The *min* Locus, Which Confers Topological Specificity to Cell Division, Is Not Involved in Its Coupling with Nucleoid Separation

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In *Escherichia coli*, nucleoid separation and cell constriction remain tightly linked when division is retarded by altering the level of synthesis of the protein FtsZ. In this study, we have examined the role of the *min* locus, which is responsible for the inactivation of polar division sites, in the partition-septation coupling mechanism. We conclude that the coupling persists in a Δ*min* strain and that its timing relative to replication remains dependent on the level of FtsZ synthesis. We suggest that the retarded nucleoid segregation observed in *min* mutants is the result of this coupling in cells with a perturbed pattern of nonpolar divisions.

The mechanisms relating termination of chromosomal DNA replication, partitioning of daughter chromosomes at the one-fourth and three-fourths positions of the cell length, and the formation of a cell constriction septum are not yet known in detail. Nucleoid partitioning and septation both require termination of DNA replication as well as an additional period of protein synthesis (reviewed in reference 9). It is also generally assumed that nucleoid separation is a prerequisite for septum formation, although only a few studies have been devoted to a direct morphological examination of these events in unperturbed *Escherichia coli* cells (16, 17). Our studies of the cell cycle under conditions of partial inhibition of synthesis of the protein FtsZ have led us to the conclusion that partition and constriction initiation are tightly linked (14). FtsZ is an essential protein which forms a cytoskeletal element, the septal ring, at the site of constriction (4). We observed that nucleoid separation and septation are similarly retarded relative to termination of DNA replication when cells are grown under conditions of *ftsZ* underexpression. This and additional experimental evidence led us to propose that nucleoid separation and septation are under a common temporal control, their timing being dependent on the rate of FtsZ synthesis (14). Since partition takes place normally in the absence of functional FtsZ protein, our model assumes that during the period between termination and partition-septation (G$_2$ period), FtsZ exerts a negative control on partitioning. This control would be lifted near the time when the protein migrates to the membrane and forms a septal ring.

Cells lacking a functional partition-septation coupling mechanism should produce DNA-less cells and display nucleoid segregation defects. Among the mutants with this sort of phenotype, we have primarily considered those with a defect in the *min* locus. The *min* operon encodes three proteins required for the proper placement of the septum at the center of the cell. One of these proteins, MinC, is a weak inhibitor of cell division which can be phenotypically suppressed by overexpression of the gene *ftsZ* (8). Point mutations in *ftsZ* yielding a minicell formation phenotype as well as resistance to MinCD

have suggested a direct interaction of FtsZ with the division inhibitor (3). MinD appears to have two functions (8). The first is to stimulate the division inhibition function of MinC, and the second is to allow MinE to suppress the MinC-dependent inhibition. MinE relieves this inhibition specifically in the middle of the cell when it is expressed at physiological levels. This finding, and the fact that the sequence of MinD resembles that of various ATPases involved in the partition of low-copy-number plasmids (6, 12), suggested a possible link between the *min* locus and nucleoid partition. Therefore, we investigated the possibility that the FtsZ-dependent coupling of partition with division is mediated by the *min* system.

In a previous study (14), the coupling of partition with septation was deduced from the fact that when cells are larger as a result of partial inhibition of FtsZ synthesis (increased division mass), nucleoids do not separate at the same time as in a noninhibited strain (i.e., do not separate at a constant partition mass soon after termination of DNA replication). Instead, the nucleoids are bigger and separate at the same time as the onset of septation (see reference 14 for details). We have repeated this sort of experiment in *min*$^+$ strain JS219 (14) and its isogenic counterpart JS964, in which the Δ*min::kan* substitution of PB114 (7) was introduced by P1-mediated transduction. Both strains were transformed with plasmid pJPB57, which expresses DicF RNA, the antisense inhibitor of *ftsZ*, under control of the wild-type *lac* promoter (15).

The strains were grown at room temperature in L broth containing 50 μg of ampicillin per ml, 500 μg of methicillin per ml, and 0.4% (wt/wt) glucose from an optical density at 540 nm (OD$_{540}$) of 0.15 to an OD$_{540}$ of 1. Then cells were diluted to an OD$_{540}$ of 0.02 into the same medium containing different concentrations of isopropyl-β-D-thiogalactopyranoside (IPTG) and grown at 32°C. When the OD$_{540}$ reached 0.6, chloramphenicol and 4,6-diamidino-2-phenylindole (DAPI) were added at 200 and 2 μg/ml, respectively, and incubation was continued for 30 min. Cells were collected by centrifugation and resuspended in L broth containing DAPI, chloramphenicol, and 1% (vol/vol) formaldehyde at an OD$_{540}$ of 10. Approximately 0.5 μl of cell suspension was spread between the slide and coverslip, and photographs were taken and analyzed as previously indicated (14).

We measured the mean length and number of nucleoids per cell of the strains grown for five mass doublings in the presence

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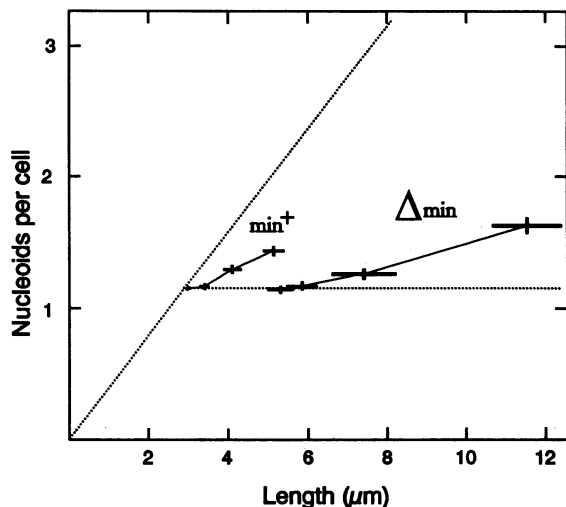
FIG. 1. Effects of a partial inhibition of FtsZ synthesis on average cell length and average number of nucleoids per cell in *min*⁺ (closed symbols) and Δ*min* (open symbols) strains. Between 175 and 250 cells were scored for each point. The crosses indicate the extents of the 95% confidence intervals of the means. Cells of increasing length were grown in the presence of 0, 0.1, 0.3, and 1 mM IPTG. The horizontal dotted line indicates the curve expected for a perfect coupling of partition with nonpolar septation. The dotted line passing by the origin corresponds to the case where partition takes place at a constant cell length.

FIG. 2. Mixed phase-contrast fluorescence pictures of *min*⁺ and Δ*min* cells. Strains JS219/pJPB57 (*min*⁺; A and B) and JS964/pJPB57 (Δ*min*; C and D) were grown without an inducer (A and C) or in the presence of 1 mM IPTG (B and D), and micrographs were taken as indicated in the text.

of different concentrations of inducer. Preliminary experiments had shown that at the temperature chosen (32°C), cells grown in the absence of an inducer were indistinguishable from those of the strains without a plasmid. Cells of either strain retained full viability when plated under the same conditions of medium and temperature on plates containing the highest IPTG concentration used (1 mM). Thus, we presumed that a new steady state of growth could be obtained at the end of the five mass doublings in the presence of an inducer.

The results of the analysis are shown in Fig. 1. In the *min*⁺ strain, there was a 73% increase in average cell length, from 2.95 μm without an inducer to 5.2 μm with 1 mM IPTG (Fig. 2A and B). Volumes were not measured, but in previous experiments involving inhibition of FtsZ, we determined that a 75% increase in length corresponded a 2.5- to 3-fold increase in cell volume (14). In contrast, the number of nucleoids per cell increased by only 24%. This value, higher than in previous experiments performed with a strain expressing DicF RNA constitutively (14), may reflect a slight deviation from steady-state conditions. Nevertheless, it confirmed the previously observed postponement of nucleoid partitioning. In the Δ*min* strain, the length increased from 5.3 μm without an inducer (Fig. 2C) to 11.5 μm in the presence of 1 mM IPTG (117% increase). No rod-shaped cells lacking DNA were observed. The number of nucleoids per cell increased by only 43%. This finding indicated that the FtsZ-dependent coupling of partition to division operates also in a Δ*min* strain.

Independently from the effect of varying *ftsZ* gene expression, the conclusion that partition is coupled to division can be proposed from a different observation. It was noticed that in untreated cells, the number of nucleoids per cell was the same in the Δ*min* strain and in its isogenic *min*⁺ counterpart (Fig. 1), yet these cells were 80% longer on the average. This finding indicated that in a Δ*min* strain, as in *min*⁺ strains, nucleoids separate at the time of constriction initiation, and therefore
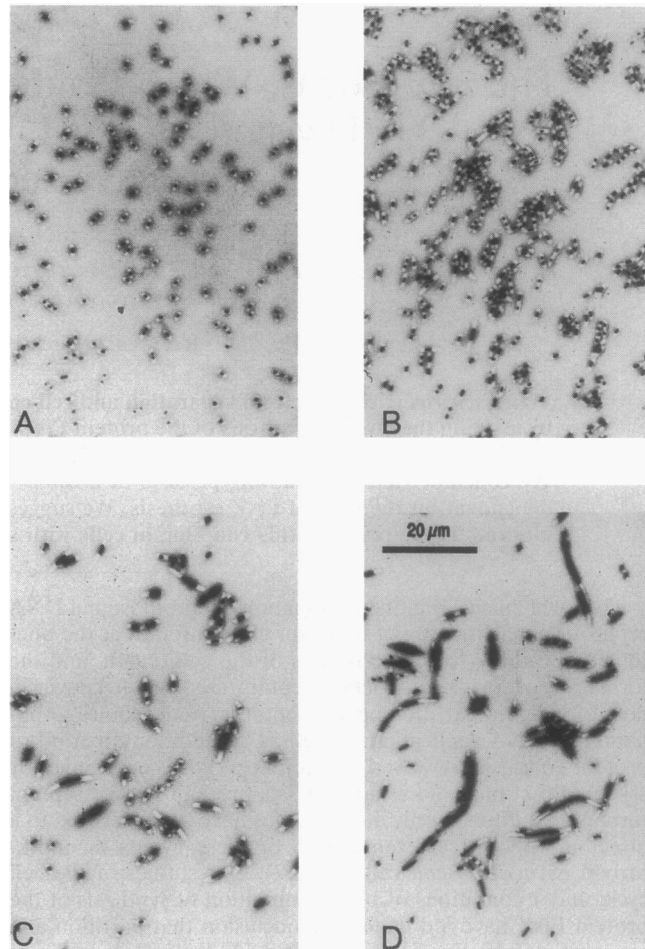
that partition is postponed together with cell division in the *min* mutant. A quantitative analysis showed the retardation of nucleoid separation more precisely (Fig. 3). For example, cells of the 3.95- to 4.95-μm size class (0.6 < log cell length < 0.7) almost always had two nucleoids in the *min*⁺ context and one in the Δ*min* background (1.96 and 1.04, respectively). As a result of retarded segregation, nucleoids appeared as large fluorescent bodies in the Δ*min* strain filaments (compare Fig. 2C and A for examples).

This aberrant nucleoid segregation, characterized by the presence of large masses of unsegregated DNA in *min* filaments, has been reported before by several groups (1, 11, 13). Mulder et al. (13) reported that plasmid DNA supercoiling is decreased in *min* mutants and compared them with *gyrB*(Ts) mutants, which generate DNA-less cells from filaments with unsegregated DNA at the nonpermissive temperature. In their view, the absence of a functional *min* gene product causes defective partitioning, which in turn leads to delayed septation at nonpolar division sites. Akerlund et al. confirmed the perturbed pattern of nucleoid segregation and found that while nonpolar divisions are significantly cell cycle regulated, polar divisions appear with a random timing (1). They proposed that if the *min* proteins are inactivated or overproduced, the
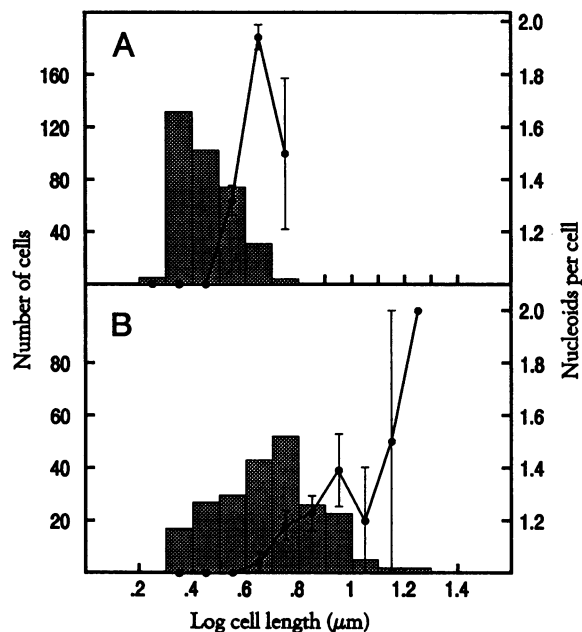
FIG. 3. Size distribution (histogram) and average number of nucleoids per cell (closed circles) in each size class for strains JS219/pJPB57 (*min*$^+$; A) and JS964/pJPB57 (Δ*min*; B) grown in the absence of IPTG. Error bars represent the 68% confidence intervals of the averages.

words, we propose that the irregular partition pattern observed in *min* mutants is a specific illustration of the general FtsZ-dependent mechanism coupling partition with septation. It should be noted that the hierarchical pattern of nucleoid segregation and nonpolar division found in *min* mutants by Jaffé et al. (10) can easily be incorporated into this model as an additional constraint of the division-partition coupling mechanism.

conformation of the nucleoid or its interaction to the partition system or the putative "divisome" is perturbed. Disturbed nucleoid interactions would block or delay division, and excess division factors would be used to generate polar divisions (2). These interpretations propose that the primary function of the *min* locus is to regulate nucleoid segregation.

However, available evidence suggests that the primary function of *min* proteins is to specifically prevent divisions within the polar cap region (3, 7, 8). Therefore, we propose the following model to explain the aberrant nucleoid segregation pattern. In *min* mutants, divisions are not prevented from taking place at the poles. We assume that these divisions have no direct effect on the segregation of neighboring DNA. They resemble normal divisions in their use of FtsZ molecules to form a septal ring (5). Thus, polar divisions would cause an abrupt and temporary decrease of the amount of FtsZ molecules available for nonpolar divisions. We propose that such a decrease has a consequence on nonpolar divisions similar to that of a reduced synthesis rate of FtsZ molecules (15), i.e., retarding septation relative to replication termination. Since polar divisions appear randomly (1), and polar septa have quite variable diameters (1), we expect the retardation of nonpolar septation to vary from cell to cell, leading to cell length heterogeneity. The FtsZ-dependent coupling of nucleoid separation with septation operating in these cells during the retardation period would lead to large nuclear bodies and, in *min* filaments, to the irregular DNA masses which have been considered as representative of a segregation defect. In other

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