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Modeling of the Dynamic Pole-to-Pole Oscillations of the Min Proteins in Bacterial Cell Division: the Effect of an External Field

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One of the most important steps in the developmental process of the bacteria cells at the cellular level is the determination of the middle of the cell and the proper placement of the septum, these being essential to the division of the cell. In *E. coli*, this step depends on the proteins MinC, MinD, and MinE. Exposure to a constant electric field may cause the bacteria's cell-division mechanism to change, resulting in an abnormal cytokinesis. To see the effects of an external field *e.g.*, an electric or magnetic field on this process, we have solved a set of deterministic reaction diffusion equations, which incorporate the influence of an electric field. We have found some changes in the dynamics of the oscillations of the min proteins from pole to pole. The numerical results show some interesting effects, which are qualitatively in good agreement with some experimental results.

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I. INTRODUCTION

Cell division is the process by which a cell separates into two new cells after its DNA has been duplicated and distributed into the two regions that will later become the future daughter cells. For a successful cell division to take place, the cell has to determine the optimal location of the cell separation and the time to start the cell cleavage. This involves the identification of the midpoint of the cell where the septum or cleavage furrow will form. For *Escherichia coli (E. coli)*and other rod-like bacteria, evidence has accumulated over the past few years which indicates that the separation into two daughter cells is achieved by forming a septum perpendicular to parent cell's long axis. To induce the separation, the FtsZ ring (Z ring), a tubulin-like GTPase, is believed to initiate and guide the septa growth by a process called contraction [1]. The Z ring is usually positioned close to the center, but it can also form in the vicinity of the cell poles. Two processes are known to regulate the placement of the division site: nucleoid occlusion [2] and the action of the *min* proteins [3]. Both processes interfere with the formation of the Z ring that determines the division site. Nucleoid occlusion is based on cytological evidence that indicates that the Z ring assembles preferentially on those portions of the membrane that do not directly surround the dense nucleoid mass [4].

The *min* proteins that control the placement of the division site are the MinC, the MinD, and the MinE proteins [3]. Experiments, involving the use of modified proteins show that inC is able to inhibit the formation of the FtsZ-ring [5]. MinD is an ATPase that is connected peripherally to the cytoplasmic membrane. It can bind to the MinC and activate the function of the MinC [6,7]. Recent studies show that MinD can also recruit MinC to the membrane. This suggests that MinD stimulates MinC by concentrating MinC near to its presumed site of activation [8,9]. MinE provides topological specificity to the division inhibitor [10]. Its expression results in a site-specific suppression of the MinC/MinD action so that FtsZ assembly is allowed at the middle of the cell, but is blocked at other sites [3]. In the absence of MinE, MinC/MinD is distributed homogeneously over the entire membrane. This results in a complete blockage of Z-ring formation. The long filamentous cells that are subsequently formed are not be able divide [8,9,11,12]. With fluorescent labeling, MinE was shown to attach to the cell wall only in the presence of MinD [13,14]. As MinD dictates the location of MinC, the latter will oscillate by itself. This will result in a concentration of the division inhibitor at the membrane on either cell end, al-

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ternating between being high or very low every other 20 s or so [8,9]. The presence of MinE is not only required for the MinC/MinD oscillation but also involved in setting the frequency of the oscillation cycle [11]. Several sets of evidence indicate that the MinE localization cycle is tightly coupled to the oscillation cycle of MinD.

Recent microscopy of the fluorescent labeled proteins involved in the regulation of *E. coli* division has uncovered stable and coherent oscillations (both spatial and temporal) of these three proteins [15]. The proteins oscillate from one end to the other end of the bacterium, moving between the cytoplasmic membrane and cytoplasm. The detail mechanism by which these proteins determine the correct position of the division plane is currently unknown, but the observed pole-to-pole oscillations of the corresponding distribution are thought to be of functional importance. Under different culture conditions and/or environment changes, *e.g.* pH, light, and external field, changes in the pole-to-pole oscillations can affect the growth of the bacteria. Here, we discuss only the effects of an electric field.

In the present work, we use a mathematical approach to investigate the influence of an external constant external field on cytokinesis mediated by pole-to-pole oscillations of the *min* protein. We propose a mathematical model and then solve it numerically to see how the *min* protein oscillation mechanism for bacteria cell division may change. We also present some comments about the connection between our mathematical approach and real-world experimental results.

II. MODEL

Sets of reaction-diffusion equations have often been used in biological applications to model self-organization and pattern formation [16]. These mathematical equations have two components. The first component is the diffusion term that describes diffusion of the chemical species. At the molecular level, the diffusion term often results in a net flow of chemical species from regions of high concentration to regions of lower concentration. The second component is the reaction term that describes the self-organization of the biological systems.

We have adopted the dynamic model of compartmentization in the bacterial cell division process proposed by Howard [17] by adding an extra term that depends on the external electric field. The dynamics of bacteria in the presence of an external field is described by a set of four non-linear coupled reaction-diffusion equations. We focus on the *E. coli* bacteria, which are commonly studied rod-shaped bacteria of approximately 2 – 6 μm in length and around 1 – 1.5 μm in diameter. *E. coli* divides roughly every hour via cytokinesis. Our starting point is the set of one dimensional deterministic coupled reaction-diffusion equations describing the dynamics of the interactions between the local concentrations of the MinD and the MinE proteins. The equations describe the time rates of change of the concentrations due to the diffusion of the MinD and the MinE and to transfer between the cell membrane and the cytoplasm. The dynamics of these *min* proteins in the presence of an external field, are described by

$$\frac{\partial \rho_D}{\partial t} = D_D \frac{\partial^2 \rho_D}{\partial x^2} + J_D \frac{\partial \rho_D}{\partial x} - \frac{\sigma_1 \rho_D}{1+\sigma_1' \rho_e} + \sigma_2 \rho_e \rho_d, \quad (1)$$

$$\frac{\partial \rho_d}{\partial t} = D_d \frac{\partial^2 \rho_d}{\partial x^2} + J_d \frac{\partial \rho_d}{\partial x} - \frac{\sigma_1 \rho_D}{1+\sigma_1' \rho_e} - \sigma_2 \rho_e \rho_d, \quad (2)$$

$$\frac{\partial \rho_E}{\partial t} = D_E \frac{\partial^2 \rho_E}{\partial x^2} + J_E \frac{\partial \rho_E}{\partial x} - \sigma_3 \rho_D \rho_E - \frac{\sigma_4 \rho_e}{1+\sigma_4' \rho_D} \quad (3)$$

and

$$\frac{\partial \rho_e}{\partial t} = D_e \frac{\partial^2 \rho_e}{\partial x^2} + J_e \frac{\partial \rho_e}{\partial x} + \sigma_3 \rho_D \rho_E - \frac{\sigma_4 \rho_e}{1+\sigma_4' \rho_D} \quad (4)$$

where ρ_D and ρ_E are the concentrations of the MinD and the MinE proteins in the cytoplasm, respectively, and ρ_d and ρ_e are the concentrations of the MinD and the MinE proteins on the cytoplasmic membrane. The first equation describes the time rate of change of the concentration of MinD (ρ_D) in the cytoplasm. The second is for the change in the MinD concentration (ρ_d) on the cytoplasmic membrane. The third is for the change of the concentration of MinE (ρ_E) in the cytoplasm. The last one is for the change in the MinE concentration (ρ_e) on the cytoplasmic membrane. Since the experimental results given Ref. 9, show that the MinC dynamics simply follows that of the MinD protein, we have not written out the equations for the MinC explicitly.

The important feature of our model is the second terms on the right-hand sides of the equations. They represent the effect of the external field in the reaction-diffusion equation [18,19] controlled by the external field parameter. We assume that a chemical substance moving in the region of an external field will experience a force that is proportional to the external field parameter J times the gradient of the concentration of that substance. In general, $J = \mu E$, where E is the field strength and μ is the ionic mobility of the chemical substance. μ, in general, will be proportional to the diffusion coefficient of the chemical substance and will depend on the total amount of free charge in that substance. In this model $J_i = \mu_i E\{i = D, E, d, e\}$ is the external field parameter for each protein types. We assume that the diffusion coefficients $\{D_D, D_E, D_d, D_e\}$ are isotropic and independent of x. The constant σ_1 represents the association of MinD to the membrane wall [12]. $\sigma_1^{'}$ corresponds to the membrane-bound MinE suppressing the recruitment of MinD from the cytoplasm. σ_2 reflects the rate that the MinE on the membrane drives the MinD on the membrane into the cytoplasm. Based on evidence for the cytoplasmic interaction between MinD and MinE [7], we

let σ_3 be the rate that cytoplasmic MinD recruits cytoplasmic MinE to the membrane and σ_4 be the rate of dissociation of MinE from the membrane to the cytoplasm. Finally, $\sigma_4^{'}$ corresponds to the cytoplasmic MinD suppressing the release of the membrane-bound MinE. Evidence points to most of the diffusion process occurring in the cytoplasm. It is, therefore, reasonable to set D_d and D_e to zero. It follows immediately that $\mu_d = \mu_e = 0$ and $J_d = J_e = 0$

In our model, we assume that the total number of each type of protein is conserved. We further assume that the *min* proteins can bind/unbind from the membrane and that the proteins do not degrade during the process. The zero-flux boundary conditions are imposed at both ends of the bacterium. The total amounts of MinD and MinE, obtained by integrating $\rho_D + \rho_d$ and $\rho_E + \rho_e$ over the length of the bacterium, are conserved.

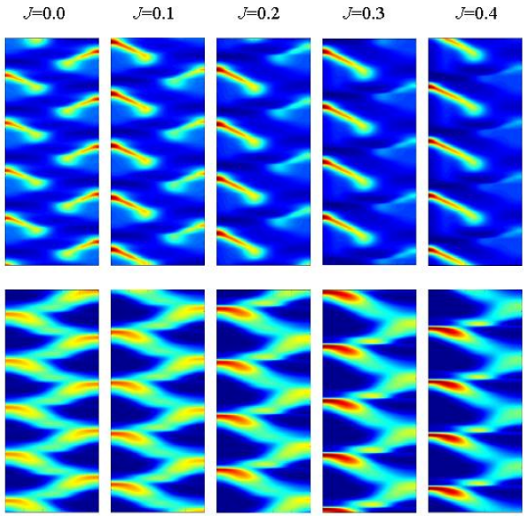

Fig. 1. Space-time plots of the total ($\sigma_D + \sigma_d$) MinD (above) and total ($\sigma_E + \sigma_e$) MinE (below) concentration for $J = 0.0$ m/s to $J = 0.4$ m/s. The color scale, running from blue to red, denotes an increase in the concentration from the lowest to the highest. The MinD depletion from mid cell and the MinE enhancement at the mid cell are immediately seen. The vertical scale spans time for 500 s. The times increase from bottom to top, and the oscillations pattern repeats infinitely as time increases. The horizontal scale spans the bacterial length (2 μm). Note the increase in the MinD and MinE concentrations at the left end of the bacterium as J increases.

III. NUMERICAL RESULTS AND DISCUSSION

Since the bacterium length is very short, it is reasonable to assume that the applied electric field has a constant value throughout the bacterium length. We have numerically solved the set of four coupled reaction-diffusion equations, Eqs. (1)-(4), by using the explicit Euler method [20]. The length of the *E. coli* is taken to be 2 μm. The total time needed for each simulation is approximately 10^4 s. In our simulations, we have discretized space and time; *i.e.*, we have taken $dx = 8 \times 10^{-3}$ μm and $dt = 1 \times 10^{-5}$ s. The space covering the bacterium is divided into 251 grid points, and the time is divided into 10^9 times steps (10^9 iteration steps). Initially, we assume that MinD and MinE are mainly at the opposite ends of the bacterium with the number of *min* molecules in each cell being 3000 for the MinD population [6] and 170 for the MinE population [21]. Since the total amount of MinD and MinE in *E. coli* must be conserved, we set the flux of MinD and MinE to zero at both ends of the bacterium. Since there are no experimental values of μ for either MinD and MinE, we work with the external field parameter J, which is proportional to E, instead of E explicitly. We also assume that $\mu_D = \mu_E$ (we assume MinD and MinE have the same type of charges). It follows immediately that $J_D = J_E \equiv J$. The values of the other parameters are: $D_D = 0.28$ μm^{2}s^{-1}, $D_E = 0.6$ μm^{2}s^{-1}, $\sigma_1 = 20$ s^{-1}, $\sigma_1^{'} = 0.028$ μm, $\sigma_2 = 0.0063$ μms^{-1}, $\sigma_3 = 0.04$ μms^{-1}, $\sigma_4 = 0.8$ s^{-1}, and $\sigma_4^{'} = 0.027$ μm. In our analyses of the numerical results, we looked at the time-averaged values of the concentrations of MinD and MinE and at the patterns of the oscillations of MinD and MinE for various J values.

In the absent of an external field, the numerical results [17] show that most of the MinD will be concentrated at the membrane and the MinE at mid cell. This results in an accurate division at mid cell. In the presence of an external field, both MinD and MinE experience a force in the same direction. This force causes a shift of the time-averaged minimum of MinD. This shifts the division site from mid cell. Our numerical solutions show that the behavior of the Min system in the presence of an external field depends on the strength of the external field parameter J.

Figure 1 shows the oscillation patterns for $J_E = J_D \equiv J = 0.0$ m/s to $J = 0.4$ m/s. It is seen that as J increase, both the MinD and the MinE concentrations in the left part of the *E. coli* become larger while the two concentrations in the right part become smaller as J is increased. This behavior is a reflection of the fact that the external force is acting in the left direction. These patterns show a shifting in the concentrations of the *min* proteins towards the left pole.

In Figure 2, we show the time-averaged concentrations of the MinD and the MinE proteins at different positions within the bacteria. In these curves, positive values of the external field parameter are used. From this Figure, we see that in the case of no external field ($J = 0.0$ m/s), the time-averaged concentrations of MinD and MinE are symmetric about mid cell. MinD has a minimum at mid cell while MinE has a maximum. When an external field is applied, we see a shift in the minima of MinD and in the maxima of MinE. The time-averaged concentration

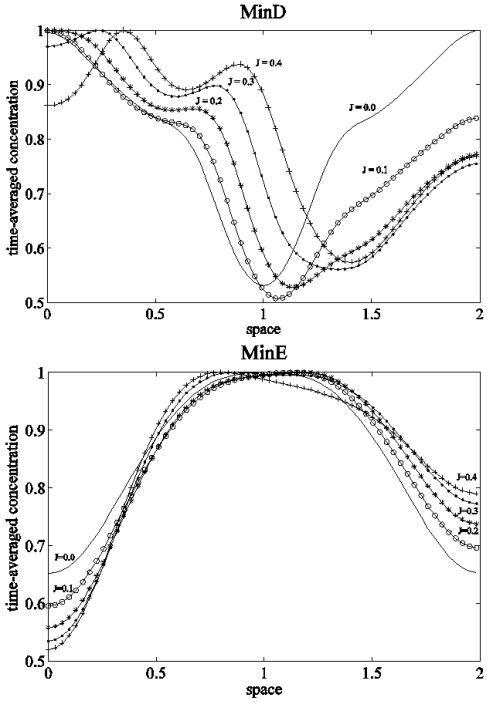

Fig. 2. Time-averaged concentration of MinD (above) and MinE (below) relative to their respective time-averaged maxima, $\langle\sigma(x)\rangle/\sigma_{max}$, as a function of the position x (in μm) along the bacterium axis under the influence of positive values of a static external field. The curves show a shift in the local minima of the MinD and the local maxima of the MinE from the mid cell that depends on the strength of the field.

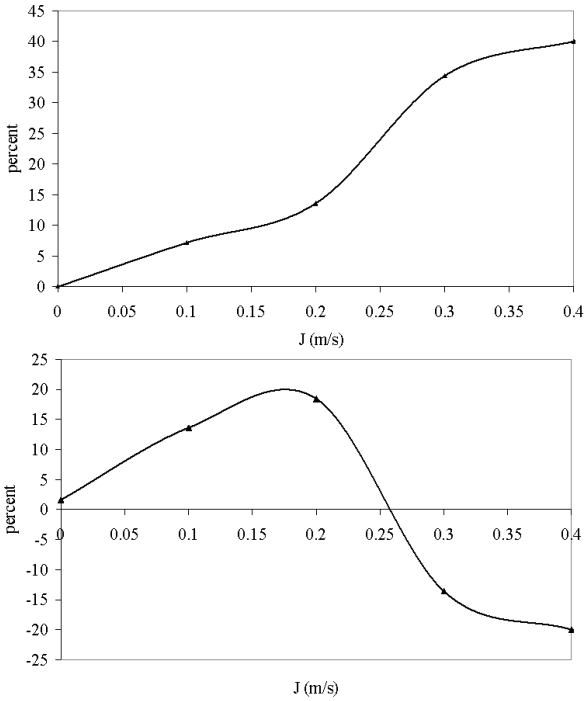

Fig. 3. Percents of the shifting of the local minima of MinD (above) and the local maxima of MinE (below) from mid cell for various values of J. Positive values denote a shift to the right pole and a negative value to the left pole.

curves are no longer symmetric about mid cell. In nature, the MinE protein looks like a ring structure that effectively positions the anti-MinCD activity [11,14]. MinCD inhibits the division process, so in nature, the bacterium divides at the site where the minimum MinD concentration occurs. The value of the MinE concentration is not maximum at the mid cell. The minimum of the MinD shifts to the right pole under the influence of positive J values.

We have measured the percent of shifting of the time-averaged concentration in the local minima of MinD and the local maxima of MinE. This is shown in Fig. 3. The figure shows that the minimum of MinD is always shifted to the right pole. This is the result of the external force pulling MinD to the left. The maximum of MinE is not always shifted to the right. When $J < 0.2$ m/s, the maximum of MinE is shifted to the right, but when $J > 0.2$ m/s, it shifted to the left of mid cell. This difference arises because of the relative magnitudes of the forces acting on the two proteins. First of all, there is an internal force between the MinD and the MinE proteins. This force causes MinE to repel MinD. In the absence of any other forces, this explains why the location of the maximum of MinE is the location of the minimum of MinD. When an external field is applied (as expressed by a non-zero value of J), then one must take into account the relative magnitudes of the two forces.

When J is large (larger than 2 m/s), the external force dominates the internal force between the MinD and the MinE proteins. The external force pulls MinD and MinE in the same direction, causing the location of the maximum of MinE to be no longer at the location of the minimum of MinD. If J is small (smaller than 0.2 m/s), the internal force between MinD and MinE dominates. This results in the two location (the maximum of MinE and the minimum of MinD) to be nearly the same. In Fig. 3, we also see that the shifts of the minimum of the MinD concentrations increase as the field parameter J increases. Since the division site will be the location where the MinD concentration is minimum, the shift in the minimum of MinD concentration to the right pole indicates that the division site must also shift to the right pole. When we let J be negative, the results are very similar to those for positive J values, as expected; the curves for the time averages of the concentrations of the *min* proteins shift in the mirror side about mid cell.

In Figs. 4(a) and 4(b), we show the concentrations of the MinD and the MinE proteins at the left end grid, the middle grid, and the right end grid versus time. In these figures, it is easy to see that when $J = 0.0$ m/s, the concentrations of MinD (or MinE) at the left end grid and the right end grid have the same patterns of oscillation with the same frequencies and amplitudes, but with a phase difference of 180°. At the mid cell grid, the frequency of the oscillation is two times greater than that

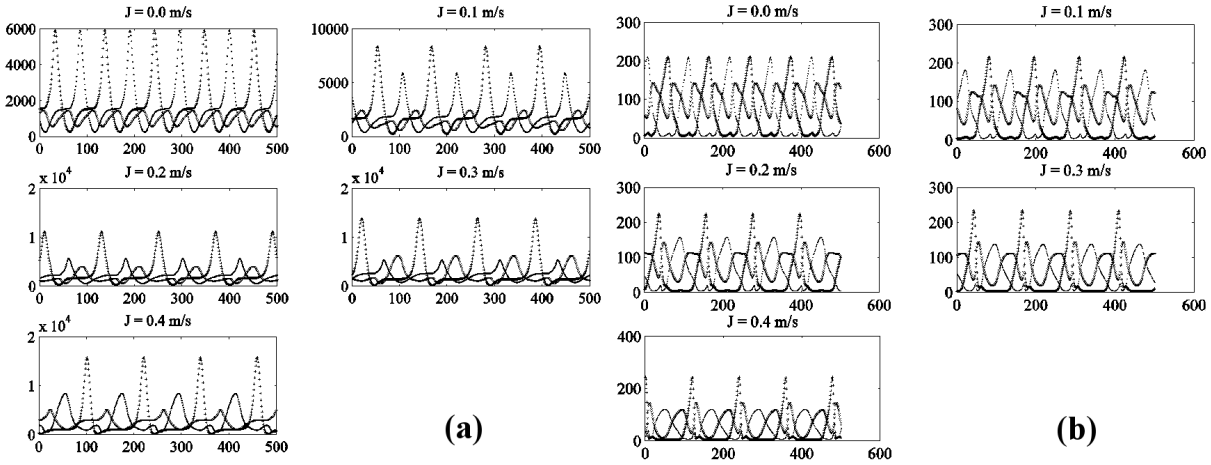

Fig. 4. (a) Plots of the concentration of MinD at the the left end grid (+), the middle grid (x), and the right end grid (·) versus time in seconds for $J = 0.0$ m/s to $J = 0.4$ m/s. The vertical scales denote concentration in molecules per meter. (b) Plots of the concentration of MinE at the left end grid (+), the middle grid (x), and the right end grid (·) as functions of time in seconds for $J = 0.0$ m/s to $J = 0.4$ m/s. The vertical scales denote concentration in molecules per meter.

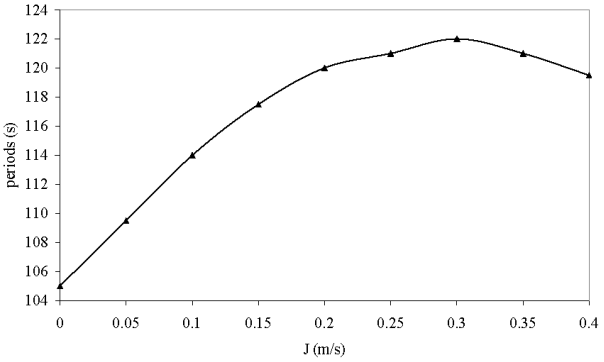

Fig. 5. Plots of the concentration of MinE at the left end grid (+), the middle grid (x), and the right end grid (·) as functions of time in seconds for $J = 0.0$ m/s to $J = 0.4$ m/s. The vertical scales denote concentration in molecules per meter.

of right end grid. When an external field is applied, the amplitudes of the oscillations at the two end grids are no longer equal, but the frequencies of the oscillations of the three grids become the same. As J is increased, the amplitude of the oscillation at the right end grid is seen to decrease while those of the left end and mid cell grids are seen to increase.

Figure 5 show the periods of oscillation for the MinD concentration at the left end grid for various value of J. In this figure, we see that for the case of no external field, the period of the oscillation is equal to 115 s, which is in good agreement with the experimental value. When an external field is applied, the period of the oscillation is seen to increase. When J is not too large ($J < 0.3$), the period of the oscillation increases as J is increased. The increase in the period of oscillation as an external field is applied indicates that in the presence of an external field, the bacterium needs a longer time to divide.

IV. CONCLUDING REMARKS

Proper divisions of bacteria require accurate definition of the division site [3]. This accurate identification of the division site is determined by the rapid pole-to-pole oscillations of MinCDE [8, 11, 22]. Using a mathematical model to describe the dynamics of the *min* pole-to-pole oscillations, Howard *et al.* [17] found that the mid cell position in the *Escherichia coli* bacteria corresponded to the point where the time-averaged MinD and MinE concentrations were minimum and maximum, respectively. They also found that the concentrations of these two proteins were symmetric about the mid cell position.

To see the effect of exposing *E. coli* bacteria to an electric field, we have added some additional terms to the reaction-diffusion equations for the pole-to-pole oscillations proposed by Howard *et al* for the *min* proteins in the *E. coli* bacteria. The additional terms are the gradient terms appearing in Eqs.(1)-(4). These terms depend on the strength of the external field and the charge of the protein. We then used a numerical scheme to solve the resulting coarse-grained coupled reaction-diffusion equations. The results are shown in Figs. 1 to 5. Our results shows deviations from the results obtained by Howard *et al.*, *e.g.*: the concentrations of MinD and MinE are no longer symmetric about the middle of the long axis, nor are the minimum and the maximum of the MinD and the MinE concentrations at the middle of the long axis. The shift in the minimum of the time-averaged concentration of MinD from mid cell should shift the division site. The shift of the minimum concentration of MinD from the midpoint appears to depend on the strength of the external field. This indicates that if the parent cell

can divide under these condition, it must divide into two filamentous cells, providing the external field is strong enough. Since an external field can shift the minimum of the time-averaged concentration of MinD, an external electric field can interfere with the division process.

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