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Symmetry and scale orient Min protein patterns in shaped bacterial sculptures

Fabai Wu, Bas G.C. van Schie, Juan E. Keymer[†], and Cees Dekker^{*}

Delft University of Technology, Department of Bionanoscience, Kavli Institute of Nanoscience, Lorentzweg 1, 2628CJ Delft, the Netherlands

Abstract

The boundary of a cell defines the shape and scale for its subcellular organisation. However, the effects of the cell's spatial boundaries as well as the geometry sensing and scale adaptation of intracellular molecular networks remain largely unexplored. Here, we show that living bacterial cells can be 'sculpted' into defined shapes, such as squares and rectangles, which are used to explore the spatial adaptation of Min proteins that oscillate pole-to-pole in rod-shape *Escherichia coli* to assist cell division. In a wide geometric parameter space, ranging from 2x1x1 to 11x6x1 μm^3 , Min proteins exhibit versatile oscillation patterns, sustaining rotational, longitudinal, diagonal, stripe, and even transversal modes. These patterns are found to directly capture the symmetry and scale of the cell boundary, and the Min concentration gradients scale in adaptation to the cell size within a characteristic length range of 3–6 μm . Numerical simulations reveal that local microscopic Turing kinetics of Min proteins can yield global symmetry selection, gradient scaling, and an adaptive range, when and only when facilitated by the three-dimensional confinement of cell boundary. These findings cannot be explained by previous geometry-sensing models based on the longest distance, membrane area or curvature, and reveal that spatial boundaries can facilitate simple molecular interactions to result in far more versatile functions than previously understood.

Introduction

Pattern formation is central to the wealth of nature's intrigue. In search for its origin, Alan Turing theoretically showed that simple reaction and diffusion of two chemicals is adequate to spontaneously form reproducible spatial patterns¹. Reaction-diffusion theory subsequently inspired broad applications in physics and chemistry, and bridged molecular interactions to morphogenesis and cellular organisation in biology^{1–8}. Reaction-diffusion systems are, however, little understood in the context of a boundary geometry, which is of

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^{*}Correspondence should be addressed to Cees Dekker (c.dekker@tudelft.nl).

[†]Present address: IEB & Department of Ecology, School of Biological Sciences, P. Catholic University, Santiago, Chile

Author Contributions F.W., J.E.K., and C.D. conceived the experiments, discussed the work and wrote the paper, F.W. and B.v.S. performed the experiments, F.W. analysed the data, derived the mechanisms, and carried out computer simulations.

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particular importance in embryos and cells, where spatial patterns such as protein gradients are established within their envelope to dictate the locations for fundamental cellular processes such as cell division^{5,9–11}. In this study, we demonstrate how reaction-diffusion patterns of Min proteins can sense and adapt to morphological features of bacterial cells – an ability that is essential for the spatial regulation for cell division and its robustness against variations in cell size and shape^{12–14}.

Min proteins in many bacteria such as rod-shape *E. coli* exhibit a fascinating phenomenon: they oscillate pole-to-pole along the cell length to result in a time-averaged concentration minimum of division inhibitors at the mid-plane^{10,11} (Fig. 1b). This oscillatory dynamics is believed to autonomously arise from MinD proteins' ability to cooperatively bind to cytoplasmic membrane, and MinE proteins' ability to unbind MinD from the membrane^{15,16} (Fig. 1a), which respectively exemplify the self-activation and inhibition kinetics in reaction-diffusion theory^{3,4,7}. The self-organising nature of dynamic Min patterns has been elegantly demonstrated by travelling waves and oscillations reconstituted *in vitro*^{17–21}, albeit exhibiting a ~10 fold larger length scale compared to Min oscillations *in vivo* due to factors remaining unclear.

Various spatial cues have been hypothesised to account for the consistent pole-to-pole orientation of the Min oscillations^{7,18,19,22–24}, including the longest travel distance through the cytosol²² or along the membrane¹⁹, a characteristic polar membrane area¹⁸, and a high membrane curvature at the poles²⁴ (Fig. 1c). Furthermore, it is unclear how Min gradients quantitatively adapt to cell size variability and growth. Here, we identify the rules of geometry sensing and scale adaptation by systematically studying the dynamic behavior of Min proteins in live cells within spatial boundaries across a broad geometric parameter space.

Bacterial cells can be sculpted into defined shapes

Bacteria maintain their cell shape by a well-regulated process of peptidoglycan insertion into the cell wall²⁵. Previous studies have shown that curved agarose micro-chambers can alter the growth of filamentous cells of *E. coli* such that they adopt a certain degree of curvature²⁶, and that sub-micron constraints imposed by silicon slits can induce *E. coli* to grow into a variety of squeezed shapes²⁷. These methods were applied to elucidate interesting mechanisms of growth and division in bacteria^{13,28}. The diversity and reproducibility of cell shapes in these methods are however limited as it employs the wild-type pathways for cell wall synthesis. Spheroplasts generated by lysozyme, on the other hand, appeared to abolish dynamic activities of proteins such as Min oscillations²⁴.

For this study, we developed a new 'cell-sculpting' method to shape living bacterial cells into user-defined geometries (Fig. 1d), which allows a quantitative and systematic study on the effect of shape and scale. Cells were inoculated into nanofabricated polydimethylsiloxane (PDMS) chambers with various lateral shapes and a fixed depth of $1.15 \pm 0.05 \mu\text{m}$ (Supplementary Fig. 1). The 10- μm -thick layer of PDMS was supported by a thin cover glass for microscopy, and the microchambers with cells were covered by a 5% agarose pad supplemented with growth medium, A22, and cephalixin. A22 inhibits rod-

shape maintenance by antagonizing the dynamics of bacterial actin MreB that regulates peptidoglycan insertion patterns; cephalaxin prevents cell wall constriction that would otherwise lead to cell division. Under these growth conditions, *E. coli* cells exhibited a surprising plasticity, growing into cell volumes more than 20 times larger than a regular cell. During several hours of growth, cells gradually adapted their shapes to the chambers with defined lateral geometries (circles, squares, rectangles, and triangles) (Fig. 1d, Supplementary Movie 1). These cells generally maintained the defined shape for 10-20 minutes (during which time we captured the Min oscillations) before they grew out of the chamber (Supplementary Fig. 2). For statistical analysis, many tens of cells (6 – 118 cells, see Supplementary Table 1) were selected for each geometric parameter; our total dataset comprised 2268 qualified cells.

Min oscillations in diverse shapes

To visualise Min protein dynamics, we inserted a green fluorescent protein fusion gene (*sfGFP-minD*) at the endogenous *minD* genomic locus (see Supplementary Methods, Supplementary Table 2 and 3). This is the first-reported endogenous fusion that rescues the MinD deletion phenotype and retains the wild-type MinD protein level, growth rate, and cell morphology (Supplementary Fig. 3, Supplementary Fig. 4, and Supplementary Movie 2). The total fluorescence intensity of sfGFP-MinD scales linearly with cell size, indicating that the MinD concentration is roughly constant during growth (Supplementary Fig. 5). Dynamic localisations of the MinD proteins in cells typically were recorded with an interval of 5 seconds for a period of 2 minutes, and presented as the standard deviation of fluorescence intensity over time calculated per pixel (Fig. 1e).

Remarkably, Min proteins are found to sustain oscillation patterns in a wide range of cell shapes and sizes (Fig. 1e, Supplementary Movie 3). These include shapes that deviate strongly from the wild-type rod shape and which possess multiple symmetry axes and highly curved regions, such as rectangles, squares, circles, and equilateral triangles. As shown in Fig. 1e, Min oscillations are found to align to the longitudinal axis in a $2 \times 4.5\text{-}\mu\text{m}^2$ rectangle, whereas by contrast, in a square of $3\text{ }\mu\text{m}$, they can occur between diagonal corners. In a circle with a diameter of $3.4\text{ }\mu\text{m}$, MinD proteins toggle between opposing ends of its diameter, while in an equilateral triangle (side lengths $4.5\text{ }\mu\text{m}$), they oscillate between one corner and its opposite side. These patterns reveal that the Min proteins preferably oscillate along one of the symmetry axes (see also Supplementary Fig. 6 for a longer time scale).

Symmetry and scale orient Min protein patterns

When exploring the same cell shape but different lateral sizes, Min proteins can display qualitatively different dynamic patterns (Fig. 2). As the size (side lengths) of squares increases from $2\text{ }\mu\text{m}$ to as large as $7.5\text{ }\mu\text{m}$, Min patterns undergo transitions from rotational motions along the lateral periphery, to diagonal pole-to-pole oscillations, to side-to-side oscillations, and further to 3-node stripe patterns along the diagonals (Fig. 2a-b, Supplementary Movie 4). At the transition points, the major angles of oscillations switch steeply between near 0° and near 45° (Fig. 2c), reflecting a strong tendency of the oscillations to align to the symmetry axes of the square shape. Note that the observed variety

of patterns cannot be explained by the longest-distance or high-curvature rules that were proposed to explain Min patterns.^{19,22,24}

The side-to-side oscillation mode dominates the pattern distribution in the sampled size range (Fig. 2b). Its occurrence as a function of cell size resembles a normal distribution, with a mean pole-to-pole distance of 4.6 μm , and a full-width-at-half-maximum value of 3.2 μm , spanning a large range of 3.0 – 6.2 μm (see yellow bars in Fig. 2b). Rotational motions occur predominantly in cells around 2 μm , where all cell dimensions are below 3 μm , indicating that the lower bound of the characteristic range (3 μm) approximates the smallest length scale for Min proteins to sustain robust oscillatory dynamics²⁹. Diagonal pole-to-pole oscillations are shown most frequently in cells around 2.5 μm , i.e. with diagonal lengths of 3–4 μm . The stripe patterns start to emerge as the cells' diagonals exceed 6 μm , and dominate when the cell widths exceed 6.25 μm , the upper bound of the characteristic range (Supplementary Fig. 7). All these observations indicate that the axis choice in square-shape cells is dictated by a characteristic length range of 3 – 6 μm .

The temporal scale of the Min oscillations is surprisingly constant, with a period (68 ± 13 seconds, mean $\pm$ standard deviation, same below) and a polar residence time (45 ± 11 seconds) that are rather insensitive to a large increase in cell size or difference in mode of oscillations (Fig. 2d, Supplementary Fig. 8, and Supplementary Fig. 9). This demonstrates that the spatial properties of the Min patterns can be regulated independent of the temporal properties.

Versatile adaptation, transversal mode, and heterogeneity

To explore the effects of symmetry selection and the characteristic length range on the robustness and versatility of the Min patterns, we sampled the Min patterns in rectangular cells from $2 \times 1 \times 1$ to $11 \times 6 \times 1 \mu\text{m}^3$ (Fig. 3a, Supplementary Fig. 10, Supplementary Movie 5 - 7). Seven modes of Min patterns emerged in this geometric parameter space (Fig. 3b), including a striking transversal mode that finds the *shortest* lateral distance in cells with widths of 3.5 – 6.5 μm , and 'zigzag' patterns that exploits an even longer path than the cell's diagonal. As can be seen from the phase diagram (Fig. 3c-d), the longitudinal modes appear to be the majority pattern, with transversal modes dominating in the wider cells. Rotational, diagonal and zigzag patterns occur at the interfaces between the spatial regimes for the major patterns, suggesting that they are less robust states that take place at the transitions between major patterns (Fig. 3c). For cells below 2.5 μm in length, Min proteins mostly exhibit stochastic fluctuations rather than stable oscillations, similar to a previous report²⁹ (Supplementary Fig. 11).

The accuracy of symmetry alignment and the consistency of axis choice are determined by the cell dimensions and the number of symmetry axes of a given cell shape, which can be illustrated from comparing the Min patterns in squares and rectangles (Fig. 3a, Fig. 3c-e, and Fig. 4a). Whereas a square has 4 symmetry axes, a slight increase in aspect ratio (AR) deforms it to a rectangle, immediately removing the diagonals as symmetry axes and leaving only one long axis and one short axis. Indeed, a small increase in AR from 1 drastically decreases the Min oscillation angle to approach the longitudinal symmetry axis with a decay

constant of 0.13 (Fig. 3b, data for cells 2.25-3.25 μm wide). The increase of AR dramatically enhances the consistency of oscillation angle, from $37^\circ \pm 27^\circ$ for $\text{AR} < 1.05$, reflecting the occurrence of both longitudinal and diagonal patterns, to $1.7^\circ \pm 2.7^\circ$ for $\text{AR} = 2.00 \pm 0.25$ with exclusive longitudinal oscillations. Such a consistent alignment to the long axis is, however, jeopardised when the short axis (cell width) increases to sizes above 3.5 μm , where the transversal mode emerges to coexist with the longitudinal modes (Fig. 3b-f). The requirement of a symmetry axis for stable Min oscillations also explains our previous observation that strongly nonsymmetric cells yielded fluctuating Min patterns¹³.

We evaluated the heterogeneity of Min patterns by scoring the Shannon entropy (a diversity index measure)³⁰ of oscillation modes for each cell size (Fig. 3e). Coexistence of patterns is not only apparent for the squares but also evident at large cell dimensions. For example, amongst all 2x9 μm cell, 15 cells (48%) oscillate pole-to-pole, whereas 16 cells (52%) show a 3-node stripe mode. In cells longer than 10 μm , the pole-to-pole mode vanishes and 4-node stripes start to emerge (Supplementary Movie 8). Also, despite the observation that the transversal mode dominates for the larger cell widths, it is not the exclusive pattern observed in any of these cell sizes. These are interesting multi-stability phenomena that are yet to be explored by pattern-formation theories.

Gradient scaling, adaptive range, and temporal stability

The longitudinal pole-to-pole oscillations are the majority mode in rectangular cells with a length of 3–8 μm and a width below 4 μm (Fig. 3d). Despite the significant variation in cell length, the standard-deviation and time-averaged intensity profiles for these cells consistently indicate a high polar residence of MinD and minima that are located at mid-cell with an average deviation of only 2.6% relative to the cell length (Fig. 4b, Supplementary Fig. 12). This is consistent with the remarkable accuracy with which wild-type *E. coli* divide at the middle of a cell with variable cell lengths^{13,31}.

Cells of 3-6 μm in length exhibit standard-deviation MinD profiles and time-averaged MinD profiles that scale surprisingly well with cell length (Fig. 4b, Supplementary Fig. 12), which is not expected from a scenario where a fixed Min wavelength would determine the gradient. MinD-binding zones are observed to always initiate at the extremities of the longitudinal axes in these cells. When cells grow longer, MinD proteins often first establish binding zones away from the poles, resulting in broad plateaus at the two ends of the standard-deviation intensity profiles that no longer scale with length. The establishment of MinD binding zones away from the cell poles can eventually lead to the formation of a middle stripe, as observed in the stripe patterns (Supplementary Fig. 13). Indeed, transitions from pole-to-pole into stripe mode can occur throughout cell lengths between 7 and 11 μm (Fig. 3e), which likely is subject to stochasticity³².

In 2 μm wide cells with a length spanning a large range of 3 - 12 μm , the oscillation period and polar residence time of Min proteins are found to be about constant, with only a small degree of variation (Fig. 4c). Similar to square shapes, these temporal scales are not affected by the number of nodes in the oscillations (Fig. 4c, Supplementary Movie 8). With a stabilised temperature of $27.0 \pm 0.3^\circ\text{C}$, the standard deviation of the period is typically

about 5 seconds (Fig. 4c). Note that the insensitivity of the temporal scale to cell length that we quantified here *in vivo* is qualitatively different from the constant velocity of the *in vitro* Turing waves that are unconfined¹⁷. The fact that the period of *in vivo* Min oscillations does not scale with cell size can be understood from the fact that the establishment of MinD polar zone is not constrained by diffusion, but by MinE sequestration^{4,7} (Supplementary Fig. 13c). While a larger MinD polar zone attracts more MinD due to cooperative membrane binding, it simultaneously enhances MinE binding and thus increases the tendency to ‘push’ MinD to the other pole.

What underlies the symmetry alignment and adaptive scaling of the Min protein patterns? These features have not been examined in previous Turing-type models which generally exhibit a fixed wavelength^{1–3,6,17,19}. To achieve symmetry alignment, spatial confinement seems required, as *in vitro* Min surface waves travel along the diagonals of rectangular lipid patches,¹⁹ apparently insensitive to the symmetry axes (Fig. 4d, middle panel). Interestingly, recent *in vitro* work on Min proteins in partly confined microwells also showed transversal oscillations²⁰, although with a different length scale and with different pattern dynamics compared to the *in vivo* behaviour here. The adaptive range could potentially be assisted by extra chemical or mechanical cues^{18,24}, but such scenarios do not fit our data quantitatively (see SI discussion, and Supplementary Fig. 14). Mechanisms proposed for pattern scaling in developing embryos rely on production-degradation feedbacks, active transport of morphogen ligands by a polarised source of inhibitors (Fig. 4d, bottom panel), or the physiological state of cells in tissues, which do not apply to our intracellular scenario^{33–36}. With current explanations lacking, we thus ask whether symmetry alignment and adaptive scaling are features that are yet to be uncovered from the Min reaction-diffusion mechanism.

Simulations capture symmetry alignment and gradient scaling

Using numerical simulations, we now show that a kinetic parameter regime can be found for the Min reaction-diffusion system that leads to both symmetry alignment and gradient scaling, when and only when Min proteins are confined to a three-dimensional geometry (Fig. 5a-d). Strikingly, this allows all the major patterns observed in our experiments (Fig. 3a) to be stabilised over the full range of cell sizes in deterministic simulations using one fixed set of kinetic parameters (Fig. 5e and Supplementary Movie 9). The model also shows that the period of Min oscillations is rather insensitive to variations in cell length within the measured range (Supplementary Movie 9, Supplementary Fig. 15).

To probe for the above-highlighted geometry-sensing properties, we carried out a parameter screening of all reaction rates within a model containing a minimal set of self-activation/inhibition interactions between Min proteins³² (shown in Fig. 1a). This model uses the rate at which membrane-bound MinD proteins recruit cytosolic MinD to constrain the strength of polar-zone binding, a finite ADP-ATP nucleotide exchange rate of MinD to delay reattachment⁴, and sequestering of the polar MinD by MinE that allows the detached MinD to escape and reattach to the membrane at a distance^{7,37}. Parameter constraints for this model that led to robust oscillatory behaviour were previously determined by Halatek and Frey using two-dimensional elliptic geometries⁷.

To explore the microscopic origin of the symmetry selection, we carried out simulations starting from an asymmetric MinD polar zone (Fig. 5a, Supplementary Movie 10). We find that the orientation of the polar zone development is most importantly affected by the MinD self-recruitment rate (k_{dD}). With a sufficiently high k_{dD} , an initial diagonal MinD gradient (e.g. in a $3 \times 2 \times 1\text{-}\mu\text{m}^3$ rectangle) is found to rapidly align to the longitudinal axis within a few pole-to-pole oscillation cycles (Fig. 5b, Supplementary Fig. 16, Supplementary Movie 11 and 12), whereas lowering k_{dD} renders a higher stability for diagonal, nonsymmetric oscillations in this rectangular shape (Supplementary Fig. 16). Importantly, symmetry selection is only achieved through oscillations in a three-dimensionally confined volume, thus explaining the lack of symmetry in unconfined two-dimensional surface waves¹⁹.

Gradient scaling of longitudinal oscillations in 3-6 μm long cells is determined by multiple kinetic parameters (including $k_{dD} = 0.05\text{ }\mu\text{m}^2/\text{s}$). Importantly, fitting the 3-6 μm scaling range simultaneously yields the transversal oscillations in cells wider than 3 μm and the sustainable longitudinal stripes in cells longer than 6 μm (Fig. 5c-d). The presence of a finite scaling range requires k_{dD} to be within a quite narrow window of 0.03-0.10 $\mu\text{m}^2/\text{s}$. For $k_{dD} < 0.03\text{ }\mu\text{m}^2/\text{s}$, polar plateaus do not appear in the SD gradients for cells of any lengths, while for $k_{dD} > 0.10\text{ }\mu\text{m}^2/\text{s}$, the gradients do not scale. A previous theoretical suggestion⁷ that transitions from pole-to-pole into stripes are not robust for k_{dD} below 0.10 $\mu\text{m}^2/\text{s}$ is in line with our experimental observation of a variable length (7-11 μm) where such transitions happen (Fig. 3f). Altogether, the analyses indicate that transitions between oscillation modes are intrinsically coupled to the scaling property of the concentration gradients. Our deterministic simulations can in future work be extended to analyse the robustness and heterogeneity of patterns against internal and external fluctuations^{32,38}, and to unravel the role of more detailed molecular interactions such as a conformational switch and membrane binding of MinE^{39,40}.

Conclusion

Taken together, the key mechanism revealed by our experiments is that Min proteins orient their oscillation patterns by sensing both the symmetry and scale of the cellular geometry. We find that Min proteins explore cellular space to select the symmetry axes in a given geometry, while the characteristic length range of 3-6 μm further restricts the choice among these axes (Fig. 4a, 4b, 4d). This range is defined by the lengths to which Min proteins can scale their concentration gradients. Notably, 3-6 μm coincides with the lengths of wild-type *E. coli* cells undergoing cytokinesis, indicating an evolved optimality of Min system in adaptation to the size of *E. coli*. As it is essential for the binary fission process to distribute the cellular material equally to its progeny, the sensing of both symmetry and scale by the Min proteins are indispensable for their role as a spatial regulator for cell division. The diverse patterns uncovered in our study provide valuable insight on how Min homologs adapt to bacteria with diverse morphologies and modes of division^{41,42}. For example, the transversal mode may well be orchestrated by Min homologs found in γ -proteobacterial symbionts that divide along their *long* axis⁴¹.

Our numerical simulations revealed that the symmetry selection and characteristic length range both derive from the self-activation/inhibition kinetics responsible for the oscillatory

dynamics of Min proteins in the three-dimensional confinement of a cell. This type of microscopic interactions was found to underlie a broad spectrum of Turing reaction-diffusion patterns, including intracellular polar protein networks and actin waves in eukaryotic cells^{5,43,44}, suggesting that the ability to sense global features of biological boundaries may be intrinsic to many spatial patterns in cells.

The bacterial cell sculpting reported here reveals that spatial boundaries can facilitate simple molecular interactions to result in far more versatile functions than previously understood. We expect that it further can be applied to unravel the fundamental mechanisms of intracellular spatial organisation, including protein/RNA/lipid localisation, cytoskeletal dynamics, and chromosome organisation⁴⁵.

Methods

Mask nanofabrication

A silicon wafer (Universitywafer.com) was spin-coated with resist NEB-22 (Sumitomo Chemical Co.)⁴⁶. Predesigned features were patterned through an electron-beam lithography system (Leica EBPG 5000+) with a beam step size of 20 nm. The exposed resist was removed by solvent Microposit MF322 solution (Rohm and Haas) and the exposed wafer surface was etched using an AMS Bosch Etcher. The remaining resist was removed through oxygen plasma.

PDMS microchamber patterning

The patterned silicon wafer is silanized by tridecafluoro-1,1,2,2-tetrahydrooctyltrichlorosilane (97%, ABCR GmbH & Co. KG), and used as a mold for creating microchambers. A 5- μ l mixture of Sylgard elastomer 184 (base to curing agent ratio 5:1) was applied onto the microstructures of the silicon surface and subsequently covered with a clean microscope coverglass (thickness 0.13-0.17 μ m) to spread the mixture throughout the area of the coverglass. The wafer attached with the coverglass was baked in an oven at 70°C for 3 hours till the solvent was evaporated, which resulted in a ~10 μ m thick layer of polydimethylsiloxane (PDMS) between the coverglass and silicon substrate. The coverglass with the patterned PDMS layer was subsequently recovered from the silicon wafer. For inspection under a scanning electron microscope (Phillips XL 30), the resulting silicon mold and PDMS structures were deposited with 10 nm gold using a Temescal deposition system.

Cell-sculpting technique

An overnight *E. coli* bacterial culture incubated at 30°C was back diluted to OD₆₀₀ = 0.02 in fresh M9 medium supplemented with 4 μ M A22 (Merk Chemicals Ltd.), and incubated for 3.5 hours at 30°C. 1 μ l of the bacterial culture was then pipetted onto the PDMS chambers on a coverglass that was clamped onto a custom-made baseplate. The droplet was then immediately covered by a 4.8% agarose pad supplemented with M9 broth, 0.4% glucose, 0.25% protein hydrolysate ampicase, 4 μ M A22 and 25 μ g/ml cephalixin (Sigma-Aldrich). A thin piece of tissue wetted with water was placed above the device to keep the humidity at a high level and a piece of stretched parafilm was enclosed the device to prevent

drying but allow air exchange. The base-plate was subsequently mounted onto the microscope stage and incubated at 26 °C during the course of the experiments.

Fluorescence imaging

Fluorescence imaging was carried out using Nikon Ti-E microscope with CFI Apo TIRF objective with an NA of 1.49. The microscope was enclosed by a custom-made chamber that was pre-heated overnight and kept at 26-27 °C. For excitation of sfGFP or NirFP signal, cells were illuminated by Nikon-Intensilight illumination lamp through a GFP filter cube ($\lambda_{\text{ex}} / \lambda_{\text{bs}} / \lambda_{\text{em}} = 450\text{-}490 / 495 / 500\text{-}550 \text{ nm}$) or a RFP filter cube ($\lambda_{\text{ex}} / \lambda_{\text{bs}} / \lambda_{\text{em}} = 540\text{-}580 / 585 / 592 - 668$). The fluorescence signal was recorded by an Andor iXon EMCCD camera. The MinD dynamics in this study was imaged with a frame time-interval of 5 seconds for 25 frames.

Bacterial strain construction and characterization

Bacterial strains were constructed via λ Red recombination⁴⁷ and P1 phage transduction. All primers, strains and plasmids used in this study are listed in Supplementary Table 2 and 3. Protein expression levels are characterized through western blot analysis using MinD and FtsZ antibodies. For more details please see Supplementary Information.

Image Analysis

Analysis of fluorescent microscopy images was carried out using Matlab. For more details please see Supplementary Information.

Shape selection and data binning

All rectangular and square-shape cells were selected for data analysis using the following criteria: 1) cell area above 90% of the smallest rectangle that contains the shape, and 2) diagonal lines having a length difference smaller than 5%. Cell widths and lengths were binned to 0.50 μm (for example, $4.00 \pm 0.25 \mu\text{m}$) for statistical analysis unless specified otherwise. Additional criteria for square shapes are: 1) an aspect ratio smaller than 1.05, and 2) a difference between measured length and width smaller than diffraction limit of around 0.25 μm . For statistics on square shapes, the denoted sizes are the average length of the sides. Larger cell sizes, i.e. with a width or length above 4.75 μm , are binned to 1.0 μm , as shown in the phase diagrams (Fig. 3d).

Note that due to the multi-fold symmetry of the square shape, all square-shape cells were rotated or reflected to show side-to-side oscillations horizontally, and diagonal oscillations no bigger than +45° with respect to the horizontal axis. Thus, for analyses in square shape, the oscillation angles are set to values between 0° and 45°. However, for consistency in comparison to the 7 modes of the oscillations in rectangular shapes (in Fig. 3), the side-to-side mode was grouped into longitudinal and transversal modes according to the quantified small length/width difference in square shapes. Hence, for example, in the case of $3.00 \pm 0.25 \mu\text{m}$ squares, whereas the fraction of side-to-side mode is higher than the fraction of the diagonal mode (Fig. 2b), the diagonal mode appears to occur more frequently than the individual fraction of either longitudinal mode or transversal mode (Fig. 3d).

Numerical simulations

The detailed set of interactions between MinD, MinE, ATP, and the cytoplasmic membrane, and the set of reaction-diffusion equations are listed in Supplementary Methods. The reaction kinetic parameters used to reproduce symmetry selection, gradient scaling, and all the major patterns are listed in Supplementary Table 4. The MinD/MinE concentration ratio and diffusion rates are set to be identical with the simulations in the Halatek-Frey model⁷, where the choices of these values are explained. Based on the set of interactions and kinetic parameters, a reaction diagram was constructed between a cytosolic compartment and the cytoplasmic membrane in the software VirtualCell⁴⁸. Three-dimensional compartments were constructed and latticed with a grid spacing of $dx = dy = dz = 0.08 \mu\text{m}$. The height of all objects was fixed to be $z = 1.00 \mu\text{m}$. For more details please see Supplementary Information.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Min proteins oscillate along the symmetry axes of *E. coli* shaped by our 'cell-sculpting' technique.

a, Schematic of interactions between Min proteins and the membrane, P: phosphate. ATP: Adenosine triphosphate. ADP: Adenosine diphosphate. **b**, From left to right: Fluorescence images of cytosolic eqFP670, sfGFP-MinD time series, time-averaged intensity and standard deviation (SD) of the sfGFP-MinD in rod shape *E. coli*. **c**, Previous geometry-sensing models from the given references. Green: cytosolic distance; red: membrane distance (line) and area (patch); d , distance; K , curvature. **d**, Left: schematic of cell-sculpting device composed of microscope coverglass (bottom), PDMS micro-chambers (middle), and an agarose pad supplemented with nutrient and drugs (top). Right: cytosolic NirFP fluorescence images of cells growing into defined shapes. **e**, Image sequence as in b, followed by illustrations of symmetry axes. Green arrows indicate cell poles defined by the Min patterns. Solid lines: long axes. Dashed lines: short axes.

Figure 2. A characteristic range dictates the Min oscillation axis choice.

a, SD patterns of sfGFP-MinD oscillations in cells with sizes as denoted. Insets at the bottom are time-lapse montages, and schematics of the modes of oscillations. **b**, Bar diagram illustrating the relative occurrence of each mode of oscillations as a function of cell size. **c**, Ball plot illustrating the dependence of oscillation angle on cell size. Angles are confined to the range 0-45° due to symmetry. Ball diameter represents the percentage of cells at a certain size. The rotational mode (magenta) is excluded in these percentage and plotted above, normalised against all cells showing pole-to-pole (blue) or stripe (red) patterns. **d**, The period (τ) and polar residence time (τ_p) of the Min oscillations as a function of cell size. Black data: pole-to-pole oscillations. Red data: stripe patterns. Grey lines: mean values for all data. Error bars denote SD. **b-d**, Sizes represent side lengths.

Figure 3. Robustness and variation of Min patterns in a wide geometric parameter space.
a, Representative Min SD patterns in rectangular cells. **b**, Time-lapse images of representative examples of the 7 oscillation modes. Each mode is presented with a colour box representing this mode in **b** and **c**, a time montage, and a schematic. **c**, Phase diagrams depicting the fraction of individual modes occurring in each rectangle size. Percentage denotes the fraction of cells with this mode, relative to the total number of cells at this rectangle size. White areas depict unsampled geometry. Lateral dimensions are indicated in microns and scaled as in **a**. **d**, Phase diagram showing the distribution of the 7 modes

mapping the patterns that exhibit maximum occupancy for each cell dimension. Colours represent different modes as indicated in b and c. **e**, Phase diagram showing the heterogeneity of oscillation patterns for each rectangle size. **f**, Bar plots presenting the fractions of rectangular cells for a given length or width that show the first 3 major modes, respectively.

Figure 4. Symmetry selection, gradient scaling, and adaptive range in Min patterns

a, Oscillation angle α versus aspect ratio in rectangular cells with widths of 2.25-3.5 μm .

Grey line shows the diagonal angles; red line is an exponential fit. **b**, sfGFP-MinD SD intensity profiles along the long axes of cells with lengths of 3-8.5 μm and widths of 2-3.25 μm . Red lines, mean profile; black error bars indicating the SD; green arrows, start of the polar plateau. All horizontal axes share the label as indicated on the left. Cell lengths are given at the bottom. Insert indicates the demands for robust longitudinal pole-to-pole oscillations. **c**, The period (τ) and polar residence time (τ_p) of the Min oscillations as a function of cell length in rectangular cells with widths of 2.25-2.75 μm . Black data: pole-to-pole oscillations. Red data: 3-node stripe patterns. Blue data: 3-node stripe patterns. Grey lines: mean values for all data. Error bars denote SD. **d**, Illustrations of patterns in a variety of rectangles for three different types of gradient formation. Red, high concentration; white, low concentration; black arrow, protein travel direction; yellow dots, inhibitor source.

Figure 5. Numerical simulations of a three-dimensional reaction-diffusion model explain the sensing of symmetry and scale by Min proteins.

a, Schematics showing the movement of MinD polar zones (contours denoted by red lines, direction by black arrows) over time for different values of k_{dD} (denoted at the bottom, unit $\mu\text{m}^2\text{s}^{-1}$) in $5 \times 3 \times 1\text{-}\mu\text{m}^3$ cells. **b**, An initially diagonal MinD gradient in a $3 \times 2 \times 1\text{-}\mu\text{m}^2$ cell develops into longitudinal pole-to-pole oscillations. **c**, SD profiles of MinD concentration in $2\text{-}\mu\text{m}$ -wide cells with various lengths (indicated at the top). Green arrow, start of polar plateau. **d**, Oscillation angle (indicating the longitudinal versus transversal oscillations) as a function of the cell width. **e**, SD images of the 2D projection of MinD concentrations from the 3D simulations at 480-600 seconds.