Min-oscillations in *Escherichia coli* induced by interactions of membrane-bound proteins

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Abstract
During division it is of primary importance for a cell to correctly determine the site of cleavage. The bacterium *Escherichia coli* divides in the center, producing two daughter cells of equal size. Selection of the center as the correct division site is in part achieved by the Min-proteins. They oscillate between the two cell poles and thereby prevent division at these locations. Here, a phenomenological description of these oscillations is presented, where lateral interactions between proteins on the cell membrane play a key role. Solutions to the dynamic equations are compared to experimental findings. In particular, the temporal period of the oscillations is measured as a function of the cell length and found to be compatible with the theoretical prediction.

1. Introduction
During division, a cell has to specify in particular the location of cleavage. In the rod-shaped bacterium *Escherichia coli*, the division plane is determined by the location of the Z-ring [1]. This structure is built from FtsZ-filaments and forms on the inner bacterial membrane. In turn the position of the Z-ring is first of all determined by the distribution of the nuclear material inside the cell. A mechanism called ‘nucleoid occlusion’ restricts the formation of the ring to regions void of DNA [2, 3]. After duplication and segregation of the chromosome, three locations of possible ring formation remain: at the cell center and close to the two cell poles. Selection of the center as the correct division site is achieved by the Min-system [4, 5]. Deletion of any of the Min-proteins results in division septa forming close to one of the two cell poles in about 50% of all divisions. In these cases, DNA-free mini-cells are formed [6].

The Min-system consists of three proteins, MinC, MinD and MinE. Out of these, MinC induces the depolymerization of FtsZ-filaments and inhibits the formation of the Z-ring [7]. The distribution of MinC on the membrane changes periodically with time such that in one half of the cycle, MinC accumulates at one pole while it accumulates at the opposite pole in the second half of the cycle [8, 9]. Formation of the Z-ring is thereby suppressed at the cell poles. The temporal period of the oscillation ranges between 40 s and 120 s in wild-type cells. In bacteria of a length that exceeds a certain threshold, a striped oscillatory pattern appears, where the number of stripes increases with increasing cell length. This observation is indicative of an intrinsic spatial wavelength of the oscillations.

The oscillations of MinC require the presence of both MinD and MinE, which themselves also oscillate [10, 11]. In fact, MinC binds to MinD and follows its dynamics [10]. In contrast, MinE is mostly localized in a ring structure which oscillates around the center of the bacterium. Remarkably, MinC is not necessary to generate oscillations, as MinD and MinE also oscillate in the absence of MinC. The behavior of MinD and MinE has been characterized by intensive biochemical and genetic studies. *In vitro* experiments have shown that the ATPase MinD has a high affinity for the inner bacterial membrane if ATP is present [12]. For concentrations of MinD exceeding a critical value, filamentous MinD aggregates are formed on the membrane [12, 13]. The formation of MinD aggregates is likely to be a two-step process, where MinD first binds to the membrane and then self-assembles [12]. Indeed, in the presence of ATPγS, a non-hydrolysable analog of ATP, MinD associated with the membrane but failed to form filaments. As for MinE, it associates with the membrane only in the presence of MinD.
There it stimulates hydrolysis of the ATP bound to MinD, which eventually drives the proteins off the membrane [12].

These in vitro results are compatible with the behavior of MinD and MinE in vivo. In MinD depleted cells, it was observed that MinE is dispersed in the cytosol, while MinD is homogenously distributed on the cytoplasmic membrane if MinE is absent [10]. Furthermore, helical MinD aggregates have been observed to form on the inner membrane [14]. The significance of the helical structures for the oscillation mechanism is still not understood. Finally, the oscillations do not depend on the synthesis and degradation of the Min-proteins [10].

Theoretical investigations of the Min-system suggest that the periodic translocations of the Min-proteins can be attributed to a collective effect of many interacting molecules resulting from a dynamic instability [15–18]. Central to all proposed mechanisms is the attachment of MinD to the cytoplasmic membrane, recruitment of MinE to the membrane by MinD, and dissociation of MinD from the membrane induced by MinE. The mechanism proposed by Meinhardt and de Boer [15] belongs to the class of classical reaction–diffusion systems with short-range activation and long-range inhibition. The synthesis and degradation of the Min-proteins play an essential role. Howard and coworkers [16] assume that MinD and MinE form complexes in the cytoplasm, which then bind to the membrane. Membrane binding is hampered by MinE present on the membrane. Furthermore, the protein number is conserved. The same holds for the mechanism presented in [17]. There, however, first MinD binds to the membrane and then recruits MinE. More importantly, aggregation of membrane-bound MinD is essential. In contrast to reaction–diffusion systems, the instability here is driven by the aggregation current of MinD. Similar to this mechanism is the one proposed more recently by Huang et al [18]. In contrast to [17], aggregation there is assumed to be a consequence of MinD binding cooperatively to the membrane. This seemingly small difference in the formation of membrane-bound MinD aggregates has remarkable consequences. Firstly, it is essential to describe the Min-dynamics in a three-dimensional geometry to obtain striped oscillatory patterns in long cells. Secondly, a finite ADP to ATP exchange rate for cytosolic MinD is a key ingredient. As transport is purely diffusive, the instability leading to the oscillations is, in this case, essentially of the same kind as in the other reaction–diffusion systems [15, 16].

In this work, we re-investigate the mechanism proposed in [17]. There, the aggregation of membrane-bound MinD was formulated in terms of a kinetic hopping model. Here, we will use a phenomenological description, which allows a quantitative comparison with experimental results. The paper is organized as follows. First, we will describe the equations governing the dynamics of the protein distributions in the cytosol and on the membrane. We then analyze the system in the limiting case of homogenous cytosolic protein distributions and discuss the oscillatory solutions. The dependence of the temporal oscillation period on the system length is compared to experimental data. Afterwards, we discuss possible mechanisms underlying the formation of the MinE-ring. Finally, we discuss our results in relation to the other proposed mechanisms as well as the implications for possible future experiments.

2. Dynamic equations

As mentioned above, the periodic changes in the distributions of the Min-proteins require the presence of MinD and MinE, but not of MinC. Therefore, in the following we will focus on the dynamics of MinD and MinE. Motivated by the observations reported above, the dynamics of the Min-proteins is assumed to be driven by four properties of the Min-proteins [17]: (i) a high affinity of ATP-bound MinD for the membrane; (ii) a high affinity of MinE for membrane-bound MinD; (iii) a MinE-induced increase of the ATP hydrolysis rate by MinD, which leads to the detachment of MinDE-complexes from the membrane; and (iv) interactions between membrane-bound proteins. The last property accounts for the formation of MinD aggregates on the membrane, which is likely to result from self-assembly of membrane-bound MinD [12]. In addition, proteins are transported by diffusion. A schematic representation of the Min-dynamics is given in figure 1.

Formally, the dynamics is given in terms of the concentrations of cytosolic MinD and MinE, $c_D$ and $c_E$, as well as the concentrations of membrane-bound MinD and $c_{DE}$, which is actually the distribution of MinE dimers. In the following, the term 'MinE molecules' refers to these dimers.

\[ c_E \text{ forms dimers} [19] \text{ and } c_E \text{ is actually the distribution of MinE dimers} \]
MinDE-complexes, \( c_D \) and \( c_{de} \). In the direction perpendicular to the long axis of the bacterium, diffusion homogenizes the cytosolic distributions on timescales that are short as compared to the temporal oscillation period. Assuming in addition that MinD aggregates into a linear structure on the membrane, the dynamical equations for the protein densities in the cell can thus be reduced such that they depend only on the position \( x \) along the long axis of the bacterium, see appendix A.

Explicitly,
\[
\begin{align*}
\partial_t c_D &= -\omega_D(c_{\text{max}} - c_d - c_{de})c_D + \omega_{de}c_{de} + D_D \nabla^2 c_D \\
\partial_t c_E &= \omega_{de}c_d - \omega_E c_E + D_E \nabla^2 c_E \\
\partial_t c_{de} &= -\omega_{de}c_d + \omega_E c_E - \partial_x j_d \\
\partial_t c_d &= -\omega_D(c_{\text{max}} - c_d - c_{de}) - \omega_E c_d c_E - \partial_x j_d.
\end{align*}
\]

Properties (i)–(iii) lead to an exchange of MinD and MinE proteins on the membrane and the coefficients \( \omega_D \) and \( \omega_{de} \) are taken into account due to the presence of MinE. Note that for an attractive interaction between MinD and MinDE-complexes. The current of membrane-bound MinD has a diffusive part \( D_d \partial_x c_d + D_d \nabla^2 c_d \).

The boundary conditions have to be specified. We impose zero flux at the boundaries, such that the total protein numbers
\[
\int_{-L/2}^{L/2} dx (c_D + c_d + c_{de}) \equiv LD
\]
are conserved. Here, \( L \) denotes the length of the system and \( L D \) and \( L \) are the total numbers of MinD and MinE molecules in the system, respectively.

3. Homogenous cytosolic distributions

We now analyze the dynamic equations (1)–(4) in the limiting case of homogenous cytosolic MinD and MinE distributions, i.e., \( c_D(x, t) = c_D(t) \) and \( c_E(x, t) = c_E(t) \). This corresponds to the case where the times needed for MinD and MinE to diffuse along the whole length of the bacterium, \( L^2 / D_D \) and \( L^2 / D_E \), respectively, are short as compared to all other relevant timescales involved. In this case, the dynamics of the cytosolic distributions is described by ordinary differential equations
\[
\begin{align*}
\frac{d}{dt} c_D &= -\omega_D(c_{\text{max}} - c_D + c_{de}) + \omega_{de}(E - c_E) \\
\frac{d}{dt} c_E &= -\omega_E(D - E + c_D + c_{de}) + \omega_{de}(E - c_E).
\end{align*}
\]

Here, the distributions of membrane-bound MinD and MinDE have been eliminated using equations (6) and (7).

Under the conditions \( 0 \leq c_D \leq D \) and \( 0 \leq c_E \leq E \), the above equations have one and only one fixed point. This point is always stable and, asymptotically, the cytosolic distributions will approach the corresponding stationary values \( C_D \) and \( C_E \), respectively. In this limit, the dynamics of the Min-proteins is described by two partial differential equations for the distributions of the proteins bound to the membrane:
\[
\begin{align*}
\partial_t c_d &= \omega_D C_D(c_{\text{max}} - c_d - c_{de}) - \omega_E C_E c_d - \partial_x j_d \\
\partial_t c_{de} &= -\omega_{de}c_d + \omega_E C_E c_d.
\end{align*}
\]

Note that the reaction terms in these equations are linear and describe relaxation to a stationary value; only the current contains non-linearities and can generate an instability. This feature distinguishes this system from classical reaction–diffusion systems, where transport is due to diffusion and where instabilities are created by the reaction terms.

The homogenous state \( c_d(x) = D - E + C_D + C_E \) and \( c_{de}(x) = E - C_E \) is a stationary state of the dynamic equations (10) and (11). It is stable, unless \( k_1 \) exceeds a critical value \( k_{1c} \). The results of a linear stability analysis for a supercritical value of \( k_1 \) are shown in figure 2(a). The stability region of the homogenous state as a function of the total MinD and MinE concentrations, \( D \) and \( E \), is shown in figure 2(b). At the instability an inhomogeneous stationary state appears if the detachment rate of MinDE-complexes from the membrane is above a certain critical value, \( \omega_{de} > \omega_{de,c} \). In the opposite case, an oscillatory state appears. Oscillatory instabilities only occur if the protein density on the membrane cannot exceed a maximal value \( c_{\text{max}} \). For an oscillatory instability the unstable mode is of the form
\[
\begin{align*}
c_d &\propto \cos(\Omega_1 t) \cos(q x) \\
c_{de} &\propto \cos(\Omega_2 t + \phi) \cos(q x).
\end{align*}
\]
This standing wave reflects the qualitative features of the observed Min-oscillations. The wave number \( q \) of the oscillatory patterns can be obtained from numerical integration of the dynamic equations (10) and (11). A typical example is shown in figures 3(a) and (b). For some time, the total MinD-distribution \( c_d + c_{de} \) is localized in one half and then switches to the other. In this process, the transition time is very short as compared to the dwell time in one half. The MinE distribution shows a similar behavior, but the transition between the two halves is less rapid. The time-averaged distribution of both, MinD and MinE, shows a minimum in the center and increases toward the system boundaries, see figure 3(c). The parameters have been chosen such that the temporal period is about 80 s, which is similar to the values observed in experiments with fluorescence-labeled MinD, see figure 4. The figure also displays the time-averaged MinD distribution with a minimum in the center. In the case displayed in figure 4(f), the minimum at the center is more pronounced than for the theoretical calculation: while experimentally the minimum is at about 50% of the maximum,
Min-oscillations in *E. coli*.  

0.1 0.2 0.3 0.4 0.5  
I(x)  
(a) 0s (b) 20s (c) 40s (d) 60s  
(e)  
(f) I  
0 0.1 0.2 0.3 0.4 0.5  
L  
Figure 4. Oscillations of MinD GFP in *E. coli*. (a)–(d) Fluorescence images of MinD GFP in a cell at subsequent time points separated by 20 s. (e) Time average of all frames during one oscillation period. Two subsequent frames are separated by 1 s. (f) Fluorescence intensity *I* obtained from a line scan of the fluorescence signal in (e). The background signal has been subtracted from the total signal which has then been rescaled with the maximum intensity during the oscillation. The slight asymmetry is due to bleaching during the observation period. Scale bar: 1 µm. The cell length is *L* = 2.3 µm.  

In the model, the transition of MinD from one half to the other can be understood as follows. If MinD is localized in one half, MinE will bind and drive MinD off the membrane. Although the distribution of cytosolic MinD is homogenous, MinD preferentially binds in the other half, because there are more available binding sites. The resulting inhomogeneity of membrane-bound MinD is then amplified by MinD aggregation. As a consequence of the homogenous distribution of cytosolic MinE, the spatial dependence of the attachment rate of MinE follows the profile of membrane-bound MinD, and the distribution of MinDE-complexes is similar to the one of MinD on the membrane, see figures 3(a) and (b). In particular, the positions of the maxima of *c*<sub>de</sub> are linked to the position of the maxima of *c*<sub>d</sub>. In the example given in figures 3(a) and (b), maxima are always located at the boundaries *x* = 0 and *x* = *L*.

As the system size is increased, the patterns change and striped patterns for *c*<sub>d</sub> and *c*<sub>de</sub> appear, see figure 3(d). This reflects the finite wave number of the critical mode. In addition to changes in the oscillation pattern, the temporal period, also changes as the system size is varied. It increases monotonically with the system size, but at certain sizes jumps back toward a lower value, see figure 5(a). The discontinuities occur for the system sizes where the oscillatory pattern acquires a new ‘stripe’. For the parameter values used here, a second stripe appears for a system size of 3 µm.

We measured the temporal period of the oscillations in *E. coli* containing MinD GFP, figure 5(b) (see appendix B). The periods fall in the range of 50 s to 120 s, even for bacteria of 10 µm in length. The data indicate large variations of the oscillation period for cells of approximately the same length. This might be due to differences in the MinD and MinE concentrations for different bacteria and thus reflect individuality of the cells. An experimental verification would require the measurement of the protein concentration in an individual cell together with the temporal period of the Min-oscillations. The oscillation periods found for the dynamic equations (10) and (11) span the same range as the experimentally observed. Furthermore, experimentally we observed striped oscillation patterns only for bacteria longer than 3 µm, however, there is no sharp transition length for which the pattern changes. This behavior could also be due to variations in the protein densities between different bacteria.

In the model, the temporal oscillation period also depends on the total MinD and MinE concentrations, *D* and *E*, see figures 5(c) and (d). It increases monotonically with the amount of MinD until it starts to descend slightly. As a function of the number of MinE molecules, the period decreases. Both dependences are compatible with experimental observations where the period has been found it is at about 70% in the numerics. This might indicate the need for further non-linearities in the theory. However, for other cells examined, the minimum is much shallower or even absent (data not shown). This might reflect deviations in the total protein density in individual bacteria from the average total protein density in a bacterial colony. Note also that in the numerics, the value of the minimum decreases with the system length up to the point the oscillation pattern acquires a new stripe. It would be interesting to experimentally test this dependence of the average MinD distribution on the cell length. Due to fast bleaching of the GFP we were not able to perform this experiment.

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to increase with the MinD concentration and to decrease with the MinE concentration [10], but only few data points have been reported and further measurements are necessary.

In conclusion, the solutions to the dynamic equations presented here are compatible with the experimental data, but further experiments are necessary in order to verify the discontinuous dependence of the oscillation period on the system length.

4. The MinE-ring

Early experiments indicated an accumulation of MinE close to the cell center [20]. This accumulation is commonly referred to as the MinE-ring. More recently, using deconvolution techniques, MinE is found to be arranged in a helix with accumulation close to the cell center and, although weaker, at the cell poles [14]. It has been suggested that the helical arrangement of MinE is induced by the helical arrangement of MinD and that the accumulation of MinE occurs at the ends of the MinD helix [14]. In cells mutant for MinE, oscillations have been observed in the absence of a MinE-ring [21]. In that case, the temporal period is larger than in non-mutant cells. Still, this experiment clearly shows that the MinE-ring is not necessary for the oscillations.

In the one-dimensional description presented above, MinE-rings correspond to maxima in the MinDE distribution. In the examples given so far, such maxima only occur at the system boundaries. For system lengths close to the value at which the pattern acquires a new stripe, maxima can be detected closer to the system’s center. However, this is unlikely to be the mechanism for MinE-ring formation in E. coli, because no dependence of the existence of the ring on the cell size has been reported. Furthermore, as argued above, in the limit of homogenous cytosolic MinD and MinE distributions, maxima in the MinDE distribution are induced by maxima in the MinD distribution. MinD-rings are not observed experimentally, though.

There are at least three other possible mechanisms that can in principle account for the observed accumulation of MinE at the ends of the MinD helix. In the first mechanism, the diffusion length of cytosolic MinE, \( l_E \), is shorter than half of the cell length. In this case, cytosolic MinE will predominantly attach before it has reached the opposite cell pole, which might lead to an accumulation close to the cell center. This seems to be the mechanism of MinE-ring formation in the models proposed in [16, 18]. Accordingly, the ring vanished in [18] when the attachment rate of MinE was reduced, leading to an increase of \( l_E \). To test whether this mechanism is supported by the equations (1–4), we studied the system for finite values of \( D_D \) and \( D_E \). In this case, cytosolic distributions \( c_D \) and \( c_E \) are not homogenous and all four equations have to be solved simultaneously. For the parameter values considered above, the oscillation patterns do not change significantly as long as the diffusion constants \( D_D \) and \( D_E \) are larger than 0.1 \( \mu m^2/s \) and no maxima of \( c_D \), independent of maxima of \( c_E \) were found. The diffusion length \( l_E \) is also influenced by the value of \( \omega_E \). For \( D_D = D_E = 2.5\mu m^2/s \), the values expected for diffusion in the cytosol, and values of \( \omega_E \) smaller than 3.2 \( \times 10^{-4}\mu m/s \) the same behavior was found. Still larger values of \( \omega_E \) destroy the oscillations. Note that by assumption the one-dimensional description is only appropriate if the diffusion length \( l_E \) is larger than the cell diameter, i.e., \( l_E \geq 1 \mu m \). We conclude that this mechanism for the formation of the MinE-ring is not
supported by the dynamic equations presented above and can be tested only in a three-dimensional description.

Two other mechanisms of MinE-ring formation are suggested by studies of kinesin-subfamily Kin13 members [22, 23]. These proteins induce the depolymerization of microtubules. In this process they accumulate at both ends of the microtubule. As MinE might act on MinD filaments in much the same way, accumulation of MinE could follow from a similar mechanism as accumulation of the Kin13-kinesins. The latter could be a consequence of a higher affinity of the microtubule end for binding the motor. Related ideas for the binding of MinE to MinD have been proposed in [15] and also in [17]. The analogy with Kin13-kinesins offers still another explanation for the accumulation of MinE, namely dynamic accumulation due to processive depolymerization [24]. The present framework for studying the dynamics of Min-proteins is not suited for studying these effects, as filaments are not explicitly incorporated. Work in this direction is in progress.

5. Conclusion and outlook

We have presented a phenomenological description of the dynamics of MinD and MinE in E. coli. The description is based on the binding of MinD to the cytosolic membrane, recruitment of MinE to the membrane by membrane-bound MinD, MinE-induced detachment of MinD, as well as an interaction between molecules bound to the membrane. For a sufficiently strong attraction between membrane-bound MinD molecules, these processes generate pole-to-pole oscillations of the Min-proteins. The phenomenological form of the current for membrane-bound MinD used in the present work captures generic features of the protein interaction and does not refer to a specific microscopic mechanism. It allows for a quantitative comparison between the oscillatory solutions of the dynamic equations and experimental findings. In agreement with the latter, oscillations with a temporal period from 40 s to 120 s can be obtained. This value is essentially determined by the detachment rate \( \omega_{DE} \) from 40 s to 120 s can be obtained. This value is essentially agreement with the latter, oscillations with a temporal period of the dynamic equations and experimental findings. In

\[ k_2 = \frac{1}{c_{max}^2} \frac{D_d}{k_BT} Ur^2 \]

(17)

and analogously for \( k_1 \) and \( k_2 \). Here, \( U \) measures the strength of the MinD interaction potential, \( r \) is a typical length scale of the interaction, and \( k_BT \) is thermal energy. These relations are valid whenever \( r \) is much smaller than the diffusion length \( l_d = \sqrt{D_d/\omega_{DE} c_{max}} \). Assuming, as done above, a diffusion constant of 0.06 \( \mu m^2 s^{-1} \) for membrane-bound MinD, which falls well in the regime of measured diffusion constants for membrane proteins [26], the values of the phenomenological coefficients used above imply values of 35\( k_BT \) for the interaction strength between membrane-bound MinD and 20\( k_BT \) between MinD and MinDE-complexes. The range for MinD–MinD interactions is then 350 nm and for MinD–MinDE interactions 10 nm. While all other values are acceptable, the range for MinD–MinD interactions is too large for pure electrostatic interactions. This points to more involved microscopic dynamics of membrane-bound MinD than discussed here.

Our analysis of the dynamic equations (1)–(4) has focused on the case of homogenous cytosolic distributions of MinD and MinE, \( c_D \) and \( c_E \). Solutions in this limit are very similar to solutions to the full equations if the diffusion constants of both MinD and MinE have the realistic value of 2.5 \( \mu m^2 s^{-1} \). This implies that the approximation of constant \( c_D \) and \( c_E \) is appropriate. Apart from providing a reduced set of equations that is more convenient to study than the four equations of the full system, this approximation might also have an important implication regarding experiments. One might expect that oscillations should be observable in a purified system containing essentially only MinD, MinE and phospholipid vesicles. The analysis presented here suggests that oscillations will show up in the presence of a homogenous distribution of cytosolic proteins. Therefore, the closed geometry of the bacterium might not be essential and an open geometry could be used instead. A second implication of our analysis is that the number of available binding sites might need to be limited in order to produce oscillations.

Other mechanisms that have been suggested for the Min-oscillations agree in the essential assumptions with the one studied here, namely the ability of ATP-dependent binding of MinD to the membrane, the recruitment of MinE to the membrane by MinD, and the release of MinD from the membrane driven by MinE. The proposed mechanisms differ, however, in essential points. Meinhardt and de Boer suggested that protein synthesis might be an essential element [15], which is not supported by experiments where the synthesis of proteins was interrupted and the oscillations still continued [16]. Howard et al assume that MinD and MinE form complexes in the cytosol and bind together to the membrane. This implies in particular an exponential increase of the temporal period of the oscillations with the system length, with a period of 1000 s for a system of length 7 \( \mu m \). This is qualitatively different from the behavior reported for the mechanism studied in this work, see figure 5(a). The experimental data presented in figure 5(b) show oscillation periods that do not exceed 120 s for bacteria of a length up to 10 \( \mu m \). However, more experiments are needed, in particular
to obtain simultaneously values for the protein densities and
the oscillation period of individual bacteria.

The system studied by Huang et al differs from that
studied here in the way MinD aggregates are formed on the
membrane [18]. There MinD aggregation follows a one-step
process: attachment to the membrane occurs with a higher rate
at locations where MinD is already bound. In contrast,
we considered a two-step process, namely, cytosolic MinD
binds first to the membrane and only then self-assembles
into a filament. This difference might at first sight seem
minor. However, it leads to striking differences in the model
behaviors. First of all, assuming a one-step process for
MinD aggregation, a three-dimensional geometry and a finite
ATP exchange rate are required to generate striped oscillation
patterns in long systems. Secondly, in the model by Huang
et al there are no oscillatory solutions for homogenous
cytosolic distributions. Furthermore, as discussed above, in
the model studied here, MinE-rings have not been found
form to by the mechanism underlying the formation of the
MinE-ring in the model by Huang et al. The differences in
the mechanism for MinD aggregation thus lead to striking
consequences for the collective behavior of the Min-proteins.
One possibility of discriminating between the two mechanisms
is by studying the dynamics of Min-proteins that are not
confined to a cell. Further analysis of the models might lead
to other possible key experiments.

In combination, all proposed mechanisms underlying the
Min-oscillations suggest new experiments that will allow us
to understand the Min-oscillations better. In order to make
closer contact with experiments, the formation of MinD helices
must be included. Fluctuations due to the moderate number of
Min-molecules might play an important role. First attempts in
studying the influence of fluctuations on the oscillations have
been undertaken [27, 28], but further work is needed and will
probably yield results of relevance for pattern formation in the
presence of noise beyond the Min-system.

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Appendix A. Dynamics in three dimensions and
reduction to one dimension

In this appendix, it is shown how the dynamics in three
spatial dimensions can effectively be reduced to a description
in one spatial dimension. The bacterium is conveniently
approximated by a cylinder with radius $R_0$ and length $L$. The
volume densities of cytosolic MinD and MinE at a given point
are $c_D(\rho, \theta, x)$ and $c_E(\rho, \theta, x)$, respectively. Here, $\rho$ and $\theta$
denote the radial and azimuthal coordinate, respectively, while
$x$ is the coordinate along the long axis. Their time evolution is
governed by

\[
\frac{\partial}{\partial \rho} c_D(\rho, \theta, x) = -\omega_D(c_{max} - c_D(\rho, \theta, x)) d_D(\rho, \theta, x) \\
\times \delta(\rho - R_0) + \omega_D c_{de}(\rho, \theta, x) \delta(\rho - R_0) + D_D \Delta_D c_D(\rho, \theta, x)
\]  
(A.1)

\[
\frac{\partial}{\partial \rho} c_E(\rho, \theta, x) = -\omega_E c_D(\rho, \theta, x) + \omega_{de} c_{de}(\rho, \theta, x) \delta(\rho - R_0) \\
+ \omega_{de} c_{de}(\rho, \theta, x) \delta(\rho - R_0) + D_E \Delta_E c_E(\rho, \theta, x).
\]  
(A.2)

Here, $c_d$ and $c_{de}$ are the surface densities of membrane-bound
MinD and MinDE-complexes, $\Delta_3$ is the three-dimensional
Laplace operator and the factors of $\delta(\rho - R_0)$ restrict
to and detachment from the cytoplasmic membrane to a region
adjacent to the cell wall.

Since the diffusion constant of cytosolic MinD and MinE
is of the order of 1 $\mu m^2 s^{-1}$, whereas the period of the
oscillations is about 1 min, it is reasonable to assume the
density of cytosolic MinD and MinE to be homogenous
perpendicular to the bacterial long axis. The volume densities
of cytosolic MinD and MinE can then be replaced by surface
densities $\bar{c}_D$ and $\bar{c}_E$ with

\[
c_D(\rho, \theta, x) = \frac{1}{R_0} \bar{c}_D(\theta, x)
\]  
(A.3)

\[
c_E(\rho, \theta, x) = \frac{1}{R_0} \bar{c}_E(\theta, x).
\]  
(A.4)

Then, the equations governing the evolution of the protein
densities read

\[
\frac{\partial}{\partial \theta} \bar{c}_D = -\frac{\omega_D}{R_0} (c_{max} - c_d - c_{de}) \bar{c}_D + \omega_{de} \bar{c}_{de} + D_D \Delta_3 \bar{c}_D
\]  
(A.5)

\[
\frac{\partial}{\partial \theta} \bar{c}_E = -\frac{\omega_E}{R_0} \bar{c}_d \bar{c}_E + \omega_{de} \bar{c}_{de} + D_E \Delta_3 \bar{c}_E
\]  
(A.6)

\[
\frac{\partial}{\partial \theta} c_d = \frac{\omega_D}{R_0} (c_{max} - c_d - c_{de}) c_d - \frac{\omega_{de}}{R_0} c_d \bar{c}_{de} - \nabla \cdot \bar{j}_d
\]  
(A.7)

\[
\frac{\partial}{\partial \theta} \bar{c}_{de} = \frac{\omega_E}{R_0} \bar{c}_d \bar{c}_{de} - \omega_{de} \bar{c}_{de}
\]  
(A.8)

where $\bar{j}_d$ is the aggregation current of MinD on the inner cell
membrane and $\Delta_3$ is the two-dimensional Laplace operator
on the cylinder surface.

It has been shown that MinD forms a filamentous structure
on the inner cell membrane [14]. Projection on this structure
yields line densities, e.g., $\tilde{c}_d(\rho, \theta, x) \approx \int_0^\infty c_d(\rho, \theta, x) R_0 d\rho$. They
are connected to the surface densities via

\[
\tilde{c}_D(\rho, \theta, x) \approx \frac{1}{2\pi R_0} \bar{c}_D(\theta, x)
\]  
(A.9)

\[
\tilde{c}_E(\rho, \theta, x) \approx \frac{1}{2\pi R_0} \bar{c}_E(\theta, x)
\]  
(A.10)

\[
c_d(\rho, \theta, x) \approx \tilde{c}_d(\theta, x) \delta(\theta - \theta(x))
\]  
(A.11)

\[
c_{de}(\rho, \theta, x) \approx \tilde{c}_{de}(\theta, x) \delta(\theta - \theta(x))
\]  
(A.12)

where $\theta(x)$ parametrizes the MinD helix on the inner cell
membrane. The dynamic equations for the line densities
$\tilde{c}_D, \tilde{c}_E, \tilde{c}_d$ and $\tilde{c}_{de}$ are then given by equations (1)–(4).
The current $\bar{j}_d$ appearing there is obtained by projection
of the surface current $\bar{j}_d$ on the x-direction. Note that a
description of the formation of MinD helices would also
require a specification of the perpendicular component of the
current $\bar{j}_d$. 

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Appendix B. Methods

Bacteria of the E. coli K12 strain JS964 were generously donated by J Lutkenhaus, University of Kansas. Bacteria taken from the freezer were grown overnight in 3 ml Luria–Bertani (LB) medium at 37 °C together with 50 µl spectinomycin. Of the overnight culture 500 µl together with 50 µl spectinomycin were given in 50 ml LB medium and grown for 2 h at 37 °C. Expression of MinD GFP was induced by 50 µl IPTG and growing the bacteria at 31 °C for at least 1 h. Bacteria were immobilized for fluorescence imagery by using silane-coated cover slips. Fluorescent images were taken at room temperature with an inverted microscope (Axiovert 200M, Zeiss) using a CCD camera from Spot Diagnostic Instruments, Inc. driven by Metavue, Universal Imaging. The frame rate for measuring the time average in figure 4 was 1 Hz and varied between 0.33 Hz and 1 Hz for the data in figure 5(b). Data were analyzed using Metamorph, Universal Imaging.

Glossary

**Mini-cell.** DNA-free small cell that is produced by E. coli dividing close to a cell pole.

**Min-proteins.** Proteins involved in the determination of the division site. Mutations in these proteins lead to the formation of mini-cells.

**Linear stability analysis.** In a linear stability analysis, the stability of a stationary state against small perturbations is assessed by linearizing the dynamic equations with respect to the stationary state.

**Reaction–diffusion system.** Several reacting substances that are transported in space through diffusion. The reactions can induce instabilities of a stationary homogenous distribution leading to the formation of spatiotemporal patterns.

**Kinetic hopping model.** Particles are confined to the sites of a lattice. Motion of the particles is described by hopping between sites.

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