CP} (μm)	L_{NP} (μm)	
LMC500 (wild type)	Steady state at 37°C			1.4 (11)
dnaX(Ts) *sfiA*	5 h at 42°C	3.2 (33)	6.5 (39)	2.1 (11)
dnaX(Ts)	2 h after shift back to 30°C	2.6 (42)	3.4 (38)	2.0 (12)
dnaA46(Ts)	5 h at 42°C	3.1 (21)	4.8 (23)	ND[b]
dnaA46(Ts)	1.5 h after shift back to 30°C	4.5 (18)	5.3 (17)	
gyrA(Am) *supF*(Ts)	3 h at 42°C	1.7 (30)	2.7 (31)	
gyrB(Ts)	2 h at 42°C	1.9 (23)	2.3 (22)	
minB	Steady state at 37°C	0.9 (25)	1.3 (19)	

[a] The percent CV is shown in parentheses.
[b] ND, Newborn cell length could not be determined.

inhibited within 40 min (Fig. 1B). Due to the presence of cells that had terminated replication, cell division continued at a low rate for about 1 h at 42°C, resulting in a 35 to 50% increase in the number of cells in various experiments. At the same time, filaments with no signs of constriction were formed due to induction of the SOS response. In these filaments, the nucleoids at first appeared to spread out, whereas after 10 min an increasing percentage of nucleoids (40% with contracted nucleoids after 2 h of growth at 42°C) became contracted [Fig. 2a shows a similar nucleoid morphology in *dnaX*(Ts) *sfiA* cells]. Within 10 min after a shift back to the permissive temperature, the contracted nucleoids became dispersed again. Subsequently, the DNA replication rate increased rapidly (Fig. 1B). The growth rate recovered very slowly but eventually reached the steady-state level (not shown). Recovery of cell division, which did not occur until 60 min after the shift back to 30°C, resulted in anucleate cell formation. The percentage of free anucleate (N^-) cells amounted to 12% after 2 h of permissive growth (Table 2).

The *dnaX*(Ts) *sfiA* mutant, in a shift experiment (30 to 42 to 30°C), behaved like the *dnaX*(Ts) mutant with respect to DNA replication inhibition and nucleoid morphology (Fig. 2a). The *dnaX*(Ts) *sfiA* mutant lacks a functional SOS-related cell division inhibition mechanism (SfiA protein) and thus formed anucleate cells at the restrictive temperature. After the shift from 30 to 42°C, cell division was nevertheless inhibited to some extent. Table 2 shows that as a result of this SOS-independent cell division inhibition (10), the percentage of polarly constricting cells (C_p) (13% after 4.5 h) was low compared with that of the recovering *dnaX*(Ts) population (C_p, 27% after 2 h). After the shift back to 30°C, the cells recovered completely, even after 5 h at 42°C. However, recovery of growth rate took two times longer after 5 h (about 4 h) than after 3 h (about 2 h) of restrictive growth.

In the *dnaA46*(Ts) mutant, the DNA replication rate decreased gradually after a shift to the restrictive temperature (Fig. 1D). The nucleoids in the *dnaA46*(Ts) mutant filaments appeared to spread out, like in the *dnaX*(Ts) mutant, but did not contract (Fig. 2c). In the present temperature shift experiments, with cells growing in minimal medium, cell division inhibition immediately set in (Fig. 1C), giving rise to filaments with multiple nucleoids (Fig. 2c). Of the few filaments that formed anucleate cells at the restrictive temperature (7% after 4.5 h; Table 2), 80% contained multiple nucleoids. The same cell division inhibition at 42°C was observed with a *dnaA*(Ts) *sfiA* strain (result not shown), confirming Monk and Gross (14) in that *dnaA46*(Ts) does not induce the SOS response. In the 30°C steady-state *dnaA46*(Ts) culture, many long cells with three or more nucleoids and asymmetrically positioned constrictions were observed, indicating that cell division is also inhibited to some extent at the permissive temperature. Therefore, the accuracy of constriction positioning determined by the variation of the newborn cell length (L_0, 3.1 μm; CV, 17%) cannot be compared with of that of the wild-type strain (1.4 μm; CV, 11%; Table 3) or of DNA-less cell formation (see below). When the culture was shifted back to the permissive temperature after 5 h of restrictive growth, DNA replication was restored (Fig. 1D) and many DNA-less cells were formed (25% after 1.5 h; Table 2).

The *gyrA*(Am) *supF*(Ts) and *gyrB*(Ts) mutants formed anucleate cells at the restrictive temperature, at a greater rate than the *dna* mutants (Table 2). In these mutants, the aberrant positioning of constrictions appeared to be related to the retardation of nucleoid segregation (Fig. 2e and f). Upon the shift to 42°C, both mutants showed an increase in growth rate, which from then on declined steadily (results not shown). The *minB* mutant also showed impaired segregation (manuscript in preparation) and formed minicells (small rounded anucleate cells) constitutively during growth under steady-state conditions at 37°C.

Comparison of positioning of constrictions in filaments with and without actively replicating nucleoids. From the examination of the various DNA-less cell-forming mutants in fluorescence micrographs as shown in Fig. 2, it appeared that most mutants preferentially positioned constrictions close to the nucleoid. Only the *dnaX*(Ts) *sfiA* and *dnaA46*(Ts) mutants at 42°C appeared to constrict on an average farther away from the nucleoid. Because of this difference, the positioning of constrictions in these filaments was analyzed in more detail.

Figure 3A shows the length distribution of anucleate cell parts (L_{NP}) of the *dnaX*(Ts) *sfiA* mutant cells at 42°C. From this distribution, a theoretical distribution of nucleoid-constriction distances (L_{NC}) was derived, assuming random positioning of constrictions between the nucleoid and the polar cap (which was assumed to extend 0.8 μm from the cell pole). Every L_{NP} length class of 0.2 μm in width contains a number of cells which may constrict with equal probability in any length class of the indicated range. Thus, the number of cells in each L_{NP} length class was distributed evenly over the L_{NC} classes between the nucleoid and $L = L_{NP} - 0.8$ μm. Summing up of the cells that thus had been assigned to every L_{NC} length class resulted in the desired length distribution. From the correspondence of the theoretical and measured L_{NC} length distributions (Fig. 3B), we conclude that positioning in the anucleate parts of the *dnaX*(Ts) *sfiA* filaments (at 42°C) is close to random. In contrast, the

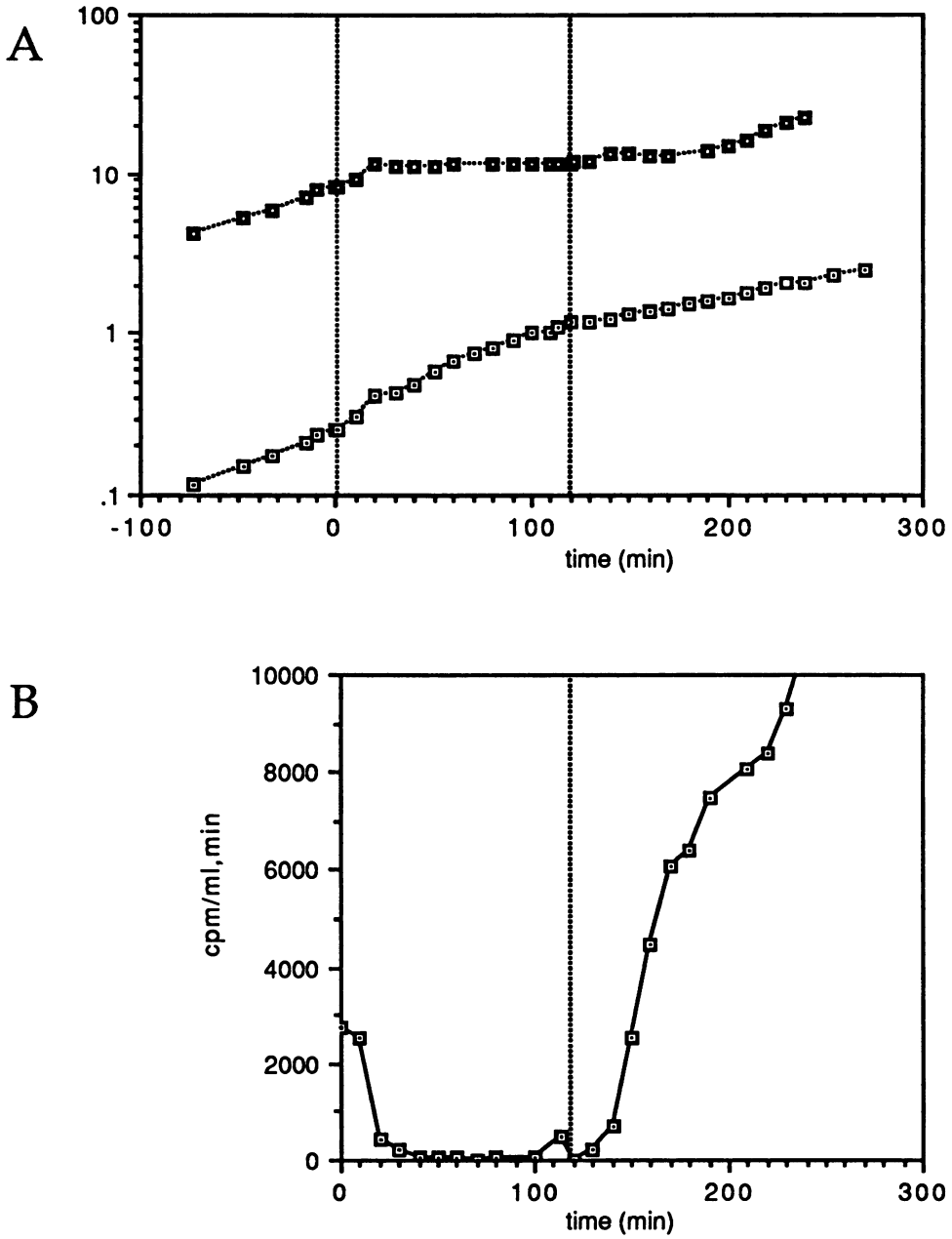


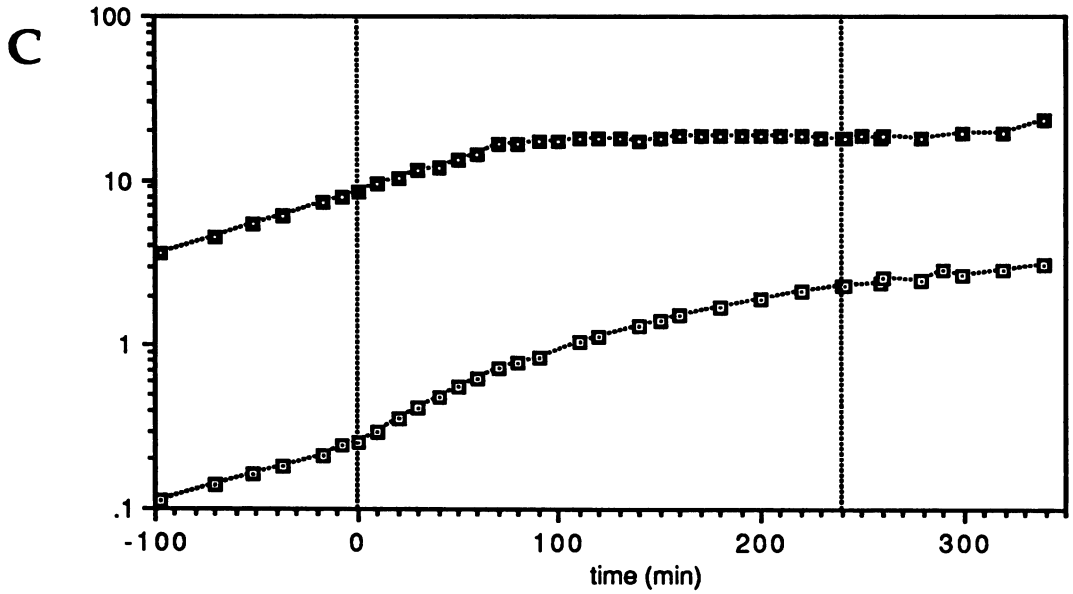
FIG. 1. Growth and changes in cell number of *E. coli* replication *dnaX*(Ts) mutant (A) and the initiation *dnaA46*(Ts) mutant (C) after a shift to the restrictive temperature (42°C) at 0 min and after a shift back to the permissive temperature (30°C); OD_{450} (□) and cell number (■). The changes in replication rate during the temperature shift experiment are depicted in panels B and D for the *dnaX*(Ts) and *dnaA46*(Ts) mutants, respectively. Shifts are indicated by dotted verticals.

dnaX(Ts) filaments, after a shift back to the permissive temperature, positioned constrictions preferentially close to the nucleoid (Fig. 4). A similar difference in positioning of constrictions relative to nucleoids was observed when isogenic *dnaA46*(Ts) cells growing at 42°C (Fig. 5) were shifted back to 30°C (Fig. 6), an observation also obtained with the *dnaA46*(Ts) mutation in another genetic background (WM1028; Table 1). However, in both *dnaA46*(Ts) strains growing at the restrictive temperature (Fig. 5B), positioning of constrictions was less random than in the *dnaX*(Ts) *sfiA* mutants (Fig. 3B).

Constriction positioning in the *gyr* mutants, which continued to replicate, was found to be nonrandom. However, due to the relatively short length of the nucleoid-free parts of the filaments, the distinction between random and nonrandom constriction positioning was less clear. The *gyrB*(Ts) mutant appeared to constrict preferentially close to the nucleoid, and the *gyrA*(Am) *supF*(Ts) mutant appeared to constrict somewhat more at random (results not shown).

We conclude from these results that the positioning of constrictions is influenced by the replication activity of the nucleoids.

Anucleate cell formation in the various DNA replication and segregation mutants. Do the above observations on constriction positioning exclude the formation of DNA-less cells of a certain uniform length? In the *dnaX*(Ts) *sfiA* filaments,



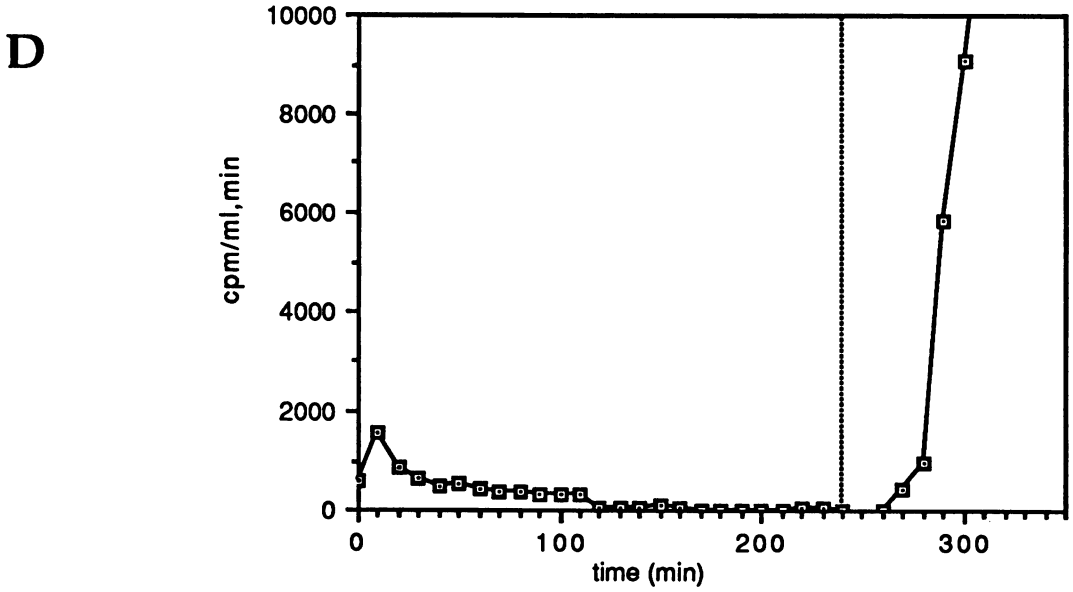


FIG. 1—*Continued.*

which did not replicate while forming DNA-less cells, constrictions were positioned nearly randomly with respect to the cell pole as well as to the nucleoid (Fig. 3B). The length distribution of the DNA-less cells that were being formed is shown in Fig. 3C and appeared to be wide (CV, 33%; Table 3) and corresponded closely with the calculated L_{CP} distribution (result not shown). The replicating filaments of the *dnaX*(Ts) mutant after the shift back pinched off DNA-less cells close to the nucleoid (Fig. 4B). Figure 4C shows that, as a consequence, these filaments pinched off anucleate cells with a length distribution similar to that of the corresponding nucleoid-free cell parts (CV, 42%; Table 3 and Fig. 4A). The length distributions of the DNA-less cells formed by the *dnaX*(Ts) and *dnaX*(Ts) *sfiA* filaments show that their great variation is probably not the result of bimodality, caused by positioning of constrictions at L_0 or $2 \times L_0$ (L_0 = 2.0 μm) from the cell pole.

The *dnaA46*(Ts) mutant, growing at 42°C, formed DNA-less cells with a narrower length distribution than expected on the basis of random constriction positioning. This can in part be explained by the formation of a few small rounded anucleate cells (minicells; Fig. 5C). The average length of these DNA-less cells (3.1 μm; Table 3) cannot be compared with the newborn-cell length of the 30°C steady-state culture (see above), but is greater than that of the wild-type strain or the *dnaX*(Ts) mutant (1.4 and 2.0 μm, respectively; Table 3); the variation of the *dnaA46*(Ts) L_{CP} distribution (CV, 21%; Table 3) was, although smaller than that of the *dnaX*(Ts) mutants (CV, 42%; Table 3), significantly greater than that of wild-type newborn cells (CV, 11%; Table 3). Table 3 also

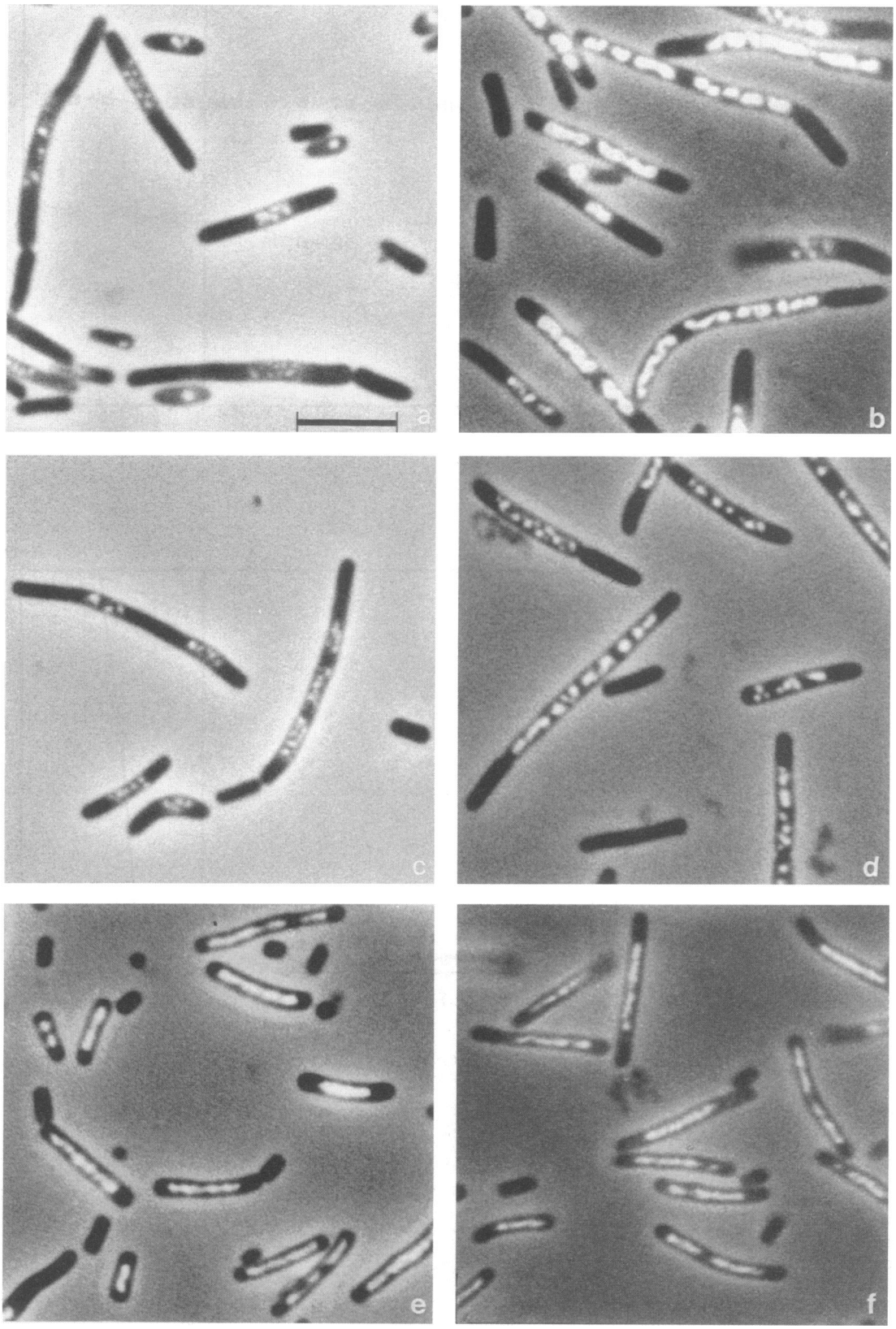


FIG. 2. Fluorescence micrographs (3,200×) of DNA-less cell-forming filaments of (a) *dnaX*(Ts) *sfiA* after 5 h at 42°C; (b) *dnaX*(Ts) 2 h after a shift back to 30°C; (c) *dnaA46*(Ts) after 5 h at 42°C; (d) *dnaA46*(Ts) 1.5 h after a shift back to 30°C; (e) *gyrA*(Am) *supF*(Ts) after 3 h at 42°C; and (f) *gyrB*(Ts) after 2 h at 42°C. Bar, 5 μm.

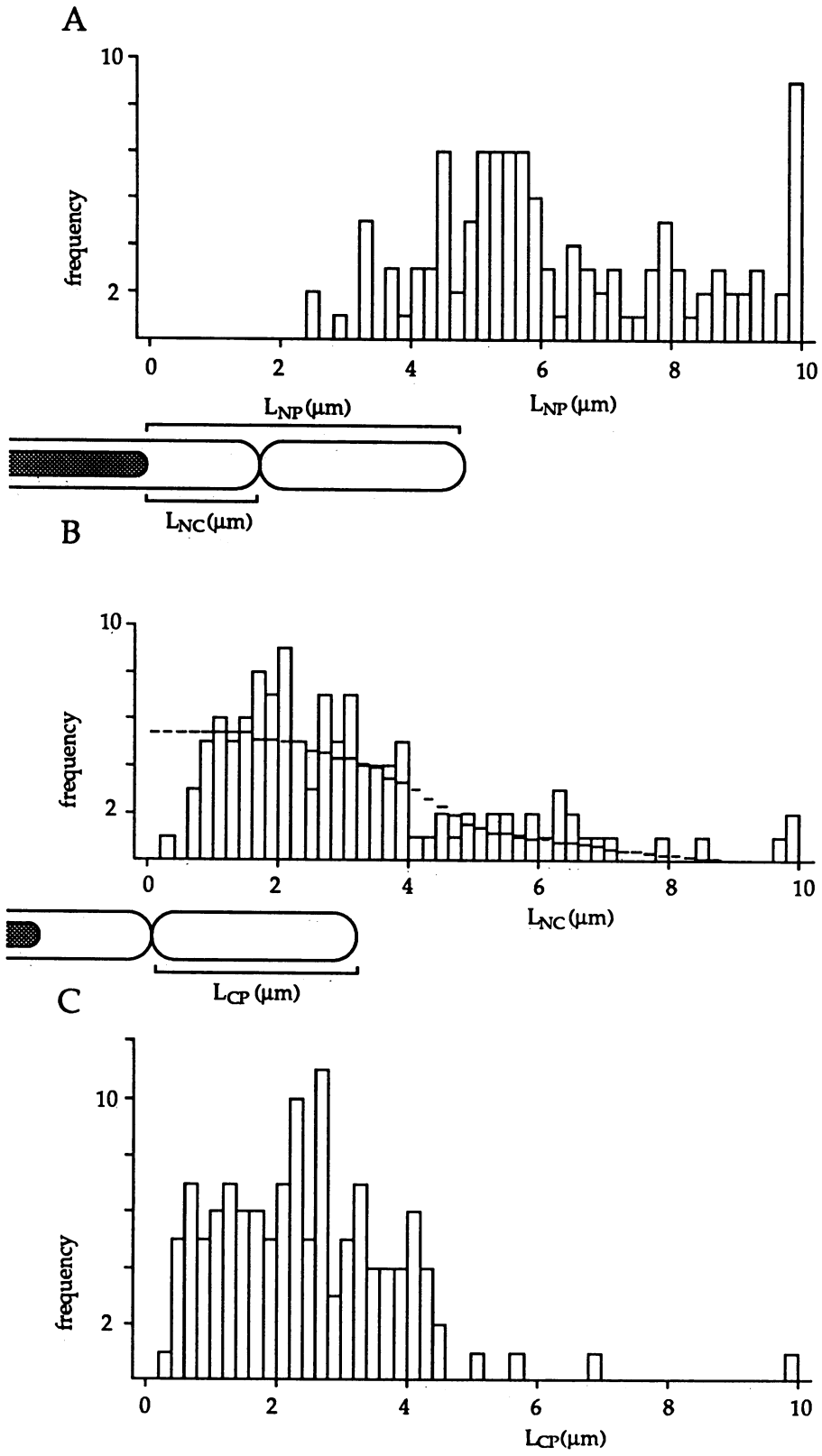


FIG. 3. (A) Distribution of nucleoid-cell pole distances (L_{NP}) measured for *dnaX*(Ts) *sfiA* filaments after 5 h at 42°C, which are pinching off DNA-less cells. (B) Plot of the theoretical distribution of the nucleoid-constriction distances (L_{NC}) (dashed line), assuming random constriction positioning (see text), superimposed on the histogram of the measured nucleoid-constriction distances. (C) Length distribution of DNA-less cells (L_{CP}) being pinched off from the filaments. All dimensions were measured from fluorescence micrographs at a final magnification of 3,200×. A total of 124 constricting filaments were measured.

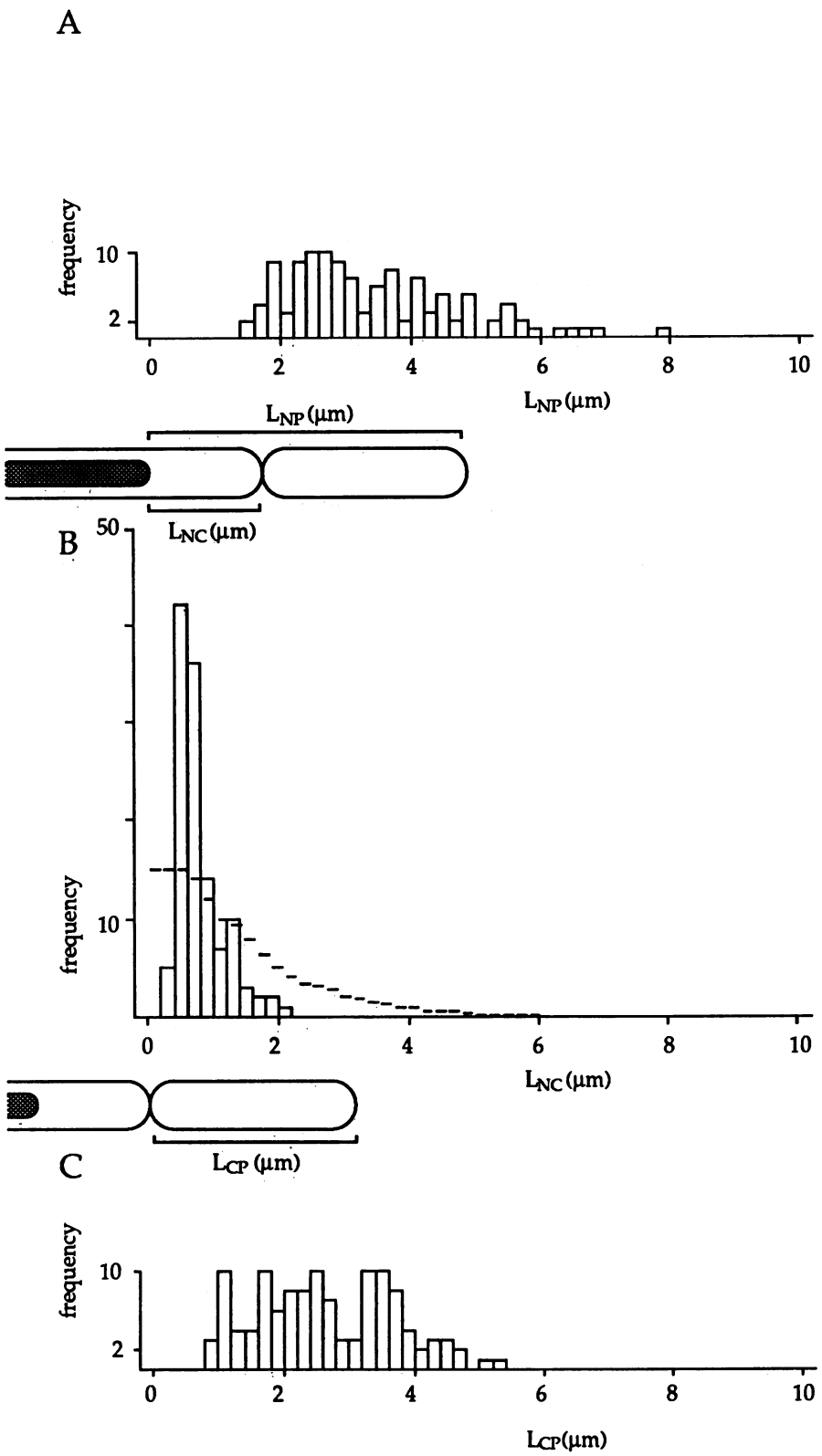


FIG. 4. *dnaX*(Ts) filaments 2 h after a shift back to 30°C. See Fig. 3 legend for details. A total of 120 filaments were measured.

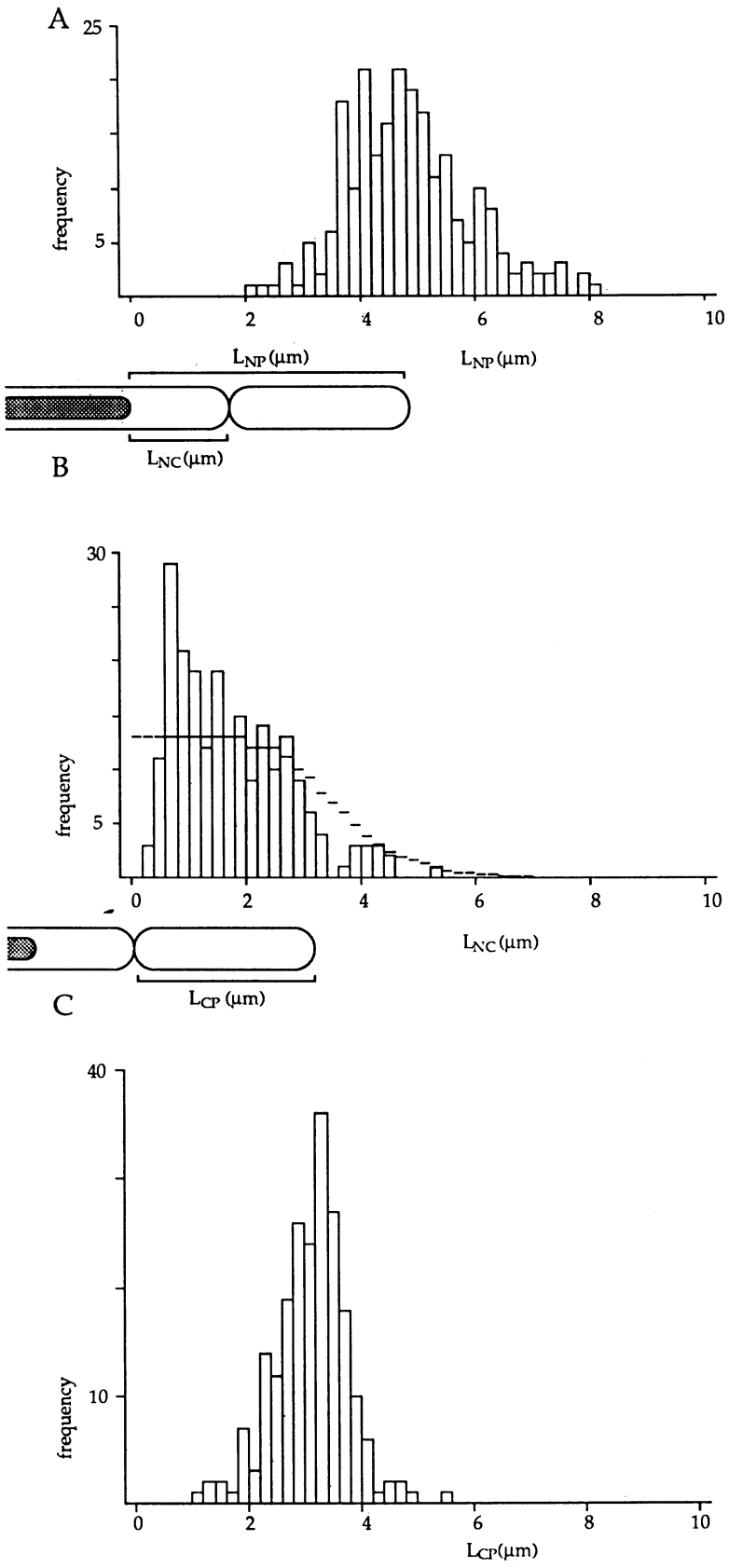


FIG. 5. *dnaA46*(Ts) filaments after 5 h at 42°C. See Fig. 3 legend for details. A total of 215 filaments were measured.

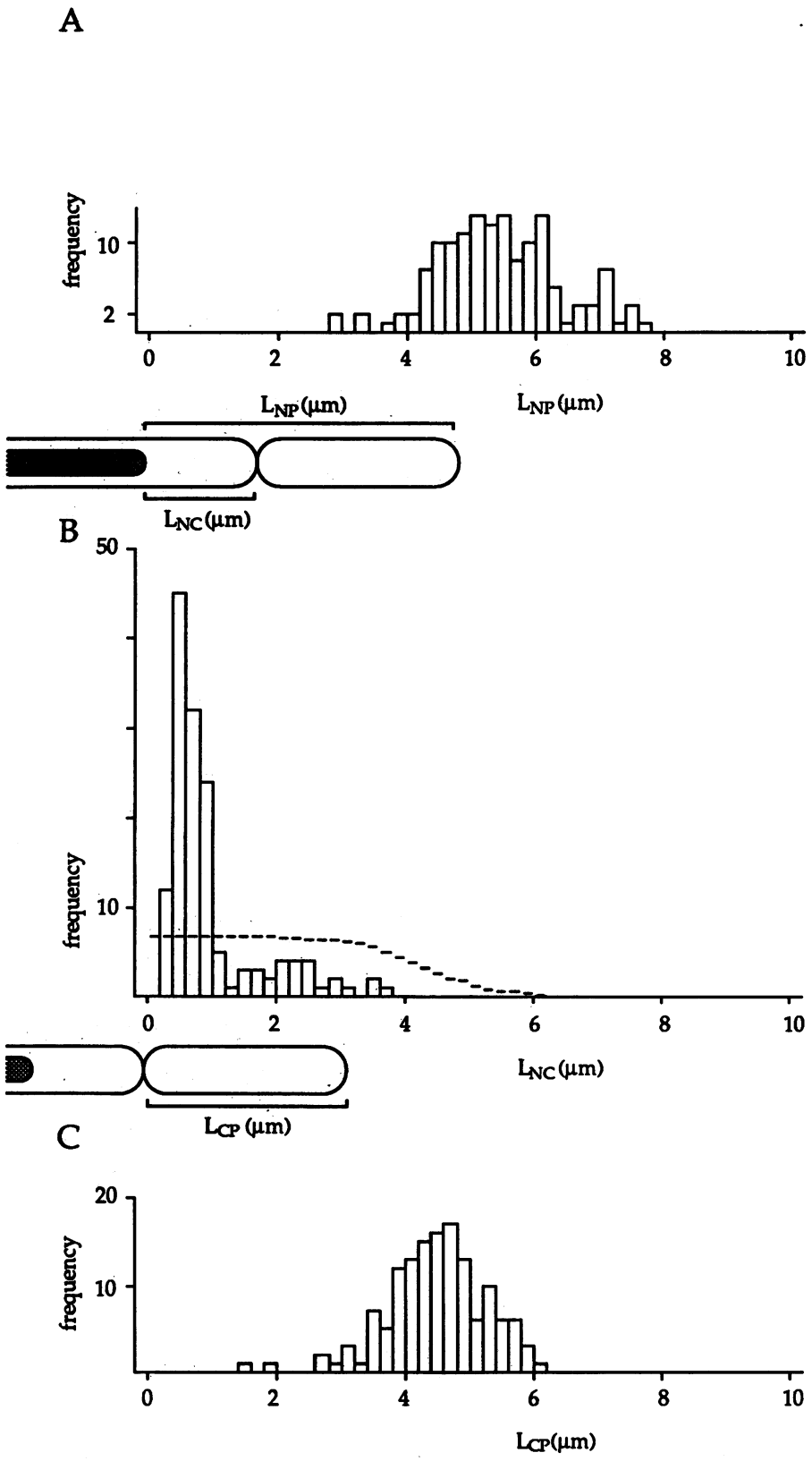


FIG. 6. *dnaA46*(Ts) filaments 1.5 h after a shift back to 30°C. See Fig. 3 legend for details. A total of 139 filaments were measured.

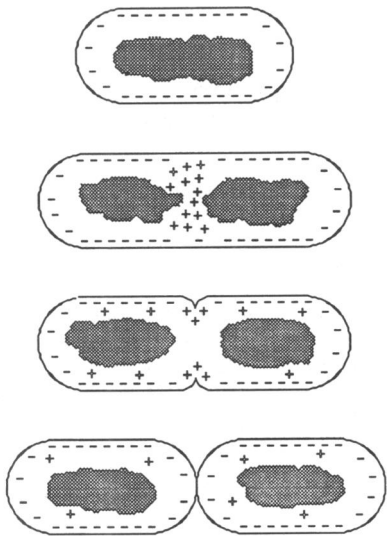


FIG. 7. Schematic representation of how, in an *E. coli* cell, a positive signal (+) that is generated upon termination of DNA replication diffuses into the cell and initiates a constriction at a site where a negative nucleoid effect (−), which is also the result of nucleoid activity, has weakened sufficiently.

shows that the lengths of the nucleoid-free parts of DNA-less cell-forming *dnaA46*(Ts) filaments had a relatively narrow distribution (CV, 27%), which, together with the slight positioning effect shown in Fig. 5A, may contribute to the smaller variation of the anucleate-cell length distribution (Fig. 5C).

From these results we conclude that no positioning of constrictions at a uniform distance from the cell pole occurs in any of these DNA-less cell-forming mutants.

DISCUSSION

Precise positioning of constrictions in the absence of segregated nucleoids depends on replication activity of the nucleoid. Filaments that do replicate, as in *dna* mutants upon a shift back to the permissive temperature and in the *gyr* segregation mutants at the restrictive temperature, preferentially position constrictions close to the nucleoid. In the absence of DNA replication, positioning of constrictions along the anucleate part of the filamentous *dnaX*(Ts) *sfiA* cells is close to random.

The *dnaA46*(Ts) mutant (Fig. 5) positioned constrictions somewhat less randomly at 42°C, i.e., on average closer to the nucleoid than those of the *dnaX*(Ts) *sfiA* mutant (Fig. 3). This difference in constriction positioning may be the result of on-going initiation of DNA replication in a subpopulation of the culture. Indeed, low but significant replication activity could be detected up to 2 h after the shift to 42°C (Fig. 1D). The synchronization effect in on-going initiations observed by Helmstetter and Weinberger (3) with the *dnaA5*(Ts) mutant at the restrictive temperature could not be observed in the *dnaA46*(Ts) mutant, which may be due to stronger inhibition of initiation.

In the literature it has been considered that constrictions may be predetermined through the localization of specific constriction enzymes in either growth zones (5) or zones of adhesion (16). Alternatively, specific peptidoglycan-synthesizing enzymes may be locally activated (1, 21). From the present observations on positioning of constrictions (Fig. 3 to 6) and the distributions of anucleate cells that were being formed (Fig. 3C, 4C, 5C, and 6C), we conclude that constriction sites are not predetermined but can be induced by local activation of peptidoglycan-synthesizing enzymes specific for constriction (1, 21). We think that such local activation could be obtained by assuming interaction of two opposing factors, which are generated by the nucleoid and interact with peptidoglycan-synthesizing enzymes in the plasma membrane.

The first, negative factor causes an overall slowing down of peptidoglycan synthesis. It is a short-range or labile factor which ensures that a constriction is never initiated in the nuclear region of the cell. Inhibition of protein synthesis by chloramphenicol (unpublished observations) or conditions of slow growth (20) appears to abolish this so-called negative "nucleoid effect," as some cells start to constrict before the nucleoids are properly segregated. Therefore, the negative nucleoid effect may be related to a transcription or translation activity of the nucleoid. A negative nucleoid effect is confirmed by the observation that in *dnaX*(Ts) filaments growing at 42°C, the rate of peptidoglycan synthesis, analyzed by autoradiography, was lower in the nuclear region than in the nucleoid-free cell ends (manuscript in preparation).

The second, positive factor is synthesized or released upon termination (e.g., the termination protein postulated by Jones and Donachie [11]). We envisage that upon termination of replication, a weak but stable activator of peptidoglycan synthesis is released, which acts either directly or via helping structures like periseptal annuli (16) or via intermediate steps like calcium fluxes (V. Norris, S. J. Seror, S. Casaregola, and I. B. Holland, J. Theor. Biol., in press). This activator can only induce constriction at a site where the influence of the first, negative factor has weakened sufficiently. During the normal cell cycle, this will ensure that constriction is only initiated when the nucleoids have segregated some distance apart.

If termination has occurred but segregation is in some way retarded, as occurs in the *gyr* mutants at the restrictive temperature and in the *minB* mutant (manuscript in preparation), the released positive factor may induce constriction just outside the inhibitory influence of the nucleoid, between the nucleoid and the cell pole. As a result, DNA-less cells are pinched off close to the nucleoid.

The *dnaX*(Ts) mutant filaments start to constrict about 60 min after the shift back to the permissive temperature, between segregated nucleoids or close to the nucleoids in the nucleoid-free cell parts. The DNA replication period for these cells has been estimated at 42 min (J. A. C. Valkenburg, C. L. Woldringh, P. Huls, and N. Nanninga, submitted for publication). The timing and positioning of constrictions indicate that these constrictions may well be induced by the putative positive factor, which is produced upon termination of replication.

The *dnaX*(Ts) *sfiA* replication mutant pinches off anucleate cells at the restrictive temperature, when DNA replication is inhibited. In these cells, no positive factor is released, and we therefore presume that in these cells constrictions are initiated spontaneously at a site where the negative factor has lost its influence. As a result, DNA-less cells are pinched off randomly in the anucleate region and at a low rate compared with *dnaX*(Ts) cells that recover from a temperature shift (Table 2). The same explanation applies to the difference in constriction positioning in the *dnaA46*(Ts) mutant growing at 42 and at 30°C.

The above concept, summarized in Fig. 7, can explain the division behavior of *E. coli* in many different physiological situations and may therefore help in further defining the factors involved in the toporegulation of cell division.

ACKNOWLEDGMENTS

We thank N. Nanninga for critical comments on the manuscript, E. Pas, J.-P. Cameron, and R. Witzenburg for technical assistance, E. van Spronsen for helping with the software, and R. D'Ari and L. I. Rothfield for stimulating discussions.

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