Proper placement of the *Escherichia coli* division site requires two functions that are associated with different domains of the MinE protein

(cell division/minicell/protein domains)

CHUN-RUI ZHAO, PIET A. J. DE BOER*, AND LAWRENCE I. ROTHFIELD

Department of Microbiology, University of Connecticut Health Center, Farmington, CT 06030

Communicated by Mary Jane Osborn, University of Connecticut Health Center, Farmington, CT, January 23, 1995

ABSTRACT The proper placement of the *Escherichia coli* division septum requires the MinE protein. MinE accomplishes this by imparting topological specificity to a division inhibitor coded by the *minC* and *minD* genes. As a result, the division inhibitor prevents septation at potential division sites that exist at the cell poles but permits septation at the normal division site at midcell. In this paper, we define two functions of MinE that are required for this effect and present evidence that different domains within the 88-amino acid MinE protein are responsible for each of these two functions. The first domain, responsible for the ability of MinE to counteract the activity of the MinCD division inhibitor, is located in a small region near the N terminus of the protein. The second domain, required for the topological specificity of MinE function, is located in the more distal region of the protein and affects the site specificity of placement of the division septum even when separated from the domain responsible for suppression of the activity of the division inhibitor.

Cell division in *Escherichia coli* normally takes place by formation of a division septum at the midpoint of the cell. The selection of the midcell site occurs with high fidelity (1), resulting in formation of two equal-sized daughter cells.

The site-selection process is complicated by the fact that the cell contains three potential division sites that can be used to support septum formation, the normal site at the midpoint of the cell plus two sites that are located adjacent to each of the cell poles (2). The sites at the cell poles are thought to represent division sites that were located at midcell in a previous division cycle and that remain at the poles after completion of the previous division event. The polar sites are competent to support further rounds of septum formation and, if reused, give rise to chromosomeless minicells (3, 4). Therefore, the cell faces the problem of distinguishing the midcell site from the polar sites to ensure that only the midcell site is used to support septum formation. This site-selection process requires the protein products of three genes, *minC*, *minD*, and *minE* (5).

Genetic studies have suggested different roles for the three gene products. All results to date are consistent with the following model for MinCDE function. MinC and MinD normally act in concert to form an inhibitor of cell division, in which MinD is believed to function by activating a MinC-dependent division inhibition mechanism. The MinCD division inhibitor is required to prevent septation at the potential division sites at the cell poles, as shown by the observation that loss of either MinC or MinD results in minicell formation (5, 6). However, the MinCD division inhibitor lacks site specificity, that is, it prevents septation at all division sites—both polar and central—unless MinE is also present. Thus, expression of

minC and *minD* in the absence of MinE leads to formation of nonseptate nonminicelling filaments (5).

The role of MinE is to give topological specificity to the MinCD division inhibitor. It does so by suppressing the action of the division inhibitor at midcell, but not at the cell poles. This permits septation to occur at the proper midcell site but not at the potential division sites at the cell poles. As a result, the normal division pattern is restored when *minC*, *minD*, and *minE* are coordinately expressed. The mechanism whereby MinE gives topological specificity to the MinCD division inhibitor is unknown.

Based on this previous work, two functions are ascribed to the MinE protein. (i) MinE is an anti-MinCD factor, capable of suppressing the activity of the MinCD division inhibitor and thereby preventing MinCD-induced filamentation. (ii) MinE is a topological specificity factor, defined by its ability to suppress the activity of the MinCD division inhibitor at midcell but not at the cell poles. This implies that MinE can distinguish between the midcell division site and the potential division sites at the poles.

In the present study we present evidence that different domains within the 88-amino acid MinE protein are responsible for each of these two functions. The first domain, responsible for the ability of MinE to counteract the activity of the MinCD division inhibitor, is located in a small region near the N terminus of the protein. The second domain, required for the topological specificity of MinE function, is located in the more distal region of the protein and can affect the site specificity of placement of the division site even when separated from the domain responsible for suppression of the activity of the division inhibitor.

EXPERIMENTAL PROCEDURE

Strains and Growth Conditions. *E. coli* PB114(λ DB173) [Δ *minCDE*(P_{lac} -*minCD*)], PB103 (the *minCDE*⁺ parent of PB114), and UT481 (*minCDE*⁺) have been described (5, 7). Overnight cultures were diluted 1:100 into LB medium; isopropyl β -D-thiogalactoside (IPTG) was then added to 0.2 mM unless otherwise stated. The cultures were grown at 37°C to midexponential growth (OD₆₀₀ = 0.5) before being examined.

Plasmids. Details of the plasmids used are in Fig. 1. Numerical superscripts attached to gene and protein symbols refer to the positions of amino acids in the *minE* gene product. Plasmids containing the constitutive P_{aadA} promoter were derivatives of the low-copy-number pSC101-derived plasmid pGB2 (8). Plasmids containing P_{lac} were derived from the moderate-to-high-copy-number ColE1-derived pUC or pMLB plasmids (9). pDB183 and pDB156 have been described (5,

FIG. 1. Effects of *minE* plasmids on division-site selection. The phenotypes of cells containing each of the indicated plasmids in host cells PB114(ΔDB173) and PB103 are indicated to the right. Cells were grown in the presence of IPTG. Similar results were obtained at IPTG concentrations between 0.05 and 4 mM for cells containing *P_{aadA}* plasmids and between 0.05 and 0.4 mM for cells containing *P_{lac}* plasmids, except for pZC57 where the range was 0.05–4 mM. When grown in LB medium containing 0.2% glucose, cells containing the *P_{aadA}* plasmids showed a minicelling (Min⁻) phenotype because of the *minCDE* deletion in the host strain, and cells containing the *P_{lac}* plasmids showed a wild-type phenotype. Phenotypes: Min⁻ (minicelling phenotype), large numbers of minicells plus cells of various lengths from normal to approximately six times normal (Fig. 2c) with internal and/or polar septa visible in ~15% of the cells; Sep⁻ (filamentation phenotype), all cells were very long filaments with no evidence of minicells or of internal or polar septa; WT (wild-type phenotype), normal division pattern. The *minE* inserts within each plasmid are shown below the diagram of *minE*⁺. Solid lines indicate *minE* coding sequences and dashed lines indicate non-*minE* coding sequences. In pZC44 and pZC28, ~3.1 kb of *lacZ* is fused in-frame to the 3' end of the *minE* fragment. DNA sequencing confirmed the predicted sequence of the *minE* and proximal *lacZ* region of the insert. In pZC80 and pZC57, 5' extensions (coding for 46 and 13 amino acids, respectively) are fused in-frame to *minE*²³⁻⁸⁸. In pZC80, a ribosomal binding site and translational start codon from pET21d (Novagen) are located between *P_{aadA}* and the *minE*²³⁻⁸⁰ insert. The terminology for the MinE protein products is shown to the right. The numbers refer to the positions of amino acids within the MinE product. P, *Pst* I; S, *Sna*BI; H, *Hpa* II.

10). Complete details of plasmid constructions can be obtained by writing to the authors.

Immunological Procedures. Anti-MinE antibody was prepared by immunizing rabbits with a synthetic peptide containing amino acids 29–38 of native MinE, coupled to keyhole limpet hemocyanin. The antibody was affinity-purified, with a column prepared by coupling the MinE-(29–38) peptide-keyhole limpet hemocyanin complex to Affi-Gel 10 (Bio-Rad), and was then absorbed with a cell extract of *minCDE* deletion strain PB114 (7). Cells were solubilized by boiling in 2% (wt/vol) SDS (11), and the supernatants obtained by centrifugation for 10 min at 13,000 rpm in an Eppendorf microcentrifuge were used as samples for immunoblot analysis. For Western blots (see Fig. 3A), samples were electrophoresed in SDS/polyacrylamide gels (16% total gel concentration/2.67% crosslinking) and then transferred to nitrocellulose membranes (7). For dot blots (see Fig. 3B), samples were serially diluted in 0.2 M Tris-HCl (pH 6.8) and 3-μl aliquots were applied directly to the membranes. For both Western and dot blot analyses, the membranes were sequentially exposed to anti-MinE-(29–38) antibody and ³⁵S-labeled donkey anti-rabbit IgG (Amersham), as described for studies with nonradioactive second antibodies (11). A Packard Instant Imager 2024 was used to quantitate radioactivity in each band or spot.

The concentration of MinE (expressed as molecules per cell) was then calculated by using known amounts of purified MinE as a standard. For the quantitation of MinE in cells that coexpressed MinE⁺ and MinE-(23–88), the primary antibody was directed against amino acids 2–21 of MinE, prepared by immunization with a MinE-(2–21) synthetic peptide.

RESULTS

Assay for MinE Functions. The ability of wild-type and mutant forms of MinE to counteract the MinCD division inhibitor was studied in host strain PB114(ΔDB173) [Δ*minCDE*(*P_{lac}::minCD*)], in which MinCD-mediated filamentation can be induced by growth in the presence of IPTG (5). Wild-type or mutant forms of *minE* were placed under control of the constitutive *P_{aadA}* promoter in the low-copy-number plasmid pGB2 and were introduced into the host strain. When *minCD* was induced by IPTG in the host strain containing the pGB2 vector, there was a complete arrest of division, as shown by formation of nonseptate filaments (Fig. 2a). In contrast, induction of *minCD* in the presence of the *P_{aadA}-minE*⁺ plasmid pDB183 restored the normal division pattern (Fig. 2b). Thus, as shown (5, 10), MinE was capable of counteracting the MinCD division inhibitor, as shown by suppression of filamentation, and did so in a topologically specific manner, as shown by the absence of polar septation events (i.e., the absence of minicell formation).

Topological Specificity Domain. We define topological specificity as the ability of MinE to suppress the activity of the MinCD division inhibitor at midcell but not at the cell poles,

FIG. 2. Effects of wild-type and mutant MinE proteins on division pattern. Cells were examined by phase-contrast microscopy (12) after growth in the presence of 0.2 mM IPTG. (a) PB114(ΔDB173)/pGB2 [Δ*minCDE*(*P_{lac}-minCD*)/*P_{aadA}*-vector]. (b) PB114(ΔDB173)/pDB183 [Δ*minCDE*(*P_{lac}-minCD*)/*P_{aadA}-minE*⁺]. (c) PB114(ΔDB173)/pZC22 [Δ*minCDE*(*P_{lac}-minCD*)/*P_{aadA}-minE*¹⁻⁵³]. (d) PB114(ΔDB173)/pZC44 [Δ*minCDE*(*P_{lac}-minCD*)/*P_{aadA}-minE*¹⁻²²]. (e) PB114(ΔDB173)/pZC80 [Δ*minCDE*(*P_{lac}-minCD*)/*P_{aadA}-minE*²³⁻⁸⁸]. (f) PB103/pZC57 [*minCDE*⁺/*P_{lac}-minE*²³⁻⁸⁸].

as described above. Loss of topological specificity is defined by the inability of certain mutant MinE proteins to prevent minicelling in cells in which *minCD* was induced, despite the continued ability of the mutant proteins to counteract the MinCD-mediated division block.

The seven C-terminal amino acids of MinE were not required for topological specificity, as shown by the observation that MinE proteins terminating at amino acids 81 and 84 (encoded by pZC118 and pZC114), like the wild-type protein, restored the wild-type division pattern to cells induced for *minCD* (Fig. 1). In contrast, more-extensive C-terminal deletions led to loss of topological specificity. Thus, plasmids pZC22 and pZC44, coding for truncated MinE proteins MinE-(1-53) and MinE-(1-22), were unable to restore the normal division pattern to cells in which *minCD* was induced by growth in the presence of IPTG (Fig. 2 *c* and *d*). Instead, the cells showed the classic minicelling phenotype.

These results suggested that at least part of the domain responsible for topological specificity was located between amino acids 53 and 81. However, it is known that the minicelling phenotype can also be induced by overexpression of *minE*⁺ (5, 9). It therefore was possible that the inability of the truncated MinE proteins to prevent minicelling was due to an increase in cellular concentration of the mutant MinE proteins rather than to loss of a domain that was directly involved in the topological specificity function. To exclude this possibility, we determined the cellular concentrations of the MinE⁺ and MinE-(1-53) proteins by quantitative immunoblot analysis of cells of PB114(Δ*DB173*) [*ΔminCDE*(P_{lac}::*minCD*)] containing pZC22 [*minE*¹⁻⁵³] or pDB183 [*minE*⁺] (which showed normal topological specificity) (Fig. 3*B* and Table 1. This showed that the concentration of immunoreactive MinE protein in *minE*¹⁻⁵³ cells was in fact somewhat lower than the concentration in the *minE*⁺ cells, showing that the failure of MinE-(1-53) to prevent minicelling was not due to an increase in the cellular concentration of MinE. Therefore, we ascribe the inability of MinE-(1-53) to prevent minicelling to the loss of a domain required for topological specificity.

Anti-MinCD Domain. Deletion analysis showed that the MinE domain required for suppression of MinCD-mediated division inhibition was located in the N-terminal region of MinE (Fig. 1). Thus, a truncated MinE molecule [MinE-(1-22), encoded by pZC44], in which the C-terminal 66 amino acids were replaced by LacZ sequences, prevented the filamentation that otherwise occurred when *minCD* was induced by growth of PB114(Δ*DB173*) [*ΔminCDE*(P_{lac}::*minCD*)] in the

Table 1. Quantitation of MinE⁺ and MinE-(1-53)

Plasmid	Relevant genotype of strain	MinE, molecules per cell
pGB2	Δ <i>minCDE</i>	<5
pDB183	Δ <i>minCDE</i> /P _{aadA} - <i>minE</i> ⁺	1000 ± 72
pZC22	Δ <i>minCDE</i> /P _{aadA} - <i>minE</i> ¹⁻⁵³	704 ± 130
—	Chromosomal <i>minE</i> ⁺	210

Strain PB114(Δ*DB173*) containing the indicated plasmids, and strain PB103 (when no plasmid is indicated), was grown as described in Fig. 3, and MinE concentrations (±SD) were determined.

presence of IPTG (Fig. 2*d*). As expected from this result, plasmids encoding MinE molecules with smaller C-terminal deletions, such as pZC22 [P_{aadA}-*minE*¹⁻⁵³] (Fig. 2*c*), also suppressed MinCD-induced filamentation.

When expressed in wild-type cells, MinE-(1-22) and MinE-(1-53) induced the minicelling phenotype when expressed under P_{lac} control (from pZC28 and pZC20, Fig. 1) and when expressed at lower levels from P_{aadA} (from pZC22, data not shown). This confirms the ability of the anti-MinCD domain of MinE to counteract the action of the division inhibitor at the cell poles when released from the constraints of the topological specificity domain.

We conclude from these experiments that the MinE domain responsible for suppression of the division inhibitory effect of MinCD is located between amino acids 1 and 22 of the native MinE protein.

Phenotypic Effect of Expression of the MinE Topological Specificity Domain. The results described above show that the MinE topological specificity domain includes determinants located in the MinE-(23-81) region of the protein. However, they do not establish whether this domain can function independently of the N-terminal region that is required for suppression of the effects of the MinCD division inhibitor.

Evidence that the putative topological specificity domain can function when disconnected from the N-terminal portion of MinE was obtained by asking whether MinE-(23-88) was capable of competing with MinE⁺ in the process of division-site localization. To accomplish this, synthesis of MinE-(23-88) was induced in wild-type cells containing pZC57 [P_{lac}-*minE*²³⁻⁸⁸] by growth in the presence of IPTG. MinE⁺ was provided from the chromosomal *minE*⁺ gene of the host cell. As shown in Fig. 2*f*, induction of *minE*²³⁻⁸⁸ resulted in a change from the wild-type division phenotype to the classic minicelling phenotype. Quantitative Western blots showed that the cellular concentration of MinE⁺ was not elevated above wild-type levels in these cells, eliminating the possibility that the minicell phenotype had been induced by an increase in MinE⁺ concentration.

Thus, the C-terminal domain of MinE affected the site-selection process, as shown by its ability to interfere with the topologic specificity function of the chromosomally encoded MinE⁺ protein, even when disconnected from the domain responsible for counteracting the MinCD division inhibitor.

Because MinE-(23-88) lacks the N-terminal domain required for suppression of MinCD-induced division inhibition, it should not prevent MinCD-mediated filamentation. This was confirmed by showing that growth of cells of PB114(Δ*DB173*)/pZC80 [*ΔminCDE*(P_{lac}::*minCD*)/P_{aadA}-*minE*²³⁻⁸⁸] in the presence of IPTG resulted in filamentation (Fig. 2*e*).

Quantitation of MinE. Wild-type strains UT481 and PB103 contained 170 and 210 molecules of MinE per cell, respectively, calculated from quantitative Western immunoblots (illustrated in Fig. 3*A*). Cells in which *minE*⁺ was expressed under P_{aadA} control [in PB114(Δ*DB173*)/pDB183] contained ≈1000 molecules of MinE per cell.

It has previously been shown that overproduction of MinE in wild-type cells leads to the minicell phenotype (9). In the

FIG. 3. Radioimmunoblot analysis of MinE in cell extracts. Extracts were prepared from cultures grown in the presence of 0.2 mM IPTG. (A) Western blots. Lanes: 1, PB114(Δ*DB173*)/pDB183 [*ΔminCDE*(P_{lac}-*minCD*)/P_{aadA}-*minE*⁺]; 2, UT481 [*minE*⁺]; 3, PB114(Δ*DB173*) [*ΔminCDE*(P_{lac}-*minCD*)]. Each lane contained 100 μg of protein. The arrows indicate the positions of 14.4-kDa (upper) and 10.6-kDa (lower) protein molecular mass standards. (B) Dot blots. Rows: a, PB114(Δ*DB173*)/pGB2; b, PB114(Δ*DB173*)/pDB183; c, PB114(Δ*DB173*)/pZC22. The amounts of protein applied were as follows: Columns: 1, 40 μg; 2, 20 μg; 3, 10 μg; 4, 5 μg.

present study, when UT481/pDB156 [*minCDE*⁺/P_{lac}-*minE*] was grown in various concentrations of IPTG for 2 hr, minicelling was induced when the MinE concentration exceeded 400 molecules per cell (IPTG $\geq 32 \mu\text{M}$). Minicelling was also induced when the MinE concentration in wild-type cells was elevated above 1000 molecules per cell by expression from P_{aadA}-*minE* (in PB103/pDB183 [*minCDE*⁺/P_{aadA}-*minE*]).

DISCUSSION

The mechanisms used by cells to ensure that topologically defined differentiation events, such as cell division, occur at their proper cellular locations are poorly understood. In bacteria, the proper placement of the division site requires that the cell be able to distinguish between the proper division site at midcell and old division sites that remain at the cell poles. The MinE protein plays an essential role in this topological discrimination function.

Previous work has defined two aspects of MinE function. First, MinE can reverse the effects of the MinCD division inhibitor, as shown by its ability to suppress the global division block that occurs when MinCD is expressed in the absence of MinE. The present study shows that this function resides entirely within a 22-amino acid segment [MinE-(1-22)] near the N terminus of the protein. It has previously been shown that although MinE can suppress the septation block induced by MinCD, it cannot suppress the division block induced when MinC is activated by an alternative activator, DicB (10). Therefore, although other scenarios are possible, MinD is likely to be the component of the division inhibition machinery that interacts with MinE. On the basis of the present results, we suggest that the MinE-(1-22) domain is responsible for this interaction.

The second aspect of MinE function, its ability to impart topologic specificity to the MinCD division inhibitor, implies that MinE, directly or indirectly, can discriminate between the potential division site at midcell and the potential division sites at the cell poles. The present study suggests that this function resides in a separate domain within MinE. Two types of evidence supported this conclusion.

(i) The 3' *minE* deletions that produced truncated proteins MinE-(1-53) and MinE-(1-22) led to loss of topological specificity, as shown by the inability of the truncated proteins to prevent minicelling despite their continued ability to suppress the action of the MinCD division inhibitor. Measurement of the cellular concentrations of wild-type and truncated MinE proteins showed that the apparent loss of topological specificity in MinE-(1-53) was not due to an increase in MinE concentration, an effect that also could have explained the inability of the C-terminally truncated proteins to prevent minicelling.

(ii) Expression of the putative topological specificity domain interfered with the topological specificity function of the native MinE protein, as shown by the ability of MinE-(23-88) to induce minicelling in wild-type cells. Thus, the topological specificity domain affects the placement of the division septum even when separated from the N-terminal domain that is responsible for counteracting the MinCD division inhibitor.

We consider two models to explain these results (Fig. 4). Both models are based on the assumption that MinE interacts, directly or indirectly, with a topological target that marks the new division site at midcell as being different from the residual division sites at the cell poles (Fig. 4A). Both models assume that a topological specificity domain within MinE has a high affinity for the topological target (illustrated in Fig. 4B). As a result, MinE is preferentially localized to the midcell site, where it can interact with the MinCD division inhibitor. This prevents the division inhibitor from blocking division at midcell but does not prevent the division inhibitor from blocking division at the cell poles. Although the figures show MinE

FIG. 4. Models for MinE function. (A–C) Model 1. Independent domain model. The topological specificity domain of MinE resides entirely in the MinE-(23–88) region of MinE. Local inhibition of septation is indicated by X. (A) Expression of *minCD* in the absence of MinE. (B) Expression of *minC*, *minD*, and *minE* from the chromosomal *minCDE* locus. (C) Expression of *minE*^{23–88} in cells expressing *minC*⁺, *minD*⁺, and *minE*⁺ from the chromosomal *minCDE* locus. Although not shown, MinE⁺ molecules in cells that coexpress MinE⁺ and MinE-(23–88) could also interact with the division inhibitor in the cytosol, thereby preventing the inhibitor from blocking septation at both polar and midcell division sites, with a similar result. (D) Model 2. Dimerization model. The topological specificity domain of MinE is created in the N-terminal region of the protein when MinE dimerizes. The dimerization determinant lies in the MinE-(23–88) region of MinE. The heterodimer [MinE⁺/MinE-(23–88)] is incapable of binding the topological target although it can still interact with the MinCD division inhibitor. T, topological target.

interacting directly with the topological target, intermediary proteins could also be involved. In both models, the site that interacts with the division inhibitor is located within the N-terminal domain [MinE-(1–22)] that is both necessary and sufficient to counteract the MinCD-mediated division inhibition reaction.

In the first model (Fig. 4A), the recognition site for the topological target is located within the MinE-(23–81) domain, consistent with the observation that deletion of this domain led to minicelling. When this domain is expressed in wild-type cells, it would compete with the wild-type MinE protein for access to the topological target (Fig. 4C). This prevents the chromosomally encoded MinE⁺ molecules from binding to the topological target, leaving them free to interact with the MinCD division inhibitor in a site-independent manner. Interaction with MinCD at a polar site will give rise to a minicell septation event, whereas interaction at the midcell site will give rise to a central division event. The resulting mixed pattern of polar and central divisions is what characterizes the minicell phenotype in classical minicell mutants [for example ΔminCDE and *minB1* mutants (4–6)] and, as shown in this

study, in cells that coexpress MinE⁺ and MinE-(23–88) (Fig. 2f).

In a second model (Fig. 4D), the MinE-(23–81) region is required only for oligomerization of MinE. In this view, oligomerization is required to create the binding site that interacts with the topological target, and formation of this binding site requires that both partners within the oligomer contain intact N-terminal domains. In this model, a single N-terminal domain can interact with the MinCD division inhibitor even when present within a mixed oligomer that is unable to interact with the topological target [for example, MinE⁺/MinE-(23–88)]. As a result, MinE⁺/MinE-(23–88) cells are capable of dividing when MinCD is expressed. However, because the hybrid protein is unable to recognize the normal topological target, it will not be functionally sequestered at midcell and, hence, will show a minicelling phenotype. This model requires that the same 22-amino acid segment [MinE-(1–22)] contain the recognition site for the MinCD division inhibitor and—when present within an oligomer—contribute to a recognition site for the topological target.

The idea that MinE can be effectively sequestered at the correct division site and thereby prevented from counteracting the MinCD division inhibitor at the cell poles implies that MinE is normally present in limiting amounts. Wild-type cells contained ≈200 molecules of MinE per cell. Therefore, if MinE was present as monomers, the cell would need only 200 copies of the putative topological target to bind all available MinE and thus ensure the normal pattern of septal placement; if MinE existed as oligomers, fewer target molecules would be needed. An increase in MinE concentration in wild-type cells to ≈400 molecules per cell induced the minicell phenotype, implying that this concentration of MinE exceeded that required to saturate the topological target, leaving the excess MinE free to prevent the division inhibitor from acting at the cell poles. In contrast, in cells that overexpressed MinCD, an increase in MinE concentration to ≈1000 molecules per cell did not induce minicelling [PB114(λDB173)/pDB183, Fig. 2b]. In this case, MinE that was present in excess of the number of topological target molecules was presumably titrated by the increased level of MinCD, thereby preventing the excess MinE from acting at the poles.

It has been suggested that the Min proteins may affect nucleoid conformation or interactions between the nucleoid and the chromosome partition system of the cell (13, 14). It is not clear how the present results would fit into such a model.

The results of the present study indicate that different sites within MinE are responsible for its interaction with the MinCD division inhibitor and for its interaction with a cellular recog-

nition site that distinguishes the potential division sites at midcell from the residual division sites at the poles. This implies that different molecular targets are used for the two MinE functions. If this idea is correct, it should be possible to identify the cellular proteins that interact with each of the two domains by the use of targeted genetic and biochemical approaches. According to our present view, the anti-MinCD domain [MinE-(1–22)] should interact directly or indirectly with MinD. The topological specificity domain that is located within the MinE-(23–88) region, or possibly within the N-terminal domain in MinE oligomers, should interact with the topological target that differentiates the proper division site at midcell from the residual division sites at the cell poles. In addition to its obvious relevance to the question of how cells decide where to divide, the identification of this target should provide information dealing with the more general question of how cells provide topological information for site-specific cellular functions.

This work was supported by Grant GM41978 from the National Institutes of Health.

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