Membrane Binding of MinE Allows for a Comprehensive Description of Min-Protein Pattern Formation

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Abstract

The rod-shaped bacterium Escherichia coli selects the cell center as site of division with the help of the proteins MinC, MinD, and MinE. This protein system collectively oscillates between the two cell poles by alternately binding to the membrane in one of the two cell halves. This dynamic behavior, which emerges from the interaction of the ATPase MinD and its activator MinE on the cell membrane, has become a paradigm for protein self-organization. Recently, it has been found that not only the binding of MinE to the membrane, but also interactions of MinE with the membrane contribute to Min-protein self-organization. Here, we show that by accounting for this finding in a computational model, we can comprehensively describe all observed Min-protein patterns in vivo and in vitro. Furthermore, by varying the system’s geometry, our computations predict patterns that have not yet been reported. We confirm these predictions experimentally.

Introduction

Nature presents an overwhelming variety of forms and patterns. While system specific conditions can play an important role for their formation, also a few general principles underlying biological pattern formation have been proposed in the past. A particularly attractive concept is the spontaneous formation of patterns in reaction diffusion systems as proposed by Alan Turing [1]. In this way, the Min system can self-organize without any additional spatial organization. Here, we show that by accounting for this finding in a computational model, we can comprehensively describe all observed Min-protein patterns in vivo and in vitro. Furthermore, by varying the system’s geometry, our computations predict patterns that have not yet been reported. We confirm these predictions experimentally.

Mathematically, subcellular pattern formation due to reactions and diffusion of just two different constituents is provided by the large-scale patterns. So far, however, only a few biological examples of bona fide Turing patterns are known [2].

An example of subcellular pattern formation due to reactions and diffusion of just two different constituents is provided by the Min system in the rod-shaped bacterium Escherichia coli [3]. This protein system forms a spatiotemporal oscillation in the cell, that is, a standing wave with a node in the cell center [4,5], see Figure 1A, which plays an essential role in division site selection in E. coli. Whereas the oscillations emerge solely from the interactions between MinD, MinE, and the membrane, the inhibitor of cell division MinC binds to MinD and is distributed similarly: it is, a standing wave with a node in the cell center [4,5], see Figure 1B, whereas in shorter cells and for slightly over-expressed Min proteins, oscillations are replaced by stochastic switching of the proteins between the two cell halves [7,8]. In Y-shaped cells, the proteins visit the different arms in a way that depends on the arms’ lengths [9].

Furthermore, in vitro studies of purified proteins found MinD and MinE to spontaneously organize into collective traveling waves [10]. Together, these observations suggest that the Min-protein patterns emerge from the intrinsic dynamics of these proteins, in particular, the exchange of proteins between the membrane, driven by the high affinity of MinD for the membrane when ATP is bound and a low affinity with ADP bound [11]. In addition, membrane-bound MinD recruits MinE, which in turn induces hydrolysis of the bound nucleotide by MinD and consequently MinD detachment from the membrane. These well-established processes are at the core of a number of computational models reproducing the Min-protein oscillations observed in E. coli [12].

The most popular mechanism studied through such models assumes that cooperative membrane-attachment of MinD is at the origin of pattern formation. In the simplest version, the rate of MinD attachment to the membrane increases in presence of membrane-bound MinD [13]. Several works on models implementing cooperative membrane attachment in various ways and complementing it by different side processes have shown that it can robustly generate the pole-to-pole observations observed in E. coli [14–16] even during septum closure [17]. Other works rather emphasized cooperative effects between already membrane-bound MinD [18,19]. However, in spite of more than a decade of
theoretical analysis, there exists to date no comprehensive description of all Min-protein patterns observed in vivo and in vitro.

Some evidence suggested that an N-terminal helix allows MinE to also interact with the membrane [20,21], however, it remained unclear if this property was important for the self-organization of the Min system. Single molecule data obtained in vitro [22] as well as genetic, physiological, and structural analysis [23] finally provided evidence that the ability of MinE to interact with phospholipids allows it to remain bound to the membrane after MinD has detached, which could lead to the subsequent removal of several MinD dimers by one MinE dimer. In analogy to molecular motors that can perform several subsequent steps on a cytoskeletal filament, we call this property “MinE processivity”. This possibility had been proposed earlier on theoretical grounds as it offers a mechanism for the formation of MinE-rings [19,24,25] and was crucial for describing the guidance of Min-protein waves on patterned substrates [26]. In the present work, we perform a computational study to explore the consequences of this molecular property for large-scale pattern formation. To this end, we use deterministic and stochastic calculations in three dimensions. We show that MinE processivity provides a key to obtain a unified description of all previously described Min-protein patterns in vivo and in vitro. In addition, our analysis predicts hitherto unknown patterns, namely traveling waves in long and moving patches in aberrantly large cells. We confirm the existence of these states by fluorescence microscopy of living E. coli cells. Beyond the Min system, our findings highlight the importance of membrane-binding for subcellular pattern formation.

Results

Min-protein dynamics

Molecular interactions. We start by detailing the molecular interactions that we consider essential for understanding Min-protein pattern formation in vivo and in vitro, see also [27]. Let us start with the ATPase MinD. After binding ATP and in the

Figure 1. Different patterns formed by MinD in living E. coli. A) Standing wave with one node; B) standing wave with two nodes. Top: DIC image followed by snapshots from a time lapse recording of MinD-GFP; bottom: corresponding kymograph. Scale bar: 3 μm. doi:10.1371/journal.pcbi.1003347.g001
presence of a lipid bilayer, an amphipathic helix is formed at the C-terminus of cytoplasmic MinD giving the protein an increased affinity for binding lipid bilayers [28–32]. Furthermore, ATP-binding leads to MinD dimerization. Only as a dimer, MinD has a sufficiently high affinity for binding to the cytoplasmic membrane. The binding kinetics of MinD shows deviations from Langmuir kinetics suggesting that MinD binding to the membrane is cooperative [30,33,34]. The molecular mechanism underlying cooperative MinD binding, though, is poorly understood.

Let us note that membrane-bound MinD can interact to form higher-ordered structures, however their exact lifetime and architecture is not known [35–37]. Experiments in vitro on vesicles incubated in a buffer containing MinD suggest a two-step process of MinD binding first to the membrane and subsequently forming clusters [35]. MinD proteins have been reported to arrange in a helical way [37]. It is not clear, though, whether aggregates of membrane-bound MinD play a functional role in Min-pattern formation. Note also, that recent works have provided evidence that the formation of MreB helices or foci of Clp Protease in E. coli were induced by attached fluorescent tags [38,39]. It remains to be seen if a similar effect is responsible for the formation of MinD helices.

MinE and MinC are recruited to the cytoplasmic membrane by membrane-bound MinD dimers. They bind to overlapping sites located at the MinD-dimer interface [32,40,41]. At the same time MinE interacts directly with the membrane through an amphipathic α-helix [23]. The binding of MinE stimulates the ATPase activity of MinD and thus triggers the detachment of MinD from the membrane [29,30]. Through its direct interaction with the membrane, MinE can reside on the membrane for a short period during which it can associate with another membrane-bound MinD dimer [22,23]. Due to the interaction of a amphipathic N-terminal helix with the membrane, MinE is able to remain attached after activation and displacement of MinD to activate another MinD dimer bound to the membrane. Since the formation of this helix of MinE depends on the formation of a complex with its substrate MinD, this behavior is comparable to processive enzymes, which are able to remain attached to their substrates and perform a large number of rounds of catalysis before dissociating [42].

Molecular processes and dynamic equations. From the molecular interactions sketched above, we inferred the dominant reaction paths governing the macroscopic dynamics of the Min-protein distributions. To keep our description simple, we only considered MinD dimers.

The processes captured in our analysis were the following: MinD in the vicinity of the membrane associates at a rate $v_D$ with the lipid bilayer, see Figure 2. Cooperative effects in the binding process lead to an increase of the binding rate if membrane-bound MinD are present nearby. We capture this effect through increasing the binding rate by $v_{dD}$ times the local density of membrane-bound MinD.

MinE binds to membrane-bound MinD and forms a MinDE complex [43]. This process occurs at a rate $v_{Ecd}$, where $cd$ is the local density of membrane-bound MinD. A MinDE complex can dissociate in two ways: either, both, MinD and MinE, detach from the membrane or only MinD leaves the membrane, whereas MinE stays on the lipid bilayer. The two processes occur at rates $v_{de}$ and $v_{de,m}$, respectively. Individual MinE dimers on the membrane associate with nearby membrane-bound MinD at rate $v_{dEcd}$ or dissociate from the membrane at rate $v_e$.

Finally, all molecules can diffuse in the cytoplasm or on the membrane. Let us emphasize, that we ignore any spatial heterogeneities due to variations in the lipid composition of the membrane.

Figure 2. Schematic illustration of the molecular processes involving MinD, MinE, and the membrane. Cytosolic MinD dimers bind to the membrane, with an increased rate in the vicinity of membrane-bound MinD (1). Note, that the molecular mechanism underlying cooperative membrane binding of MinD has not been characterized yet and it is still unclear whether or not membrane-bound MinD form clusters. Cytosolic MinE bind to membrane-bound MinD and form MinDE complexes (2). MinDE complexes dissociate in one of two different ways: MinD and MinE detach simultaneously from the membrane (3) or MinD detaches whereas MinE remains on the membrane (4). There it can rebind to another MinD protein (5) or detach (6).

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membrane, to cytoplasmic crowding in the region of the nucleoid, or to the possible formation of MinD clusters on the membrane. We expect these effects to be of minor importance compared to the processes we consider [44].

To study the patterns resulting from these processes theoretically, we employed two different approaches. On one hand we used a mean-field approach that leads to a system of partial differential equations. On the other hand we used a particle based stochastic model. In this model, each dimer is represented by a particle that moves randomly in space and the processes mentioned above occur stochastically. The corresponding reaction schemes are

\[
\text{MinD}_{\text{cyt}} \xrightarrow{\epsilon_D} \text{MinD}_{\text{mem}}
\]

\[
\text{MinD}_{\text{cyt}} + \text{MinD}_{\text{mem}} \xrightarrow{\epsilon_D} 2 \text{MinD}_{\text{mem}}
\]

\[
\text{MinE}_{\text{cyt}} + \text{MinD}_{\text{mem}} \xrightarrow{\epsilon_E} \text{MinDE}
\]

\[
\text{MinDE} \xrightarrow{\epsilon_{DE}} \text{MinD}_{\text{cyt}} + \text{MinE}_{\text{cyt}}
\]

\[
\text{MinDE} \xrightarrow{\epsilon_{D,mem}} \text{MinD}_{\text{cyt}} + \text{MinE}_{\text{mem}}
\]

\[
\text{MinE}_{\text{mem}} + \text{MinD}_{\text{mem}} \xrightarrow{\epsilon_{cD}} \text{MinDE}
\]

\[
\text{MinE}_{\text{mem}} \xrightarrow{\epsilon_E} \text{MinE}_{\text{cyt}}.
\]

Furthermore, we include the fact that the density of membrane-bound MinD is limited such that the rate of MinD attachment to some membrane area is proportional to the number of free binding sites in that area.

In the mean-field approach the state of the system is given by densities for the various protein states. The volume densities \(c_D\) and \(c_E\) denote the cytosolic concentrations of MinD dimers and MinE dimers, respectively. The surface densities of membrane-bound MinD, MinE, and MinDE complexes are denoted by \(c_{de}\), \(c_e\), and \(c_{de}\), respectively. The time evolution of these densities is governed by the following dynamic equations

\[
\dot{c}_D = D_D \Delta c_D + \omega_D c_D c_e - \omega_{de} c_{de} - \omega_e c_e
\]

\[
\dot{c}_E = D_E \Delta c_E + \omega_E c_E c_d + \omega_{de} c_{de} - \omega_e c_e - \omega_{cD} c_d c_e
\]

\[
\dot{c}_{de} = D_{de} \Delta c_{de} + \omega_{cD} c_d c_e + \omega_{de} c_{de} - \omega_{de} c_{de}
\]

\[
\dot{c}_e = D_e \Delta c_e + \omega_{de} c_{de} - \omega_{cD} c_d c_e
\]

The densities \(c_d\), \(c_e\), and \(c_{de}\) are defined only on the surfaces representing the membrane. In Equations (10)–(12), \(\Delta\) denotes the Laplace-operator on the surface and \(D_D\), \(D_E\), and \(D_{de}\) are the respective diffusion constants of membrane-bound MinD, MinE, and MinDE. Furthermore, \(c_{\text{max}}\) is the maximal MinD density on the membrane. In Equations (10) and (11), the densities \(c_D\) and \(c_E\) are evaluated at the same points as the surface densities. In Equations (8) and (9), \(\Delta\) denotes the Laplace-operator in three dimensions and \(D_D\) and \(D_E\) are the diffusion constants for cytosolic MinD and MinE, respectively. The dynamic equations for cytosolic MinD and MinE are complemented by boundary conditions on the diffusion currents that account for protein binding to and detachment from the membrane: The components of these currents orthogonal to the membrane equal the net attachment rate. Formally, we have

\[
- D_D \nabla L c_D = \epsilon_D (\omega_D + \omega_{D,mem} c_D)
\]

\[
(\epsilon_{\text{max}} - c_d - c_{de})/\epsilon_{\text{max}} - (\omega_{de,mem} + \omega_{de} c_{de})
\]

\[
- D_E \nabla L c_E = \omega_E c_E c_d - \omega_e c_e - \omega_{de} c_{de}.
\]

Here, \(\nabla L\) denotes the outward gradient normal to the boundary. Note, that these equations conserve the total protein number.

**Min-protein patterns in cellular geometries**

We first studied the behavior of Min protein patterns in cellular geometries. To this end, we solved the stochastic and deterministic dynamic equations in a cylindrical domain with hemispherical caps. The parameters used in this section are given in Table 1. The values of the cytosolic diffusion constants have been measured in Ref. [45]. While there is no direct measurement of the diffusion constants for membrane-bound MinD, MinE, and MinDE, diffusion on membranes is usually two to three orders of magnitude smaller than in the bulk [46]. For larger values of these constants, the resulting patterns are broader and less well defined. Decreasing their values does not affect the patterns significantly. To determine the value of the maximal density of membrane-bound proteins, we use that close packing of MinD on the membrane would yield a density of about 1/lateral extension of a MinD dimer), with the latter being approximately \(4 \times 10^8 \mu m^{-2}\). To account for crowding of the membrane by other molecules we use a value roughly 10 times smaller, \(c_{\text{max}} = 5.4 \times 10^8 \mu m^{-2}\). The values of the various attachment and detachment rates have been chosen to match the experimentally observed patterns. Note, that for the parameter values given in Table 1, the dominant path for MinE-induced MinD detachment involves MinE staying on the membrane. This corresponds to a high MinE processivity. Finally, we mostly considered the Min patterns in geometries of fixed size. Even under optimal growth conditions, \(E. coli\) gains only about 100 nm per oscillation period. As we show below, the patterns are robust against such changes.

**Pole-to-pole oscillations - Standing waves.** The pole-to-pole oscillations described in the Introduction are physiologically the most important patterns formed by the Min proteins. In Figure 3A and Movie S1, we show that for total protein concentrations similar to those in wild type \(E. coli\) and for a cell length of 3.8 \mu m, our dynamic equations reproduce this pattern. The oscillation period is about 50s, comparable to experimental.
values. The pattern does not change qualitatively as long as the system length $L$ obeys $L \leq 5 \mu m$. In agreement with previous work [47,48], stochastic simulations of the processes described in Eqs. (1)–(7) show, that molecular noise does not destroy this pattern.

If the cell length is increased beyond 5\mu m, then the pattern changes. In this case, the Min proteins still form a standing wave, but the number of nodes is larger than one, see Figure 3B and Movies S2, S3. This result agrees with the experimentally observed Min-protein patterns in long cells. The appearance of multiple nodes has its origin in the characteristic length scale of the Min-protein patterns that is also evident from the in vitro patterns reported in Ref. [10], which we discuss below.

In Figure 3C and D, we present the oscillation period as a function of the total MinE concentration $C_E$ and of the system length, respectively. It decreases approximately linearly with increasing $C_E$, reflecting the increasing activity of MinE removing MinD from the membrane. The dependence on cell length is non-monotonic. Overall, the dependence of the period on the system length is less pronounced than its dependence on $C_E$. Combining the data from Figure 3C, D we conclude that the oscillation period is not a robust feature of the Min system. This conclusion is in line with experimental measurements of the oscillation period as a function of cell length in vivo, which showed significant differences between different cells [4,19].

**Traveling waves.** Changes in the self-organized Min-protein pattern can also be induced by changing the total MinD and/or MinE concentrations. As shown in Figure 4 and Movie S4, for a total concentrations of $C_D=2.2 \times 10^3 \mu m^{-3}$ and $C_E=1.5 \times 10^4 \mu m^{-3}$ compared to $C_D=1.4 \times 10^4 \mu m^{-3}$ and $C_E=9.7 \times 10^3 \mu m^{-3}$ used above, we find traveling waves in cells of 4.8\mu m length. In these states, the Min proteins assemble at one cell pole and then travel along the membrane towards the opposite pole. Then, the proteins detach from the membrane and move through the cytoplasm back towards the original pole where they assemble again on the membrane and restart the process. In longer systems, the traveling wave breaks up into packets moving in the same direction reflecting the wave length inherent to the dynamic system. As expected on the ground of the system’s symmetries, we occasionally observed in the stochastic simulations a change in the direction of motion of the traveling waves, see Figure 4A.

Table 1. Parameter values used for the numerical solutions of the deterministic dynamic equations (8)–(14) and for the simulations of the stochastic dynamics (1)–(7).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>in vivo</th>
<th>in vitro</th>
<th>growing cell (1d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$D_P$</td>
<td>$1.4 \times 10^{-4} \mu m^2/s$</td>
<td>$5 \times 10^{-4} \mu m^2/s$</td>
<td>$1.4 \times 10^{-4} \mu m^2/s$</td>
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<tr>
<td>$D_E$</td>
<td>$1.4 \times 10^{-4} \mu m^2/s$</td>
<td>$5 \times 10^{-4} \mu m^2/s$</td>
<td>$1.4 \times 10^{-4} \mu m^2/s$</td>
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<tr>
<td>$D_0$</td>
<td>$0.06 \mu m^2/s$</td>
<td>$0.3 \mu m^2/s$</td>
<td>$0.06 \mu m^2/s$</td>
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<tr>
<td>$D_r$</td>
<td>$0.3 \mu m^2/s$</td>
<td>$1.8 \mu m^2/s$</td>
<td>$0.3 \mu m^2/s$</td>
</tr>
<tr>
<td>$D_c$</td>
<td>$0.3 \mu m^2/s$</td>
<td>$1.8 \mu m^2/s$</td>
<td>$0.3 \mu m^2/s$</td>
</tr>
<tr>
<td>$D_v$</td>
<td>$0.3 \mu m^2/s$</td>
<td>$1.8 \mu m^2/s$</td>
<td>$0.3 \mu m^2/s$</td>
</tr>
<tr>
<td>$c_{max}$</td>
<td>$2.7 \times 10^{-4} \mu m$</td>
<td>$2.7 \times 10^{-4} \mu m$</td>
<td>$2.7 \times 10^{-4} \mu m$</td>
</tr>
<tr>
<td>$\omega_P$</td>
<td>$0.1 \pi \mu m/s$</td>
<td>$5 \times 10^{-4} \pi \mu m/s$</td>
<td>$0.1 \pi \mu m/s$</td>
</tr>
<tr>
<td>$\omega_D$</td>
<td>$8.8 \times 10^{-3} \pi \mu m/s$</td>
<td>$3.18 \times 10^{-3} \pi \mu m/s$</td>
<td>$8.8 \times 10^{-3} \pi \mu m/s$</td>
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<tr>
<td>$\omega_E$</td>
<td>$1.5 \times 10^{-4} \pi \mu m/s$</td>
<td>$3.16 \times 10^{-4} \pi \mu m/s$</td>
<td>$6.96 \times 10^{-4} \pi \mu m/s$</td>
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<tr>
<td>$\omega_D,E$</td>
<td>$0.139 \pi \mu m/s$</td>
<td>$4.9 \times 10^{-5} \pi \mu m/s$</td>
<td>$0.139 \pi \mu m/s$</td>
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<tr>
<td>$\omega_{D,E}$</td>
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<td>$0.16 \pi \mu m/s$</td>
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<tr>
<td>$\omega_{D,CE}$</td>
<td>$1.5 \pi \mu m/s$</td>
<td>$2.52 \pi \mu m/s$</td>
<td>$1.5 \pi \mu m/s$</td>
</tr>
<tr>
<td>$\omega_{E}$</td>
<td>$0.5 \pi \mu m/s$</td>
<td>$0.5 \pi \mu m/s$</td>
<td>$0.5 \pi \mu m/s$</td>
</tr>
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For the cellular geometries, the ‘cell’ diameter was 0.8\mu m. The total MinD and MinE concentrations, $C_D$ and $C_E$, and the system length varied between simulations and are given in the corresponding figure captions. 

doi:10.1371/journal.pcbi.1003347.t001
Figure 3. Standing wave patterns from simulations in a bacterial geometry. A) Pole-to-pole oscillations for a system of length 3.8 μm. B) Standing wave with two nodes for a system of length 7 μm. The diameter is 0.8 μm in both cases. Top rows: Distributions of membrane-bound MinD for the deterministic system; middle: same for the stochastic system; bottom: corresponding kymographs. C) Deterministic oscillation period as function of the total MinE concentration with $C_D \sim 1.15 \cdot 10^3 \text{μm}^{-3}$. D) Deterministic period as function of the system length with $C_D = 1.4 \cdot 10^3 \text{μm}^{-3}$, $C_E = 9.7 \cdot 10^2 \text{μm}^{-3}$. The dashed vertical line indicates the length at which the pattern changed from pole-to-pole oscillations to a standing wave with two nodes. doi:10.1371/journal.pcbi.1003347.g003

Figure 4. Traveling Min-protein waves in bacterial geometries. A) Traveling wave solutions to the deterministic (top) and stochastic (middle) dynamic system for total protein concentrations of $C_D \sim 2.2 \cdot 10^3 \text{μm}^{-3}$ and $C_E \sim 1.5 \cdot 10^3 \text{μm}^{-3}$. Bottom: corresponding kymographs. B) Distribution of MinD-GFP in a living cell of length 6 μm. C) Kymograph of the MinD distribution in a simulated growing one-dimensional cell. The total protein concentrations are $C_D = 8.4 \cdot 10^2 \text{μm}^{-3}$ and $C_E = 5.8 \cdot 10^2 \text{μm}^{-3}$. D) Distribution of MinD-GFP in a growing cell. doi:10.1371/journal.pcbi.1003347.g004
lations are suppressed [33]. Instead small spots of membrane-bound MinD form stochastically on the cytoplasmic membrane. Furthermore, our analysis of the mean-field equations (8)–(12) had shown the existence of mirror-symmetric stationary states in short cells. In a stochastic system one might expect that the proteins switch stochastically between these two states. Indeed, there is a critical cell length below which the Min proteins do not oscillate, but switch stochastically between the two cell poles in case MinD and MinE are overexpressed [7,8], see Figure 6B and Movie S7.

As in experiments, the switching time is very short compared to the time the proteins spend in one cell half. In Figure 6C, we present the distribution of the corresponding residence times. The distribution decays algebraically with a slope of $-2.06 \pm 0.27$. This value is very similar to the experimental value of $-2.1$. In Figure 6D, we show the dependence of the mean residence time on cell length. Two regimes can be distinguished. For system lengths between 1.6μm and 2.4μm the mean residence time decays exponentially with a characteristic length of 1.45μm. It then turns sharply into an exponential dependence with a characteristic length of 3.0μm. Before the transition, the standard deviations of the distributions of residence times are comparable to the respective mean values. After the transition the standard

Figure 5. Phase diagram. Min protein patterns in cellular geometry with length 4.8μm for varying total MinD and MinE concentrations (A) and for varying total MinD concentration and length with $C_E/C_D = 0.69$ (B). Symbols represent pole-to-pole oscillations (red triangles), traveling waves (green circles), standing waves with two nodes (light blue squares), spatially heterogeneous steady states (yellow pentagons), and standing waves with three (dark blue diamonds) and four nodes (purple triangles). Parameters see Table 1.

doi:10.1371/journal.pcbi.1003347.g005

Figure 6. Stationary patterns and stochastic switching. A) Pattern for $C_D = 2.2 \times 10^3 \mu m^{-3}$ and $C_E = 1.5 \times 10^3 \mu m^{-3}$ in a system of length 1.8μm obtained from the deterministic (top) and the stochastic system (middle). Bottom: corresponding kymographs. B) Distribution of MinD-GFP in a living cell of length 2.1μm. C) Distribution of residence times in the stochastic switching regime from simulations of 12000s in systems of 1.6μm, 1.8μm, 2.0μm, 2.2μm and 2.4μm length. D) Average residence time (blue dots) and standard deviation of the corresponding distributions (open squares) obtained from simulations as a function of cell length. Lines represent exponential functions with characteristic lengths of 1.45μm and 3.0μm, respectively.

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deviation decreases more rapidly than the average residence time, indicating an increasing regularity of the pole-to-pole oscillations. This is qualitatively similar to observations in vivo [7]. The characteristic lengths agree within a factor of three with the experimental values.

**Min patterns in aberrantly thick cells.** All patterns in the bacterial geometry discussed so far were invariant under rotations with respect to the system’s long axis. One might expect that the Min-protein patterns will break this symmetry if the cell diameter is sufficiently large. It is possible to increase the cell diameter by Min-protein patterns will break this symmetry if the cell diameter is of motion. This is different for the stochastic solution, see Figure 7C, where the spot frequently changes direction after passing a cell pole.

**Min protein patterns in open geometries**

A major breakthrough in the understanding of Min-protein pattern formation has been achieved by studying the Min-dynamics in open geometries [10,22,26,54]. Experimentally, in vitro studies using supported lipid bilayers have allowed us to clearly establish the propensity of the Min proteins to self-organize [10]. Structural analysis suggested that binding to the membrane can also occur for MinE not associated with MinD [23], providing a natural explanation for guiding Min-protein waves on structured surfaces [26].

In Figure 8 we present the result of a numeric solution of the dynamic equations (8)–(12), where we have employed periodic boundary conditions in the x- and y-directions. Parameter values are given in Table 1. The differences between these values and those used for the in vivo geometries reflect differences in the environmental conditions, notably the presence respectively absence of other macromolecules. Similar to the experimental observations, the Min proteins self-organize into traveling waves. The calculated wave profile presents the same features as in the experiment: the MinD profile increases at the wave front and then saturates until it sharply drops. The density of MinE increases more slowly than that of MinD. Towards the wave’s trailing edge it exhibits a sharp increase and then drops rapidly. The parameter $c_{	ext{max}}$ is increased in comparison to the value determined in the section ‘Min-protein patterns in cellular geometries’. The presentation of the distribution’s z-dependence in Figure 8C shows that the pattern is confined to a layer of about 15μm above the membrane. This result justifies a posteriori the use of effective 2d descriptions for the Min-protein dynamics [10,26] even though it is not obvious how to formally obtain the 2d equations from the 3d system.

**Intuitive picture of Min-protein patterns**

The propagation of the wave fronts can be understood by interpreting the space coordinate in Figure 8B as time: First cytosolic MinD binds to the empty membrane. The nonlinearity in the MinD binding term then leads to an increased binding rate and thus to an accelerated increase of the MinD density on the membrane. As soon as membrane-bound MinD is present, MinE starts to attach. As the MinE binding sites are abundant, the increase of the MinE density is roughly linear. With increasing MinE density, the net rate of MinD attachment decreases. Eventually, the MinE-induced detachment rate exceeds the
attachment rate and the density of membrane-bound MinD decreases. This decrease is sharp at the waves trailing edge, because MinE processivity leads to an accumulation of MinE in this region.

The sequence of Min protein patterns in vivo upon changing the system length can be intuitively understood from the mechanism underlying traveling waves in vitro. To this end, we introduce the diffusion length $\ell_{\text{diff}}$, which is the length a molecule typically diffuses before attaching to the membrane. For a diffusion constant $D$ and an attachment rate $\omega$ it is given by $\ell_{\text{diff}} = D/\omega$. Now, consider a wave in a cell propagating in the direction of the long axis. The wave is sustained by molecules binding to the wave's leading edge after they have been released from the trailing edge. When the wave reaches a pole, the MinD dimers released from the membrane at the trailing edge can no longer bind at its leading edge. Instead, they diffuse away from the cell pole. If the cell length is on the order of $\ell_{\text{diff}}$, the proteins will preferentially bind at the opposite pole [55], see Figure 9A. Similarly, with some delay, MinE released from the original wave, will bind at this pole, too, and a new wave traveling in the same direction as the original one is generated.

If the system size is shorter, MinD binding will occur in a zone extending further from the new pole to the cell center because the ratio of diffusion length to the cell length has increased. As the affinity for MinE binding to MinD on the membrane is large, MinE will preferentially bind to the part of the MinD zone proximal to the cell center and the wave will move into the opposite direction compared to the original wave, see Figure 9B, thus giving rise to pole-to-pole oscillations. For even shorter cells, the distribution of cytosolic MinD and MinE is essentially homogenous as the diffusion lengths significantly exceeds the cell length. MinD and also MinE thus bind preferentially to zones of the highest MinD concentrations on the membrane and a stationary profile emerges, see Figure 9C.

The picture presented here is thus somewhat different from the mechanism underlying the pole-to-pole oscillations proposed in Ref. [15] as we discuss below. Let us finally note, that it is harder to get an intuitive picture of the dependence of the Min-protein patterns on the total protein concentration and we refrain here from discussing this topic further.

**Discussion**

In this work, we presented a computational study of self-organized pattern formation by MinD and MinE from E. coli. The equations, which notably account for membrane-binding of MinE, generate the patterns previously observed in living cells as well as Min protein waves on flat surfaces observed in reconstitution experiments. In addition, our analysis yielded two patterns that had not been reported before: In sufficiently long cells and for elevated protein levels, traveling waves emanating from one cell pole and propagating to the opposite pole should emerge. Secondiy, in aberrantly large cells, the rotational symmetry of the pattern should be lost and a moving spot should form instead. Both predictions were confirmed experimentally. We conclude that the membrane-binding of MinE is an essential molecular feature to comprehensively describe large-scale pattern formation of the Min proteins.

In vitro experiments on micropatterned membranes suggest an important role of MinE processivity for Min-protein pattern formation [26], but it remains to be seen whether this is the case in vivo. In fact, comparing our system to the one proposed by Huang et al. [15] shows that MinE processivity can at least in part be replaced by a high rate of MinE binding to membrane-bound MinD (they chose a rate orders of magnitude higher than we did). This leads to a different mechanism underlying the pole-to-pole oscillations and requires a finite MinD-ADP to MinD-ATP exchange rate for stabilizing standing waves with several nodes. It will be interesting to test experimentally which of the two possibilities is realized in living E. coli.

Our description neglects many molecular details. For example, we did not consider explicitly a MinD dimerization step or the finite exchange rate of ADP for ATP for cytosolic MinD. Also, different expressions accounting for the binding of cytosolic MinE to membrane-bound MinD might be used. We analyzed several different expressions describing the effect that a single MinE dimer can induce detachment of several MinD dimers from the membrane. While these modifications led to quantitative differences, their analysis also revealed that details of the corresponding expressions are rather unimportant for the overall behavior of the system.
As a consequence of the relatively simple reaction terms employed in our description, our model reveals some quantitative discrepancies compared to experimental observations. For example, the fluctuations present in the kymographs in Figure 3A and B are apparently larger than in the experimental kymographs in Figure 1. In addition, the wave profile shown in Figure 8 differs from the experimentally determined [22]. However, complete quantitative agreement likely requires knowledge of more molecular details of the reactions involved. Note, however, that a quantitative comparison on the single cell level also requires precise measurements of the corresponding amount of MinD and MinE, which are currently not available. On a coarser level, though, our description seems to match the topology of the phase space. That is, we present one set of parameters, that correctly reproduces the sequence of patterns as cells grow and also correctly describes the appearance of stochastic switching and traveling waves in living cells with increasing protein levels. In contrast, the exact transition points differ in general from those observed in experiment and any coincidence would be fortuitous. Let us also emphasize that, experiments are now very much needed to constrain possible parameter values. Only with such data we can expect to make further significant progress in understanding Min protein patterns.

In agreement with previous work, our analysis also showed that molecular noise has only a minor effect on the Min-protein patterns. Macroscopic signatures of molecular noise were only found under special conditions, namely, in short cells presenting stochastic switching and in large cells, where the Min proteins formed a rotating patch with a stochastically switching sense of rotation. Our description of the Min-protein dynamics can now be used to design new experiments, for example, to test the interplay between the Min oscillations and Z-ring assembly in vivo or to determine conditions to generate Min-protein patterns inside vesicles in vitro. Such experiments could present important steps on the way to synthesize a system that is able to divide autonomously, that is, a minimal synthetic cell.
Materials and Methods

Experiments

We used cells of the *E. coli* strain JS964 containing the plasmid pAM238 encoding for MinE and GFP-MinD under the control of the lac-Promoter [5]. Bacteria were grown overnight in a 3ml LB medium at 37°C. Cells were induced with Isopropyl-β-D-thiogalactopyranosid (IPTG) at a concentration of 200μM and incubated for 3-4 hours prior to measurements. During 1-2 hours prior to measurement, cells were kept at 30°C for better fluorescence. The optical density was less than 0.6. During measurements, cells were in the exponential growth phase. The samples were kept at a temperature of 30°C using a Bachoffler chamber. To keep bacteria from moving under the cover slip, we put them on an agar pad (1% agar solution in LB medium with a reduced yeast extract fraction, 10%, in order to lower background fluorescence). The fluorescence recordings were taken with an Olympus FV 1000 confocal microscope, at an excitation wavelength of 488 nm from a helium laser at low power. We used an Olympus UPLSAPO 60×, NA 1.35 oil immersion objective and recorded a frame every 3s. A measurement lasted 40min. During this period, the focus was manually readjusted at irregular intervals. A22 (S-(3,4-Dichlorobenzyl)isothiourea, HCl) was purchased from Merck Millipore. Cells were imaged 2–3 hours after adding 10μg/ml of A22.

Numerical solutions of the dynamic equations

We solve the dynamic equations (8)-(14) in the *in vitro* as well as in the *in vivo* geometry by using Comsol Multiphysics 4.1 which is a solver for partial differential equations based on the finite element method (FEM). All computations with exception of those for Figure 4C were performed in 3d and no assumption was made about the symmetries of the solutions. For the calculations for the patterns in a bacterial geometry the maximal grid size was 1.5μm. For the calculations in the *in vitro* geometry, we used a maximal grid size of 2μm in the surface domain and of 15μm in the buffer domain. As initial condition we used homogeneous distributions of cytosolic proteins with a random perturbation of 5–10%. The initial surface densities were chosen to be zero for the *in vivo* geometries. For the *in vitro* geometry the surface densities were different from zero in a semi-annulus to rapidly induce a spiral.

The calculations for the growing cell presented in Figure 4C, the system length was increased by adding discrete pieces at one end of the interval. For the pattern shown in Figure 4, the rate of growth was $2 \times 10^{-7} \text{μm s}^{-1}$. On the added pieces, the protein densities of cytosolic MinD and MinE were initialized with the values $C_D = 8.4 \times 10^2 \text{μm}^{-3}$ and $C_E = 5.8 \times 10^2 \text{μm}^{-3}$, respectively, whereas the densities of membrane-bound proteins were initially set to zero.

To simulate the stochastic reaction diffusion kinetics (1)-(7) in three dimensions, we used MesoRD [36], a tool to solve the stochastic Master Equation using a reaction diffusion Master Equation. It is based on the Next Subvolume Method [57].

Supporting Information

Text S1 Lower dimensional versions of the dynamic equations and Min-protein pattern formation in growing cells. (PDF)

References


Video S1 (Theory) Pole-to-pole oscillation for a system of length 3.8 μm. The total protein concentrations are $C_D = 1.4 \times 10^3 \text{μm}^{-3}$ and $C_E = 9.7 \times 10^2 \text{μm}^{-3}$. (AVI)

Video S2 (Theory) Standing wave with two nodes for a system of length 7 μm. The total protein concentrations are $C_D = 1.4 \times 10^3 \text{μm}^{-3}$ and $C_E = 9.7 \times 10^2 \text{μm}^{-3}$. (AVI)

Video S3 (Theory) Standing waves with 3 and 4 nodes. The systems have a length of 10 μm and 13 μm. The total protein concentrations are $C_D = 1.4 \times 10^3 \text{μm}^{-3}$ and $C_E = 9.7 \times 10^2 \text{μm}^{-3}$. (AVI)

Video S4 (Theory) Traveling waves in a cell of 4.8 μm length. The total protein concentrations of $C_D = 2.2 \times 10^3 \text{μm}^{-3}$ and $C_E = 1.5 \times 10^3 \text{μm}^{-3}$. (AVI)

Video S5 (Experiment) Time-lapse fluorescence microscopy of MinD-GFP showing traveling waves in living *E. coli* cells. (AVI)

Video S6 (Theory) Traveling wave with two wave packets. The length of the cell is 12 μm and the total protein concentrations are $C_D = 2.2 \times 10^3 \text{μm}^{-3}$ and $C_E = 1.5 \times 10^3 \text{μm}^{-3}$. (AVI)

Video S7 (Theory) Stationary pattern and stochastic switching. The length of the system is 1.8 μm and the total protein concentrations are $C_D = 2.2 \times 10^3 \text{μm}^{-3}$ and $C_E = 1.5 \times 10^3 \text{μm}^{-3}$. (AVI)

Video S8 (Experiment) Time-lapse fluorescence microscopy of MinD-GFP showing traveling waves in a living *E. coli* cell after treatment with A22, see Materials and Methods. (AVI)

Video S9 (Theory) Min-protein pattern formation in a aberrantly large cell. The system has a length of 2.7 μm and a diameter of 2 μm. In the deterministic simulation, the total protein concentrations are $C_D = 9.9 \times 10^2 \text{μm}^{-3}$ and $C_E = 6.9 \times 10^2 \text{μm}^{-3}$ and in the stochastic simulations $C_D = 8.0 \times 10^2 \text{μm}^{-3}$ and $C_E = 5.5 \times 10^2 \text{μm}^{-3}$. (AVI)

Video S10 (Theory) Simulation of Min-protein pattern formation in the 3D *in vitro* geometry. (A) z-dependence of the cytosolic MinD and MinE densities. (B) Densities of membrane-bound MinD and MinE on a planar membrane with periodic boundary conditions. (AVI)

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Author Contributions

Conceived and designed the experiments: MB EFF ML PS KK. Performed the experiments: EFF. Analyzed the data: MB EFF ML PS KK. Wrote the paper: MB EFF ML PS KK. Implemented code for simulation: MB.

Min-Protein Pattern Formation


