Min-protein oscillations in round bacteria

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Abstract
In rod-shaped Escherichia coli cells, the Min proteins, which are involved in division-site selection, oscillate from pole-to-pole. The homologs of the Min proteins from the round bacterium Neisseria gonorrhoeae also form a spatial oscillator when expressed in wild-type and round, rodA− mutants of E. coli, suggesting that the Min proteins form an oscillator in N. gonorrhoeae. Here we report that a numerical model for Min-protein oscillations in rod-shaped cells also produces oscillations in round cells (cocc). Our numerical results explain why the MinE-protein rings found in wild-type E. coli are absent in round mutants. In addition, we find that for round cells there is a minimum radius below which oscillations do not occur, and a maximum radius above which oscillations become mislocalized. Finally, we demonstrate that Min-protein oscillations can select the long axis in nearly round cells based solely on geometry, a potentially important factor in division-plane selection in cocci.

Introduction
In the rod-shaped bacterium Escherichia coli, the proteins MinC, MinD and MinE are observed to oscillate from pole to pole [1–4] every ~20 s [2]. Both MinD and MinE are required for oscillations [2]; MinC is not required, but is observed to oscillate because it forms complexes with MinD on the membrane [5]. MinD is an ATPase which associates to the membrane and forms helical polymers there [6] in its ATP-bound form (MinD:ATP) [7]. MinE is recruited to the membrane by MinD, where it activates hydrolysis of MinD:ATP resulting in dissociation of MinD from the membrane [7]. The oscillations concentrate MinCD complexes near the poles of the cell, where MinC blocks formation of a ring of FtsZ protein [8]. Formation of this FtsZ ring is required to initiate septum formation and cell division [9], so the net effect of the Min oscillations is to block cell divisions near the poles (minicelling). Placement of the FtsZ ring is also regulated by nucleoid occlusion [10–12], a mechanism which blocks FtsZ ring formation in the vicinity of a nucleoid.

Homologs of the Min proteins have also been identified in round cells. In the coccus Neisseria gonorrhoeae, loss of MinDNg results in abnormal cell division and morphology, and decreased cell viability [13]. MinDNg and MinENg from N. gonorrhoeae were observed to oscillate when expressed in E. coli lacking its own MinD and MinE [14]. To date, there has been no direct observation of Min-protein oscillations in N. gonorrhoeae because of resolution limits associated with the small size of the bacteria, typically around 0.5 µm in radius [13]. However, in round E. coli cells, resulting from disruption of the rodA gene, both native MinD [15] and MinDNg from N. gonorrhoeae [14] have been observed to oscillate. Interestingly, while oscillations in wild-type E. coli display a ring of MinE protein, no MinE ring is observed in the round rodA− cells. Taken together, the close homology of the E. coli and N. gonorrhoeae Min proteins, the complementation experiments and the Min oscillations in round E. coli cells suggest that the Min proteins also form an oscillator in N. gonorrhoeae [14].

Here we demonstrate that a numerical model for Min-protein oscillations in rod-shaped cells [17] also leads to oscillations in round cells. The model is shown schematically in figure 1, and involves only in vitro observed interactions of MinD and MinE [2, 7]. In contrast to earlier modeling attempts [18–20], the model in [17] is fully three-dimensional,
so that extension to round cells is straightforward. The oscillations are driven by a cycle in which MinD:ATP binds to the membrane, preferentially at locations of high pre-existing concentrations of MinD:ATP. MinE then attaches to the membrane-bound MinD:ATP, activates ATP hydrolysis and MinE then attaches to the membrane, preferentially where other MinD:ATP is bound. (2) MinE in the cytoplasm attaches to a membrane-associated MinD:ATP complex. (3) MinD:ATP complex, and releasing (a) MinD:ADP, (b) MinE and (c) phosphate into the cytoplasm. (4) MinD:ATP is converted back to MinD:ATP by nucleotide exchange. In wild-type cells, MinE is likely active as a homodimer [16].

**Reaction–diffusion equations**

The equations describing the time evolution of MinD and MinE concentrations in a spherical cell of radius \( R \) (see schematic of reaction cycle in figure 1) are

\[
\begin{align*}
\frac{\partial \rho_{D,ADP}}{\partial t} &= D_D \nabla^2 \rho_{D,ADP} - \sigma_{D,ADP\rightarrow ATPE} \rho_{D,ADP} + \delta (r-R) \rho_{de} - \delta (r-R) \rho_{de} \\
\frac{\partial \rho_{D,ATP}}{\partial t} &= D_D \nabla^2 \rho_{D,ATP} + \sigma_{D,ATP\rightarrow ADP} \rho_{D,ATP} - \delta (r-R) \rho_{de} \times [\sigma_D + \sigma_{ADP} (\rho_d + \rho_{de})] \\
\frac{\partial \rho_E}{\partial t} &= D_E \nabla^2 \rho_E + \delta (r-R) \rho_{de} - \delta (r-R) \sigma_{E,PE} \rho_{de} \\
\frac{\partial \rho_{de}}{\partial t} &= -\sigma_{de} \rho_{de} + \sigma_S \rho_{PE} (R) \\
\end{align*}
\]

where \( \rho_{D,ADP}, \rho_{D,ATP}, \rho_E, \rho_{de} \) are the concentrations in the cytoplasm of MinD:ADP complexes, MinD:ATP complexes, and MinE and \( \rho_d, \rho_{de} \) are the concentrations on the membrane of MinD:ATP and MinD:MinE:ATP complexes.

**Results and discussion**

**Min-protein oscillations with no MinE ring**

The results of numerical integration in time of the model equations (cf figure 1 and equations (1)–(5)) for a spherical cell with radius \( R = 0.5 \mu m \) are shown in figure 2. In the spherical cell, any initial protein distributions (except those with perfect spherical symmetry) generate an oscillation which is rotationally symmetric about a ‘north–south’ axis. The choice of oscillation axis does depend on initial conditions; in figure 2 we have restricted the initial concentrations to be uniform in the azimuthal direction. The oscillation cycle has a period of 81 s. As in cylindrical cells [17], zones of MinD:ATP grow from and shrink to the poles of the cell. However, in contrast to cylindrical cells, there is a notable absence of a MinE ring; rather, the MinE spreads throughout the entire MinD:ATP polar zone. The bottom row of panels in figure 2 shows the quantities in columns (a–c) averaged over one complete oscillation. The minimum for membrane-bound MinD:ATP occurs at the equator, indicating the most likely division site due to lower concentrations of the FtsZ ring-inhibitor MinC. Periodic oscillations occur for a wide range of model parameters. The period can be increased without limit by reducing the hydrolysis rate \( \sigma_{de} \). Increasing \( \sigma_{de} \) reduces the
MinD and (MinE in red). The concentrations as a function of polar angle \( \theta \)–axis on each plot ranges from 0 to 500 \( \mu \text{m} \). To mimic the membrane-associated concentrations as a function of polar angle \( \theta \). MinD:ATP in blue and MinE in red. The \( y \)-axis on each plot ranges from 0 to 500 \( \mu \text{m} \). We approximate the new polar zone as the southern hemisphere. For a cylinder with radius 0.5 \( \mu \text{m} \) and length 4 \( \mu \text{m} \), we approximate the new polar zone by a region of constant density, as shown in figures 3(c) and (d), with the densities shown in figures 3(e) and (f). In the sphere the MinE density is nearly uniform throughout the southern hemisphere, while in the cylinder the MinE density shows a clear central peak, i.e., a MinE ring.

**Oscillations in nearly round cells**

Real cells are not perfectly round. In nearly round rodA+ mutants of *E. coli*, Min-protein oscillations were almost always observed to orient along the long axis [15]. While this orientation could be due to membrane targets or scars, we ask whether the geometry of the cell alone could be sufficient to orient the Min oscillations. In figure 4, we show the results of numerical integration in time of equations (1)–(5) for an elliptoidal cell with semi-major axis of length \( R_1 = 0.525 \mu \text{m} \) and semi-minor axes of length \( R_2 = 0.5 \mu \text{m} \), starting from a completely random distribution of proteins. All diffusion constants, rate constants and mean volume concentrations are the same as for the spherical cell in figure 2. Within a few oscillation periods, a stable oscillation is established precisely along the long axis of the ellipsoidal cell with a period of 80 s.

**Minimum radius for oscillations**

We find that spherical cells below a minimum radius \( R_{\text{min}} \) do not support oscillations. The exact value of \( R_{\text{min}} \) depends on the choice of parameters; for our choice \( R_{\text{min}} = 0.45 \mu \text{m} \). The non-oscillating solution of equations (1)–(5) is spherically symmetric and has uniform concentrations of MinE and total MinD in the cytoplasm, which lacks sources or sinks of these proteins. However, the concentrations of the two species MinD:ADP and MinD:ATP can individually vary in the radial direction due to local exchange with the membrane. To analyze stability, we solve for the static concentrations: \( \rho_i^0(r) \) where \( i \in S = \{D; \text{ADP}, D; \text{ATP}, E, d, de\} \), and then perturb about this static solution:

\[
\rho_i^0(r) \rightarrow \rho_i^0(r)\left[1 + \varepsilon P_l(\cos \theta)\right],
\]

where \( P_l \) is the \( l \)th Legendre polynomial, which is an eigenmode of the angular part of the diffusion equation in spherical coordinates with eigenvalue \( \nu_l = l(l + 1)/r^2 \). In
where for the most unstable perturbation in practice, we define the cytoplasmic densities on a grid of size $n_r = R/dr$.

$$
\rho_i^0(r) \rightarrow \left( \rho_i^0 \right)^{R_\text{min}}_{r=1}.
$$

The perturbations in equation (6) evolve in time according to

$$
\rho_i = \rho_i^0(r)[1 + e^{\Lambda_1 + i\Lambda_2}],
$$

where the exponent $\Lambda \equiv \Lambda_1 + i\Lambda_2$, is an eigenvalue of the matrix $H - \nu_1 D$. The Hessian matrix $H$ is

$$
H = \begin{bmatrix}
\frac{\partial}{\partial r} (\rho_i^0)_{\eta}
\frac{\partial}{\partial r} (\rho_i^0)_{\nu}
\end{bmatrix},
$$

where $i, k$ run over species in $S$ and $j, m = 1$ if $i, k \in \{d, e\}$ and $j, m = 1, \ldots, n_r$ otherwise. The diffusion matrix is

$$
D = [D_{ij}],
$$

where $D_{ij} = \delta_{ij} D_i$ and $D_i$ is the diffusion constant of species $\rho_i$. Any mode with instability exponent $\Lambda_1 > 0$ represents an unstable (growing) perturbation about the static solution, and indicates that the solution of equations (1)–(5) is oscillatory.

In figure 5(a), we plot the period of full nonlinear oscillations and in figure 5(b) the growth-rate exponent $\Lambda_1$ for the most unstable perturbation ($\sim \cos \theta$) from our linear-stability analysis. Both panels indicate a minimum radius for oscillations of $R_{\text{min}} = 0.45 \mu$m.

### Conclusion and outlook

Min-protein oscillations in rod-shaped *E. coli* cells block cell division at the poles, thereby preventing minicelling. The Min system may also contribute to the accuracy of cell division. Wild-type *E. coli* forms an FtsZ ring at the cell center with an accuracy better than $\pm 1\%$ of cell length, and this accuracy is lost in $\min^{-}$ mutants [11]. An alternative view is that this loss of accuracy may simply reflect the onset of minicelling, rather than indicating a direct role for the Min system in division accuracy [23]. (Indeed in *Bacillus subtilis*, homologs of MinC and MinD statically localize to the poles to prevent minicelling and have been shown to be dispensable for division accuracy [24].) Division-site placement in *E. coli* is regulated both by the Min-protein system and by nucleoid occlusion [10]. Certainly, in rod-shaped cells, Min oscillations and division accuracy, possibly based on nucleoid occlusion, could be independent, with both relying on the obvious long axis of the cell.

In round cells (cocc) such as *N. gonorrhoeae* there is no obvious long axis. Nevertheless, cocci divide accurately along an equatorial plane into two daughter cells. If the Min proteins oscillate in *N. gonorrhoeae*, as experiments [13, 14] and our numerical results suggest, then for the Min system to perform its usual function of blocking polar divisions requires that the poles and equator of the cell be...
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Figure 4. Time slices in 14 s increments of one complete MinD,E oscillation in an ellipsoidal cell with semi-major axis of length $R_1 = 0.525 \mu m$ and semi-minor axes of length $R_2 = 0.5 \mu m$. The quantities shown in (a), (b) and (c) are the same as in figure 2. Starting from a random initial distribution of proteins, pole-to-pole oscillations along the long axis become established within 3 oscillation periods. The $y$-axis on each plot in (c) ranges from 0 to 600 $\mu m^{-1}$.

Figure 5. (a) Oscillation period as a function of cell radius. For cells with a radius smaller than $R_{\text{min}} = 0.45 \mu m$, no oscillations are possible. (b) Instability exponent $\Lambda_1$ as a function of cell radius for the most unstable perturbation ($\sim \cos \theta$) superimposed on a static, spherically symmetric solution.

Figure 6. Schematic of pre-division round cell. (a) The alignment of the Min oscillations and nucleoid segregation ensures proper formation of the FtsZ ring. (b) Misalignment of the axial direction of the Min oscillations and the equator defined by the segregated nucleoids prohibits FtsZ-ring formation.

consistently defined. This point is made in the schematic in figure 6, with the schematic nucleoid segregation representing the full machinery of accurate cell division. The requirement for consistency between poles (MinCD accumulation zones) and equator (division plane) suggests that Min oscillations and division accuracy are directly coupled in *N. gonorrhoeae*. Experiments in round *E. coli* mutants [15] indicate that Min oscillations can pick out the long axis in slightly asymmetric cells. Our numerical simulations in ellipsoidal cells support this conclusion, and it is tempting to speculate that for cell division in *N. gonorrhoeae*, the Min oscillations exploit small variations in cell shape to define the poles and equator of the cell.

The ability of the Min oscillations to select the long axis of an ellipsoidal cell reflects the length dependence of the oscillatory instability. As shown in figure 5(b), the instability exponent $\Lambda_1$ increases with radius. Therefore, in asymmetric cells the oscillation pattern with the longest wavelength will become established. In the case of an ellipsoidal cell, the longest wavelength pattern corresponds to oscillations along the long axis of the cell. In both ellipsoidal and rod-shaped cells, a protein oscillator which prefers long wavelengths can provide a general mechanism for polar targeting of proteins.

In the most spherical of their round *rodA*− *E. coli* cells, Corbin et al [15] observed rapid reorientation of Min oscillations of the MinD$_{Ng}$ proteins. Ramirez-Arcos *et al* [14] also observed uncoordinated oscillations in large *rodA*− *E. coli* cells, but saw consistent pole-to-pole oscillations in smaller *rodA*− cells. In so far as the Min oscillations have a preferred wavelength for oscillations [17], it is
not surprising that qualitatively different oscillation patterns were observed in differently-sized round cells. Indeed, our numerical results suggest that the mislocalized oscillation patterns observed in large round cells reflect the instability of higher-order oscillation modes. Specifically, we found that larger cells, with radii \( R > 0.7 \mu m \), starting from a north–south-symmetric protein distribution, support an oscillatory solution where the MinD distribution alternates between simultaneous accumulations at both poles and an equatorial accumulation. Moreover, in our simulations of cells with radii larger than ~1.2 \( \mu m \), we observe oscillations in which the MinD accumulates at intermediate locations between the pole and equator rather than at the pole. These patterns, which can be understood as a combination of pole-to-pole oscillations with higher-order oscillation modes, would be sensitive to fluctuations and nucleation effects in real cells. Indeed, the specific movement of the MinD `pole' in large rodA+ \( E. coli \) cells is likely a consequence of nucleation effects, i.e., in the regime of cell sizes where accumulation can occur near the equator, the first significant accumulation of MinD away from the old pole becomes the new pole. This nucleation effect is beyond the scope of our current mean-field model for Min oscillations. Even for rod-shaped cells, it remains an open question how fluctuations affect the stability and character of Min oscillations. The recent observation by Shih \textit{et al} [6] that MinD accumulates in helical polymers which remain present even at the `empty' pole may help explain why fluctuations do not lead to uncoordinated oscillations in wild-type cells. The role of Min oscillations in nucleoid segregation and cell-division accuracy remains an important open question. We are currently focused on imaging Min proteins in \( N. gonorrhoeae \) to verify that the Min system forms a spatial oscillator. Two-color fluorescence microscopy experiments, labeling MinD and FtsZ, will test whether the Min oscillations properly align with the division plane to achieve accurate nucleoid segregation. The correlation of the oscillation axis with the cell shape will demonstrate whether elongation of the cell orients the Min oscillations.

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**Glossary**

\textit{GFP-fusion.} Translational fusion to Green Fluorescent Protein (GFP) used to tag a specific protein for visualization with subcellular fluorescence microscopy.

\textit{Linear-stability analysis.} Method of determining whether the steady-state solution of a system of equations (e.g. reaction-diffusion equations) is stable with respect to a given perturbation.

**Ellipsoid.** A distorted sphere which has been elongated in one direction. The surface of an ellipsoid with major axis along the z-axis satisfies the equation

\[
\left( \frac{x}{R_{\text{minor}}} \right)^2 + \left( \frac{y}{R_{\text{minor}}} \right)^2 + \left( \frac{z}{R_{\text{major}}} \right)^2 = 1.
\]

**Protein targeting.** The direction of proteins to particular cellular regions, e.g. the cell poles.

**Nucleoid targeting.** Separation of the nucleoids to opposite halves of the cell prior to cell division to allow for septum formation.

**Cell-division accuracy.** How accurately the cell divides into two equal daughter cells, i.e. how close cell division is to midcell. In units of cell length, wild-type \( E. coli \) divides with accuracy 0.50 ± 0.01 [11].

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