The Min System as a General Cell Geometry Detection Mechanism: Branch Lengths in Y-Shaped *Escherichia coli* Cells Affect Min Oscillation Patterns and Division Dynamics[∇]

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In *Escherichia coli*, division site placement is regulated by the dynamic behavior of the MinCDE proteins, which oscillate from pole to pole and confine septation to the centers of normal rod-shaped cells. Some current mathematical models explain these oscillations by considering interactions among the Min proteins without recourse to additional localization signals. So far, such models have been applied only to regularly shaped bacteria, but here we test these models further by employing aberrantly shaped *E. coli* cells as miniature reactors. The locations of MinCDE proteins fused to derivatives of green fluorescent protein were monitored in branched cells with at least three conspicuous poles. MinCDE most often moved from one branch to another in an invariant order, following a nonreversing clockwise or counterclockwise direction over the time periods observed. In cells with two short branches or nubs, the proteins oscillated symmetrically from one end to the other. The locations of FtsZ rings were consistent with a broad MinC-free zone near the branch junctions, and Min rings exhibited the surprising behavior of moving quickly from one possible position to another. Using a reaction-diffusion model that reproduces the observed MinCD oscillations in rod-shaped and round *E. coli*, we predict that the oscillation patterns in branched cells are a natural response of Min behavior in cellular geometries having different relative branch lengths. The results provide further evidence that Min protein oscillations act as a general cell geometry detection mechanism that can locate poles even in branched cells.

Bacteria have a fundamental need to apportion their components consistently between daughter cells at division (42). The need is both genetic, because the replicated genomes must be segregated evenly, and biochemical, because the daughters are better off if each receives approximately equal amounts of cytoplasmic and membranous material (42). In *Escherichia coli*, at least two mechanisms restrict septation to the centers of predivisional cells—regular oscillation of the MinCDE proteins from pole to pole prevents preseptal FtsZ rings from forming at sites other than midcell (7, 12, 29, 30), and the SlmA protein inhibits FtsZ polymerization in regions directly around the bulk nucleoid (1). Other bacteria use different systems for comparable purposes (21, 38). Regardless of the biochemical specifics, all these mechanisms will distribute cellular components most efficiently if the cells have a uniform, symmetrical morphology (42). For example, the simple rod shape of *E. coli* allows the MinCDE and SlmA proteins to localize cell division near the geometric centers of growing cells, which increases the likelihood of partitioning material evenly and creating two nearly identical newborn daughters. Because the partitioning process is so important, it is of great interest to know more about the interplay between cell shape and the mechanisms that regulate where cell division occurs.

One of the most impressive characteristics of the Min system is its highly dynamic mechanism of action, first discovered by

Raskin and de Boer (29, 30). In normal rod-shaped *E. coli* cells, the Min proteins are in constant motion within the bacterial cytoplasm, oscillating from pole to pole with a time-averaged gradient of MinC that is most highly concentrated at the poles and is least concentrated at the cell's midpoint. Because MinC inhibits polymerization of the division initiator FtsZ, productive cell division is restricted to this “minimum MinC zone” near the cell's center. The basic biochemical stages of the process by which the MinC gradient is generated and maintained are well established (reviewed in references 31 and 36). MinC associates with helical filaments of MinD that coil around the inner surface of the cytoplasmic membrane, beginning at a pole and extending toward the center of the cell. MinE attaches to the leading edge of the helical complex, triggers the hydrolysis of MinD-ATP to MinD-ADP, and releases MinC and MinD from the membrane, which then diffuse and accumulate at the opposite pole, where the process is repeated (31, 36).

Mathematical models have demonstrated that interactions among the Min proteins are sufficient to explain their repetitive oscillatory behavior and suggest that nothing more is required for this system to function properly in localizing the division site (5, 7, 9–11, 13, 17, 18, 20). These models predict that the observed oscillations are generated by a reaction-diffusion linear instability, with a preferred wavelength that leads to an alternating accumulation of Min proteins at the two poles of rod-shaped cells. So far, most of these models have been applied only to uniformly rod-shaped wild-type cells, although one model accurately reproduces the observed oscillations in filamentous rod-shaped cells and in round or ellipsoidal cells (13, 14). A more stringent test of the assumptions

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TABLE 1. *E. coli* strains and plasmids

Strain or plasmid	Genotype or characteristics	MinCDE ^a	PBPs deleted	Reference or source
Strains				
MG1655	<i>ilvG rfb-50 rph-1</i>	+	None	W. Margolin
AV23-1	MG1655 <i>dacA::res pbpG::res</i>	+	5 and 7	This study
AV23-1K	MG1655 <i>dacA::res pbpG::Kan</i>	+	5 and 7	39
AV42-2K	AV23-1 $\Delta(\lambda attL-lom)::bla lacI^q$ P207-ftsZ-gfp	+	5 and 7	This study
AV62-1K	AV23-1 <i>minCDE::Kan</i>	—	5 and 7	This study
EC448	MC4100 $\Delta(\lambda attL-lom)::bla lacI^q$ P207-ftsZ-gfp	+	None	40
WM1032	MG1655 <i>minCDE::Kan</i>	—	None	W. Margolin
Plasmids				
pFX9	<i>P_{lac}-gfp::minD minE</i> (Amp ^r)			32
pFX55	<i>P_{lac}-minC minD minE::yfp</i> (Amp ^r)			35
pYLS49-2	<i>P_{lac}-gfp::minC minD minE</i> (Amp ^r)			33
pYLS68	<i>P_{lac}-yfp::minD minE::cfp</i> (Amp ^r)			33

^a +, present; —, absent.

underlying these computational representations requires that the models account for the behavior of Min proteins in cells of different geometries.

E. coli mutants lacking certain penicillin binding proteins exhibit aberrant shapes, including cells that have uneven widths, are bent, or are branched, with three or more poles (4, 23, 24, 26, 41). We utilized these cells as miniature reactors in which to visualize the behavior of MinCDE to determine if the proteins maintained a predictable pattern of oscillation as explained by current models. Surprisingly, we observed two different patterns of MinC and MinD oscillation. In one case, the Min proteins oscillated symmetrically between the two ends of each cell, regardless of minor intervening shape abnormalities. However, in cells with at least three distinct branches, MinC and MinD more often moved in a clockwise or counterclockwise direction among the poles. We applied an oscillatory-instability model to several cells with different branch lengths and found that clockwise or counterclockwise MinDE oscillations were the preferred pattern of oscillation in cells with three different branch lengths, while symmetric oscillations were preferred in cells with two short branches of approximately the same length, results that were in very good agreement with the experimental observations. The spontaneous reorientation of Min oscillations in ellipsoidal cells (13, 14) and the ability of the Min proteins to respond with specific spatio-temporal dynamics in abnormally shaped cells (this work) suggest that the Min proteins comprise a general cell geometry detection mechanism that can dynamically reorganize division site placement in response to changes in cell shape.

MATERIALS AND METHODS

Strains, plasmids, and growth conditions. The *E. coli* strains used in this study are listed in Table 1. *E. coli* AV23-1 was derived from AV23-1K by transient expression of RP4 resolvase to remove the kanamycin resistance cassette (3, 16). *E. coli* WM1032 served as the source of the *minCDE::Kan* mutation, which was transduced by phage P1 (22) into *E. coli* AV23-1 to give strain AV62-1K. Strain AV42-2K was constructed by moving $\lambda(attL-lom)::bla lacI^q$ P207-ftsZ-gfp from strain EC448 into AV23-1 via P1 transduction. Cells were incubated in LB medium (22) supplemented, when required, with 0.4% glucose and 100 μ g/ml ampicillin. *E. coli* AV42-2K was maintained on LB agar plus 0.2% glucose and 25 μ g/ml ampicillin. Plasmids pFX9, pFX55, pYLS49-2, and pYLS68 were provided by L. Rothfield and were maintained by including ampicillin at a 100- μ g/ml final concentration.

Fluorescence microscopy. The in vivo distributions of green fluorescent protein (GFP)- and yellow fluorescent protein (YFP)-tagged Min proteins were observed by fluorescence microscopy. Overnight cultures were diluted 1:100 in LB broth supplemented with 10 μ M isopropyl β -D-thiogalactoside (IPTG) to induce the expression of GFP- and YFP-tagged proteins and were incubated at 37°C for 2 h as described previously (35). For time-lapse microscopy of individual cells, 2 μ l of this culture was transferred onto the surface of LB agar (containing 10 μ M IPTG) that had been solidified in wells of chambered microscope slides (Lab-Tek chamber slide 177402). When CFP- and YFP-tagged proteins were to be observed simultaneously, cultures were grown in 0.4% glucose at 30°C until they reached exponential phase to minimize leaky readthrough expression from the plasmids. These cells were diluted 1:5,000 in LB plus 10 μ M IPTG, incubated overnight at 30°C, diluted 1:50 in the same medium, incubated at 30°C for 2 to 3 h (33), and prepared for time-lapse microscopy as described above.

A slightly different protocol was followed to visualize FtsZ-GFP expressed in *E. coli* AV42-2K. Overnight cultures were diluted 1:100 in LB plus 10 μ M IPTG and 25 μ g/ml ampicillin, after which the culture was incubated at 37°C for 2 h. A small amount of culture (2 μ l) was transferred to the surface of LB agar (containing 10 μ M IPTG) that had been solidified in the wells of a chambered microscope slide, and FtsZ-GFP ring assembly was followed during continued incubation at 37°C. (We note here that FtsZ-GFP rings were not visible with lower concentrations of IPTG in this strain background.)

In all cases, cells were viewed with a Zeiss Axio Imager.Z1 microscope, employing a 100 $\times$ differential interference contrast (DIC) 1.45-numerical-aperture objective fitted with neutral-density filters to minimize the exposure of growing cells to UV light. The temperature during the experiment was maintained by means of a stage heating element, and images were captured with a Zeiss AxioCam cooled charge-coupled device digital camera. Photographs were taken every 15 to 20 s when GFP- or YFP-tagged proteins were monitored individually or every 45 s when CFP- and YFP-tagged proteins were monitored simultaneously. We noted that CFP fluorescence was bleached away much more rapidly than was YFP fluorescence during repeated observations in the same experiment. Thus, in some cases where bleaching became problematic, whenever photographs were to be taken at longer intervals, the samples were observed (but not photographed) very briefly at intermediate times to be sure that significant changes were not missed.

Monte Carlo simulations of Min oscillations in aberrantly shaped *E. coli* cells. The numerical simulations in this work are similar to stochastic simulations (6, 15) derived from the deterministic model of Huang et al. (13, 14). In brief, each protein molecule was represented as a point diffusing in a cell with three cylindrical branches of user-defined lengths, capped with hemispheres. In all simulations, we assumed a fixed radius identical to the wild-type cross-sectional radius of 0.5 μ m. The probability of each reaction was determined from the bulk rate using the time step (dt) of 10^{-5} s and the concentration of each species, as averaged over a cylindrical grid with the following spacings: $dz = dr = 0.05$ μ m and $d\theta = \pi/10$. In a collision of a cytoplasmic protein with the membrane that did not result in binding, the protein was assumed to rebound from the membrane.

The set of chemical reactions involving the cytoplasmic (c) species MinD-ADP(c), MinD-ATP(c), and MinE(c) and the membrane-bound (m) species MinD-ATP(m) and MinD-MinE-ATP(m) was described previously (13, 14).

These reactions are reproduced below, with (m) representing complexes that bind to a patch of bare membrane.

The reaction probabilities k_{1-5} were determined from the bulk deterministic rates as follows: $k_1 = \sigma_D^{ADP \rightarrow ATP} dt$; $k_2 = \sigma_D dt$; $k_3 = \sigma_{AD}(\rho_d + \rho_{de})[(\rho_{\max} - \rho_d - \rho_{de})/\rho_{\max}] dt$; $k_4 = \sigma_E \rho_d dt$; $k_5 = \sigma_{de} dt$, where the bulk rates are $\sigma_D^{ADP \rightarrow ATP} = 1/s$, $\sigma_D = 0.025 \mu\text{m/s}$, $\sigma_{AD} = 0.0015 \mu\text{m}^2/s$, $\sigma_E = 0.3 \mu\text{m}^2/s$, and $\sigma_{de} = 0.6/s$. ρ_d (ρ_{de}) are the local concentrations of MinD:ATP (MinD:MinE:ATP) on the membrane, and the maximal concentration of MinD on the membrane is given by $\rho_{\max}$. The diffusion constant of all cytoplasmic proteins (D) is $2.5 \mu\text{m}^2/s$, and membrane-bound proteins were assumed not to diffuse. The number of proteins was determined for each cell from the total concentrations of $1,000/\mu\text{m}$ and $350/\mu\text{m}$ for MinD and MinE, respectively.

RESULTS

MinC oscillates in rotational or symmetric patterns in branched cells. To evaluate the predictive and explanatory powers of current models of Min action and division control, it was necessary to examine the behavior of Min proteins in non-rod-shaped cells. *E. coli* AV23-1 (with penicillin binding proteins [PBPs] 5 and 7 deleted) grows as branched and aberrantly shaped cells (39) and provided a suitable suite of non-uniform shapes in which to observe the behaviors of these proteins. Especially useful were those cells that exhibited branches of different lengths. In these cells, four modes of Min oscillations were possible (Fig. 1). The Min proteins could rotate clockwise or counterclockwise from branch to branch (Fig. 1A), back and forth between two branches (Fig. 1B), symmetrically between two halves of the cell (Fig. 1C), or randomly among any of the branches (Fig. 1D). To determine which of these oscillatory modes predominated, a GFP-MinC fusion protein encoded on plasmid pYLS49-2 was expressed in *E. coli* AV23-1 and its behavior over time was observed in cells whose shapes were significantly different from that of normal uniform rods. Of these, we analyzed only those cells in which there were two or more complete cycles of oscillation that could be captured before fluorescence bleaching reduced the signal to an unacceptable level.

In branched cells having three or more distinct poles, GFP-MinC moved around the cells in one of two patterns: either MinC rotated from branch to branch in a repetitive cycle that progressed clockwise (Fig. 2A) or counterclockwise (Fig. 2B) or MinC oscillated symmetrically from one half of the cell to the other (Fig. 2C). Out of 24 cells that exhibited repeated oscillatory cycles, in 67% (16) MinC moved either clockwise or counterclockwise and in 33% (8) MinC oscillated symmetrically. In no case did the direction of GFP-MinC reverse itself

FIG. 1. Potential Min protein oscillation patterns in branched *E. coli* cells. (A) Clockwise and counterclockwise oscillations. The red and green arrows denote completely independent Min motions. (B) Back-and-forth movement between two branches. Min proteins proceed from one pole to a third (red arrows) and then return to the original pole (green arrows). (C) Symmetric oscillation between two halves of a cell, regardless of abnormalities (the dashed line represents the plane of symmetry). (D) Random movement among branches. The Min proteins move from pole to pole in no specified order.

over the time of observation (4 min). That is, once the direction of oscillation was established in a particular cell, MinC movement did not change from clockwise to counterclockwise or vice versa. In addition, GFP-MinC oscillated in the classical manner (moving symmetrically from one end of the cell to the other and dividing the cell into halves) in cells with simple bifurcations of a pole and in a few cells in which the branches formed distinct tubular extensions from the main body (Fig. 2C). The only discernible trait that determined the pattern of MinC oscillations (clockwise/counterclockwise versus symmetrical) was the length of the branches. Oscillation was symmetrical if two of the visible poles were very close to one another (e.g., in cells with bifurcated poles); otherwise, MinC moved from pole to pole in sequence. In a few cells with symmetrical oscillations, MinC sometimes exhibited a slightly longer “dwell time” at one branch at the bifurcated end of the cell (Fig. 2C), suggesting that there may be an intermediate stage in which symmetrical oscillations transition to the clockwise or counterclockwise mode.

Regarding the motion of Min proteins in branched cells, the clockwise and counterclockwise motions are equivalent because movement between branches is relative to the observer. That is, the observed direction depends on whether the cell landed “face up” or “face down” on the agar surface—in one case the observer would see the Min proteins travel clockwise, and in the other case they would move counterclockwise. In either case, the Min proteins could choose an oscillation direction at random or there might be a rule governing the exact sequence with which the proteins visited each branch. An example of the latter possibility would be if Min movement

FIG. 2. GFP-MinC oscillation patterns in abnormally shaped *E. coli* cells. *E. coli* strains were transformed with plasmid pYLS49-2, from which GFP-MinC and untagged MinD and MinE were induced by the addition of 10 μ M IPTG. Each strain was incubated at 37°C in the presence of 10 μ M IPTG for 2 h, the cells were transferred to the surface of a chambered microscope slide containing LB agar plus IPTG, and individual cells were observed by time-lapse fluorescence light microscopy. Images were captured every 15 to 20 s, and selected images from each series are shown, with the number in each image indicating the time in seconds. (A) Clockwise oscillation of GFP-MinC in *E. coli* AV23-1 (with PBPs 5 and 7 deleted) (wild-type *minCDE*⁺). (B) Counterclockwise oscillation of GFP-MinC in *E. coli* AV62-1K (with PBPs 5 and 7 deleted) (*minCDE*::Kan) in the branched cell at the right. (C) Symmetric oscillation of GFP-MinC in *E. coli* AV62-1K (with PBPs 5 and 7 deleted) (*minCDE*::Kan). At 135 min, there was a slightly longer “dwell time” of GFP-MinC at one of the two branches at the bifurcated end of the cell, possibly indicating the nascent evolution of counterclockwise movement.

proceeded directionally from the shortest branch to the middle-size branch and finally to the longest and then back again to the shortest. However, when we measured the lengths of branches among cells exhibiting clockwise or counterclockwise oscillation, there was no correlation of branch length and rotation of Min within the cell. That is, the number of cells with Min oscillations progressing from short to intermediate to long branches was approximately the same as the number of cells in which oscillations moved from long to intermediate to short branches. It appeared, then, that the choice of rotation was either random or, at the very least, not robustly dictated by branch length.

A final possibility was that the observed oscillatory patterns were due to an artifact arising from the mixing of GFP-MinC fusion proteins with the wild-type (nonfused) MinCDE proteins expressed from the chromosome. To rule this out, we

observed the oscillation of GFP-MinC in *E. coli* AV62-1K, from which the chromosomal *minCDE* genes had been deleted. The same patterns of oscillation were seen in these cells (Fig. 2B) as in *E. coli* AV23-1, which expressed wild-type Min proteins from the chromosome (Fig. 2A). Thus, the fusion proteins were reporting Min oscillations accurately regardless of subunit mixing between the two forms.

MinD mimics the oscillation patterns of MinC. In normal rod-shaped *E. coli* cells, MinD oscillations match those of MinC, although the accumulations are more dispersed (31, 36). To confirm that MinC and MinD maintained the same relationship in branched cells with multiple poles, we observed the oscillation of GFP-MinD in *E. coli* AV23-1 (with PBPs 5 and 7 deleted). As expected, MinD movement mimicked the patterns exhibited by MinC in cells exhibiting two or three cycles of oscillation (Fig. 3). In 7 of 12 cases, GFP-MinD

FIG. 3. GFP-MinD oscillation patterns in abnormally shaped *E. coli* cells. *E. coli* strains were transformed with plasmid pFX9, from which GFP-MinD was induced by the addition of 10 μ M IPTG, and the cells were treated as described in the legend to Fig. 2. The number in each image indicates the time in seconds. (A) Clockwise oscillations of GFP-MinD in *E. coli* AV23-1 (with PBPs 5 and 7 deleted) (wild-type *minCDE*⁺ background). (B) Clockwise oscillations of GFP-MinD in *E. coli* AV62-1K (with PBPs 5 and 7 deleted) (*minCDE*::Kan). (C) Symmetric oscillations of GFP-MinD in two cells of *E. coli* AV23-1 (with PBPs 5 and 7 deleted) (wild-type *minCDE*⁺ background).

FIG. 4. MinE-YFP oscillation patterns in *E. coli* AV23-1 (with PBPs 5 and 7 deleted). *E. coli* strains were transformed with plasmid pFX55, from which MinE-YFP was induced by the addition of 10 μ M IPTG, and the cells were treated as described in the legend to Fig. 2. The number in each image indicates the time in seconds. (A) Counterclockwise oscillations of MinE-YFP in a cell with three long branches. The asterisks mark the branch having little or no MinE-YFP during stages of the oscillation cycle. (B and C) Symmetric oscillations of MinE-YFP in cells with two relatively short branches and one much longer branch.

rotated from branch to branch in a repetitive cycle that progressed clockwise or counterclockwise in cells having three or more poles (Fig. 3A and B), while in the remaining 5 instances, GFP-MinD moved symmetrically from end to end in cells with small bifurcations at one pole (Fig. 3C). MinD was not as clearly confined to the poles as was MinC (Fig. 3). Instead, as MinD moved from pole to pole, the protein extended further toward the middle of the cell, whereas MinC was concentrated strongly at the polar tips (Fig. 2). Once again, the patterns were identical in strains with or without wild-type MinCDE proteins expressed from the chromosome (Fig. 3B). Thus, MinD oscillations consistently followed the behavior of MinC in abnormal cells, as it did in normal rod-shaped cells (not shown).

MinE oscillates in rotational or symmetric patterns. The MinE protein helps disassemble the MinCD complex, thereby driving the oscillatory behavior of MinCD in normally shaped cells (31, 36). Since the motions of MinC and MinD were similar to one another in branched cells, we expected that MinE oscillations would follow the same patterns. To determine if MinE movement paralleled the rotational motions of MinCD, we expressed MinE-YFP in *E. coli* AV23-1 and observed how it functioned in abnormally shaped cells.

In cells with long branches of approximately equal lengths, MinE moved around the cells in a clockwise or counterclockwise manner (Fig. 4A). However, the behavior of MinE-YFP differed somewhat from that of MinCD. Instead of being confined mostly to a single branch, MinE concentrations remained high in two branches simultaneously, with the third branch exhibiting a much lower intensity of MinE. This “low MinE concentration” migrated from branch to branch in a rotational manner (e.g., counterclockwise in Fig. 4A), while MinE protein was distributed between the other two branches in a manner that followed the cyclical oscillations of MinD. Except for

this quantitative difference, the rotational behavior of MinE followed a pathway qualitatively similar to that of MinC and MinD.

In contrast to the above, in misshapen cells with one or two short branches (in 13 of 13 cases) MinE-YFP oscillated symmetrically from one half of the cell to the other half (Fig. 4B and C). The distribution of MinE in these cells was distinguished from the distributions of MinC and MinD in that during the course of its oscillation, MinE-YFP often covered more than half of the cell (Fig. 4B and C), whereas MinC and MinD were confined within less than one half of these types of cells. It seemed possible that MinE might not reproduce the motions of MinCD because it moved more slowly than the other two proteins in abnormally shaped cells. If so, then its period of oscillation would be expected to be significantly different. However, each of the Min proteins required approximately 60 to 120 s to complete one oscillation cycle (the time required to return to the starting pole) (not shown). The concordance of these times suggested that the movements of all three Min proteins were coordinated even in oddly shaped cells (8).

One other trivial explanation for the discrepancies between the patterns followed by MinE and MinCD could have been that fusing the protein to YFP made MinE behave differently than when MinC and MinD were fused to GFP. Therefore, we examined the oscillations of YFP-MinD and MinE-CFP simultaneously in the same cell. When the two fusion proteins were expressed from plasmid pYLS68 in a cell having one very short branch between two very long ones, both MinD and MinE oscillated in a “back-and-forth” motion with a peak of concentrated MinE lagging behind in the branch just vacated by MinD (Fig. 5). These results were consistent with the behavior of each protein when tested individually, indicating that the

FIG. 5. Oscillation patterns of YFP-MinD and MinE-CFP expressed simultaneously in branched *E. coli* cells. *E. coli* strains were transformed with plasmid pYLS68, from which the expression of both YFP-MinD and MinE-CFP was induced by the addition of 10 μ M IPTG, and the cells were treated as described in the legend to Fig. 2. The images were captured with a $63\times$ 1.25-numerical-aperture objective lens (Zeiss). MinE-CFP is pseudocolored blue, and YFP-MinD is pseudocolored yellow. The numbers in the frames indicate the time in seconds. The CFP channel photograph was acquired first, followed 1 s later by the YFP channel. The lag of MinE movement in the 90-s frame is unexplained by this time period. (A) Oscillation of Min proteins in *E. coli* AV23-1 (with PBPs 5 and 7 deleted). (B) Schematic representation of the oscillation of YFP-MinD and MinE-CFP as observed in the series of frames in panel A.

relative motions of MinE and MinD remained similar in each other's presence and were not artifacts related to the type of fusion protein.

Placement of division sites in branched cells. To determine how cell morphology affected localization of the division plane in aberrantly shaped *E. coli* cells, we observed individual cells growing on an agar surface by using time-lapse photomicroscopy. In cells having at least three prominent branches (Y shaped), division always occurred near the center of the cell at the junction of these branches, usually via asymmetric invagination of the division furrow near the base of one branch (Fig.

6). In addition, successive division events sequentially separated one branch at a time from the original cell (Fig. 6A and C). This was especially clear when a single cell could be followed through three or four divisions (Fig. 6C). In this case, the cell completed its first division at 40 min (at the junction of the three branches), division in a second branch was completed at 68 min, and release of the third branch occurred at 96 min (Fig. 6C). This sequence of events left a curiously shaped, slightly pinched, rectangular cell whose sides were derived mostly from previous septation events (Fig. 6C, 108 min). This central localization of division sites was not surprising because

FIG. 6. Cell division in aberrantly shaped *E. coli* cells. Overnight cultures of *E. coli* AV23-1 (with PBPs 5 and 7 deleted) were diluted 1:50 in LB medium and incubated for 1 h at 37°C. A cell suspension was transferred onto the surface of LB agar in a chambered microscope slide incubated at 37°C, and the division of individual cells was followed by time-lapse photomicroscopy. DIC images were captured at 4-min intervals. (A) Cell division in a branched (Y-shaped) cell. (B) Cell division in an abnormally shaped (but not branched) cell. (C) Division site selections in cells of a microcolony derived from a single cell. The number in each frame indicates the time in minutes, and the arrows point to locations where division furrows begin. The arrow in panel C at 108 min indicates the misshapen cell that remains after multiple division events.

FIG. 7. FtsZ ring assembly in branched and aberrantly shaped *E. coli* cells. Overnight cultures of *E. coli* AV42-2K were diluted 1:100 in LB medium containing ampicillin (25 μ g/ml). After 30 min of incubation at 37°C, 10 μ M IPTG was added to induce expression of FtsZ-GFP from the plasmid, and incubation was continued for an additional 1.25 h. A cell suspension was transferred onto the surface of LB agar plus IPTG in a chambered microscope slide at 37°C, and the formation of FtsZ-GFP rings in individual cells was followed by time-lapse photomicroscopy. The upper photograph of each pair is a DIC image, while the lower is a fluorescence image of the same cells. Selected images are presented for the development of two typical cells (A and B). The black arrows indicate the positions of developing division furrows, and the white arrows indicate the positions of FtsZ rings. See the text for a more detailed description of events. The number in each frame indicates the time in minutes.

one would expect septation to occur where the concentration of MinC was at its minimum, and therefore, the observed results were consistent with the pattern of Min protein oscillation.

What could not be answered by the above-mentioned approach was how the specific septation sites were chosen so that branches were released one at a time. Since the positioning of FtsZ polymers determines the location of the division site, we followed the time-dependent placement of FtsZ-GFP rings in aberrantly shaped *E. coli* AV42-2K cells (with PBPs 5 and 7 deleted). Figure 7 presents two typical examples of how FtsZ rings formed in this population. In one case, FtsZ rings began to form at each of two sites near the branch points (Fig. 7A, 4-min frame). However, soon afterward, the FtsZ ring confined itself to only one of the branches (Fig. 7A, 6-min frame), and this ring constricted to release the branch as a newborn cell (Fig. 7A, 10- to 20-min frames).

Similarly, in a second example, an FtsZ ring formed near the center of a branched cell and division occurred close to the junction of the three branches (Fig. 7B, 0-min frame). An interesting phenomenon was captured in the Y-shaped daughter cell that resulted from this division event. In this cell, two FtsZ rings began to form at the junction of the three branches (Fig. 7B, 30-min frame), but one ring disappeared and left a single Z ring at right angles to one of the branches (Fig. 7B, 36-min frame). Surprisingly, this ring did not persist but

“flipped” from one branch to the other (Fig. 7B, 36- and 42-min frames), after which the cell initiated constriction (Fig. 7B, 60-min frame) and completed septation (Fig. 7B, 78-min frame). Finally, the two-branched daughter cell from this second division event formed two FtsZ rings near the branch junction of the original cell (Fig. 7B, 90-min frame). In this case, both rings persisted, but constriction and cell division took place at only one ring at a time. The first FtsZ ring constricted and released the branch as a daughter cell (Fig. 7B, 96-min frame), after which the second FtsZ ring began to constrict (Fig. 7B, 108-min frame), leading to a second division event (Fig. 7B, 114-min frame). This event was consistent with the previous observation that successive divisions released each branch in turn.

The results suggest that MinCD oscillation in branched cells creates a broad MinC-free zone that gives the cell leeway to place FtsZ rings at more than one position. In this centralized area, a single ring may move from one branch to the other before constriction begins. On the other hand, if multiple rings form, one may disappear while the other persists and constricts, or (in larger cells) both may persist with constriction proceeding at each one in turn.

Relative branch lengths determine Min protein dynamics in the reaction-diffusion model. Mathematical modeling has been useful in mimicking the motions of Min proteins in simple rod-shaped cells and cocci (9, 11, 13, 14, 17, 18, 20). Specifi-

cally, a deterministic numerical reaction-diffusion model accurately reproduces the experimentally observed oscillatory patterns in both wild-type and filamentous rod-shaped cells and cocci (13, 14). We applied a stochastic version of this model to predict the movement of Min proteins in branched cells to see if this approach could accurately describe the rotational and symmetric oscillatory behaviors in cells having more complicated geometries. As in the study by Kerr et al. (15), the reaction-diffusion cycles were implemented as stochastic processes with probabilities proportional to their rate constants (13, 14). We chose as case studies cell geometries that we expected would result in each of the first three oscillatory patterns in Fig. 1, since these cell types typified the observed behaviors of Min proteins in aberrantly shaped *E. coli* cells. The cases fell into three broad categories: (i) cells with three branches that were roughly equal in length and that exhibited clockwise/counterclockwise Min movement (Fig. 8I), (ii) cells with one long and two short branches that exhibited symmetric movement (Fig. 8II), and (iii) cells with one short and two long branches that exhibited back-and-forth motion (Fig. 8III).

First, we modeled the movements of MinD and MinE in a cell with three branches of nearly equal lengths (Fig. 8I). Monte Carlo simulations followed the positions of the five different states of these proteins within the cytoplasm or on the membrane along the length of the virtual cell as time progressed (Fig. 8IB). These data were also used to predict the microscopically visible patterns that would be observed when using fluorescent versions of MinD (Fig. 8IC) or MinE (Fig. 8ID), assuming that the fluorescence intensity from a single molecule has a Gaussian distribution with a width of 200 nm. In this simulation, movement of MinD and MinE from branch to branch progressed in a clockwise direction (Fig. 8IB to D). (Note that if viewed from the rear, the projected motion would be clockwise, which is consistent with what is observed for real cells, which can land on an agar surface in either of two orientations. More importantly, though, other simulations with the same initial conditions displayed counterclockwise rotations, indicating that noise can play a role in determining the direction of the oscillations.) The majority of MinD proteins start in the longest branch at time zero and migrate in succession to the shortest and middle branches with a pole-to-pole time of about 30 s (Fig. 8IC). This rotational pattern was similar to the MinC and MinD oscillations observed in real cells (Fig. 2A and Fig. 3A, respectively), and as noted, the distribution of MinD was not always completely confined to the poles (Fig. 3A and 8IC at 30 and 90 s).

The physical origin of these oscillations can be understood using the same conceptual framework developed for explaining Min behavior in rod-shaped cells (9, 11, 13, 17–19). A linear instability in the coupled reaction-diffusion system has a characteristic wavelength that generates (i) the accumulation of Min proteins at alternating poles in wild-type cells and (ii) a repetitive pattern of Min protein accumulation at sites spaced every 8 to 10 μm along the length of filamentous cells (13, 20, 30). In the branched cell of Fig. 8IC, once an oscillation is initiated by fluctuations in local protein concentrations, the rotational pattern persists, because as MinD moves from pole 1 to pole 2, the residual and relatively high concentration of MinE at the first pole biases MinD to bypass pole 1 and accumulate instead at pole 3. This mechanism is evident in Fig.

8IC ($t = 30$ s), where MinD is found predominantly in the shortest branch while a high concentration of MinE remains in the longest branch (Fig. 8ID, $t = 30$ s), effectively blocking the return of MinD to the longest branch and thereby perpetuating the rotational direction of oscillation. Similar situations are visible in Fig. 8I at 60 s and 90 s.

The projected movement of MinD and MinE was different in a virtual cell having two branches of equal length plus a third that was much longer (Fig. 8II). Here, the majority of MinD proteins began in the long branch ($t = 0$ s and 30 s), migrated away from this pole to populate both short branches at the opposite end of the cell ($t = 60$ s), and returned to the pole of the long branch after a total period of about 90 s. Though partitioning of the MinD population into the two short branches was not equal, both received a significant fraction of the MinD proteins. The difference in length between the long and short branches mandates that MinD accumulations in the short branches are disassembled before a new MinD polar zone is completed in the long branch, thereby perpetuating the symmetry of the oscillations. This symmetric pattern was similar to MinD and MinE oscillations observed in real cells (Fig. 3C and 4B, respectively).

Finally, a third pattern of oscillation was observed in simulated cells having two long branches of roughly equal lengths plus a third that was much shorter (Fig. 8III). In this case, the Min proteins moved alternately between the poles of the two long branches, but with a temporary (very brief) accumulation in the short branch as the proteins traveled to each of the far poles, similar to real cells (Fig. 5). In the simulation, MinD and MinE molecules were initially distributed equally between the two long branches, but the system rapidly evolved into the alternating pattern shown. This back-and-forth movement (Fig. 1B) between the distal poles of a long cell with an intervening short branch is in direct contrast to the “doubled” pattern of oscillations observed in previous experiments using uniformly smooth rod-shaped filaments $\sim 10\ \mu\text{m}$ long. There, in cells with no intervening branch, the Min proteins did not move from one end of the elongated cell to the other. Instead, the oscillation pattern was dramatically different in that the proteins behaved as though the structure consisted of two cells, each approximately one-half the length of the whole. In such elongated cells, the Min proteins accumulated simultaneously at both poles, followed by accumulation at a site near the middle of the cell, after which the Min molecules returned to the two poles, thereby creating parallel oscillations within a single filament (13, 30). We verified that in simulated rod-shaped cells of the same length, equivalent and symmetrically “doubled” oscillations occurred around the centers of these elongated cells when MinD and MinE were initially distributed equally near the two poles (not shown). These results show that the “doubled” oscillation pattern in a single elongated cell can be broken in a cell possessing an additional branch. In sum, then, the simulations and experimental observations strongly suggest that Min protein oscillations depend on and respond to changes in the geometry of bacterial cells.

DISCUSSION

The behavior of the MinCDE proteins in abnormally shaped *E. coli* cells indicates that the Min system can function as a

FIG. 8. Stochastic simulations of clockwise (I), symmetric (II), and back-and-forth (III) MinD/E oscillations in branched cells. In each of the sections is a cartoon of the shape of the model cell in which the motion of the Min proteins was simulated. In case I, the cell has three cylindrical branches of lengths 2.0, 1.5, and 1.0 μm , each with a radius of 0.5 μm and capped by a hemispherical pole. In case II, the branch lengths are 3, 1, and 1 μm . In case III, the branch lengths are 5, 4, and 1 μm . (A) Colored spheres represent the different MinD and MinE species shown in the Monte Carlo simulations in panel B. The MinE molecules and ADP/ATP-bound MinD molecules are located either in the cytoplasm (*cyto*) or associated with the inside of the inner membrane (*mem*). (B) Positions of the five states of MinD and MinE at various times during an oscillation. In case I, the zone of highest Min protein concentration moves from pole to pole in a clockwise fashion. In case II, the zone moves from the long branch to both short branches nearly simultaneously. In case III, the zone moves from one of the long branches to the short branch and then to the opposite long branch, repeating in a back-and-forth manner. (C and D) Simulated fluorescence from GFP-tagged MinD (C) and MinE (D), using the positions of the molecules as predicted in panel B. In all cases, time zero was chosen arbitrarily to begin a few hundred seconds after initiation of the simulations to eliminate any artifacts stemming from the initial protein distributions.

general “cell geometry detection mechanism” in cells with different morphologies. In line with this capability, it is conceivable that such a system could help localize proteins to the extremities of any cell regardless of its specific morphology. The results argue that the use of aberrantly shaped *E. coli* cells as miniature reaction vessels is useful for testing in vivo the spatiotemporal predictions that arise from biological models and from mathematical simulations of cellular biochemistry. Finally, not only does this protein system restrict cell division to a central zone in a broad range of cell shapes, it may also serve to rescue bacteria by restoring relatively normal shapes to cells with otherwise exceptional, and perhaps lethal, abnormalities.

Pole detection by Min oscillation. In *E. coli*, a defined (and brisk) period of oscillation is important for inhibiting undesirable division events at either pole (30). The impact of failing to oscillate rapidly enough is obvious in cells that express a truncated form of MinE, where the period of Min oscillations slows significantly from a pole-to-pole period of 40 to 90 s to a period of ~ 10 min (32). The longer dwell time of MinCD at one pole leaves the opposite pole unoccupied and unprotected, allows division to take place at this site rather than at midcell, and results in the creation of chromosome-free minicells at a high rate (32). Thus, even in relatively normal geometries (rod-shaped or quasispherical cells), the ability of the Min system to select the division plane accurately depends on appropriate timing.

In cells with multiple branches (and multiple poles), it would clearly be important for Min protein oscillation to adjust so that the elaboration of additional branches does not increase the formation of undesirable minicells at these new poles. Growth of a new, short branch (as in Fig. 5) changes the cell's geometry and effectively slows the diffusion of Min proteins in this region, which increases the local concentration of MinCD near the intersection of the three branches and prevents FtsZ ring formation near the new pole. Once the additional branch grows longer, MinCD oscillates evenly among all polar sites so that no single pole is left unprotected for too long. Regular rotation of MinC among branches evidently ensures that even remarkably misshapen cells rarely produce minicells. Of course, this behavior could also be used to “detect and mark” the position of each of a cell's extremities for the purposes of localizing other molecules without relying on preexisting chemical targets, though there is as yet no evidence that the Min proteins play such a role.

A complete cycle of Min oscillations in normal rod-shaped cells occurs in 40 to 90 s (5, 30, 36), so one might expect the oscillation period to be longer in the grossly atypical cells reported here. Instead, the cycling times in these cells ranged from 60 to 120 s, well within the range of that reported for normal rods, which seems to be sufficient to prevent excessive minicell formation. In explaining why similar periods exist in normal and abnormal cells, simulations predict that, due to the fast diffusion of proteins inside the cell, the length of time required for a complete oscillation cycle depends more on the accumulated concentration of Min proteins than on the length of the cell or its branches (not shown). Thus, in normal rod-shaped cells, the oscillatory period is relatively insensitive to elongation from 2 to 4 μm (30). Moreover, when a normal cell grows longer (e.g., up to 10 μm), the Min proteins begin to

oscillate in a “doubled” fashion; that is, the molecules oscillate in two separate polar zones within a single cell (30), which is why the period in the longer cell is roughly the same as in the unit cell. In sum, the choice of oscillation dynamics in branched cells is determined by the lengths of the branches, and the distance traveled by the proteins from one MinD accumulation to the next is consistent with the wavelength of pattern formation in filamentous *E. coli*.

Potential interconversion of Min oscillatory patterns. We found that when an oscillation pattern was adopted, its direction remained stable. That is, Min proteins moving clockwise among three branches of a cell did not suddenly reverse direction and move counterclockwise. However, there are at least two reasons why this observation cannot be considered an ironclad rule. The first is that we could observe cells for only a few cycles. It is therefore possible that reversals in direction might occur after longer periods than we could monitor or that they occur with low frequency. In fact, in occasional numerical simulations, a large fluctuation of the concentration of MinD at the “wrong” (out-of-order) pole did reverse the direction of oscillation (not shown), implying that this phenomenon might occur in real cells if monitored for a long enough time.

The second reason for not considering the oscillation direction to be completely stable is that the cells we observed did not grow appreciably over the course of the experiment. It is easy to imagine that the pattern of Min oscillations evolves in growing cells as their geometry changes. Consider, for example, how a back-and-forth oscillation might evolve into a clockwise or counterclockwise oscillation in a cell with one short branch. As depicted in Fig. 2C and 3C and as illustrated schematically in Fig. 8IIIC, the Min proteins leave the pole of one of the long branches but converge briefly into the short branch before proceeding to the distal pole, after which the pattern is repeated in reverse. This alternating oscillatory pattern could change into a clockwise/counterclockwise pattern when the short segment grows into a sufficiently long branch, with the final direction being determined randomly by the direction of Min movement at the time when the proteins begin accumulating in the third branch on equal footing with the other two branches. Thus, oscillation switching would be triggered by the number and relative lengths of branches of a cell. Simulations show that in rod-shaped *E. coli* cells there is a minimum cell length of ~ 2 μm below which oscillations do not occur (13), and this dependence on length may play a role in the transition from symmetric oscillations to other patterns. In particular, in cells with two short branches, oscillations between these two poles may be disfavored until the branches grow longer. The bottom line is that the Min proteins maintain relatively stable oscillation patterns, but these should not be considered permanent.

The predictive power of mathematical modeling. By applying mathematical modeling to cells with abnormal shapes, we found that a clockwise/counterclockwise MinCD motion was the preferred pattern of oscillation in cells with branches of longer but differing lengths, whereas symmetric oscillations were preferred in cells with two short branches of approximately the same length. The results closely match experimentally observed patterns for cells with these types of morphologies. Other groups have successfully applied this model in studying the effects of stochasticity on Min oscillations using

molecular-level Monte Carlo simulations (6, 15, 25). The model accurately reproduces known experimental features observed in rod-shaped cells, the oscillatory and division phenotypes of filamentous cells, the behavior of mutant strains with truncated MinE proteins (13, 32), and the oscillations of Min homologs in cocci (2, 14, 28, 37). In particular, the model demonstrates that the rapid reorientation of the oscillatory direction of Min proteins in nearly round cells can be stabilized along a single axis if there is a slight elongation in that direction (13, 14). In short, the modeling demonstrates that experimentally observed oscillatory patterns can develop spontaneously and persist throughout the cell cycle.

All the results, from experiments and theoretical modeling, indicate that Min oscillations are sensitive to cell geometry and suggest that the system can select the plane of division in cells of many different shapes. In addition, the concordance between the observed behavior of the Min proteins and that predicted by mathematical simulations lends credence to the underlying assumption that the Min proteins are a self-contained system that can self-organize into the observed pattern formations. In light of these findings, we propose that mutants of different shapes should continue to be employed to test whether specific mathematical models accurately capture the real-life behavior of proteins in different circumstances.

Aggravating shape abnormalities. One of the original motivations for pursuing these experiments was to address the question of whether oddities in Min protein oscillation forced particular *E. coli* mutants to adopt aberrant shapes. This does not seem to be the case, because changes in Min protein oscillation appear to follow rather than produce changes in cell shape. Also, we saw no appreciable difference in the shapes of mutants with or without MinCDE (not shown). Instead, the localization of FtsZ rings may propel bacterial morphology in opposing directions, both aggravating and restraining the production of oddly shaped cells.

The genesis of misshapen cells in PBP mutants seems to lie in geometric inaccuracies that occur during septation, which in turn arise because the orientation of FtsZ rings can be skewed from their normal perpendicular relationship with the side-walls (for examples and discussion, see reference 27). MinC and the nucleoid occlusion protein SlmA (1) restrict the formation of FtsZ rings to the middle of the cell for proper division. However, there is evidently some “wobble room” for the precise localization of the FtsZ ring within this permissive zone. That is, although ring formation may be restricted to a general area where the concentrations of MinC and SlmA are low, the exact geometry of the FtsZ ring is probably not specified by the shape of this inhibitor-free region. Thus, for example, if the zone is wide enough, FtsZ rings may tilt slightly with respect to the long axis of the cell. Though unusual, such lopsided rings do occur in some mutants and under some circumstances, suggesting that additional proteins or attributes of the cell wall fine tune the placement of Z rings so that they develop at near-perfect 90° angles to the lateral walls (27). The ability of FtsZ rings to form at multiple positions near the centers of branched *E. coli* cells certainly indicates that these cells, at least, have a broad inhibitor-free area in which polymerization is allowed. Although altered Min oscillations do not appear to create the shape changes in these strains in the first place, any morphological abnormalities that do appear could

be perpetuated and exacerbated by Min oscillation, which may exaggerate small irregularities and turn them into larger ones by allowing broader zones for septation.

Restraining shape abnormalities. The flip side of the above discussion is that FtsZ ring placement also seems to impose a restraining tendency on cell shape. One way this phenomenon may arise is from the dynamic behavior of the Min system. For example, in spherical *E. coli* cells the Min proteins can oscillate in any direction with respect to the plane of the segregating nucleoids (2, 14, 28, 34), but numerical modeling suggests that elongation of a spherical cell in one direction can stabilize the oscillatory axis of the Min proteins and reenforce the cell to define a consistent division plane (14, 34).

Similar to the case for spherical cells, the location of the FtsZ ring appears to return grossly misshapen cells to a more normal mode of rodlike extension and division. A good example is the behavior of the extremely branched *E. coli* cells described here. In these cells, division occurs preferentially at a junction where one or more branches meet, with the subsequent release of a daughter cell having a more nearly normal rodlike shape than its immediate parent. A series of such divisions can produce multiple near-normal daughters, leaving behind a single seriously deformed cell that becomes more aberrant with each division cycle (e.g., the results of sequential divisions shown in Fig. 6C). Since many near-normal daughters may arise from one misshapen cell, the culture continues to grow at an almost normal rate and produces a high percentage of virtually normal cells. We have observed this sequence of events numerous times in different PBP mutants (not shown). The phenomenon is probably driven by the fact that a productive FtsZ ring may form and function only in cells having a certain diameter (42), while the location of the ring depends on the configuration of an inhibitor-free zone. Therefore, although the indeterminate placement of FtsZ rings may aggravate some shape problems, the restrictions on Z-ring formation may also act as a natural brake that generates cells having more normal morphologies.

As an aside, the behavior of FtsZ rings in aberrantly shaped *E. coli* cells suggests that even though multiple FtsZ rings may form, only one of the rings may constrict at a time. If two rings form, either one disappears and the other constricts (Fig. 7A, 4- to 10-min panels) or, in a larger cell, both rings may persist with one constricting first, followed by the other (Fig. 7B, 0- to 114-min panels). In the first case, one Z ring may siphon off FtsZ monomers from the second, whereas in the second example, competition for some other limiting factor(s) may prevent the two rings from constricting simultaneously. Both situations are intriguing and indicate there are as-yet-undiscovered rules that govern the selection of FtsZ polymerization sites and FtsZ ring constriction.

Summary. Overall, the data suggest that in these PBP mutants, cell shape probably changes by a sequence of uneven division events leading to oddly shaped poles that develop into lateral branches. These alterations, though slight in themselves, may be exaggerated over time by branch growth, which then alters the diffusion patterns of MinCDE, which may open up larger permissive zones for FtsZ polymerization and thereby lead to a greater latitude in septum formation and an increased asymmetry in localizing the plane of cell division. As growth continues, constraints on FtsZ ring formation may per-

form the opposite function of producing rodlike daughter cells from aberrantly shaped progenitors. The two opposing tendencies may drive the oscillatory fluctuations of overall cell shape in these mutants.

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