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Synthetic Biology

Reconstitution of Pole-to-Pole Oscillations of Min Proteins in Microengineered Polydimethylsiloxane Compartments**

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Cell division is a highly regulated process, and even "simple" organisms such as the bacterium *Escherichia coli* use complex mechanisms to control their temporal and spatial organization during cytokinesis. *E. coli* typically divides by binary fission, a process that is mediated by the divisome, a protein complex that assembles into a ringlike structure in the middle of the cell. The placement of the divisome is spatially regulated by the Min system, which consists of the three proteins MinC, MinD, and MinE. A particularly interesting feature of these proteins is their ability to self-organize and dynamically oscillate from pole to pole,[1–6] thereby generating, on time average, a non-uniform concentration profile of Min proteins with the highest concentration at the cell poles and the lowest concentration in the middle of the cell.[3,5–7] During the last three decades, the roles of MinC, MinD, and MinE in spatially regulating cell division in *E. coli* have been studied intensively. Experiments with deletion mutants and fluorescently labeled proteins revealed that MinE and MinD account for the mechanism of the dynamic pole-to-pole oscillations, while MinC is a division inhibitor which follows the movement of the other Min proteins by binding to MinD, and thereby prevents cell division near the cell poles.[2,4,8–10] The biochemical basis of the Min system was elucidated by in vitro studies with purified Min proteins.[11–14] It was shown that MinD is an ATPase and that MinD.ATP binds to membranes through an amphipathic helix.[12,15,16] The association of MinE with MinD activates the ATPase activity of MinD, thereby resulting in a dissociation of the Min proteins from the membrane.[13,14] Subsequently, MinD.ADP is converted into MinD.ATP, and in repeated circles of cooperative membrane attachment and detachment the Min proteins self-organize and form patterns.[3,5,6] Recently, Loose et al. demonstrated that purified Min proteins are capable of self-organizing in a synthetic environment on supported lipid membranes.[17] The Min proteins were found to form dynamic wavelike patterns along the lipid membranes, thus suggesting that the mechanism responsible for the protein waves in vitro is the same as for the Min oscillations in vivo.[17] But what are the mechanistic requirements for the appearance of regular, pace-making oscillations? Studies with aberrantly shaped *E. coli* cells as well as theoretical models indicated that cell shape plays a role in Min pattern formation.[18–22] To date, however, oscillations of Min proteins have not been reconstituted in minimal systems and, thus, a well-defined model system to study the relationship between compartment geometry and the ability of oscillations to act as a spatial cue for cell (i.e., compartment) division is still missing.

Here we show for the first time that Min protein oscillations can be generated in a synthetic system. We use a device composed of model membranes and micrometer-sized reaction compartments and demonstrate that Min oscillations occur in restricted sample volumes with bacteria-like shape. Furthermore, we investigate the influence of geometry on Min pattern formation in synthetic systems and compare our results to live cell studies that showed the influence of aberrantly shaped bacteria on the dynamics of Min proteins. Our findings demonstrate that pattern formation in round and filamentous cells can be reproduced in vitro, and provide further evidence that cell geometry plays a pivotal role in biological pattern formation and pace-making.

Min pattern formation in spherical and filamentous *E. coli* cells is modified compared to that in wild-type cells.[3,18,23] Therefore, we hypothesized that oscillatory Min patterns in *E. coli* require a specific size and shape of the bacterium, and that the same is true for an in vitro system. To test this hypothesis, we produced a biomimetic device with restricted reaction volumes of well-defined geometries, in which dynamic Min pattern formation could be investigated. To restrict the sample volume to micrometer-sized dimensions, we produced microfabricated polydimethylsiloxane (PDMS) chambers. Their dimensions were scaled, according to the approximately ten times longer Min wavelengths in vitro than in live bacteria. Resist micropatterns with rodlike shapes and 10 μm height were produced by standard photolithographic techniques on Si wafers, against which PDMS was molded. After curing the set-up at 80 °C for three hours, the PDMS (Figure 1a) was peeled off, which resulted in a solid PDMS layer containing microcavities that was utilized as the support for lipid membranes. *E. coli* lipid bilayers that adopt the topography of the underlying PDMS structures were produced by vesicle fusion, and the two-dimensional mobility of the membrane was verified by fluorescence recovery after photobleaching (FRAP) experiments (data not shown). The formation of self-organizing surface waves, similar to the patterns reported by Loose et al.,[17] were observed when purified MinD (1 μM), MinE (1 μM), and Alexa488-labeled MinE (0.1 μM) were added to the buffer reservoir on top of

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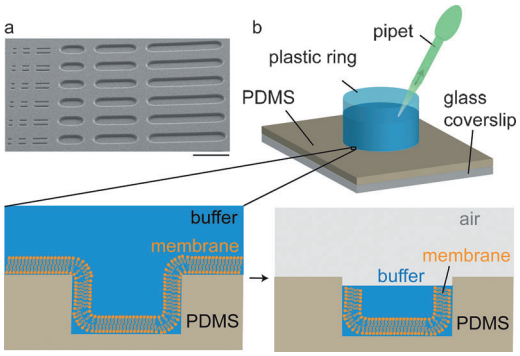

Figure 1. Experimental setup of a synthetic model system for Min protein oscillations. a) Electron micrograph of a microstructured PDMS membrane support. Scale bar: 100 μm b) Membrane bilayers were produced on PDMS microstructures by vesicle fusion techniques. MinD and MinE were added to the buffer and subsequently the buffer level was reduced below the upper rim of the microcavities. The image is not to scale.

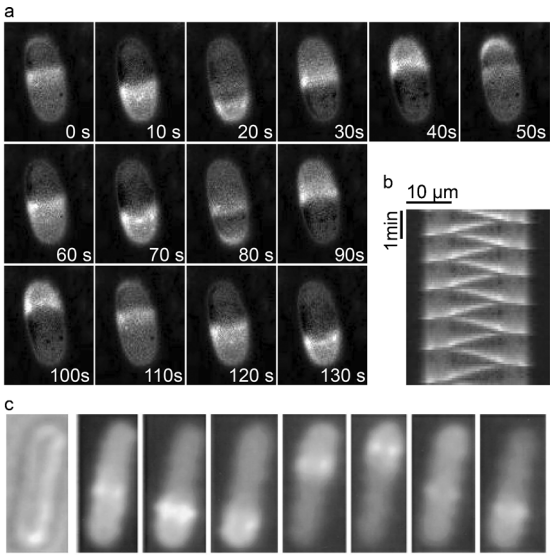

Figure 2. Synthetic Min protein oscillations are comparable to pattern formation in vivo. a) Confocal time-lapse images of purified Min proteins on a lipid membrane in a PDMS microcompartment. The PDMS structure is 10 μm wide, 25 μm long, and 10 μm high. 1 μM MinD, 1 μM MinE doped with 10% Alexa488-labeled MinE. Images were modified by subtracting the average intensity of time-lapse images, thereby removing nondynamic fluorescent background signals. b) Kymograph for MinE-Alexa488 along the long axis of the compartment in (a). c) Min protein oscillations in vivo. Images reproduced from Ref. [5] (Copyright 2001, The National Academy of Sciences, U.S.A).

the bilayer (see movie 1 in the Supporting Information). After formation of the Min protein waves, the buffer level was decreased to a level below the upper rim of the PDMS to produce picoliter sample volumes (Figure 1 b).

To investigate Min pattern formation in restricted volumes with well-defined geometry we observed the fluorescent signal from MinE-Alexa488 in rod-shaped microcavities of 10 μm height, 10 μm width, and 25 μm lengths by using confocal time-lapse microscopy. Strikingly, the confinement to small volumes and a rod-shaped geometry indeed led to regular oscillations of the fluorescent signal. In other words, MinE-Alexa488 rapidly oscillated between the two poles of the PDMS cavity in a manner strongly resembling Min oscillations in vivo (Figure 2). In repeating cycles, the Min proteins disassembled from the membrane when they reached one pole of the microcompartment, then reassembled near the middle of the compartment and moved towards the opposite pole. Oscillatory Min patterns were formed by repeating cycles of cooperative membrane association, wave propagation towards the pole, and membrane dissociation. To better compare the oscillations in vitro with oscillations in *E. coli* we determined the average oscillation period in the synthetic system. The oscillation period in *E. coli* is 0.5 to 2 min, which is well below its generation time of about 20 min under optimal growth conditions.[3,5,6] This short oscillation period allows the Min proteins to perform several oscillations between two successive cell divisions and thus enables the cell within its generation time to build a time-averaged protein concentration profile with the lowest protein concentration in the middle of the cell. In accordance with the literature values of 0.5 to 2 min for the oscillation period in *E. coli*, the average oscillation period of $n = 6$ synthetic samples was 70 s with a standard deviation of 13 s. Taking into account that Min proteins form surface waves before the sample volume is reduced and the proteins start to oscillate, our findings provide evidence that wavelike patterns indeed underlie the same mechanism as the oscillatory pattern, and that the size and geometry of the reaction volume is indeed constitutional for oscillations to occur. It should be noted that in contrast to a three-dimensional cell, the reaction volume is not completely enclosed by a membrane, but has the upper side exposed to air. Thus, although the lipid membrane and a restricted sample volume are required for the occurrence of Min oscillations, the encapsulation of Min proteins in closed membrane compartments is not absolutely necessary.

As mentioned above, the wavelength of Min patterns on flat membranes is typically an order of magnitude larger than in vivo (3.0–4.0 μm).[24] Thus, PDMS compartments significantly larger than bacterial cells could be used to observe the same patterns. Although the oscillations can now be controlled in vitro, it is still an open question as to how the size of Min patterns is determined exactly. The patterns were suggested to be influenced by viscosity,[17] but other regulating factors, such as increased biochemical competition for the Min proteins in the cellular environment, cannot be excluded. Currently, experimental evidence for a key parameter that modulates the size of Min patterns is still missing and should be addressed in future studies. Nevertheless, the possibility to reproduce Min protein patterns in larger departments than a bacterial cell is highly convenient, because cavities of tens of micrometers can be easily produced by photolithographic techniques, and the observation of the dynamic processes is much simpler, well above the optical resolution limit.

Having observed oscillating Min patterns in rod-shaped cavities, we rationalized whether our approach could be applied to mimic Min patterns in filamentous cells that disclose multiple dynamic MinE rings (Figure 3 a).[5,6,24] To study the dependency of pattern formation on the compartment length the Min pattern in 45 μm long compartments,

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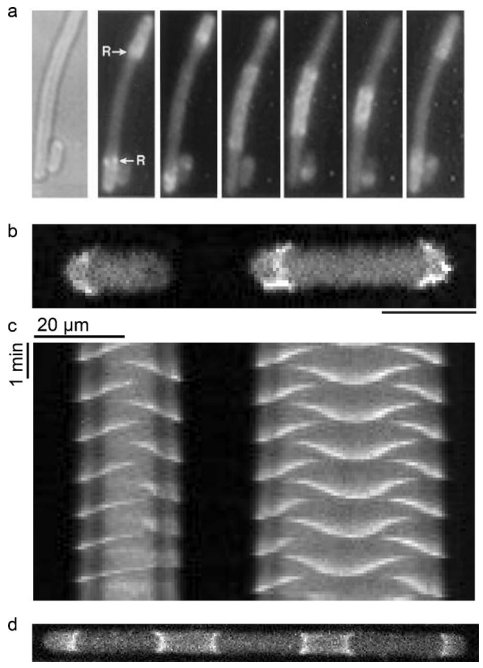

Figure 3. Min pattern formation is modulated by the length of the reaction compartment. a) Min protein pattern in filamentous *E. coli* cells. Reproduced from Ref. [5] (Copyright 2001, The National Academy of Sciences, U.S.A). b) Confocal image of Min protein patterns in a short and long microcavity. Both structures are 10 μm high and 10 μm wide. The cavity length is 25 μm and 45 μm, respectively c) Kymograph for MinE-Alexa488 along the long axis of the cavities in (b). Confocal image of Min protein patterns in a microcavity with a length of 195 μm. The structure is 10 μm high and 10 μm wide.

instead of the aforementioned 25 μm long compartments, were investigated. Pole-to-pole oscillations of MinE is still observed in some of the long compartments (see movie 2 in the Supporting Information). Intriguingly, however, the MinE ring patterns in many of the 45 μm long compartments indeed mimicked the patterns of filamentous cells: similar to short bacterial filaments, two distinct MinE regions were detected in the synthetic system (Figure 3b) and they repeatedly passed through the following sequence of steps. The Min regions moved towards the cavity poles and subsequently disassembled from the membrane and reassociated between the poles and the center. Then the fluorescent MinE regions moved in the opposite direction towards the cavity center, disassembled from the membrane, reassembled again between the center and the poles, and moved towards the poles of the cavity. The movement of MinE patterns thereby appears to be highly coordinated, with one MinE structure mirroring the other with respect to a symmetry plane in the center, perpendicular to the long axis of the cell. When compartments even longer than 45 μm were investigated, the percentage of patterns with one bright oscillating area was reduced, in favor of patterns with multiple bright areas. In extremely long cavities of 195 μm, we even observed dynamic patterns with up to four bright areas (Figure 3d). It seems clear from the different patterns obtained in short and long synthetic cavities (but otherwise under the same conditions) that the compartment length can modulate Min patterns. In line with studies in filamentous cells,[5,6,24] our data suggest that multiple Min rings in filamentous bacteria arise as a consequence of the length of the cells, and only geometrical cues are needed to account for the formation of multiple versus single mobile MinE rings.

To further elucidate the influence of the sample geometry on pattern formation we investigated Min patterns in round, that is, cylindrical PDMS cavities with a height of 10 μm and a diameter of 40 μm. If the directionality of the Min patterns is determined by the length axis of the reaction volume, no preferential direction of the Min protein movement should be detected in cylindrical cavities. As expected, no unique directionality of traveling Min patterns could be observed in such reaction volumes. We mainly observed three kinds of Min patterns in cylindrical cavities. First, oscillations with switching oscillation axes were detected (Figure 4). Second,

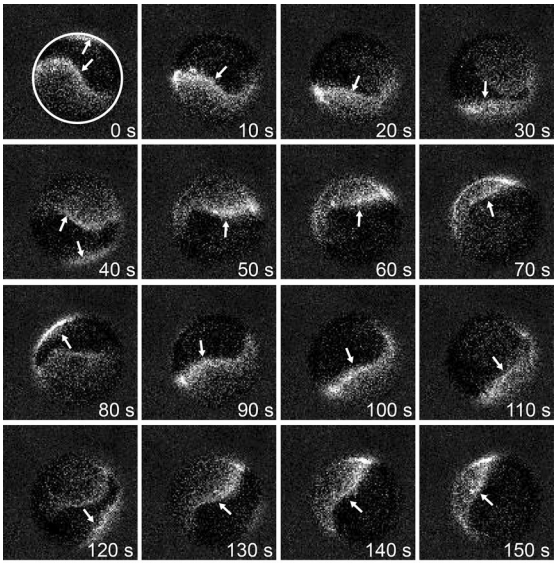

Figure 4. Self-organization of purified Min proteins in round cavities. 1 μM MinD and 1 μM MinE doped with 10% Alexa488-labeled MinE self-organize in cavities of 10 μm height and a diameter of 40 μm. The images were modified by subtracting the average intensity of time-lapse images, thereby removing nondynamic fluorescent background signals.

patterns moving around a circle were detected (see movie 3 in the Supporting Information). Finally, we observed oscillations along a fixed axis (see movie 4 in the Supporting Information); however, the angle distribution of cavities with a fixed axis seemed to be random, and we cannot exclude a switching of the axes after the acquisition times of time-lapse series. The findings in cylindrical artificial systems are in accordance with studies in spherical cells, in which Min proteins were reported to move along drifting or fixed axes or to perform circular sweeping motions.[18] We observed movements along one axis exclusively in all of the aforementioned elongated cavities. Therefore, our investigations of Min patterns in round and elongated cavities demonstrate that Min patterns can be produced in geometries without a long axis, but that elongated geometries result in high fidelity in movements along a fixed axis.

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In summary, we have shown that dynamic Min protein patterns can be modulated by geometrical cues in synthetic minimal systems composed of microstructured membrane supports, lipid bilayers, and purified Min proteins. The emergence of Min oscillations similar to those in wild-type, filamentous, and spherical cells was observed by adapting the size and geometry of the reaction volume. Our experimental results suggest that the cell shape of wild-type *E. coli* is optimal to give rise to oscillatory behavior, and provide evidence that variations in cell shape lead to variations in Min protein patterns and the occurrence of pace-making oscillations. Thus, although the geometry of *E. coli* membranes is rather simple compared with many other cells, cell shape seems to play a pivotal role in Min pattern formation and, therefore, in spatial and temporal regulation of cell division. We believe that our approach to investigating the role of cellular shape on Min pattern formation provides an attractive assay for studying spatial regulation during cell division and might open up new channels in the field of bottom-up synthetic biology.

Experimental Section

Protein purification and labeling: His-MinD and His-MinE were purified with an N-terminal His tag, as previously described in Ref. [17]. MinE was labeled with AlexaFluor488 C_5 maleimide (Molecular Probes) according to the manufacturer's instructions.

Microfabrication of PDMS devices: Photoresist patterns (ma-P 1275, micro resist technology GmbH) on top of Si wafers (Si-Mat, Kaufering) were produced by photolithography by using a chrome mask (Compugraphics Jena GmbH). The wafer with the resist structures was coated with chlorotrimethylsilane (Sigma–Aldrich). PDMS (Sylgard184, Dow Corning) was mixed at a monomer/cross-linker ratio of 9:1, degased in a vacuum, and cured on top of the wafer for three hours at 80 °C. The cured PDMS was carefully peeled off and stored at room temperature until further use. Before the microstructured PDMS was used as a membrane support, it was sonicated for 5 min in ethanol, washed with water, air dried, and treated with an oxygen plasma.

Supported lipid membranes (SLBs): SLBs were produced by using standard vesicle fusion techniques. *E. coli* polar lipid extract in chloroform was purchased from Avanti Polar Lipids. The lipids were dried under a nitrogen flow and placed in a vacuum for 30 min. Subsequently, the dried lipids were dissolved in buffer A (25 mM Tris-HCl pH 7.5, 150 mM KCl, 5 mM $MgCl_2$), incubated at 37 °C for 30 min, sonicated for 15 min, and applied to the PDMS support at a concentration of 0.5 mg mL^{-1}. 2.5 μM $CaCl_2$ was added to facilitate vesicle rupture. The vesicles were incubated for 20 min at 37 °C to form lipids membranes, which were subsequently washed with buffer A. For the experiments with Min proteins, 1 μM MinD, 1 mM MinE doped with 10% Alexa488-labeled MinE, and 2.5 mM ATP were added.

Microscopy: Image acquisition was performed on a ZEISS (Jena, Germany) LSM780 confocal laser scanning microscope equipped with a ZEISS Plan-APO 25x/NA 0.8 objective.

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